The Genetic Contribution to Muscular Strength and Power in Elite Rugby Athletes

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Collaborators

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Professor Liam Kilduff and his research team at Swansea University provided the Isometric Mid-Thigh Pull (IMTP) rig, the force plate and associated interface equipment that were originally used with the elite athletes (participants in this thesis) for the countermovement jump (CMJ) and IMTP tests. This same equipment was used to conduct the same aforementioned tests with the non-athlete participants of this thesis at Crewe campus of Manchester Metropolitan University. Dr Dan Cunningham who forms part of Professor Kilduff's team, took the responsibility for the familiarisation session with the equipment, and for extracting the strength/power measures from the IMTP and CMJ force-time histories produced by the non-athletes.

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Publications

Investigations in this thesis will be published in due course. Accordingly, this thesis will be embargoed for two years. Permission for embargo was obtained from the postgraduate department of the Graduate School at Manchester Metropolitan University.

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LIST OF ABBREVIATIONS

Abbreviation	**Definition**
ACE	angiotensin converting enzyme
ACE	angiotensin I converting enzyme gene
ACSA	anatomical cross-sectional area
ACTN2/3	alpha-actinin 2/3
ACTN3	alpha-actinin 3 gene
ADC	analogue to digital converter
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMPD1	adenosine monophosphate deaminase 1 gene
ATP	adenosine triphosphate
BMI	body mass index
BW	body weight
CG	centre of gravity
CMJ	counter movement jump
COD	change of direction
CSA	cross-sectional area
cM	centimorgan
DNA	deoxyribonucleic acid
DMTP	dynamic mid-thigh pull
FTO	alpha-ketoglutarate dependent deoxygenase gene
g	acceleration due to gravity
GPS	global positioning systems
GRF	ground reaction force
GWAS	genome wide association study
h^2	narrow-sense heritability
H^2	broad-sense heritability
HIF1A	hypoxia inducible factor 1 subunit alpha gene
HPTA	hypothalamic-pituitary-thyroid axis
HRST	high resistance strength training
H-W	Hardy-Weinberg (Hardy-Weinberg equilibrium)
Hz	herz
IMTP	isometric midthigh pull
IPF	isometric peak force
KDR	Kinase insert domain receptor gene
kg	kilogram
kN	kilo newtons
LD	linkage disequilibrium
LOD	logarithm of the odds
m	metre
MAF	minor allele frequency
min	minute/s
mL	millilitre
ms	millisecond
N	newtons
NO	nitric oxide
NOS3	nitric oxide synthase gene

eNOS	endothelial nitric oxide synthase
P	power
PRL	prolactin
PTK2	Protein Tyrosine Kinase 2 gene
QTL	quantitative trait locus
RAS	renin angiotensin system
PCSA	physiological cross-sectional area
RFD	rate of force development
RL	rugby league
RM	repetition maximum
rpm	revolutions per minute
rs	reference single nucleotide polymorphism
RU	rugby union
s	seconds
SD	standard deviation
SNP	single nucleotide polymorphism
TGS	total genotype score
TRHR	thyrotropin releasing hormone receptor gene
TSH	thyroid stimulating hormone
VGRF	vertical component of the ground reaction force
W	watts
WGS	whole-genome sequencing

ABSTRACT

Muscular strength and power are key determinants of performance in elite rugby and accordingly, established measures of strength/power have been used to assess and monitor athletes, inform training interventions and training prescription, and discriminate athletes between levels of competition. Multiple genetic variants are thought to influence strength/power; exerting a combined effect that in addition to environmental factors produces the inter-individual variation observed in diverse populations. To date, ~62 genetic variants have been found to influence strength/power phenotypes, however, how these genetic variants combine to affect strength/power phenotypes in athletic populations is unclear. Furthermore, there is a scarcity of investigations that have considered the exploration of genotype-phenotype associations in elite athletes – in particular, whether specific genetic variants have an influence on the athletes' strength/power, or on their performance during competition. Consequently, the overall aim of the current thesis was to characterise strength/power and the genetic characteristics of elite rugby athletes using established measures of strength/power and 10 single nucleotide polymorphisms, and investigate whether the 10 variants, individually and collectively, were associated with measures of strength/power and/or in-game performance variables. 567 elite rugby athletes and 1138 non-athletes were genotyped for 10 polymorphisms found in 9 genes (***ACE*** rs4341, ***ACTN3*** rs1815739, ***AMPD1*** rs17602729, ***FTO*** rs9939609, ***HIF1A*** rs11549465, ***NOS3*** rs2070744, ***KDR*** rs1870377, ***PTK2*** rs7460 and rs7843014, ***TRHR*** rs7832552) and data of isometric mid-thigh pull (IMTP) and countermovement jump (CMJ) were collected for 263 athletes and 14 non-athletes. Differences in CMJ and IMTP variables were observed between athletes and non-athletes and between playing positions, whilst polymorphisms within *ACTN3, FTO, HIF1A* and *TRHR* were found to be associated with athlete status, with the latter two also associated with playing position. In addition, polymorphisms within *NOS3* and *TRHR* were associated with IMTP whilst those within *FTO* and *PTK2* were associated with CMJ. In-game performance data were acquired for 291 athletes during eight seasons (2012–13 to 2019–20) of rugby union competition in the highest professional competitive leagues, and associations were observed between polymorphisms within *ACTN3* and *TRHR* and in-game variables, namely involvement in tackling, frequency of carries and the ability to gain territory. Furthermore, the polygenic influence of seven of the SNPs expressed as a Total Genotype Score was associated with involvement in tackling within the forwards. Accordingly, forwards possessing 8–9 or 10–14 favourable alleles were involved in 9.5% and 16.5% more tackles compared to those possessing 1–7 favourable alleles, respectively. In addition, backs possessing 9–14 favourable alleles gained 26.3% more territory than those possessing 1–8 favourable alleles. Most of these results identify novel genetic associations in an elite rugby context. In conclusion, there appears to be a genotype-dependent influence on strength/power phenotypes and competitive performance within elite rugby athletes that varies with positional roles. Further research is needed to replicate the associations observed in comparable and larger athletic cohorts. Nonetheless, the work presented here has added to our understanding of the genetic contribution to strength/power and competitive performance of elite rugby athletes, which when combined with physiological data, may have implications for management and performance enhancement of elite rugby athletes in future.

CHAPTER 1
LITERATURE REVIEW

LITERATURE REVIEW

1.1 Introduction

Muscular strength and power are multifactorial traits that show a Gaussian (continuous) variation in the population, which depends on environmental and genetic factors. The main factors which contribute to muscular strength and power (e.g. muscle mass; neuromuscular activation) and intermediate contributing factors (e.g. number of muscle fibres; fibre cross-sectional area; number of motor neurons), all demonstrate a continuous variation in the population, and are also accepted to be influenced by both the environment and genetic variation. Multiple genetic variants (polygenic in nature) are thought to influence sport- and exercise-related phenotypes, yet how the relevant polymorphisms combine to influence muscular strength and power in individuals and populations is unclear (Hughes et al., 2011).

Heritability estimates for strength- and power-related phenotypes such as leg strength (Zhai et al., 2004) and muscle fibre-type composition (Simoneau and Bouchard, 1995) have values congregating around the 50% value, with heritability for mesomorphy (Peeters et al., 2009) and lean mass (Hsu et al., 2005; Souren et al., 2008; Bogl et al., 2011) estimated at approximately 60–80%. Nevertheless, pioneers in the field of sports genomics agree that relatively little is known about the molecular variations in the DNA sequence and the mechanisms that contribute to the heritability of complex sport- and exercise-related phenotypes such as muscular strength and power. Consequently, an increasing focus of research in recent years was to take genetic research from the classical indirect approach to an era that uses a molecular genetic approach; by identifying the specific DNA sequence variations that contribute to sport- and exercise-related traits (Williams et al., 2014).

Hughes et al. (2011) identified 22 common genetic polymorphisms which were associated with either a strength and power performance phenotype such as hand grip strength and one repetition maximum (1RM), or a physiological attribute of strength and power such as fibre-type distribution. More recently, Ahmetov et al. (2016) demonstrated that the total number of genetic markers identified which contribute in some way to elite athlete status is ~155, with ~62 of these related to strength and power athlete status. Two studies conducted by Heffernan and colleagues as part of the ongoing RugbyGene project (Heffernan et al., 2015) investigated three polymorphisms found in three separate genes (*ACTN3* and *ACE* (Heffernan et al., 2016), and *FTO* (Heffernan et al., 2017)), with the overall aim of those studies serving as initial efforts in elucidating the genetic influence in an elite rugby context. These authors have investigated other polymorphisms (previously associated with soft tissue injuries and elite athlete status) in elite rugby athletes (Heffernan et al., 2017; Brazier et al., 2019), that could (indirectly) contribute to the exceptional performance characteristics (including strength and power) observed in elite athletes.

The scarcity of published literature that focuses on the genetics of elite athletes, combined with the potential of elucidating the genetic influence to strength and power in an elite athletic population such as rugby, where strength/power phenotypes have a major contribution to elite athletic performance, suggest further research efforts in this area are warranted. Since rugby union athletes perform under a well-defined set of rules and parameters which are ubiquitous across all playing positions, they present an ideal cohort via which to study the genetic contribution to complex phenotypes such as strength and power that are fundamental for elite athletic performance (Heffernan et al., 2015). Thus, as part of the ongoing RugbyGene project, this thesis aims to investigate the genetics of muscular strength and power in an elite rugby context. Elite rugby union athletes and non-athlete

controls were genotyped for ten polymorphisms (previously associated with strength and power phenotypes in the literature) found in nine separate genes. Measures of strength/power in the countermovement jump (CMJ) and isometric mid-thigh pull (IMTP), and in-game performance data were obtained from some of the athletes. This work aims to identify any associations that may exist between the ten polymorphisms (individually and collectively) and the established measures of strength and power (CMJ and IMTP), and between the polymorphisms and in-game performance data. The findings from this work can serve to (i) strengthen the body of literature that have shown a relationship between the variants investigated and strength and power, and between the variants and elite performance during rugby match-play (ii) ultimately combine the acquired genetic data with the more commonly used physiological data of elite rugby athletes, to aid athletes' monitoring and management, and talent identification in future.

1.2 Muscular Strength and Power

Human strength and power are complex phenomena that have generated a large body of scientific research concerning their various aspects in different areas of study and population groups. In sport, a plethora of scientific research have demonstrated the importance of specific strength and power characteristics (according to the sport concerned) in achieving success, especially at the highest levels of competition. As such, the requisite for scientific research that addresses the numerous lacunae in understanding the complexity of muscular strength and power in sport have increased dramatically. In particular, research efforts are required to identify novel genetic variations (in addition to those identified to date – whether common or rare variants) that influence the strength and power of high calibre athletes, and to understand the mechanisms by which these genetic variations exert their combined effect to

produce the exceptional strength and power characteristics observed in elite strength/power athletic populations.

The large body of scientific literature evidencing the importance of muscular strength and power capabilities in sport, may have (arguably) been the main factor in generating further interest in the strength and conditioning literature. To this end, several forms of the terms 'strength' (e.g. explosive strength) and 'power' (e.g. critical power; explosive power) have emerged in the literature; however, with inconsistencies in their definition and application. Over 30 years ago, Kroemer (1986) pointed out that different definitions, terms, measurement strategies, and exertion techniques used in the topic of 'human muscle strength' have caused incomplete, incompatible and even contradictory information (Kroemer, 1986). More recently, Knudson (2009) criticised the inconsistencies in the usage of terminology related to 'muscular performance variables' in the literature, focusing on the colloquial use of the term 'power' in that paper. Other authors (Moffroid and Kusiak, 1975; Knuttgen, 1978; Sapega and Drillings, 1983; Knuttgen and Kraemer, 1987; Harman, 1993; Cronin and Sleivert, 2005; Schilling et al., 2008; Winter et al., 2016) have also accentuated the inconsistency in the use of terminology related to muscular strength and power; attributing some of the flawed measurement methodologies and calculation errors that exist in the exercise science literature to this inconsistency.

Although it is not the aim of this thesis to critically review the scientific literature on the use of terminology related to muscular strength and power, the author believes that due to the germane of the terms 'strength' and 'power', clear workable definitions of the terms are of utmost importance for reference throughout this work. These are provided in the next Section

(Section 1.2.1), followed by a discussion related to the fundamental relationships that exist between strength and power (1.2.2).

1.2.1 Defining muscular strength and power

There is a general consensus in the contemporary scientific literature that 'strength' is the ability of exerting force, whilst 'power' is the rate of doing work. Various meanings, definitions and ideas of 'strength' have been proposed by pioneers in the scientific literature as from the early 19th century. On the other hand, 'power' has been more specifically defined (in the scientific literature) and accepted to be the ratio of work versus time. Nevertheless, the vague use of the terms 'strength', 'power' and related terminology have fostered misunderstandings, problems of interpretations, and even conflicting results in the literature. In addition, the concepts of 'strength' and 'power' have become dichotomised, in the sense that 'strength' is usually associated with movements at low speeds, and 'power' with movements at high speeds (Knudson, 2009; Maud and Foster, 2006). In fact, forces cause mechanical work when movement is present and, therefore, mechanical power flow is present in most human movements (Knudson, 2009; Enoka, 2008; Winter et al., 2016) at any given speed. If this fundamental concept were accepted in its entirety, understanding and defining the terms would be greatly simplified, and their application facilitated.

One early definition of strength was proposed by Steindler and Keene (1936), stating that 'strength is the maximum display of contractile power.' Their definition was used by Delorme and his co-workers (DeLorme, 1945; DeLorme et al., 1948) and adopted by many others during that era, although the authors (Steindler and Keene) have substituted one unexplained concept (the term 'power') for another. Strength was associated with a more general concept by Henry and Whitley (1960), who proposed the idea of 'strength in action.'

Another broad, however unusual concept of the term ‘strength’ was proposed by Jones (1974), who viewed strength and muscular endurance as the same quality.

A somewhat clearer definition of strength was proposed later by Clarke (1966), stating that ‘muscular strength is the tension that muscles can apply in a single muscular contraction.’ Although less ambiguous than the definition by Steindler and Keene (1936), the definition by Clarke (1966) initiated the idea that ‘strength’ refers to the internal tension generated by a muscle (or muscles); excluding any of the external forces impressed by a limb on an external resistance, and ignoring the fact that strength is an ability. Interestingly, this early definition by Clarke seems more adage to the term ‘muscle specific force,’ which is the force developed by a muscle per unit physiological cross-sectional area (PCSA), expressed in $N \cdot cm^{-2}$, with certain factors (e.g. agonist muscle activation and antagonist muscle co-activation; tendon moment arm length) taken into account for its estimation (Maughan et al., 1983; Erskine et al., 2009; Stebbings et al., 2014). The concept of ‘maximality of strength without movement’, emanated from the definition of Muller (1970), who stated that ‘strength is the maximum force that can be exerted against an immovable resistance by a single contraction.’ At that same period, Kroemer (1970) (considered a pioneer in human factors engineering) proposed a similar definition of strength by stating that ‘strength is the maximum force muscles can exert isometrically in a single voluntary effort.’ In that paper, Kroemer emphasised that strength refers to the muscular capacity to exert force under static conditions, and is measured as the external effect of internal isometric muscle efforts, modified by the mechanical advantages of the body members included (Kroemer, 1970). Atha (1981) concurred with the definitions of ‘strength’ by Muller (1970) and Kroemer (1970), however, he pointed out that the definition by Muller (1970) is incomplete as strength may be measured as a force, but it is not a force in itself (Atha, 1981). Enoka (1988) endorsed a definition of static strength (presented by Atha

(1981)), which states that ‘strength is the ability to develop force against an unyielding resistance in a single contraction of unlimited duration.

It is arguable that although the definitions of static (isometric) strength tend to simplify the meaning of ‘strength’ (potentially facilitating its measurement), they avoid consideration of the complex interaction of force development, and the velocity of concentric and eccentric muscle actions which are prevalent in human performance (Maud and Foster, 2006). Knuttgen and Kraemer (1987) provided a definition of strength that takes into account the dynamic nature of force exertion (even at relatively low speed movements), while capturing the intimate interrelation between muscular strength and power, stating that; ‘strength is the maximal force a muscle or muscle group can generate at a specified velocity’ (Knuttgen and Kraemer, 1987), as power is a direct mathematical function of the force generated and the speed of movement. However, of note here is that the importance of *quasi* static (or low speed) muscular actions of maximal (or near maximal) force exertions during sport should not be underestimated. For example, Quarrie and Wilson (2000) found that rugby props were able to apply a force of ~1420 N (equivalent to the force exerted on the ground by a person of ~144 kg body mass, without noticeable movement) each, for several seconds against a bespoke scrum machine (used for testing purposes in that study), in a bent-over position that is normally adopted during scrummaging (Quarrie and Wilson, 2000). The iron cross in gymnastics (a rings position in which the arms are stretched fully to the sides, supporting all the body weight while the performer’s body is vertical) requires extreme isometric strength of the upper body, including the main ‘core’ musculature (transversus abdominus; internal obliques; multifidus). Numerous other examples of the importance of isometric strength capacity can be given for judo, wrestling, etc. Furthermore, one must consider that many dynamic, high-speed movement skills in sport require the coactivation of several muscles

(requiring static or quasi static muscle actions) that serve to stabilise certain body segments to permit the execution of the skill. As such, for the purposes of the work presented in this thesis, reference will be made to the definitions proposed by Atha (1981) and Knuttgen and Kraemer (1987), for isometric strength and dynamic strength, respectively. Additionally, due to the requirement for the frequent use of the phrase 'muscular strength and power' throughout the thesis, 'strength and power' or 'strength/power' will sometimes be used interchangeably with the aforementioned whole phrase.

1.2.2 Relationship between strength and power

A plethora of scientific research have demonstrated the positive relationship that exists between various measures of muscular strength and power, and physical performance, in different settings and population groups. The general consensus is that; (i) an increased ability to maximise muscular power typically results in enhanced athletic performance (Kraemer and Newton, 2000; Newton and Kraemer, 1994; Baker, 2001; Sleivert and Taingahue, 2004; Young et al., 2005) and, (ii) the ability to generate force is a fundamental component of performance in most sports and other physically demanding activities (Fisher and Jensen, 1992; Komi et al., 1988; Cormie et al., 2011; Cormie et al., 2011). This implies that by increasing the magnitude of force application and/or speed of movement (e.g. as a result of specific training) in completing a particular manoeuvre (or skill) that is pertinent to performance, with a concurrent increase in power production, an increase in performance is likely observed. The manoeuvre may be the performance itself (e.g. snatch in weightlifting), part of it (e.g. deadlift as part of the or clean and jerk), or a movement for which a positive relationship with performance has been established (e.g. correlation between 1 repetition maximum (RM) squat and sprinting performance). Furthermore, the scientific literature demonstrated that stronger individuals are capable of producing higher power outputs (Baker

and Nance, 1999; Cormie et al., 2011; Cormie et al., 2011); reflecting the strong intra-individual relationship that exists between the ability to produce force and power production, as muscular power varies in direct proportion with the generated muscular force and speed of movement (or velocity when representing a vector quantity), although this latter contention is 'encapsulated' by the physiological limits and the interrelation between strength and speed, and the force-velocity relationship of skeletal muscle (Huxley, 1957; Sugi and Ohno, 2019; Alcazar et al., 2019).

In elucidating the complexity of the neuromuscular function in generating force and producing power during different skills, extensive research has focussed on fundamental principles of skeletal muscle physiology (e.g. modelling of the force-velocity relationship of skeletal muscle) and human mechanical output properties (e.g. determining peak mechanical power production during various skills). Since human movement is determined by the interaction between the forces generated by the muscles, and the exertion of such forces on the environment, movement is governed by the laws of physics whether at the cellular level (e.g. force generated by series/parallel sarcomeres; stiffness of muscle-tendon unit) or at the whole organism level as human mechanical output. As such, the application of Newton's laws of motion to this interaction (at any stage of force generation and transmission) can explain the relationship that exists between the ability of the neuromuscular system to generate force (strength) and the mechanical power output produced by the generated force being exerted on the environment. It is, therefore, unsurprising that an extensive body of scientific literature have documented a strong relationship between several measures of strength, and measures of power in different settings and athletic populations (Cormie et al., 2011; Cormie et al., 2011). In other words, these relationships just confirm the inherent nature of the two quantities (force and power flow), in that mechanical power cannot exist

without a force acting on an object and causing a movement; with the strength of correlation reflecting the collective intra-individual differences between the two measures for specific movements (or skills) (e.g. squat 1RM and sprinting speed) and population group, occurring due to differences in the force-velocity relationships for the two movements.

Different sports require that certain magnitudes of strength, speed, and power output are achieved to produce the required movements that constitute the skills that determine performance; the temporal magnitude and interrelation of which (strength; speed; power) depending primarily on the skill being executed, level of competition, and characteristics (physiological and biomechanical) of the performer. Empirical evidence supported by previous research demonstrated that the most important neuromuscular function in producing movements pertinent to sport performance is the ability to generate maximal muscular power (Newton and Kraemer, 1994; Kraemer and Newton, 2000; Baker, 2001; Sleivert and Taingahue, 2004; Young et al., 2005). From a practical perspective, maximal power represents the highest instantaneous power during a single movement performed with the goal of producing maximal velocity at take-off, release or impact (Newton and Kraemer, 1994; Kraemer and Newton, 2000). This encompasses generic movements such as sprinting, jumping, changing of direction, throwing, kicking and striking and, therefore, applies to the vast majority of sport (Cormie et al., 2011) where the trajectory of a projectile determines performance. This implies that on many occasions in sport the critical factor that determines performance is the final velocity of the object (e.g. horizontal velocity of the body at foot take-off from ground in sprinting; the shot velocity at the instant it leaves the hand for the shot-put event). According to Newton's second law of motion, the acceleration of the object (that determines the final velocity) is directly proportional to the resultant force acting on the object (in the direction of movement) divided by the mass of the object. Therefore, the

greater the force applied to the object and/or the longer the period of force application, the greater are the magnitudes of the acceleration experienced by the object, and the final velocity and momentum of the object; expressed mathematically by the impulse-momentum relationship. Of note is that by increasing the duration of application of a force of constant magnitude (resulting in constant acceleration) throughout a movement, the greater is the impulse and final velocity of the object, with a concurrent increase in the average power produced during that period of force application. It has been argued in some scientific reports (mainly by biomechanists) that impulse can better reflect the performance of various skills than maximum power output (Winter et al., 2016; Knudson, 2009), based on the fact that impulse reflects the magnitude and duration of force application throughout a skill, both of which contributing to the final velocity achieved by the object (or human body). However, due to the short period of force application (and short contact time that depends on the biomechanical characteristics of the skill) that is available during the performance of the aforementioned generic skills (for example), and the time taken to develop force during that period (that depends on the rate of force development), the rate of force development and the maximum force achieved are imperative for performance. Furthermore, the greater the magnitudes of the force and rate of force development, and the velocity achieved (due to an increase in the acceleration), the shorter becomes the contact time available for force application during certain skills. As such, the general consensus remains that the most important physiological attribute to sport performance is the ability to develop maximal muscular power at specific time epochs during the manoeuvre concerned.

Despite the importance of maximal power production in sport, at certain times the critical factor that determines performance is the ability to apply maximal or near maximal force during slow speed (e.g. level change in Olympic wrestling, used to force a reaction from

opponent; scrummaging in rugby) or quasi static (e.g. support position on parallel bars in gymnastics, with both hips flexed at 90 degrees and legs extended; supporting the loaded bar overhead with extended arms during the final stage of the clean and jerk in weightlifting) movements; during which maximal neuromuscular power is generally neither achieved or required. During these skills, the opponent (in wrestling and rugby), performer's body (in gymnastics) or the overhead load (in weightlifting) provide a relatively large force that opposes that produced by the performer and, therefore, the resultant movement is of low speed or *quasi* static. Of note is that the same athletes in the sport mentioned above (and other sport) that require an ability to apply relatively high forces at slow movement speeds, are also required (and able) to apply force at high movement speeds during other skills required in their respective sport. However, regardless of the magnitude of the force applied and speed of movement, external mechanical power (a scalar quantity) is produced during all movements at any speed, and is expressed mathematically as the product of two vector quantities – force and velocity.

1.3 Inter-Individual Variability of Muscular Strength and Power

Muscular strength and power are evaluated on a continuous scale of measurement; with their distributions in the normal population showing a Gaussian, or skewed, distribution – typical for quantitative multifactorial traits (Beunen and Thomis, 2004). An individual's strength generation and power production capabilities are influenced by a descending cascade of factors ranging from the control initiatives generated in the central nervous system, to the force application at the point of contact between a body segment and the environment; including the effects of the neurological, morphological, and hormonal factors in between (Enoka, 2008). It is unsurprising, therefore, given the complex interaction of these factors which affect strength and power, that a degree of inter-individual variability persists in otherwise homogenous population groups.

The inter-individual variability in strength and power phenotypes represents the spread of values produced by subjects within a sample population, and is dependent on environmental factors and the individual's genetic profile (Thomis et al., 1998; Beunen and Thomis, 2004; Tiainen et al., 2005). An extensive study by Silventoinen et al. (2008) reported handgrip strength in over one million 16- to 25-year old males. Force measurements recorded on the dynamometer were 50–999 N, exemplifying the large inter-individual variability in strength amongst a young non-athletic population (Silventoinen et al., 2008). Hubal et al. (2005) studied the response to a 12 week progressive strength training programme in 585 non-athletes (342 women; 243 men) and found that some individuals did not respond with any gain in muscle size (cross-sectional area of the biceps brachii measured by magnetic-resonance imaging) or strength (isometric maximum voluntary contraction (MVC)), and dynamic (1 repetition-maximum (RM)) of the elbow flexors, whereas others exhibited profound changes, increasing muscle size by 10 cm^2 and doubling their strength (Hubal et al.,

2005). The investigators have demonstrated that non-athletic men and women exhibit wide ranges of response to resistance training (Hubal et al., 2005).

Strength and power are influenced by gene-environment interaction, with training and nutrition being the main environmental factors that interact with the genome to affect muscular strength and power phenotypes (Roth, 2007). The mechanical load and tension imposed across a muscle generate signals (via a mechanoreceptor) that ultimately produce an adaptation that elicits changes in the structural and functional characteristics of the muscle (Baar and Wackerhage, 2014). More specifically, exercise generates signals that are sensed by sensor proteins, and conveyed and computed by signal transduction proteins; whilst adaption regulators regulate the transcription, translation (protein synthesis), protein degradation and other cellular functions such as the cell cycle (Burniston et al., 2014). Sections 1.3.1 and 1.3.2 provide a brief overview of the heritability of strength and power, and gene-environment interaction, respectively. Section 1.3.3 describes the signal transduction pathway of adaptation in response to strength training.

1.3.1 Heritability of strength and power

Heritability is the quantification of the phenotypic variation across a population that is attributable to genetic factors, and is formally defined as a ratio of variances (Roth and Wackerhage, 2014). More specifically, narrow-sense heritability (h^2) is the proportion of total phenotypic variance in a population, taken at a particular time or age, that is attributable to variation in additive genetic factors (the sum of the effect of each allele at all loci) (Visscher et al., 2008). On the other hand, broad-sense heritability (H^2) is the proportion of total phenotypic variance in a population that is attributable to total genetic factors (additive and

non-additive genetic factors; the latter including the interaction between alleles at the same locus or at different loci) (Visscher et al., 2008).

The genetic basis of muscular strength and power can be studied by two basic approaches: the unmeasured genotype approach (top-down), and the measured genotype approach (bottom-up) (Bouchard et al., 1997). In the absence of the measured genotype, inferences about the genetic influences on strength and power phenotypes are based on statistical analyses of the distributions of phenotypic measures in related individuals and families, and on the theoretical framework of biometrical genetics (Neale and Cardon, 1992). The classical twin study has been the most popular study design in estimating heritability. Previous twin studies have used data from monozygotic and dizygotic twins to identify both genetic and environmental factors unique to the individual and shared within families (Beunen and Thomis, 2004). Since monozygotic twins have an identical genetic background, it is expected that they display a higher intra-pair correlation in a phenotype that is under genetic control than dizygotic twins, who share ~50% of their genes (Roth and Wackerhage, 2014). More extended family studies have been used to elucidate the genetic, cultural transmission and maximum transmissibility of strength and power phenotypes (Beunen and Thomis, 2004).

Peeters et al. (2009) provided an overview of 38 studies that have examined the heritability of muscular strength phenotypes, and that have used family or sib-pair analyses to estimate heritability. Collectively, these studies have shown that the heritability of static, dynamic, and explosive strength (involving various populations and testing protocols) ranges from 14–83% (25 studies), 29–90% (5 studies) and 34–97% (8 studies), respectively (Peeters et al., 2009) – remarkably wide ranges. More recently, Zempo et al. (2017) conducted a systematic review of the literature (24 most relevant articles involving 330,902 participants for studies

conducted between 1976 and 2008) that have examined the heritability of strength and power phenotypes, and a meta-analysis to estimate the inheritance of the phenotypes (Zempo et al., 2017). The phenotypic subgroup (by type of measure, involving all subgroups) analysis of the heritability estimates using a total of 58 strength and power measurements showed that the heritability estimates of isometric grip strength, other isometric strength measures, isotonic strength, isokinetic strength, jumping ability and other measurements of muscular power were 0.56, 0.49, 0.49, 0.49, 0.55, and 0.51 respectively (Zempo et al., 2017) – within a much narrower range than some of the individual studies. The investigators reported a weighted mean for the heritability of strength and power that congregates around the 0.52 figure; and concluded that the overall heritability of strength and power, and the environmental contribution to the phenotypic variation in strength and power phenotypes are somewhat comparable (Zempo et al., 2017).

1.3.2 Gene-environment interactions that influence strength and power

Variance in muscular strength and power that is attributable to the environment depends on factors such as nutritional habits, level of habitual physical activity, nonheritable intrauterine influences, and various other lifestyle components and factors from the social and physical environment (Simoneau and Bouchard, 1995). These factors can be broadly categorised as shared environmental factors (e.g. common environmental factors affecting all family members, such as socioeconomic status and cultural activities) and unique environmental factors (e.g. differences in parental treatment, prenatal environment, and life events) (Roth, 2007). Environmental factors are known to interact with the genome to affect the strength and power abilities of individuals (Bouchard et al., 1990; Roth and Wackerhage, 2014).

Observations in anthropomorphic characteristics (which are easily visible even in homogeneous populations) led to the initiation of family-based investigations seeking to determine rough estimates of the genetic vs. environmental contributions to the variability in biological traits (Bouchard et al., 1997). With regards to muscular strength, there is evidence that >50% of the variation in baseline strength and lean body mass is heritable (Nguyen et al., 1998; Calvo et al., 2002). Subsequently, more quantitative studies that have examined individual differences in response to cardiorespiratory (Bouchard and Rankinen, 2001) and resistance (Hubal et al., 2005) exercise training have made their way into the literature; to quantify the variability in trainability (gene-training interaction) of these vital physiological phenotypes. For complex traits with a genetic basis such as strength and power, it is generally accepted that several to many genes likely act cumulatively and/or concurrently to influence the trait, with each gene individually explaining a small portion of the variance in the trait (Bray et al., 2011). A genetic influence can be either dominant or recessive: for a dominant influence only one allele copy is necessary, while for a recessive influence an allele must be present on both gene copies to exert an effect on the trait – when both alleles contribute equally to the trait, they are considered additive (Roth, 2007; Wackerhage, 2014). Importantly, beyond dominant and recessive influences, genetic factors may only influence a phenotype when in interaction with another gene or environmental factor (Roth and Thomis, 2011). Figure 1.1 shows important intermediary traits (also known as sub-phenotypes or endophenotypes (Roth, 2007)) that contribute to muscular strength and power. Each of these intermediary traits are complex traits (polygenic) which are also influenced by both genetic and environmental factors, and their interaction (Wackerhage, 2014). The influence of the genome on the trainability of strength (genotype-training interaction) is shown by the horizontal arrow in the lower part of Figure 1.1. From a conceptual point of view, the variance in training response (trainability) represents one of the most striking examples of a

genotype–environmental effect, in this case a genotype–training interaction effect (Roth, 2007; Sarzynski et al., 2011; Wackerhage, 2014).

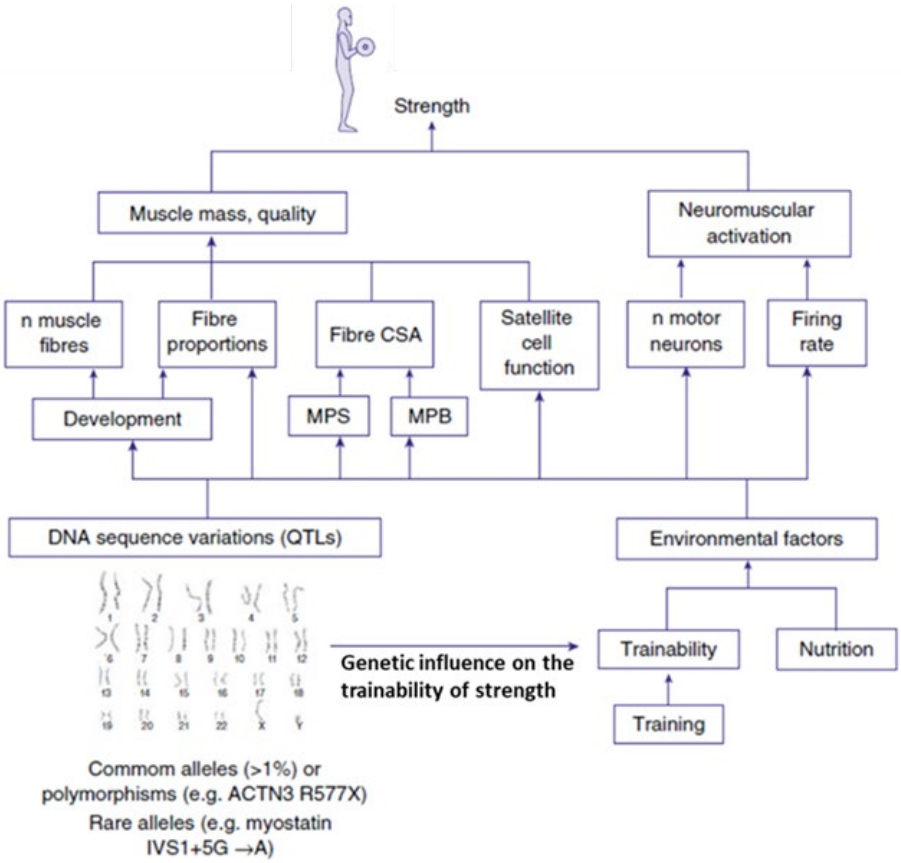


Figure 1.1 Genetic and environmental factors that contribute to muscular strength and power phenotypes. Note the horizontal arrow that represents the genetic influence on the trainability of strength. **CSA**: cross-sectional area; **MPS**: muscle protein synthesis; **MPB**: muscle protein breakdown; **QTLs**: quantitative trait loci. Adapted from (Wackerhage, 2014).

Although the role of genetic factors in the response to resistance training has not been examined extensively (Sarzynski et al., 2011; Wackerhage, 2014), it appears that this response is genetically determined (Barh and Ahmetov, 2019). An early study by Thibault et al. (1986) that involved five pairs of monozygotic twins submitted to a 10-week strength training program suggested that the response to strength training was independent of the genotype (Thibault et al., 1986). In contrast, Thomis et al. (1998) found significant gene-

training interactions for dynamic strength phenotypes in 25 pairs of monozygotic twins subjected to a 10-week resistance training program for the elbow flexors (Thomis et al., 1998). The results indicated that twin pairs responded similarly to strength training (F ratio: 3.5 for 1 RM increase with training; 1.83 for maximal isometric contraction at 110° response) (Thomis et al., 1998). In addition, model-fitting procedures indicated that about 20% of the variation in post-training 1 RM, isometric strength at 110° and concentric strength at $120°\cdot s^{-1}$ was explained by training-specific genetic factors that were independent from the genetic factors that explained variation in the pre-training phenotype (Thomis et al., 1998). It appears that there are separate genetic components affecting performance-related phenotypes at baseline in the untrained state, and in response to regular exercise (Sarzynski et al., 2011). More recent research efforts have been made to explore the associations between gene polymorphisms (e.g. *ACE* I/D (Charbonneau et al., 2008; Erskine et al., 2014), *ACTN3* R577X (Gentil et al., 2011; Pereira et al., 2013; Erskine et al., 2014), and *PTK2* (rs7460 and rs7843014) (Erskine et al., 2012)), and the response to resistance training.

Although there is substantial evidence for a role of genes in modulating the status of athletic performance determinants (Bouchard and Hoffman, 2011; Wackerhage, 2014; Barh and Ahmetov, 2019) (e.g. various strength and power phenotypes (Zempo et al., 2017)) in the untrained state, the elite athlete needs to be an obligatory, highly responsive individual to regular concurrent training protocols and practice (Jones et al., 2016). Therefore, the identification of potential elite athletes should be based on both performance in the untrained state, and on individual responsiveness to exercise training (Sarzynski et al., 2011). Determination of performance in the untrained state could involve measuring baseline performance in established assessments of vital traits that determine athletic performance. On the other hand, the potential of training response would most probably be more challenging to

measure and determine prior to the actual exercise training however, it could be the most potent tool for talent identification in future (Heffernan et al., 2015; Pitsiladis et al., 2016). In that eventuality, a panel of genetic markers for training response would be needed to determine which individuals are more likely to be high responders to training and thus likely to exhibit greater performance over time compared to low responders. Understanding the genomic determinants of the responsiveness to resistance training would allow for individualized approaches to exercise recommendations and prescription in an athletic context; and augment the training methods of talented and trainable individuals whose ultimate aim is to perform at the highest possible athletic level.

1.3.3 Signal transduction pathway for increased muscular strength

Human traits are determined by the selective utilisation of an individual's unique genotype upon exposure to environmental stimuli, such as exercise (Puthucheary et al., 2011). Resistance exercise is an excellent means to manipulate the size and force production of skeletal muscle in mammals (Widrick et al., 2002). It has been shown that resistance exercise increases skeletal muscle signalling and protein synthesis acutely and for up to several days after exercise (Miller et al., 2005), which is a phase duration of elevated protein synthesis that is much longer than that occurring after a meal (Bohé et al., 2001). There is evidence that protein synthesis responses after resistance exercise critically depend on the mammalian target of rapamycin (mTOR) pathway; an important anabolic signal transduction pathway that increases protein synthesis and muscle hypertrophy (Baar and Wackerhage, 2014).

Physiological adaptations are changes that occur in response to external factors such as exercise, nutrition, and a multitude of other environmental factors. At a whole organism level, inputs from the external environment are sensed by specific sensor organs (e.g. eye,

ear), and information is relayed via endocrine and nervous systems which act as specialized adaptation systems for the whole organism (Tiidus et al., 2012). However, cells also have an intrinsic ability to sense and adapt to changes in their local environment (Tiidus et al., 2012). This is elegantly illustrated by the specificity of resistance training, where hypertrophy of the muscle fibres recruited during training occurs in the absence of measurable effects in muscle fibres that were not recruited during exercise (Burniston et al., 2014); suggesting that exercise-induced muscle hypertrophy cannot be due only to systemic factors such as endocrine hormones. Conceptually, the intracellular events that link the acute responses to exercise with long-term adaptation involve three main steps: i) the sensing of exercise-related signals (sensor proteins detect changes in Ca^{2+}, AMP, glycogen, pO_2, amino acids, force, neurotransmitters and hormones), ii) signal transduction (proteins form pathways and networks that convey and compute the sensed input) and, iii) effector process (effector proteins regulate transcription, translation, protein degradation and other cellular functions such as the cell cycle) (Burniston et al., 2014). This results in changes in the concentrations of metabolic enzymes or contractile proteins (Tiidus et al., 2012) and thus, the function of cells and organs – the final step by which exercise changes the human organism via adaptation. Figure 1.2 illustrates a simplified general model of signal transduction of adaptation to exercise.

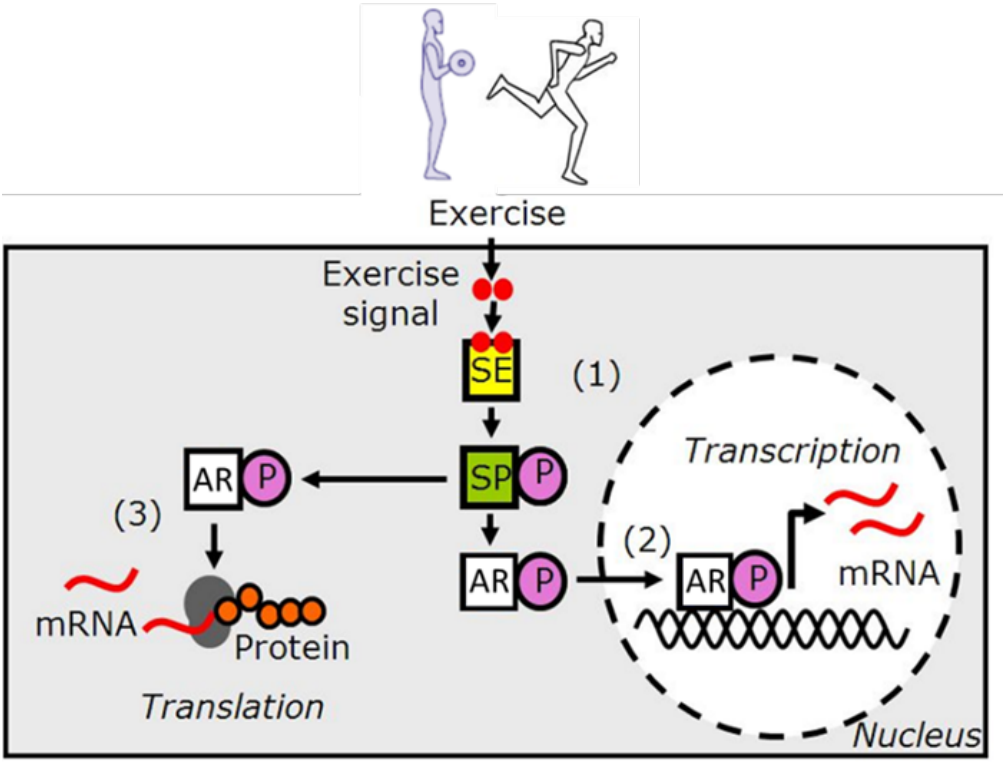


Figure 1.2 Schematical drawing depicting a simplified general model that explains adaptation to exercise. (1) Exercise leads to physical (e.g. tension) and chemical (change of the $Ca2^{+}$ concentration) changes which regulate the activity of sensor proteins (**SE**). Sensor proteins then regulate the activity of signalling proteins (**SP**) by phosphorylation and other modifications. Regulation of adaptation regulators (**AR**) by signal transduction pathways can be; (2) activation or inhibition of transcription factors and changes in gene expression, (3) the regulation of translation regulators which up- or down-regulate translation or protein synthesis and (4) the regulation of protein breakdown by various mechanisms. Adapted from Burniston et al. (2014).

Important to note is that the poorly understood mechanoreceptor that senses muscle mechanical loading and links it to protein synthesis after resistance exercise (Hornberger, 2011) is one of the most important receptors from the point of view of exercise physiology, in particular with regards to resistance training. After sensing exercise signals such as mechanical loading, signal transduction involves the computation of the signals detected by sensor proteins. The sensed information is conveyed and computed using several mechanisms that include protein-protein interactions (binding between proteins and other molecules that allows information to be conveyed from one protein to the next), protein modifications

(covalent modifications that include phosphorylation, acetylation, glycosylation etc., change the shape of the protein and thus, their activity), translocation (movement of signal transduction proteins between the membrane, cytoplasm, nucleus, organelles and from outside to inside the cell and vice versa allow signals to be transported through space), and synthesis and degradation (changes in the concentration of signalling proteins can amplify or terminate intracellular signals) (Tiidus et al., 2012; Burniston et al., 2014).

Protein-protein interactions are essential in signal transduction as proteins need to be in contact with each other so that they can modify one another and convey information (Tiidus et al., 2012). Further, many cellular functions rely on multimeric protein complexes, and mTOR is such a protein complex (serine/threonine kinase) that is a key regulator of ribosomal translation implicated in nutrition (Hamilton et al., 2014) and exercise-induced protein synthesis (Burniston et al., 2014). mTOR increases protein synthesis (the translation of mRNA into protein by the ribosome) and ribosome biogenesis (the capacity of a cell for protein synthesis), and in some cells inhibits a form of protein breakdown termed autophagy (Baar and Wackerhage, 2014). mTOR got its name from the fact that it is selectively inhibited by the macrolide antibiotic rapamycin (Davies et al., 2000), and its pathway is considered as the central node within our muscles that integrates multiple inputs and determines the degree to which protein synthesis and thus, muscle hypertrophy occur (Baar and Wackerhage, 2014). The regulation of muscle protein synthesis by the mTOR pathway is illustrated in Figure 1.3.

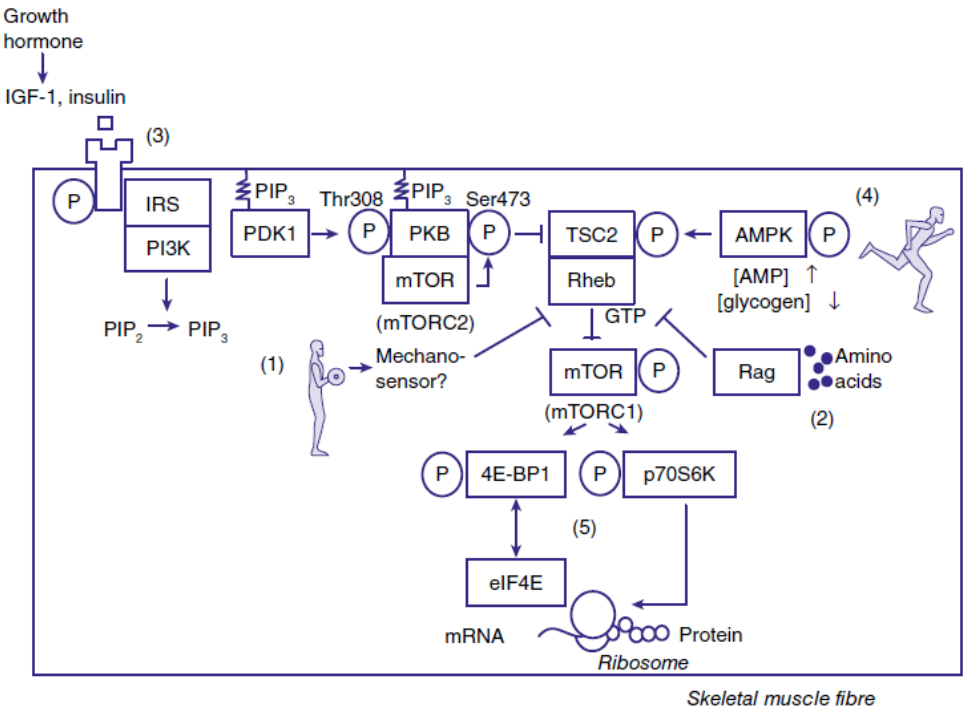


Figure 1.3 Regulation of muscle protein synthesis by the mTOR pathway. Model of the regulation of mammalian target of rapamycin complex 1 (mTORC1) by (1) resistance exercise, (2) amino acids, (3) growth hormone via IGF-1, muscle secreted IGF-1 and insulin, and (4) endurance exercise via AMPK. (5) Active mTORC1 phosphorylates 4E-BP1 and p70S6k, and 4E-BP1 detaches from eIF4E which increases protein synthesis (translation).

mTORC2: mammalian target of rapamycin complex 2; **Rheb**: Ras homologue enriched in brain; **IRS**: insulin receptor substrates; **Rag**: regulator-rag complex; **PI3K**: phosphoinositide 3-kinase; **PDK1**: phosphoinositide-dependent kinase-1; **PKB**: protein kinase B; **TSC2**: Tuberous Sclerosis Complex 2; **AMP**: adenosine monophosphate; **AMPK**: AMP-activated protein kinase; **4E-BP1**: Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 is a member of a family of translation repressor proteins (Qin et al., 2016); **p70S6K**: Ribosomal protein S6 kinase (serine/threonine) beta-1 (Chung et al., 1994); **EIF4E**: Eukaryotic translation initiation factor 4E (Pelletier et al., 1991). Adapted from Baar and Wackerhage (2014).

The mTOR pathway was first identified as being activated by resistance exercise in rats (Baar and Esser, 1999), who demonstrated that the magnitude of muscle hypertrophy following six weeks of exercise training was directly related to the degree of phosphorylation of p70S6K1 which is a target of mTOR (Baar and Esser, 1999). Drummond et al. (2009) showed that those volunteers who received 12 mg of rapamycin before training (11 sets of 10 repetitions each of leg extensions at 70% 1 RM) did not show an increase in muscle protein synthesis after exercise, whereas those who got a saline injection have shown the expected classic

increase in protein synthesis (Drummond et al., 2009). Further, Goodman et al. (2010) demonstrated that activating mTOR on its own was enough to cause muscle hypertrophy. The researchers injected DNA into muscle to genetically activate mTOR and removed the muscles after seven days. The muscle fibres in which they had activated mTOR had grown by ~40%, whereas a control injection had no effect on muscle size (Goodman et al., 2010). Collectively, the evidence shows that when muscles are mechanically loaded mTOR is activated, and that the activation of mTOR is sufficient and required for load-induced skeletal muscle hypertrophy (Baar and Wackerhage, 2014; Gehlert et al., 2015).

From the perspective of resistance exercise, mTORC1 is the centre of the mTOR pathway (Figure 1.3). There are other independent ways that activate mTORC1 however, all of them end with mTOR being activated by a small G-protein (Rheb), as illustrated in Figure 1.3. Growth factors such as IGF-1 and insulin activate mTOR via a cascade involving the IGF-1 or insulin receptor, insulin receptor substrates (IRS), PI3K, PDK1/mTORC2, PKB/Akt, PRAS40 (proline-rich Akt substrate of 40 kDa), TSC2 and Rheb (Coleman et al., 1995; Bodine et al., 2001). Amino acids activate mTOR via the Rag small G-proteins and the regulator, that bring mTOR to Rheb and thereby activate mTOR (Kim and Guan, 2009). With regards to resistance exercise, mechanical loading of muscle activates mTOR via a mechanoreceptor that inhibits TSC2, resulting in the activation of Rheb and thus, mTOR (Baar and Wackerhage, 2014). Important to note is that endurance exercise, possibly through high AMP or NAD^+ and low glycogen, can potentially inhibit mTOR activation in part through AMPK, TSC2 and Rheb (Inoki et al., 2006); ensuring that muscle is not built when vital energy is needed to fuel endurance exercise (Figure 1.3).

Subsequently, activated mTORC1 regulates several cellular functions, with the most important of these being the increase of protein synthesis after resistance exercise. Proteins are synthesized by the ribosome – an organelle made from ribosomal RNAs and 79 proteins (Tiidus et al., 2012). mTORC1 signalling activates translation via the phosphorylation of the regulatory proteins 4E-BP1 (Baar and Esser, 1999) and p70S6K (Dreyer et al., 2006) (Figure 1.3). Phosphorylation of 4E-BP1 detaches it from eIF4E (Holz et al., 2005), and this allows the ribosome to bind to the mRNA to initiate translation. Importantly, mTORC1-dependent signalling also regulates the movement of the ribosome along the mRNA (translation elongation) which results in the synthesis of a peptide chain on the basis of the mRNA blueprint (Baar and Wackerhage, 2014). The phosphorylation of several of the activity-related proteins of the mTOR pathway, specifically TSC-2 (Inoki et al., 2006), mTOR (Kubica et al., 2005), p70S6k (Baar and Esser, 1999) and 4E-BP1 (Dreyer et al., 2006), is observed in the acute phase following resistance exercise. In addition, the magnitude of initial mTOR-related signalling after resistance exercise correlates with increased protein synthesis and skeletal muscle hypertrophy (Baar and Esser, 1999; Terzis et al., 2008). Consequently, the phosphorylation pattern of activity-related sites of the mTOR network is frequently investigated to assess the efficiency of various resistance exercise types and resistance exercise determinants in inducing early states of skeletal muscle adaptation (Tannerstedt et al., 2009; Camera et al., 2010).

1.4 Muscular Strength and Power in Elite Rugby

Elite rugby athletes possess a high performance ability that reflects their highly developed physical, physiological, and psychological characteristics. These athletes are able to produce the various skills required at the highest level of competition, while tolerating training of high volume and intensity. Performance in rugby depends on complex traits (e.g. maximal aerobic and anaerobic power; muscular strength and power; speed; agility) (Duthie et al., 2006; Brooks and Kemp, 2008; Mellalieu et al., 2008) that, in addition to being influenced by the environment (the most obvious being training and diet), are known to have a genetic component (Heffernan et al., 2015) (Section 1.3).

Rugby union and rugby league are characterized as high intensity intermittent collision sports, requiring athletes to perform repeated running actions, collisions, and static efforts of differing work to rest periods (Duthie et al., 2003; Gabbett et al., 2008). Successful performance in rugby is dependent, in part, on well-developed physiological capacities, that allow players to exhibit high levels of skill under pressure and fatigue (Gabbett, 2002). Accordingly, the superior playing performance of elite level rugby players (in comparison to their sub-elite counterparts) is attributed to the greater physiological capacities of these athletes (Gabbett, 2005). Rugby performance requires both generic athletic skills (e.g. sprinting; change of direction) and specific rugby skills (e.g. high intensity exertion during a scrum; tackling) (Meir et al., 1993; Brewer and Davis, 1995; Duthie, 2006; Brooks and Kemp, 2008; Gabbett et al., 2008; Mellalieu et al., 2008); that require high magnitudes of muscular force and velocity. Thus, muscular strength and power are crucial physiological traits in rugby, as the abilities to generate high levels of muscular force and to produce high mechanical power output are fundamental to enable rugby athletes to perform more

effectively during tackling, lifting, and pushing and pulling tasks that occur during match play in both rugby league (Gabbett et al., 2008) and rugby union (Duthie et al., 2003).

This Section provides an overview of the role of strength and power in elite rugby and highlights key literature that addresses the role of strength and power in rugby performance. Rugby league has many similar rules and movement patterns to rugby union; however, unlike rugby union, rugby league does not have line-outs, rucks and mauls, and involves 13 players (6 forwards and 7 backs) per team (15 players in rugby union – 8 forwards and 7 backs) and an immediate play-the-ball after each tackle (Gabbett, 2005; Gabbett et al., 2008). Due to the many similarities between the two codes of rugby in terms of physical characteristics, movement patterns and rules (Brazier et al., 2020), reference will be made to relevant key literature within the two codes.

1.4.1 Requirements of elite rugby athletes

Muscular strength expressed in both absolute terms (regardless of body mass) and relative to body mass is critical to success in rugby. The strength and power training practices of contemporary elite rugby athletes are dictated, in part, by the strength and power requirements of the modern game for the different positional groups. There is substantial evidence showing that the athletes' strength and power abilities (and other vital physiological qualities required in rugby) have increased over time (reviewed by Brazier et al. (2020)) and are of highest magnitudes in contemporary elite rugby players. Consequently, increases in performance at the elite level were observed, and in turn, it appears that the physical and physiological requirements for the contemporary game increased in concert. The literature that quantifies these requirements of elite rugby and the influence of training interventions on the athletes' physical and physiological status can serve to gain a greater understanding of the

physiology of rugby, and potentially prescribe more effectively for their players (Jones et al., 2016). Notably, there is sparse literature that provides detailed information for the prescription of long term (e.g. preseason, in-season or a full year) strength and power training programmes for elite rugby athletes and thus, the evaluation and interpretation of the available literature is important for serving coaches and sport scientists involved in elite rugby.

One way for quantifying the strength and power requirements of contemporary elite rugby is by measuring specific strength and/or power qualities of the athletes using established assessments normally employed in the elite athletic environment. Maximal strength is a frequently assessed strength quality that is expressed by the one-repetition maximum (1 RM) using specific exercises such as the bench press and back squat. Both exercises are probably the most frequently used for this purpose within both rugby league (Meir, 1994; Meir et al., 2001; Fernandes et al., 2019; Daniels et al., 2019) and rugby union (Smart et al., 2013; Smart et al., 2014). The bench press and back squat possess high reliability in experienced athletes (Daniels et al., 2019) and offer appropriate measurements of muscle strength (coefficient of variation (CV) = 2.9–4.5%) (Weakley et al., 2017). Data reported for English and Australian professional rugby league players for the 1 RM bench press (mean (SD)) were 123 (11.8) kg for the forwards and 114 (17.0) kg for the backs (Meir et al., 2001). Similarly for upper body strength but in elite rugby union Crewther et al. (2009) reported a 1 RM bench press of 143.6 (10.9) kg for the forwards and 130.6 (17.9) kg for the backs (Crewther et al., 2009). More recently, Smart et al. (2013) reported that rugby union props (from amateur, semiprofessional, and professional competitive standards) display the highest 1 RM bench press (133 (24.0) kg) compared to other playing positions in rugby union (Smart et al., 2013). Recently, Daniels et al. (2019) reported a 1 RM bench press of 130 (17.0) kg in a squad of

professional rugby league players after a 7-week preseason training period (Daniels et al., 2019), whilst Fernandes et al. (2019) reported a 1 RM bench press of 135.2 (16.2) kg for an entire squad of first grade Super League rugby league plays (Fernandes et al., 2019). Regarding maximal strength in the back squat using free weights (barbell and weight plates, but not smith machine), data for a full squad of elite English rugby league players were 170.6 (21.4) kg before an 8-week strength and power intervention during the preseason, and 200.8 (19,0) kg after the intervention (Comfort et al., 2012). Data for 1 RM squat reported by Smart et al. (2014) in rugby union props of mixed competitive standard was 184 (34) kg. More recently, Fernandes et al. (2019) reported a 1 RM back squat (predicted from 3 RM back squat (Baechle and Earle, 2008)) of 183.3 (20.6) kg for an entire squad of first grade Super League rugby league plays (Fernandes et al., 2019). Interestingly, Daniels et al. (2019) reported a 3 RM back squat of 199 (25.0) kg in a squad of professional rugby league players after a 7-week training intervention (Daniels et al., 2019), indicating the trend for increased strength in contemporary elite rugby league athletes.

The most common method for assessing or estimating lower body muscular power in rugby players is via jump height (Gabbett, 2000; Gabbett, 2002; Gabbett, 2006; Gabbett et al., 2011). Although jump height is not a direct power measurement, it is widely used to estimate peak power from jump squats (Sayers et al., 1999; Baker, 2002; Maud and Foster, 2006; Baker and Newton, 2008). Within both rugby codes, backs generally produce greater vertical jump performances compared to forwards (Duthie et al., 2003; Gabbett, 2002) which is, in part, likely due to the backs' lower body mass (Gabbett, 2000; Gabbett, 2006; Darrall-Jones et al., 2015; Brazier et al., 2020; Stoop et al., 2019) rather than their higher peak power production. Notably, lower body power output has been negatively associated with sprint times over 5 m, 10 m, and 30 m (Cronin and Hansen, 2005), and positively associated with

dominant tackles during tackling (Gabbett et al., 2011) in elite rugby league players. In addition, muscular power data from jump squats (average power output) assessments can discriminate between competitive levels in rugby league with elite players achieving significantly higher results (1897 (306) W) than semi-professional players (1,701 (187) W) (Baker and Newton, 2008). This corresponds with previous reports that have demonstrated increases in muscular power as competitive standard increased (Gabbett, 2002; Gabbett, 2002). Similar findings but in rugby union have been reported by Argus et al. (2012) with elite athletes producing greater absolute peak power output than semiprofessional and academy players in the jump squat using a smith machine (professional 5,240 (670) W; semi-professional 4,880 (660) W; academy 4,430 (950) W) (Argus et al., 2012). Regarding upper body power the same authors reported higher absolute peak power also in the bench press throw for the elite athletes than semi-professional and academy standard athletes (professional 1,140 (220) W; semi-professional 880 (90) W; academy 800 (110) W) (Argus et al., 2012). The bench press throw is a commonly used assessment to achieve power-related data of the upper body and have been used previously with professional rugby league players. Baker (2002) reported an average mechanical power output (during the concentric phase of a bench press throw against a resistance of 20 kg) of 341 (24) W, 316 (32) W and 283 (20) W for national league professional, college aged and senior high school rugby players, respectively. Important to note is that due to methodological differences between studies, the results presented here should be interpreted with caution in that they should be used only as normative data achieved using specific methods of assessment with rugby cohorts.

1.4.2 Discriminative ability to distinguish competitive standard

The first evidence of the importance of strength and power for rugby performance comes from research that have investigated the discriminative ability of strength and power

measures to distinguish playing standard. For example, muscular strength (1 repetition maximum (RM) bench press) and power (bench press throw and jump squat) have been shown to be potent discriminators of competition level in rugby league with differences found between elite professionals, college aged, and high school (senior, junior, and non-trained juniors) players that were aligned to the same club (Baker, 2002). In addition, Baker and Newton (2008) found that the full squat (considered a measurement of strength) and the jump squat (considered a measurement of power) were better discriminators of which players were in the elite first division national rugby league or second division state rugby league squads; than acceleration in the 10-m sprint, maximal speed in the 40-m sprint, or in a 40-m agility test (Baker and Newton, 2008). More recently, Baker (2017) found that strength, as measured by the 1RM bench press and 1 RM full squat could distinguish between players from the same professional rugby league club as those who attained selection and played in the team that won the grand final of the National rugby league competition, and the remaining as those who did not attain selection (Baker, 2017). Furthermore, differences in lower-body strength and power parameters (derived from the force-time and power-time curves of a loaded countermovement jump squat) were able to discriminate between playing level in elite senior and junior rugby union players (Hansen et al., 2011); and differences in sub-maximal strength have also been observed between playing standards in adolescent rugby union players, with academy players performing better than school players in the 3 RM bench press and 3 RM neutral grip pull-up (Jones et al., 2018). Most recently, Fernandes et al. (2019) compared the load-velocity and load-power relationships (using the bench press and squat with external loads of 20, 40, 60 and 80 kg) among first grade (n = 26, age 22.9 ± 4.3 years), academy (n = 23, age 17.1 ± 1.0 years), and scholarship (n = 16, age 15.4 ± 0.5 years) Super League rugby league players. The authors concluded that peak velocity and power are key physical qualities to be developed that enable progression from junior elite

rugby league to first grade level. Notably, first grade players also demonstrated greater absolute and relative upper- and lower-body strength than academy players which in turn, were stronger than scholarship players (Fernandes et al., 2019). The authors suggested that resistance training should emphasize both maximal strength and velocity components to optimise upper- and lower-body power in professional rugby players (Fernandes et al., 2019).

1.4.3 Role of strength and power for tackling ability in rugby

Due, in part, to the large number of injuries that occur during tackles and collisions (Gabbett et al., 2010; King et al., 2011; King et al., 2012), the tackle contest has received considerable attention in rugby. Additionally, the ability to control and dominate the tackle contest is considered as one of the most important components for success in rugby (Gabbett and Kelly, 2007; Van Rooyen et al., 2014) and this might have contributed to an increased research interest on tackling performance. A greater understanding of the factors that limit tackling performance is considered as critical, from both an injury-prevention and a performance perspective (Gabbett, 2009). Superior rugby performance is dependent, in part, on the ability to tolerate physical collisions, and the skill to 'win' the tackle contest in both rugby league (Gabbett and Ryan, 2009; Austin et al., 2011) and rugby union (Hendricks et al., 2014; Hendricks et al., 2014). Accordingly, it appears that successful teams are involved in fewer 'ineffective' tackles than unsuccessful teams (Van Rooyen et al., 2014), and this may contribute to fewer metres conceded in defense by successful teams (Gabbett, 2014). Furthermore, Gabbett and Ryan (2009) have shown that higher-skilled tacklers make a greater proportion of dominant tackles and miss a smaller proportion of tackles than lesser-skilled tacklers (Gabbett and Ryan, 2009).

The significance of a high physical or physiological ability is reduced if the physiological parameter does not transfer to superior playing performance. For example, an increase in lower body muscular power is redundant unless the enhanced physiological capacity transfers to improved leg drive in tackles, a greater play the-ball speed following a tackle, or to exhibit a high level of a skill (that requires high lower body muscular power) while fatigued (Gabbett et al., 2011). Thus, the strongest evidence of the importance of strength and power in rugby could, arguably, be provided by investigations that found a significant relationship between a strength and/or power derived parameter and a key performance indicator in rugby. With regards to the role of strength and power for tackling proficiency, Gabbett et al. (2010) have shown that the ability to produce high muscular power was required to provide leg drive in tackles, whilst leg driving was a key tackler characteristic associated with positive tackle outcome in rugby union (Hendricks et al., 2014). Gabbett et al. (2010) have investigated the relationship between some physiological (acceleration in 10-m sprint, change of direction speed in 505 test, and lower body muscular power in the vertical jump) and anthropometric (stature, body mass, and sum of 7 skinfolds) qualities, and tackling ability under fatigued conditions in junior elite and sub-elite rugby league players. Interestingly, the strongest individual correlates of tackling ability were acceleration and lower body muscular power (Gabbett et al., 2010), while there were no significant relationships between tackling ability under fatigued conditions and any other physical quality. These findings provide the evidence that lower-body strength protects against fatigue-induced decrements in tackling ability in rugby players. Gabbett et al. (2011) found that lower-body power as measured by the vertical jump test was positively associated with the number of tackle attempts and tackles completed, and negatively associated with the proportion of missed tackles in high-performance rugby league players that were selected to play in the National rugby league. In addition, Gabbett et al. (2011) have shown that lower body power (also estimated using a vertical jump test) was a

main contributor to tackling proficiency in rugby league players. More recently, Gabbett (2016) found that semi-professional rugby league players with greater relative lower body strength as measured by the 4 RM squat had the best tackling ability under fatigued conditions, and concluded that the development of lower-body strength should be a priority to facilitate the development of robust tackling skills that are maintained under fatigue (Gabbett, 2016). Furthermore, Speranza et al. (2016) found that training-induced improvements in lower-body strength were also associated with improvements in tackling ability. More recently, Speranza et al. (2017) found that upper- and lower-body strength (using the bench press and squat, respectively) were significantly related to success in the tackle contest.

1.4.4 Role of strength and power for sprinting ability in rugby

Sprinting ability is a fundamental component of performance and success for both rugby league (Gabbett, 2002; Gabbett, 2002) and rugby union (Wheeler et al., 2010; Austin et al., 2011). Speed and acceleration (the rate of change of velocity) are the fundamental components of sprinting ability, with running speed over short distances considered important for success in team sport (Baker and Nance, 1999; Sayers, 2000). In rugby, players need to accelerate over short distances or accelerate and sprint to make position (Cunniffe et al., 2009) by varying their acceleration and/or sprint movement patterns in terms of change of direction, to achieve the required ball carriage and opponent avoidance outcomes (Duthie et al., 2006). Notably, during competition rugby union players rarely cover the necessary distance to achieve top speed (West et al., 2013) and therefore, the ability to accelerate is considered more fundamental to success than maximum velocity (Delecluse et al., 1995; Cronin and Hansen, 2005). However, despite acceleration predominating, there is also a need to develop maximal velocity so that a player has a higher endpoint of acceleration (Duthie, 2006). Moreover, time–motion analysis has demonstrated that players regularly achieve more

than 90% of maximal velocity during rugby union competition (Duthie et al., 2006). Collectively, these factors make sprinting in rugby match-play qualitatively and quantitatively different from straight-line track sprinting (Sayers, 2000) and thus, it appears that rugby players require specific strength training and preparation to improve both their acceleration and maximal sprinting speed (West et al., 2013; Seitz et al., 2015).

Lower body strength is often considered of utmost importance for sprinting ability in rugby players (Barr et al., 2014), and accordingly, improving lower body strength relative to body mass (lower body relative strength), and decreasing ground contact time have been suggested as ways of improving the sprinting speed of athletes (Stone et al., 2007; Weyand et al., 2010; Mann, 2015). The rationale for this improvement is that decreasing ground contact time (foot- to-ground contact period), particularly at maximal sprint speed, is considered the most important kinematic change for improving sprinting (Weyand et al., 2010; Barr et al., 2014), and this can be achieved by increasing the force applied by the foot to the ground to achieve the relatively shorter contact time (Weyand et al., 2010; Mann, 2015). Notably, ground contact times are relatively much longer during initial and mid-acceleration phases when compared with contact times at maximal velocity (Barr et al., 2013), however, relatively lower ground contact times are also important for high velocities during all the acceleration phases of a sprint (Lockie et al., 2011). Collectively, these findings imply that the ability to develop force quickly, a characteristic of the most powerful athletes (Tillin et al., 2013), can potentially be advantageous for sprint performance due to the achievement of the shorter ground contact times throughout all phases of the sprint. Important to note is that acceleration and maximal sprint velocity are considered as different entities (Barr et al., 2013; Cunningham et al., 2013), mainly due to the different ground contact times typical throughout all the phases of a sprint. Mann (2015) reported that the vertical velocity of the center of

gravity changes from -0.5 to 0.5 $m \cdot s^{-1}$ during the maximal velocity sprinting stride, and maintaining this vertical velocity of the center of gravity while at the same time decreasing ground contact time would require an increase in the average force production (Weyand et al., 2010; Mann, 2015). Thus, all else (e.g. maximal velocity stride length, and flight time) being equal, a rugby player with a body mass of 100 kg who is able to shorten the ground contact time from 0.12 to 0.10 seconds must hypothetically increase the average vertical force during foot- to-ground contact from 1,814 N to 1,981 N (an increase of 14.7 %) to attain a vertical velocity of the center of gravity of 0.5 $m \cdot s^{-1}$ in between each stride (Mann, 2015). This would hypothetically increase the athlete's sprint speed from 9.2 to 10 $m \cdot s^{-1}$ – a change in magnitude that would mean an improvement from average to exceptional for a professional rugby player (Duthie et al., 2006; Higham et al., 2013). However, there is evidence showing that this scenario is not always realistic with elite rugby athletes that have an extensive strength and power training background (Barr et al., 2014) due, in part, to the principle of diminishing returns (Haskell, 1994); meaning that certain strength qualities (e.g. maximal strength, relative strength (1 RM/body mass), and strength deficit [the difference between the force produced at 1RM and any other force value achieved against lighter relative loads (Suchomel et al., 2018; Badillo, 2017)] may no longer be trainable in a manner that leads to transfer for sprinting performance in experienced athletes (Barr et al., 2014). On the other hand, the relatively less experienced rugby players have better chances to improve the different speed qualities (acceleration phases and maximal sprint velocity) to meliorate sprinting performance by means of specific resistance exercise training that targets the different strength qualities required for reducing the typical ground contact times during the acceleration phases (initial, mid-acceleration, and later stages of acceleration) and maximum velocity of a sprint (Barr et al., 2014).

1.5 Training for Strength and Power in Elite Rugby

Increased professionalism in rugby has resulted in national unions developing high-performance models for elite player development of which physical preparation is an important component to ensure the athletes' success in future years. Competition movement patterns and athletes' physical profiles are generally used as the basis of athlete development frameworks to reinforce the repeated high-intensity nature of rugby (Duthie, 2006). Training programming is implemented within the yearly plan, with the basic training principles of overload, specificity, reversibility, and progression implemented via a periodized approach through each phase of the season (Gamble, 2004). Generally, there is a progressive increase in training load during the preseason phase, with a main purpose of loading endurance, resistance, and speed training to have players near peak condition at the start of the season (Duthie, 2006). Subsequently, training is modified to maintain fitness during competition while accommodating match load and related fatigue (Duthie, 2006; Johnston et al., 2015; Tavares et al., 2017; Owens et al., 2019). During the off-season, training is focused on minimizing reversibility in fitness levels, and have players prepared for the pre-season training load.

There is a general agreement that elite rugby is challenging to support because in contrast to many other sports, rugby requires differing and, in some cases, contrasting physical qualities for successful performance (Till et al., 2016; Owen et al., 2020; Watkins et al., 2021). This is mainly due to the differing movement requirements between positional roles and the various physical qualities (e.g. aerobic power; aerobic endurance; muscular strength) required by players from different playing positions (Brazier et al., 2020). For example, whilst previous research has indicated that both absolute and relative strength and power are important physical qualities in elite rugby (Loturco et al., 2021; Watkins et al., 2021; Redman et al.,

2021), players may be required to cover ~6 km (with large variations between positional groups and competition levels for both codes) during a competitive rugby union (Quarrie et al., 2013) and rugby league (Twist et al., 2014) match, respectively. Thus, elite rugby athletes require highly developed physical qualities such as aerobic, fatigue resistance capabilities, and strength (Brazier et al., 2020) which are challenging to develop concurrently (Jones et al., 2016; Weaving et al., 2020). This may present coaches and sport scientists with problems when programming training, as the responses to strength and power training can be 'muted' as a result of endurance type stimulus (Hickson, 1980; Kraemer et al., 1995; Häkkinen et al., 2003; Jones et al., 2013) (Section 1.3). Accordingly, the main challenge is to develop a base of endurance or aerobic fitness, together with anaerobic and strength qualities such that players can attain and reproduce high levels of work output during the repeated high-intensity efforts of the match (Duthie, 2006). Important to note is that the inhibited strength development or 'interference effect' (Hickson, 1980) associated with concurrent training warrants particular consideration during training phases such as pre-season, in which coaches have limited time to develop strength and power phenotypes of their athletes (Duthie, 2006). Jones et al. (2016) explored (via questionnaires) some elements associated with concurrent training practices used by strength and conditioning coaches and sport scientists who worked with international level and/or professional rugby union athletes. Forty-three (41 men, 2 women; age: 33.1 ± 5.3 years) of 52 (83%) responded to the questionnaire and believed that strength training benefits rugby performance and thus; strength training was regularly prescribed to, and performed by their athletes. In addition, 38 coaches (88%) reported they have used periodization strategies, and 42 (98%) conducted physical testing. Interestingly, 33 (77%) indicated they considered the potential interference effect associated with concurrent strength and aerobic training when programming for their athletes (Jones et al., 2016).

The literature quantifying the physical demands and the influence of conditioning interventions in elite rugby has allowed sport scientists to gain a greater understanding of the physiology of rugby. For example, research has examined methods that are commonly implemented in the elite athletic environment to develop specific neuromuscular qualities that underpin strength and power (Cormie et al., 2011; Cormie et al., 2011). In addition, researchers have examined the influence of standardized and controlled conditioning interventions (Bevan et al., 2009; Bevan et al., 2010; West et al., 2013) and the influence of pre-performance strategies such as post-activation potentiation (Bevan et al., 2010; Kilduff et al., 2007) and hormonal priming (Gaviglio et al., 2014) on muscle performance phenotypes that are vital to rugby performance. Of particular consideration is that according to the law of diminished returns, developing strength and power in elite athletes can be progressively harder to achieve (Pyke, 1980; Cormie et al., 2011). Therefore, the identification of training strategies that can effectively and efficiently improve muscular strength and power of experienced athletes is of critical importance for coaches and sport scientists working in elite rugby. The following Sections provides details on the physiology of post-activation potentiation (PAP) and the use of PAP within elite rugby. If effectively utilized, PAP could be implemented into a strength and power training programme to enhance the training stimulus of a ballistic (e.g. plyometric) exercise (Robbins, 2005; Docherty and Hodgson, 2007). In addition, inducing PAP prior to competition might also prove better than conventional warm-up techniques at enhancing performance of explosive sports activities such as jumping, throwing and sprinting (Güllich and Schmidtbleicher, 1996), which are vital qualities to rugby performance.

1.5.1 Post-activation potentiation (PAP)

Sport scientists and coaches in elite sports are constantly seeking to use innovative and advanced training strategies to efficiently improve the strength and power in already highly trained athletes. For over two decades there has been considerable interest and research into the functional significance of post-activation potentiation (PAP) on sport performance. The interest has evolved around the potential for enhancing acute performance or the long term training effect, typically in the form of complex training (Docherty and Hodgson, 2007). Complex training is a training strategy that involves the execution of a heavy resistance exercise prior to performing a ballistic movement with similar biomechanical characteristics (Hodgson et al., 2005; Freitas et al., 2017). This mode of training is based on the physiological condition referred to as PAP, which is defined as an acute enhancement of muscle function after a PAP stimulus (Hodgson et al., 2005); the latter commonly referred to as 'conditioning activity' (Sale, 2004) or 'conditioning contraction' (Tillin and Bishop, 2009) in the literature. The presence of PAP in skeletal muscle has been recorded by many studies in both mammals (Manning and Stull, 1982; Moore and Stull, 1984) and humans (Stuart et al., 1988), prompting a discussion amongst review articles over the mechanisms that elicit PAP (Sale, 2002; Hodgson et al., 2005; Robbins, 2005), and the potential application of PAP to sports performance (Hodgson et al., 2005; Docherty and Hodgson, 2007). Indeed, the identification of the precise physiological mechanisms mediating alterations in force production after a PAP stimulus can be utilised to promote the development of innovative strategies that are effective in optimising training methods and performance in elite rugby.

It is well-established that the contractile history of a muscle has a profound effect on the muscle's ability to generate force (Docherty and Hodgson, 2007). Accordingly, the response of muscle to volitional or electrically induced stimuli is affected by its contractile history,

with neuromuscular fatigue being the most obvious effect (Sale, 2002). Neuromuscular fatigue is defined as the force decrement observed after a period of repeated muscle activation (Rassier and Macintosh, 2000), and is reflected by the inability of a muscle to generate an expected level of force (Hodgson et al., 2005). In contrast to the effects of neuromuscular fatigue and the associated impairment of force production, the contractile history of skeletal muscle may facilitate the volitional production of the force the muscle produces – with the phenomenon known as PAP (Sale, 2002). Thus, the response of a muscle following some form of contractile activity is the net balance between simultaneous processes: those causing fatigue and those resulting in potentiation (Hodgson et al., 2005). The measured force output following contractile activity may therefore, reflect the net balance between the processes that enhance force development and those that diminish it (Vandenboom et al., 1993). Although fatigue and potentiation have opposing effects on force production, these mechanisms can coexist (Rassier and Macintosh, 2000). It can also be said that the coexistence of fatigue and PAP may result in a net potentiated state, a net attenuated state or a constant state of the muscle's capacity to produce force; as compared to its capacity at the pre-stimulus stage (Robbins, 2005).

Previous research have applied the principles of PAP to short-term motor performance, whilst more recently, numerous studies have used PAP as a rationale for producing long-term neuromuscular changes through complex training – a training mode that requires an individual to work against a heavy load (e.g. 1 set of 5 RM back squat) followed by a lighter load such as body mass (e.g. 4–6 countermovement jumps using only body mass (Gourgoulis et al., 2003)). This exercise combination (commonly referred to as a complex pair in the literature (Docherty et al., 2004)) is repeated for a number of sets, and is postulated that over a period of time it will produce long-term positive changes in the ability of a muscle to

generate force and power (Docherty and Hodgson, 2007). Further, it has been suggested that the performance of a heavy pre-load exercise used to potentiate a muscle (e.g. via mechanisms that underpin PAP) may also be used to enhance short-term performance and possibly, this strategy could be integrated into the warm-up prior to competition that requires strength/power (Güllich and Schmidtbleicher, 1996; Docherty and Hodgson, 2007). The findings from previous studies that have examined the short-term effects of PAP on subsequent performance were equivocal. The discrepancies among the results of previous studies were due, in part, to differences in design and methodology; with particular reference to the mode and intensity of the high resistance exercise, the length of the rest interval within and between the complex pairs, the type of ballistic activity, the participants' training history, and the nature of the examined dependent variables (Docherty and Hodgson, 2007; Cuenca-Fernández et al., 2017). In addition, it is important to note that numerous studies (both previous and more recent) that have examined PAP have not included any measures (e.g. muscle twitch response or H-reflex (see below)); to determine whether the muscles of interest were in a potentiated state via the classical mechanisms of PAP (Cuenca-Fernández et al., 2017; Prieske et al., 2020). This is despite the fact that such measures were used to measure neuromuscular output and to quantify the acute effect of previous activation history on subsequent force production in previous research on PAP (Hodgson et al., 2005; Tillin and Bishop, 2009). Therefore, the inconsistencies of research that have examined PAP can be attributed to both the complex interaction of factors that influence acute performance following a conditioning stimulus (Sale, 2002; Robbins, 2005; Docherty and Hodgson, 2007; MacIntosh, 2010), and to the methodological inconsistencies such as those that failed to measure the acute potentiated state of muscle (Blazevich and Babault, 2019); but have attributed the observed performance enhancement (referred to as post-activation performance

enhancement (PAPE) in recent literature (Cuenca-Fernández et al., 2017; Blazevich and Babault, 2019; Prieske et al., 2020)) to the physiological mechanisms that underpin PAP.

It has been proposed that two main principal mechanisms are responsible for PAP. The first is the phosphorylation of myosin regulatory light chains (Hamada et al., 2000; Chiu et al., 2003; Sale, 2004; Baudry and Duchateau, 2007), and the second is an increase in the recruitment of higher order motor units (Güllich and Schmidtbleicher, 1996; Chiu et al., 2003; Hodgson et al., 2005; Tillin and Bishop, 2009). There is also evidence suggesting that changes in pennation angle may contribute to PAP (Tillin and Bishop, 2009).

1.5.2 Myogenic mechanisms of PAP - phosphorylation of regulatory light chains

A muscle twitch is a brief muscular contraction that occurs in response to one presynaptic action potential or one synchronised volley of action potentials (Latash, 2008). Twitch contraction force is increased following: (i) a sustained maximal voluntary contraction (MVC) (Gossen and Sale, 2000; Hamada et al., 2000; Vandervoort et al., 1983); (ii) an evoked tetanic contraction (O'Leary et al., 1997); or (iii) repeated sub-fusion stimuli (MacIntosh and Willis, 2000). In addition to enhancing peak twitch force, these preceding forms of contractile conditioning have been shown to increase the rate of force development (RFD) in a twitch response and subsequently, decrease its time to peak force (Sale, 2002; Grange et al., 1993). This effect, known as twitch potentiation, is a well-established and reproducible phenomenon (Hodgson et al., 2005), although its functional relevance to the enhancement of neuromuscular performance in humans is less clear. Figure 1.4 illustrates an example of post-activation potentiation (PAP). Note the increased twitch force and its shortened time course (right of diagram) after the PAP 'conditioning stimulus' or 'conditioning contraction' (left of diagram).

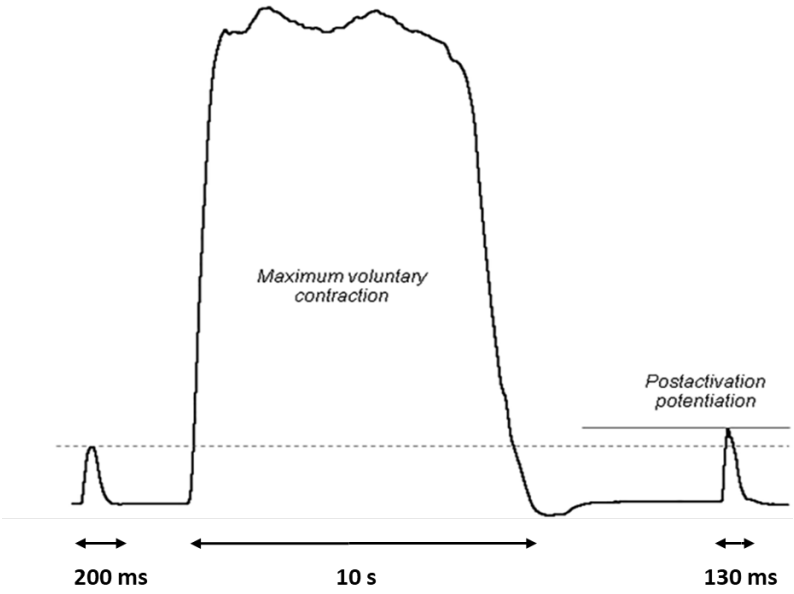


Figure 1.4 Example of post-activation potentiation (PAP). A baseline twitch is evoked in a muscle that has been at rest for a period of time. After the twitch, a conditioning contraction such as an electrically evoked tetanic contraction or a MVC is executed. A twitch contraction evoked soon after the conditioning contraction shows the increased force and shortened time course typical of PAP. The increment from baseline twitch peak force (dashed line) to post-contraction twitch peak force (solid line) corresponds to the extent of PAP. For an example of actual twitch recordings see Hamada et al. (2000). Adapted from Sale (2002).

One proposed mechanism of twitch potentiation is the phosphorylation of myosin regulatory light chains via myosin light chain kinase, which theoretically renders actin-myosin interaction more sensitive to released Ca^{2+} from the sarcoplasmic reticulum (Sweeney et al., 1993). A myosin molecule is a hexamer composed of two heavy chains (Szczesna-Cordary, 2003), with the amino termini of each heavy chain (classified as the myosin head) containing two regulatory light chains (Vandenboom et al., 1993; Szczesna-Cordary, 2003) (see Figure 1.5). Each regulatory light chain has a specific binding site for incorporation of a phosphate molecule (Tillin and Bishop, 2009). Regulatory light chain phosphorylation is catalyzed by the enzyme myosin light chain kinase, which is activated when Ca^{2+} molecules (released from the sarcoplasmic reticulum during muscular contraction) bind to the calcium regulatory

protein calmodulin (Manning and Stull, 1982; Szczesna et al., 2002; Szczesna-Cordary, 2003). The activated myosin light-chain kinase phosphorylates a specific portion of the S-1 myosin head near its hinge region with the S-2 component (Hodgson et al., 2005). It is suggested that phosphate binding induces a structural or conformational alteration in this portion of the myosin molecule that leads to an increased rate by which myosin cross-bridges move from a non-force producing state to a force producing state (Grange et al., 1993; Sweeney et al., 1993).

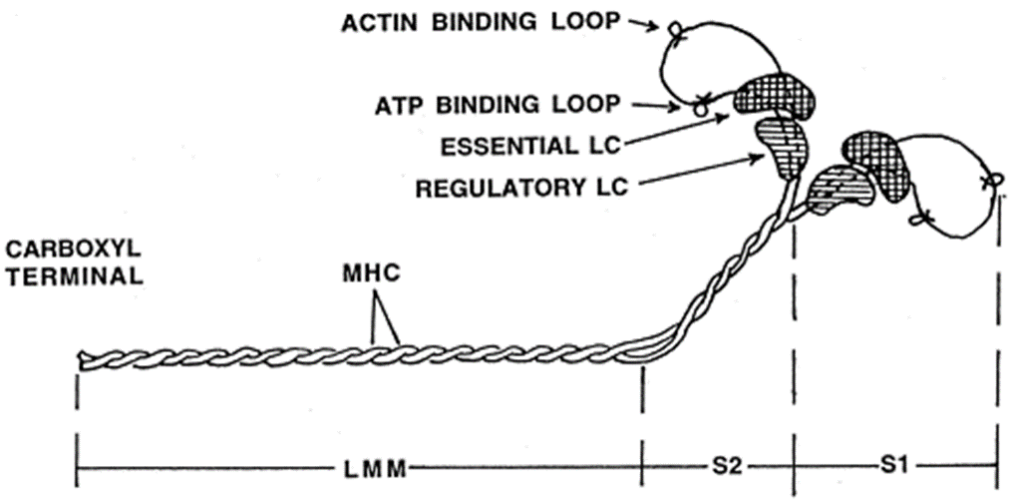


Figure 1.5 One myosin molecule, with each myosin molecule composed of two myosin heavy chains. Regulatory light chain (LC) represents a pair of regulatory light chains positioned at the neck of a myosin head. Each regulatory light chain can incorporate a phosphate molecule, altering the structure of the myosin head. At each myosin head there is an actin and adenosine triphosphate (ATP) binding site. Adapted from Eddinger (1998).

When myoplasmic Ca^{2+} levels are relatively low, such as is the case during a twitch and low frequency tetanic contractions, the increased Ca^{2+} sensitivity exerts its greatest effect however; when Ca^{2+} levels are saturated as during those levels associated with high frequency tetanic contractions, increased Ca^{2+} sensitivity has no observable effect (Vandenboom et al., 1993; Abbate et al., 2000). Thus, it is important to recognize that the

type of muscle contraction (after the conditioning stimulus) affects both the force-frequency relation and the range of frequencies over which PAP occurs post-stimulus (Sale, 2002). Thus, with respect to the force-frequency relationship, twitch potentiation increases the force and rate of force development (RFD) of low frequency tetanic isometric contractions. On the other hand, it appears that twitch potentiation does not increase peak force of high frequency tetani however, it enhances only RFD (Vandenboom et al., 1993). Specifically, in concentric contractions, especially those at higher velocities, the force-frequency relation is shifted to the right compared with isometric contractions and thus, higher frequencies are needed to evoke a given percentage of maximum force (Abbate et al., 2000). Further, there is evidence that PAP extends to higher frequencies in concentric versus isometric contractions (Abbate et al., 2000). Of note is that most activities such as swimming, rowing, and cycling involve primarily concentric contractions, whilst running, jumping, and weightlifting involve coupled eccentric-concentric contractions (Enoka, 2008). Therefore, the performance-enhancing effect of PAP may extend beyond of what would be expected based on its effect on isometric contractions (Sale, 2002). Figure 1.6 shows the interaction between the force-frequency relation, PAP, and contraction type. Note the potentially greater role of PAP in concentric contractions compared with isometric contractions.

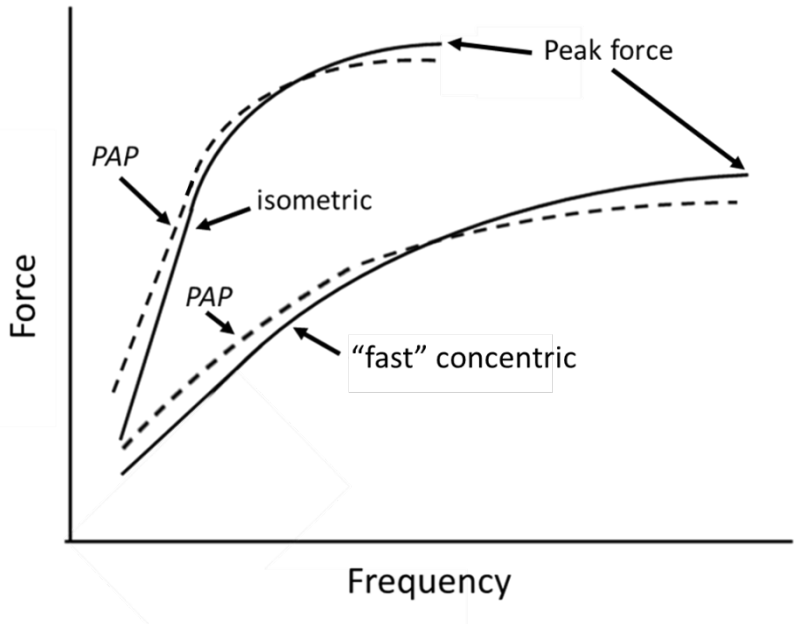


Figure 1.6 Effect of contraction type on the force-frequency relation and range of frequency over which PAP extends. An isometric and a "fast" concentric contraction condition are compared. A higher frequency is required to attain the plateau or peak force (solid lines) in the concentric contraction. Also, PAP induced by a conditioning activity (dashed line) extends to a higher frequency in the concentric contraction. Note that the maximum isometric force is greater than the maximum concentric force, in accordance with the force-velocity relation. Also, in this example, fatigue produced by the conditioning activity caused a decrease in high frequency force (see also Abbate et al. (2000)). Adapted from Sale (2002).

When the contraction conditioning that induces a twitch potentiation is a voluntary contraction, the magnitude of twitch potentiation is dependent on the duration and intensity of voluntary effort (Vandervoort et al., 1983) and muscle fibre type (Vandervoort and McComas, 1983; Hamada et al., 2000). The twitch potentiation in human plantar flexor and tibialis anterior following maximal voluntary isometric contractions were found to be maximized after contractions of ~10 s in duration, after which the effect on potentiation was partially suppressed by fatigue (Vandervoort et al., 1983). The authors found that maximal (vs. submaximal) voluntary contractions lasting ~10 s induce the greatest twitch potentiation (Vandervoort et al., 1983). With respect to the intensity of the PAP stimulus, voluntary contractions that were <75% of maximal voluntary contraction (MVC) produced minimal or no potentiation (Vandervoort et al., 1983).

Another important and notable feature of PAP is that the induced twitch potentiation is greater in fast, type II muscle fibres because fast fibres undergo greater phosphorylation of myosin regulatory light chains in response to previous contractile conditioning (Sweeney et al., 1993). Accordingly, the extent of twitch potentiation in response to tetanic stimuli was significantly greater in the human gastrocnemius muscle (which exhibits a higher percentage of type II fibres) than in the soleus (Vandervoort and McComas, 1983). In addition, a study that examined the correlation between fibre type distribution and twitch potentiation in human knee extensor muscles demonstrated that individuals with shorter twitch contraction times and a higher percentage of type II fibres showed greater twitch potentiation (Hamada et al., 2000). Of note is that an individual's fiber-type distribution is determined primarily by genetic factors (Simoneau and Bouchard, 1995), but may also be influenced by age and activity level. Specifically, Simoneau and Bouchard (1995) discovered that the genetic component for the proportion of type I fibres in human muscles is of the order of 40–50%, indicating that muscle fibre type composition is determined by genetic factors and environmental factors (Simoneau and Bouchard, 1995). These findings shed light on the importance of genetic factors for the potential role of PAP and complex training in enhancing strength and power performance. In light of these findings, it is reasonable to expect that PAP offers the greatest potential for performance enhancement in brief, maximal intensity activities requiring maximal strength, speed, and power (the product of strength and speed) that depend on fast type II muscle fibres. However, in these activities, motor unit firing rates are likely to be at their highest, in the very range where PAP's effect on force (attributable to phosphorylation of regulatory light chains) is negligible or absent (Sale, 2002). Thus, taken in this light, it appears that greater PAP in fast type II fibres cannot be utilised to enhance performance and therefore, it would be better for slow type I muscle fibres to have greater PAP because they are typically involved in low-intensity activities in which motor unit firing

rates are relatively low - the range in which PAP is greatest (Sale, 2002). However, previous research has shown that PAP can potentially increase the recruitment of higher order motor units and thus, this effect might be most pronounced in fast type II muscle fibres. This additional effect that involves the central nervous system has been proposed as potentially beneficial in enhancing strength and power performance, during which motor units are firing at high rates.

1.5.3 Neural mechanisms of PAP - increased recruitment of higher order motor units

Evidence from research on animals has shown that an induced tetanic isometric contraction (evoked by stimulating specific afferent neural fibres, which in turn activate adjacent α-motoneurons via an afferent neural volley; Figure 1.7), increases the transmittance of excitation potentials across synaptic junctions at the spinal cord (Güllich and Schmidtbleicher, 1996). This accommodating state of elevated transmittance can last for several minutes following the tetanic isometric contraction (Güllich and Schmidtbleicher, 1996). Consequently, there is an increase in post-synaptic potentials for the same pre-synaptic potential during subsequent activity (Gossard et al., 1994; Lüscher et al., 1983).

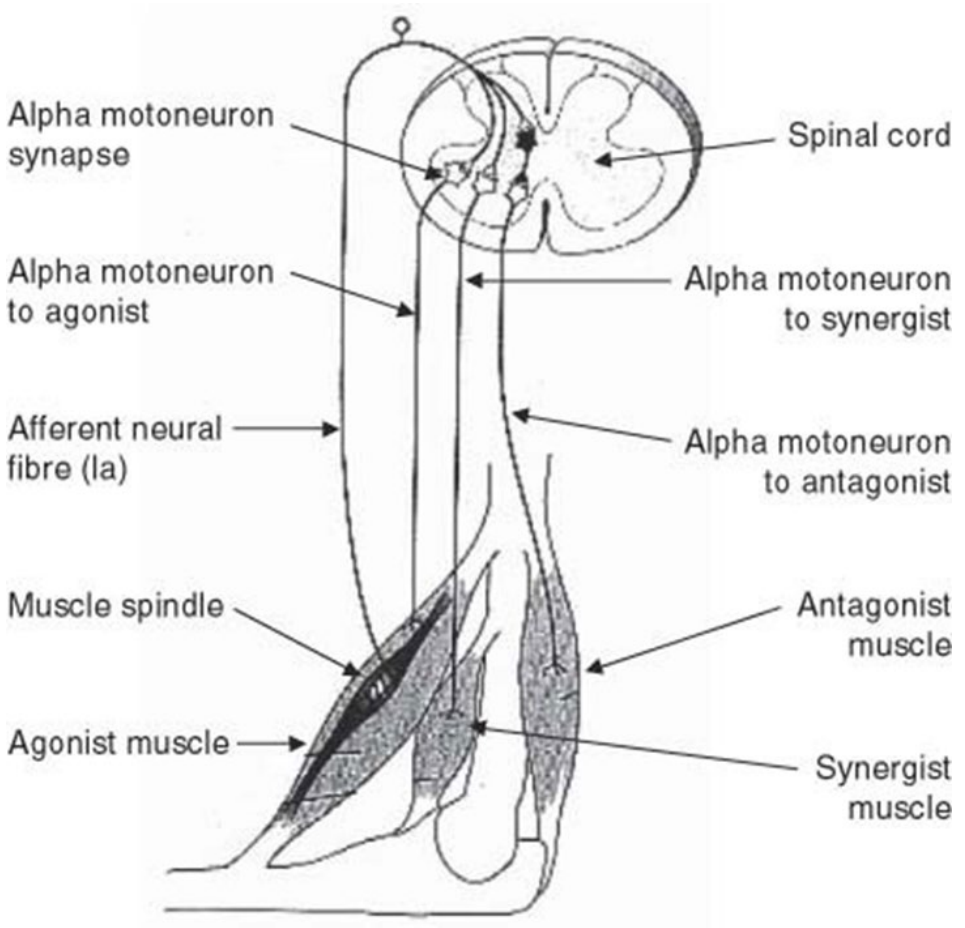


Figure 1.7 The neural volleys of a Ia afferent fibre. An action potential generated at the Ia afferent neural fibre travels to the spinal cord, where it is transferred to the adjacent α-motoneuron of the agonist muscle. The action potential then travels directly to the agonist muscle and initiates the processes of muscular contraction. Adapted from Tillin and Bishop (2009).

A possible mechanism underlying the elevated transmittance of action potentials across synaptic junctions at the spinal cord was proposed by Lüscher et al. (1983). For each parent Ia neural fibre numerous synapses project onto each α-motoneuron (Enoka, 2002). Recruitment of α-motoneurons afferent inputs proceeds in an orderly fashion according to the size principle; i.e. from smallest to largest (Henneman et al., 1965; Zehr, 2002), whereby pre-synaptic transmitter release must coincide with the post-synaptic receptor sensibility (Lüscher et al., 1983). A common occurrence during normal reflex or voluntary responses is transmitter failure. This occurs at various synaptic junctions, possibly due to an autonomously protected activation reserve (Hirst et al., 1981; Lüscher et al., 1983). It was

suggested that an induced tetanic contraction decreases transmitter failure during subsequent activity via a single response or a combination of several possible responses which include; i) an increase in the quantity of released neurotransmitter; ii) an increase in neurotransmitter efficacy and; iii) a reduction in axonal branch-point failure occurring along the afferent neural fibres (Enoka, 2002).

Evidence supporting a decrease in monosynaptic transmitter failure during subsequent activity was provided by Hirst et al. (1981). The researchers stimulated cat afferent neural fibres (tetanic isometric contraction for a duration of 20 s) and observed an increase of 54% in excitatory post-synaptic potentials for the same pre-synaptic stimulus (Hirst et al., 1981). Greater excitatory post-synaptic potentials represent greater depolarization of the α-motoneuron membrane (Enoka, 2002). This would increase the probability of that α-motoneuron reaching the threshold required to initiate an action potential, and subsequently contract the muscle fibres of that motor unit (Enoka, 2002). Lüscher et al. (1983) also measured excitatory post-synaptic potentials at cat α-motoneurons in response to electrical stimulation and found a significant positive correlation between; i) motoneuron input resistances and amplitude of excitatory post-synaptic potentials for a standard stimulus, and ii) input resistance and the size of α-motoneuron (relatively smaller input resistance represented a larger motoneuron) (Lüscher et al., 1983). This finding suggests that monosynaptic transmitter failure is greater at larger motoneurons that are responsible for activation of higher order or fast-twitch motor units (Tillin and Bishop, 2009). In contrast, when a twitch was stimulated following a 10 s tetanic contraction, the researchers found a significant negative correlation between excitatory post-synaptic potentiation and motoneuron input resistance (Lüscher et al., 1983). This demonstrates that a tetanic contraction decreased the transmitter failure occurring primarily at larger motoneurons,

which considerably induced a PAP effect at these α-motoneurons. If an increase in higher order motoneuron recruitment could be induced by a conditioning stimulus in humans, the effect might theoretically enhance subsequent explosive activity via an increase in fast-twitch fibre contribution to that explosive activity (Güllich and Schmidtbleicher, 1996).

Previous studies have measured the H-reflex in humans to investigate the effects of a conditioning stimulus on α-motoneuron recruitment (Güllich and Schmidtbleicher, 1996; Trimble and Harp, 1998). The H-reflex is traditionally defined as a monosynaptic reflex which is induced by an electrical stimulation of group Ia afferents of the muscle nerve (Latash, 2008). The H-reflex is recorded at the muscle fibres using electromyography, and is the result of an afferent neural volley in response to single-pulse submaximal stimulation of the relevant nerve bundle (Enoka, 2002) (Figure 1.8). An increase in H-reflex following a conditioning stimulus may therefore represent a decrease in transmitter failure at synaptic junctions, and a subsequent increase in higher order α-motoneuron recruitment (Tillin and Bishop, 2009).

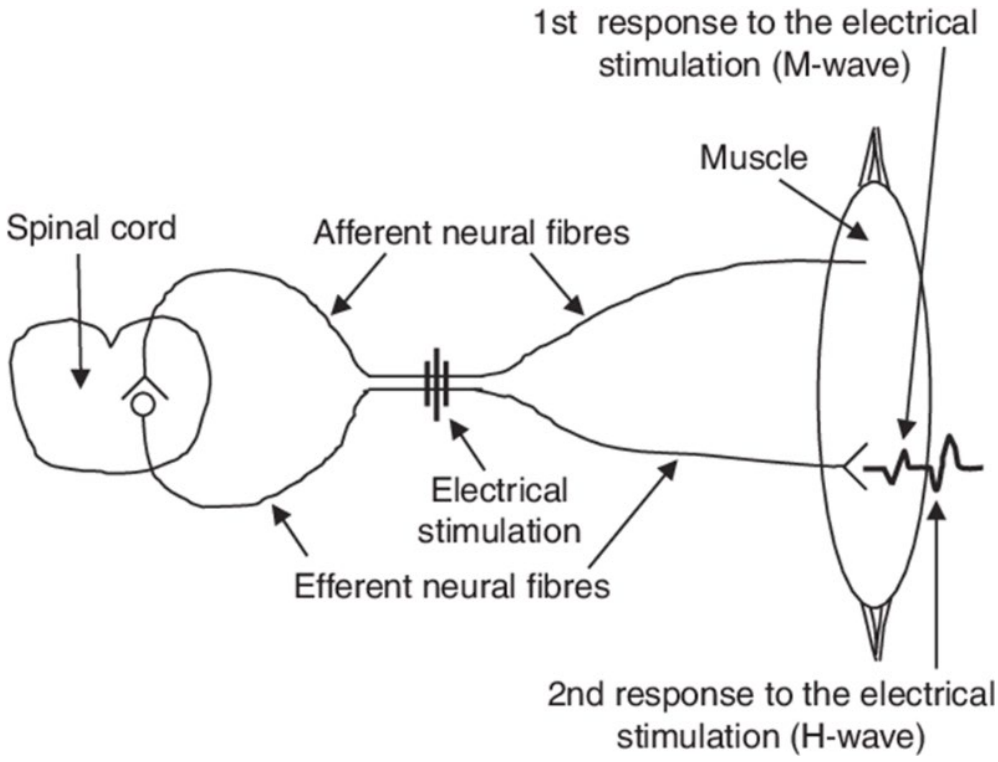


Figure 1.8 Elicitation of an M- and H-wave. Stimulation of a nerve with a single sub-maximal electrical impulse evokes two electrical responses at the muscle. The first response is the M-reflex, which is the result of an action potential travelling directly down the **efferent** neural fibres (α-motoneurons). The second response is the H-reflex, which is the result of an action potential travelling along the **afferent** neural fibres to the spinal cord, where it is **transmitted to adjacent efferent** neural fibres, and down to the muscle. Adapted from Tillin and Bishop (2009).

Although the H-reflex was viewed as a purely monosynaptic reflex, it was shown that only the rising edge of the H-reflex waveform is monosynaptic (Burke et al., 1984). The researchers provided this evidence by modifying the H-reflex amplitude via oligosynaptic pathways such as Ib inhibitory effects from golgi tendon organs and large cutaneous afferents (Burke et al., 1984). Further evidence indicating modulation of H-reflex through mechanisms that effect levels of presynaptic inhibition has further disconfirmed its use as a direct measure of α-motoneuron excitability (Zehr, 2002). In addition, due to a number of methodological issues that have been found to influence the interpretation of the H-reflex and also undermine its validity as a measurement (Hodgson et al., 2005), it was suggested that rigorous

experimental controls must be implemented to avoid a multitude of confounding factors that can affect the accurate interpretation of H-reflex measurements (Zehr, 2002).

Following volitional activation, the two main effects that have been found to modulate H-reflex were post-activation depression and post-activation potentiation (Hodgson et al., 2005). After voluntary contraction, post-activation depression of H-reflex has been consistently documented (Enoka et al., 1980; Güllich and Schmidtbleicher, 1996; Trimble and Harp, 1998; Crone and Nielsen, 1989). Post-activation depression is presumed to be caused by mechanisms acting at a presynaptic level which are most probably related to the phenomenon of a reduced transmitter release from previously activated fibres (Hultborn et al., 1996). Post-activation depression develops immediately upon muscle relaxation (Hodgson et al., 2005) and, depending on the nature of the preceding contractile activity, the time course of this phenomenon can be relatively brief in the range of 10–60 s (Enoka et al., 1980; Gollhofer et al., 1997; Crone and Nielsen, 1989) or can persist for several minutes (Güllich and Schmidtbleicher, 1996; Trimble and Harp, 1998). On the other hand, H-reflex potentiation induced via high-frequency electrical stimulation of the Ia afferents of the homonymous muscle, known more commonly as post-tetanic potentiation, has been previously observed in the human soleus muscle (Corrie and Hardin, 1964; Van Boxtel, 1986; Kitago et al., 2004). Following tetanic stimulation of the test muscle nerve, reflex potentiation develops after several seconds and can last from 1–16 min, depending on the subject and the specific parameters of the tetanic stimulation employed (Corrie and Hardin, 1964; Van Boxtel, 1986; Kitago et al., 2004). Stimulation frequencies of >100 Hz are required to produce post-tetanic potentiation of the H-reflex in humans (Corrie and Hardin, 1964; Van Boxtel, 1986; Kitago et al., 2004). In contrast, lower frequencies of electrical stimulation have been shown to be insufficient in eliciting H-reflex potentiation (Corrie and

Hardin, 1964). The post-tetanic potentiation mechanism has been attributed to the effects of a residual elevation in presynaptic Ca^{2+}, which is responsible for a corresponding increase in the probability of neurotransmitter release from the presynaptic membrane terminal (Zucker and Regehr, 2002).

It has been theorized that if some attributes of tetanic electrical stimulation (such as the sustained recruitment of high-threshold motor units) are reflected in the volitional conditioning stimulus, the reflex potentiation could occur in response to previous voluntary activation (Trimble and Harp, 1998). This effect was demonstrated in two studies by Güllich and Schmidtbleicher (1996), and Trimble and Harp (1998). Following a cyclical concentric-eccentric exercise of the triceps surae muscles that consisted of 8 sets of 10 repetitions, every subject was found to demonstrate an initial depression of the lateral gastrocnemius and soleus H-reflex immediately post-conditioning (Trimble and Harp, 1998). The duration of this depression lasted 10–60 s, which is consistent with earlier reports of post-activation depression following volitional activation (Enoka et al., 1980; Crone and Nielsen, 1989). After post-activation depression, a significant H-reflex amplitude potentiation of the lateral gastrocnemius muscle was reported by Trimble and Harp (1998). Although non-statistically significant, potentiation of the soleus H-reflex was also reported by the same authors (Trimble and Harp, 1998). Notably, despite these findings this effect was found in only halve of ten subjects, and the data revealed marked inter-subject variability regarding the time course of post-activation depression and the onset and duration of potentiation (Trimble and Harp, 1998). The findings by Trimble and Harp (1998) regarding the temporal profiles of H-reflex modulation post-conditioning are consistent with previous literature that have examined reflex potentiation using either electrical stimulation or volitional activation (Corrie and Hardin, 1964; Enoka et al., 1980; Van Boxtel, 1986; Güllich and Schmidtbleicher, 1996).

In a similar study conducted by Güllich and Schmidtbleicher (1996), H-reflex amplitude was measured in the lateral gastrocnemius and soleus muscles pre- and post-performance of a series of isometric maximum voluntary plantarflexions that consisted of 5 repetitions of MVC of 5 s each with a 1 min recovery interval between contractions. Subjects were differentiated by their level of training status as either physical education students or elite speed-strength athletes. Concordant with the findings of Trimble and Harp (1998), the immediate post-conditioning H-wave amplitude was significantly depressed for all subjects. The researchers reported a significant depression in H-reflex amplitude (-24%) 1 min after the MVCs, but a significant potentiation of H-reflex amplitude (+20%) 5–13 min after the MVCs (Güllich and Schmidtbleicher, 1996). Following the initial post-activation depression, H-reflex amplitude was significantly potentiated in the lateral gastrocnemius muscle of the trained speed-strength athletes, but not for the non-athlete physical education students (Güllich and Schmidtbleicher, 1996). These findings might indicate an adaptation inherent to spinal reflex processing that seems to exist only in highly trained athletes. In accordance with previous findings (Corrie and Hardin, 1964; Enoka et al., 1980; Van Boxtel, 1986; Güllich and Schmidtbleicher, 1996; Trimble and Harp, 1998), the time course and peaks of post-activation potentiation varied to a great extent between participants. The approximate time for the onset and period of H-reflex potentiation among speed-strength athletes was between 4–11 min (Güllich and Schmidtbleicher, 1996) which is also consistent with the data from Trimble and Harp (1998). A main limitation of the study by Güllich and Schmidtbleicher (1996) was that the H-wave was not normalized to maximal M-wave, where the M-wave is the electrical counterpart of the activation of all motor units in the pool (Maffiuletti et al., 2001). Therefore, other factors not relating to central activation such as increased activity of the Na^+-K^+ pump at the muscle fibres (Hamada et al., 2000; Enoka, 2002; Hamada et al., 2003) may be responsible for the observations by Güllich and Schmidtbleicher (1996). Nevertheless, other investigations have

reported a potentiation in normalized H-reflex amplitude 3–10 minutes after eight sets of dynamic MVCs (Trimble and Harp, 1998) and 5–11 minutes after a 10 s isometric MVC (Folland et al., 2008). Further, Enoka et al. (1980) reported only H-reflex depression following a 5 s maximal effort plantar-flexion however, the duration of the H-reflex recording period post-conditioning was limited to 1 min. Although the findings are consistent with the time course of post-activation depression following voluntary activation, the onset of potentiation in the studies previously discussed did not evolve until at least 3 min post-activation. Therefore, had a longer recording period post-conditioning (e.g. 10 min) been employed, a potentiating effect may have been observed by Enoka et al. (1980). Collectively, these results suggest that PAP increases H-reflex amplitude in humans, possibly after sufficient recovery, and this may be the result of increased higher order motoneuron recruitment at the spinal cord.

The effect of isometric MVCs on subsequent voluntary motoneuron recruitment has been assessed using the interpolated twitch technique. The interpolated twitch technique can facilitate measurement of motoneuron activation (Folland and Williams, 2007) by comparing maximal twitch amplitude at rest with that evoked when superimposed upon a MVC (Folland and Williams, 2007; Shield and Zhou, 2004). Behm et al. (2004) used the interpolated twitch technique and reported a significant decrease in voluntary muscle activation following 10 s MVCs (Behm et al., 2004). Although these results are in contrast to the proposed mechanism of PAP, they may demonstrate the dominance of central fatigue observed throughout the study by Behm et al. (2004).

1.5.4 Muscle architecture contribution to PAP - increased pennation angle

There is evidence suggesting that changes in pennation angle may contribute to PAP (Tillin and Bishop, 2009). The pennation angle of a muscle is the angle formed by the fascicles and the inner aponeurosis of skeletal muscle (Figure 1.9), and is defined as the angle between the orientation of a fascicle and the attached tendon axis (i.e. the line of action) (Lee et al., 2015). Thus, pennation angle is one determinant of force transmission to bones via tendons (Fukunaga et al., 1997; Folland and Williams, 2007). The sum of the forces of all individual fibres being applied to the relevant tendon during muscular contraction is reduced by a factor of cos θ; where θ = pennation angle (Fukunaga et al., 1997). Therefore, with regards to force transmission to the tendon, larger pennation angles have a mechanical disadvantage in terms of decreased force (Fukunaga et al., 1997).

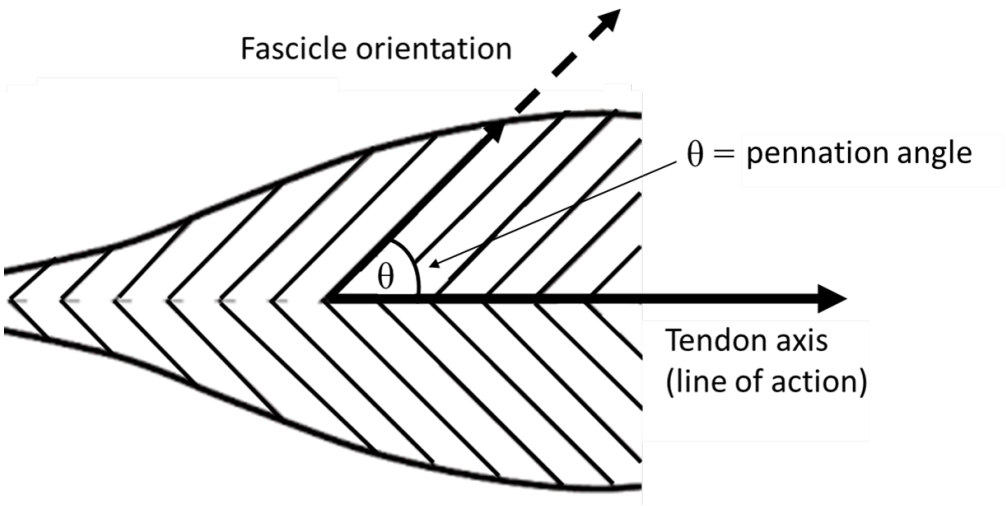


Figure 1.9 Schematic for the definition of pennation angle. Note the pennation angle (θ) formed between the fascicle orientation and the tendon axis. An increase in pennation angle results in a decrease in the force transmitted to the tendon. Adapted from Lee et al. (2015).

Mahlfeld et al. (2004) measured resting pennation angle of the vastus lateralis using ultrasonography before and after three sets each of 3 s isometric MVCs. Immediately after the MVC pennation angle was 15.7º, with a relatively small change observed compared to the

pennation angle before the MVC exercise (16.2º). However, after 3–6 minutes following the MVCs, pennation angle had decreased significantly to 14.4º (Mahlfeld et al., 2004). Although this change would only be equivalent to ~0.9% increase in force transmission to the tendons (Tillin and Bishop, 2009), it is possible that changes in pennation angle following MVCs may contribute to muscular potentiation. On the other hand, there is evidence that conditioning contractions might increase connective tissue or tendon compliance (Kubo et al., 2001), and this effect may counter any increase in force transmission to the tendon resulting from a decrement in pennation angle (Tillin and Bishop, 2009). The extent and direction of the contribution to PAP via architectural changes in skeletal muscle warrants further investigation.

1.5.5 Application of PAP in rugby

There is a growing body of literature in sport indicating that previous voluntary muscular contractions can enhance force production of subsequent exercise (Wilson et al., 2013) in part, via the effects of post-activation potentiation. The coupling of a potentiating stimulus (potentiating activity or conditioning contraction) with a post-stimulus exercise performance is often referred to as a complex pair (Docherty et al., 2004) or strength-power potentiating complex (Stone et al., 2008; Seitz and Haff, 2015). Different complex pairs have been shown to stimulate enhancements in horizontal jumping (Ruben et al., 2010), vertical jumping (Chiu et al., 2003; Chiu and Salem, 2012; Seitz et al., 2014) and sprint (Chatzopoulos et al., 2007; Mcbride et al., 2005; Seitz et al., 2014) performances, when such performances were executed between 4 and 19 min after the completion of a potentiating stimulus containing *quasi* maximal power cleans and back squats. With regards to upper body performance, previous studies have reported enhancement in bench press throw 7–8 min after the performance of a *quasi*-maximal bench press (Kilduff et al., 2007; Esformes et al., 2011).

The neuromuscular rationale underpinning the improvement of these athletic performances after a voluntary conditioning stimulus has often been referred to as PAP in the strength and conditioning literature, with the proposed mechanisms responsible for the performance enhancement attributed to PAP, and thought to occur at the central and peripheral levels (detailed earlier in this Section). From an applied perspective, complex training is used to complement the training of rugby athletes in an attempt to optimise strength, speed and power development, which are vital qualities in rugby (Duthie et al., 2003; Gabbett et al., 2009; McMaster et al., 2013; Johnston et al., 2014). Therefore, one aim of applied strength and conditioning research is to explore the most effective ways on how best to implement complex training with rugby athletes of different competitive levels, and integrate this training system in the athletes' preparation programmes.

A broad range of exercise intensities have been used to provide a potentiating stimulus designed to elicit a PAP response within rugby athletes. Protocols using traditional resistance exercise as potentiating stimulus have typically used heavy loaded back squats (3 RM or 87–91% 1 RM) (Kilduff et al., 2007; Kilduff et al., 2008; Bevan et al., 2010; Crewther et al., 2011; Seitz et al., 2014), power cleans (90% 1 RM) (Seitz et al., 2014), and bench presses (3 RM, 87% 1 RM, or maximal voluntary efforts) (Kilduff et al., 2007; Bevan et al., 2009; Esformes et al., 2011). In addition, there are limited studies that have used potentiating stimuli that contain 'accommodating resistance exercises' (mechanical means of modifying the resistance through the entire range of motion or part of it) of 68% 1 RM in the box squat with an additional resistance equivalent to 6–20% 1 RM by means of an elastic band (average total resistance of 81% 1 RM through the entire range of motion) (Baker and Newton, 2008) and 65% 1 RM in the bench press with an additional 12% 1 RM by means of a chain resistance (total resistance at full arm extension of 77% 1 RM) (Baker, 2009).

Scientific research have demonstrated that the exercise/s selected to be used as the potentiating stimulus can affect the magnitude of the PAP response. For instance, Seitz et al. (2014) compared the effects of potentiating stimuli (which contained 3 repetitions at 90% 1 RM) in either the back squat (high force and low velocity movement) or power clean (explosive movement), on the 20 m sprint performance of elite rugby league athletes. While both potentiating stimuli enhanced sprint performance, greater improvements in 20 m sprint time (3.1 vs. 2.2%), velocity (3.2 vs. 2.3%), and average acceleration (6.6 vs. 4.6%) were observed when power cleans were used as the potentiating stimulus (Seitz et al., 2014). With regards to the upper body, Esformes et al. (2011) compared the acute effects of performing a bench press (isometric (7 s); 3 RM concentric and eccentric; 3 RM concentric; 3 RM eccentric) on bench press throw performance. The researchers have shown that the isometric bench press was the only mode (used as a potentiating stimulus) that induced statistically significant increases (2.8%) in subsequent performance (Esformes et al., 2011). In terms of optimal rest interval between exercises that constitute the complex pair, longer rest periods appear necessary to elicit PAP after the performance of potentiating stimuli using traditional exercises (4–12 min, 3–16 min and 7 min; in the back squat, bench press and power clean, respectively; (Seitz and Haff, 2015)). Of note is that using accommodating resistance exercises may be advantageous from a training perspective since it appears that PAP can be elicited with a considerably shorter rest periods (~90 s between the potentiating stimulus and subsequent performance) and maintained across multiple sets of complex pairs (Seitz and Haff, 2015).

There is evidence showing that the magnitude of performance enhancement via PAP is influenced by the strength level of the performer, with stronger rugby athletes expressing a

higher degree of potentiation during vertical jumping (Kilduff et al., 2007; Kilduff et al., 2008; Seitz et al., 2014), sprinting (Seitz et al., 2014), and bench press throw (Kilduff et al., 2007) performances. Seitz et al. (2014) reported a statistically significant correlation between relative 1 RM back squat and maximum PAP effect on absolute (r = 0.775) and relative (r = 5 0.775) peak power output, and jump height (r = 0.740) during a squat jump in elite rugby league players (Seitz et al., 2014). Similarly, elite rugby union players that have displayed greater absolute (Kilduff et al., 2007) and relative (Kilduff et al., 2007; Kilduff et al., 2008) 3 RM back squat were shown to express a significantly greater PAP performance enhancement on peak power output during a countermovement jump (r = 0.489–0.631).

Considering the collective findings of studies that have investigated PAP within rugby, it could be speculated that long-term complex training programmes should result in greater training adaptations than the 'more' traditional training interventions, since players would train more effectively in terms of magnitudes of mechanical power output produced during potentiated states via PAP effects. In addition, there is evidence that complex training revealed the largest beneficial effects on sport-specific performance compared with other modes of resistance training in young adolescent athletes (Lesinski et al., 2016), showing that complex training could potentially be used to develop strength and power qualities of young athletes during the various phases of athlete development pathway in rugby. Important to note is that due to both the multitude of variables that constitute complex training (e.g. exercise selection and intensity of potentiating stimulus; inter-set rest between complex pairs) and the methodologies used (e.g. study designs; population investigated; quantification of performance enhancement attributed to PAP effects), the translation of findings from studies investigating PAP to the field of strength and conditioning should be interpreted with caution.

1.6 Talent Identification and development in Rugby

Although often used interchangeably, the concepts of talent identification and talent development are separately defined. Talent identification is the process of recognizing current participants with the potential to excel in a particular sport, whereas talent development is the process of providing the most appropriate learning environment to realize this potential (Williams and Reilly, 2000). The fundamental concept is that elite success is based on junior identification and development pathways progressing to senior ranks and eventually international competition. In practical terms, talent identification and development are related since the effectiveness of one concept can directly affect the outcomes of the other (Till and Baker, 2020). The rationale for this interconnection is that a player's progression to elite rugby is multi contextual and multi factorial and therefore, practitioners continue to search for the unique and dynamic factors responsible for optimum identification and developmental outcomes in this sport. Nevertheless, the complex nature of predicting youth trajectories towards eliteness in sport remains challenging for investors and coaches selecting athletes into talent development pathways.

Various methodological approaches have been used in sport science research to explore the processes of talent identification and talent development within both codes of rugby. In rugby league, a substantial number of studies have provided recommendations on how to advance the talent pathway (Spamer and Hare, 2001; Gabbett, 2006; Till et al., 2010; Waldron et al., 2014; Dobbin et al., 2018; Cupples et al., 2018). Although some of the recommendations might be applicable also to rugby union, it appears that less research endeavored to recommend ways to advance the talent pathway specifically in rugby union. Considering that rugby union is a sport in continuous evolution, there is the constant necessity to optimize the talent path in this sport via the application of sport science research (Till et al., 2020). Efforts

to accomplish this might have been reflected, in part, in national governing bodies and professional rugby union clubs investing a large portion of their financial budget on the identification and development of talented youth athletes (Jones et al., 2018). England has its own structure of youth rugby union, known as age-grade rugby, whereby players participate within annual age categories (e.g. under-13 years of age; under-18 years of age) (Till et al., 2020). England rugby union's national governing body, the Rugby Football Union (RFU), governs age-grade rugby in relation to participation within the game alongside the identification and development of young talented players (Till et al., 2020). Talent identification and development programmes are delivered via fourteen regional academies – normally aligned with professional rugby union clubs. Typically, players are identified from community or school rugby and invited to train within a regional academy from 15 years of age, before being in a potential position to sign a professional contract at eighteen years of age.

Early research revealed that development programs for junior players were often based on the content and practices of elite senior players (Duthie et al., 2003). However, recent research evidence suggests that a structured pathway for rugby should entail the development of vital physical fitness qualities throughout the course from juniors to elite athletes (Cupples et al., 2018; Till et al., 2020). Accordingly, substantial resources and emphasis should be directed toward developing and maintaining these vital qualities in players from an early age. Indeed, differences in physical fitness have been suggested as key discriminative functions between playing standards in rugby union (Jones et al., 2019) and rugby league (Dobbin et al., 2018). Nevertheless, it appears that the general agreement is that young players who aim to become professional in rugby are required to possess a wide range of skills such as effective psychological and technical characteristics, in addition to those which are considered vital

physical fitness qualities (Davids et al., 2013; Till et al., 2020). The processes of talent identification and development have been studied extensively in other sports, particularly in soccer. A recent systematic review by Sarmento et al. (2018) addressed talent identification and development in soccer and identified three important major areas of research in the talent pathway: task constraints, performer constraints, and environmental constraints – further sub-categorized into seven areas in that review (Sarmento et al., 2018). More recently, Dimundo et al. (2021) reviewed talent identification and development research in a rugby union context in an attempt to explore the most researched areas in the talent pathway within this sport.

The current Section reviews talent identification and development in rugby using five topics: participation history, birth date and associated relative age effects, psychological factors, anthropometric characteristics, and physiological characteristics (Sections 1.6.1–1.6.5). Section 1.6.6 addresses the potential role of sports genetic research for identifying talent.

1.6.1 Participation history

Participation in sport activities that youth athletes engage in is one of the key variables influencing later attainment of expert performance (Winn et al., 2017). In rugby union, there appears that no research has attempted to explore the practice and participation history profiles of professional rugby union players (Dimundo et al., 2021). Previous research in sport development has identified three main pathways towards senior expertise: early sampling, early engagement, and early specialization (Ford and Williams, 2017). Participation is an obvious integral factor mediating these pathways and therefore, the level of deprivation from participation that a young child or adolescent experiences might negatively influence his/her chances to attain their athletic potential, and subsequent professional career in sport. Deprivation can create many barriers to sports participation,

which can be broadly categorized into practical and knowledge barriers (Winn et al., 2017). Practical barriers include not being able to afford the costs to participate in sports and having little access to facilities, whilst knowledge barriers include lack of education in the importance of physical activity with a consequent lack of exposure to the health benefits associated with sport participation. The scientific literature that explored the effects of deprivation on sport participation during childhood is equivocal, with some reports finding no effect of deprivation on participation (Voss et al., 2008), whereas others suggest that numerous factors related with deprivation decrease access to participation in sport (Nezhad et al., 2012; Payne et al., 2013). Collectively, the scientific research suggests that deprivation is an important consideration while examining who is at risk of lower access to structured practice and other sport activities that are beneficial for the development of young rugby athletes. As such, those involved within youth rugby union are encouraged to offer more equitable access to player development pathways that may provide a larger selection cohort of prospective talent in the future (Till and Baker, 2020).

1.6.2 Birth date and associated relative age effects (RAE)

The relative age effects, whereby earlier birthdate children within a selection year are more commonly selected as talented, has been highlighted in the literature (Barnsley et al., 1985; McCarthy and Collins, 2014). These skewed birthdate distributions among youth players favoring those born near the start of the cut-off date for an age group have been well documented (Webdale et al., 2020). For example, those born in birth quarter 1 of an annual age group in England (i.e. September, October, November) are more likely to be endowed with superior anthropometric and physiological characteristics, and cognitive skills; and have an older training age compared to their later born birth quarter 4 peers (i.e. June, July, August; (Hancock et al., 2013)). Roberts and Fairclough (2012) examined the Northwest of

England representative rugby union squads from under-13 to under-16 and revealed a significant overrepresentation of those born in birth quarter 1 compared to those born in birth quarter 4 (46% vs.14%). Similarly, Lewis et al. (2015) found consistent relative age effects across all Welsh age grade and district rugby union cohorts from under-7 to under-19 (e.g. birth quarter 1 = 29% vs. birth quarter 4 = 22%). The researchers also found an increasingly pronounced relative age effect at under-16 representative levels where regional and national selection occurs (e.g. birth quarter 1 = 44% vs. birth quarter 4 = 12%). Further, McCarthy and Collins (2014) identified a significant overrepresentation of birth quarter 1 compared to birth quarter 4 (48% vs. 8%) in a single English Premiership rugby union academy (McCarthy and Collins, 2014). Collectively, these results suggest that relative age effects are prevalent throughout youth rugby union in the UK, with an increasingly skewed birth quarter distribution at higher playing levels. Interestingly, McCarthy et al. (2016) investigated this initial apparent bias that mediates the relative age effects in professional rugby union academies, and found a reversal of these effects. The researchers revealed that relatively young athletes were less likely to be selected into their respective national academy systems, but more likely to transit into senior professional squads (McCarthy et al., 2016). Although the bias in terms of selection inequality normally favors relatively older and earlier maturing athletes via the relative age effects, research within rugby union (McCarthy et al., 2016), rugby league (Till et al., 2016) and soccer (Ostojic et al., 2014) has shown greater attainment at the senior professional level for relatively younger and later maturing athletes, further substantiating the existence of the reversal relative age effects during a later stage in an athlete's career.

1.6.3 Psychological factors

Psychological skills have been broadly investigated as factors of achieving success in high performance sport, and acknowledged as key determinants in the realization of potential and long term athletic success (Rees et al., 2016), in particular in rugby union (Hill et al., 2015). Despite this, the prevalence of systematic psychological inquiry into both senior and youth populations worldwide in sport is scarce (Till et al., 2020), with very limited research investigating the psychological factors that may influence talent identification and development in rugby union (Dimundo et al., 2021). Hill et al. (2015) used a retrospective research design to identify a range of psychological characteristics that may impact the talent identification and development processes within English rugby union academies. The researchers interviewed 15 English rugby union academy directors and coaches to identify the positive and negative psychological constructs mediating talent development. The researchers found that professional rugby union coaches perceived commitment, self-regulation, resilience, realistic performance evaluation, growth mind set, and being proactive, as key psychological characteristics that discriminate successful players in a professional rugby union environment (Hill et al., 2015). The researchers continued to suggest that these constructs are vital because they provide young athletes with the essential competencies required to face the challenges associated with sport and developmental opportunities (Hill et al., 2015). In contrast, certain dual characteristics such as obsessive passion and perfectionism were found to have a negative impact on athlete development (Hill et al., 2015). Collectively, the findings by Hill et al. (2015) provide support to previous research that examined psychology in sport. For example, self-regulation has been found to be an important positive construct that might influence talent identification; as individuals without this skill tend to rely on others, attributing failures to maladaptive causes, and are not able to be in charge of their own development (Karoly, 1993; Petlichkoff, 2004). The limited

research that has addresses the psychology within male age-grade rugby union indicates that it may be useful for coaches and practitioners to assess the psychological profiles of young rugby athletes to identify their strengths and weaknesses; and attempt to meliorate those skills that appear vital for the players' continued development. Further, future research should explore the psychological profiles of the athletes and consider effective methods to continue to inform coaches on how to facilitate the athletes' psychological development.

1.6.4 Anthropometric characteristics

Anthropometric factors have been shown to play important roles in elite rugby league (Till et al., 2014; Brazier et al., 2020) and rugby union (Sedeaud et al., 2012; Brazier et al., 2020). Research have shown that rugby players are becoming heavier, taller, and leaner (Brazier et al., 2020) and accordingly, anthropometric characteristics have received particular attention in relation to talent identification and development within both rugby league (Till et al., 2017; Dobbin et al., 2019) and rugby union (Jones et al., 2019; Till et al., 2020). In a 20-year (1988 to 2008) longitudinal study, Sedeaud et al. (2013) examined anthropometric factors in senior (n = 2051), under-21 (n = 145) and under-15 (n = 448) elite French rugby union players. The authors revealed that senior, junior and youth players selected to play in national rugby union academies and professional clubs became progressively heavier and taller compared to those selected during the previous years of their investigation (increases in body mass and height: senior forwards 12.3 kg and 2.9 cm; backs 12.0 kg and 5.4 cm; under-21 forwards 11.1 kg and 4.4 cm; backs 9.9 kg and 6 cm; under-15 forwards 4.7 kg and 4.7 cm; backs 6.5 kg and 5.1 cm) (Sedeaud et al., 2013). Similarly, a comparison between top level Argentinian rugby union players and the general population demonstrated that front row forwards had greater muscle mass and larger skeletal structure (Holway and Garavaglia, 2009). These findings suggests that anthropometric characteristics are becoming increasingly important during

identification, development, and selection processes of potentially distinctive athletes. Interestingly, the body mass and height of under-21 English players (Darrall-Jones et al., 2015) appear similar to those reported by Lombard et al. (2015) for South African under-20 players, and greater than those reported by Vaz et al. (2016) in under-19 Portuguese forwards and backs.

Distinct anthropometric profiles have been found between rugby union forwards and backs in under-15 and under-21 French (Sedeaud et al., 2013) and under-16 Irish (Delahunt et al., 2013) players. In both studies, forwards were heavier, taller, and older with body mass being the significant predictor of positional role classification. Similarly, English age-grade rugby union forwards are heavier and taller than their backs counterparts (Darrall-Jones et al., 2016). There is also evidence of differences in percent body fat between playing positions in rugby union, with backs showing a lower percentage body fat than forwards (Holway and Garavaglia, 2009). These differences in anthropometrics between forwards and backs could be attributed, in part, to differences in physical requirements between positional groups in professional senior (Roberts et al., 2008; Cunniffe et al., 2009; Quarrie et al., 2013), schoolboy and academy (Read et al., 2017; Read et al., 2018) rugby union players. Interestingly, Holway and Garavaglia (2009) found that forwards develop anthropometric components later than backs, whilst Sedeaud et al. (2013) showed that forwards were older than backs among the same age group athletes. These findings suggest that anthropometric factors can be predictive of selection at older ages for forwards compared to backs. Thus, a more longitudinal screening that considers the interplay between chronological age and biological maturation might be more suitable to account for anthropometric differences between forwards and backs during the talent identification and development processes in rugby union. To the author's knowledge, only one study by Howard et al. (2016) has

examined the anthropometric characteristics of English under-16 rugby players while considering height, mass and maturity status within the 14–17 year old range. This study revealed that youth rugby union players were above the 75th and 90th reference percentiles for height and mass, respectively. These results suggest that advanced size and maturity may be advantageous during selection processes within rugby union – consistent with previous findings in Australia by Patton et al. (2016) who suggested assessing players' anthropometrics prior to registration for potential player dispensation and grading (Patton et al., 2016). Tables 1.1a-d present 20 peer-reviewed studies (categorized by the physiological characteristics investigated – see Section 1.6.5) that have examined athletes' characteristics (anthropometric and physiological) solely in male age-grade rugby union players in relation to talent identification and development. The Tables include the aims, number of participants and their age group/s, characteristics investigated, and the main findings of the studies.

Table 1.1a Studies that have examined anthropometric, and strength and power characteristics in male age-grade rugby union players.

Authors/reference	Aim	Participants	Characteristics examined	Results
Grobler et al. (2017)	To determine the prevalence of RAEs in South African schoolboy rugby union players, and whether RAEs were related to physical fitness parameters.	Schoolboy U14 to U16 (n = 281)	RAEs and anthropometric and physiological factors. **Strength and power**	Stronger and players with superior physicality were most likely to be selected. RAEs were also prevalent in all age groups, with the first two quartiles of the year overrepresented. U15s demonstrated a significant relationship between stature, handgrip strength, and upper body muscular endurance and RAEs.
Hansen et al. (2011)	To study the discriminative ability of rebound jump squat force–time and power–time characteristics in differentiating speed performance and competitive standard in academy and professional rugby union players.	Professional (n = 25) Academy (n = 15)	Anthropometric and physiological factors. **Strength and power**	Force and power differentiated competitive standards. Parameters of lean mass assisted in the transition from academy to professional status.
Pienaar et al. (1998)	To identify the physical qualities, rugby specific skills, and anthropometric variables that enable coaches to identify 10 year old schoolboys who possess the potential to become distinct rugby union players.	Schoolboy U10 (n = 218)	Technical and tactical skills and, anthropometric and physiological factors. **Strength and power**	Anthropometric parameters (stature), physical characteristics (strength and speed), and some rugby specific skills such as passing for accuracy were qualities that predicted selected and deselected young players.
Spamer and De la Port (2006)	To identify the characteristics of South African U16 and U18 schoolboy rugby players with reference to anthropometric variables, physical and motor abilities, and game specific skills.	Schoolboy U16 (n = 71) U18 (n = 75)	Technical and tactical skills and anthropometric and physiological factors. **Strength and power**	U18s were heavier, taller, and leaner than U16s. In addition, U18s were stronger, more agile, and had superior aerobic endurance than U16s. In contrast, U16s were faster than U18s. During the study game specific handling skills decreased in both age categories.
Maria van Gent and Spamer (2005)	To compare playing positions in terms of anthropometric, rugby specific skills, physical, and motor components among U13, U16, U18, and U19 provincial players.	Provincial U13 (n = 21) U16 (n = 22) U18 (n = 18) U19 (n = 19)	Technical and tactical skills and anthropometric and physiological factors. **Strength and power**	Significant differences were observed between playing positions for anthropometric, rugby specific skills, physical, and motor components. Forwards developed later in terms of anthropometric components. The older the players, the fewer the differences in rugby specific skills, physical, and motor components.
Howard et al. (2016)	To evaluate the mediating effect of biological maturation on anthropometric measurements and performance indicators, and subsequent selection in a cohort of academy rugby union players.	Professional regional academy U14 to U17 (n = 51)	Anthropometric and physiological factors. **Strength and power**	Evidence of selection bias towards early maturing players. This prevalence appears to result from the superior anthropometric attributes exhibited by the early maturing players, which likely contributed towards superior components of speed, anaerobic power, and momentum.

Note. U = under; RAEs = relative age effects. Physiological characteristic investigated in **bold** under column named 'Characteristics examined.'

Table 1.1b - Studies that have examined anthropometric, and maximal speed and acceleration in male age-grade rugby union players.

Authors/reference	Aim	Participants	Characteristics examined	Results
Jones et al. (2018)	To compare the physical qualities between academy and schoolboy rugby union players.	Professional regional academy U18 (n = 55) Schoolboy U18 (n = 129)	Anthropometric and physiological factors. **Maximal speed**	Academy players were heavier, taller, and stronger; and possessed superior 20 and 40 m sprint, 10 m momentum, and aerobic fitness compared to schoolboy players.
Parsonage et al. (2014)	To examine conditioning specific movement tasks and physical fitness characteristics of U16 rugby union players. Also, to perform an exploratory analysis that classified players into groups by their conditioning specific movement tasks ratings, after which scores were compared between groups.	Regional and national U16 (n = 156)	Anthropometric and physiological factors. **Maximal speed**	Training conditioning specific movement tasks (Romanian deadlift, overhead squat, double leg to single leg landing, single leg squatting, sprinting, and jumping) improved sprinting over 40 m and endurance running. Effective training intervention after movement screening can facilitate players' long term development.
Pienaar et al. (1998)	To identify the physical, rugby specific skills, and anthropometric variables that enable coaches to identify 10 year old schoolboys who could become successful rugby union players.	Schoolboy U10 (n = 218)	Technical and tactical skills and anthropometric and physiological factors. **Acceleration**	Both South African and English groups did not demonstrate significant differences in anthropometric characteristics. English players demonstrated significantly inferior qualities in the physical and motor abilities, while the South African players performed better in game specific skills.
Smart et al. (2013)	To examine the between player differences and within player changes in physical performance in rugby union players.	Senior professional and provincial (n = 1,161)	Anthropometric and physiological factors. **Acceleration**	Small to moderate differences between players selected and not selected for provincial teams, and small to large differences between provincial and professional players.
Wood et al. (2018)	To provide normative data of physical fitness in elite adolescent Irish rugby union players, and determine the differences in the physical capacities between forwards and backs.	International U18 (n = 89)	Anthropometric and physiological factors. **Acceleration**	Forwards had greater anthropometrics than backs. In addition, forwards showed a significantly lower CMJ height, triple hop for distance score, and 150 m shuttle test score on their right leg. Further, forwards had a significantly higher 10 m sprint time than backs.

Note. U = under; RAEs = relative age effects. CMJ = countermovement jump. Physiological characteristic investigated in **bold** under column named 'Characteristics examined.'

Table 1.1c - Studies that have examined anthropometric, and momentum in male age-grade rugby union players.

Authors/reference	Aim	Participants	Characteristics examined	Results
Darrall-Jones et al. (2016)	To evaluate the anthropometric, sprint, and high intensity running profiles of English regional academy rugby union players by playing positions.	Professional regional academy U16 (n = 29) U18 (n = 24) U21 (n = 15)	Anthropometric and physiological factors. **Momentum**	Forwards demonstrated significantly different anthropometric and sprint momentum compared to backs. Body mass and sprint momentum had the largest differences at consecutive age categories for playing positions.
Darrall-Jones et al. (2015)	To evaluate the anthropometric and physical characteristics of English regional academy players by age category.	U21 (n = 15) Professional regional academy U16 (n = 29) U18 (n = 23) U21 (n = 15)	Anthropometric and physiological factors. **Momentum**	Anthropometric and physical characteristics were more developed in older groups. Physiological characteristics also improved with age. Sprint times, aerobic profile, and ASR appear to remain stable across age categories.
Fontana et al. (2017)	To examine whether and to what extent specific anthropometric and functional characteristics could accurately predict subsequent career progression in rugby union.	U16 to senior national and international (n = 531)	Anthropometric and physiological factors. **Momentum**	Players' success was predicted using a linear combination of anthropometric and physical characteristics, among which a lower percent body fat and higher speed over a 15 m sprint provided the most important predictors of the highest career success.
Jones et al. (2019)	To compare anthropometric and physical characteristics between current professional and amateur rugby union players. Also, to determine which anthropometric and physical characteristics were predictive of competitive standards.	Professional and amateur (n = 60)	Anthropometric and physiological factors. **Momentum**	Professional players showed superior anthropometric and physical characteristics than amateur players. The sum of the eight skinfolds, power, and CMJ peak velocity were predictive of competitive standard.
Quarrie et al. (1996)	To describe the anthropometric and physical performance characteristics of a cohort of professional rugby union players, in an attempt to highlight differences between playing positions.	Professional (n = 94)	Anthropometric and physiological factors. **Momentum**	Forwards' anthropometrics differed significantly between playing positions. For example, locks and loose forwards were taller than front row forwards (props and hookers).

Note. U = under; RAEs = relative age effects. CMJ = countermovement jump. ASR = anaerobic speed reverse; (ASR = maximum velocity – maximal aerobic speed (Buchheit and Laursen, 2013)). Physiological characteristic investigated in **bold** under column named 'Characteristics examined.'

Table 1.1d - Studies that have examined anthropometric, change of direction, speed endurance, aerobic characteristics, and speed, agility and jump performance in male age-grade rugby union players.

Authors/reference	Aim	Participants	Characteristics examined	Results
Spamer et al. (2009)	To conduct a comparative study between elite U16 New Zealander and South Africa rugby union players, by examining game specific skills and anthropometric and physical profiles.	Provincial U16 (n = 88)	Technical and tactical skills and anthropometric and physiological factors. **change of direction**	New Zealanders outperformed South Africans in favorable anthropometric qualities, game specific tests, and physical abilities.
Read et al. (2018)	To compare the physical characteristics of academy and schoolboy U18 rugby union players by playing position (i.e., forwards vs. backs).	Regional academy and schoolboy U18 (n = 66)	Anthropometric and physiological factors. **speed endurance**	Academy players covered greater total distance, and greater jogging distance than school players. In addition, academy forwards had greater sprinting distances than schoolboy forwards, and academy backs accumulated greater player load than schoolboy backs.
Scott et al. (2003)	To evaluate differences in aerobic fitness between forwards and backs from a professional rugby union team.	Professional (n = 28)	Anthropometric and physiological factors. **oxygen uptake**	Backs had a higher peak oxygen uptake per kilogram body mass than forwards.
Pienaar and Spamer (1998)	To determine the reason/s why certain 10 year old players who were initially identified as having talent were selected in a high performance primary school programme.	Schoolboy U10 (n = 31)	Technical and tactical skills and anthropometric and physiological factors. **skill speed, agility, and jump performance**	The successful group was significantly better in rugby skills and motor abilities. These included: passing for distance, passing for accuracy, throwing over the crossbar, rolling and picking up of the ball running speed, agility run, sit and reach, and vertical jump.

Note. U = under; RAEs = relative age effects. Physiological characteristic investigated in in bold under column named 'Characteristics examined.'

1.6.5 Physiological characteristics

Young players that aim to become professional in rugby union, are required to possess a range of skills such as effective psychological and technical characteristics (Davids et al., 2013). Nevertheless, differences in the physical qualities (anthropometric and physiological) have been suggested as key discriminative functions between playing standards and age categories in rugby union (Jones et al., 2019) and accordingly, have received particular attention in relation to talent identification and development in rugby. Some of the most important physiological capacities that have been examined in relation to talent identification and development in rugby union include: maximal speed (Parsonage et al., 2014; Jones et al., 2018), acceleration (Pienaar et al., 1998; Smart et al., 2013; Wood et al., 2018), momentum (Quarrie et al., 1996; Darrall-Jones et al., 2015; Darrall-Jones et al., 2016; Fontana et al., 2017; Jones et al., 2019), maximal strength (Grobler et al., 2017; Hansen et al., 2011; Pienaar et al., 1998; Spamer and De la Port, 2006; Maria van Gent and Spamer, 2005), peak power (Howard et al., 2016), agility and change of direction performance (Spamer et al., 2009), speed endurance and aerobic qualities (Read et al., 2018; Scott et al., 2003), and skill speed, agility and jump performance (Pienaar and Spamer, 1998). The aims, number of participants and their age group/s, characteristics investigated, and the main findings of these studies are presented in Tables 1.1a-d above as follows: Table 1.1a (strength and power); Table 1.1b (maximal speed and acceleration); Table 1.1c (momentum); Table 1.1d (change of direction; speed endurance; aerobic characteristics; speed, agility, and jump performance). These studies examined anthropometric, and some physiological characteristics solely in male age-grade rugby union players in relation to talent identification and development and are peer-reviewed.

Recently, Till et al. (2020) reviewed the literature in relation to the applied sport science of male-age-grade rugby union players in England. The authors suggested that a broad range of physical qualities including body size, muscular strength and power, speed, change of direction speed, and high-intensity running ability are important for player development while considering injury prevention and thus, these qualities should be strongly accounted for within the programmes of age-grade rugby union players to optimize long term player development and participation within the sport (Till et al., 2020). With regards to strength and power, the authors highlighted six studies that have presented strength and power data specifically in age-grade rugby union players in England, with data acquired via the Wattbike peak power output (Howard et al., 2016), countermovement jump (Weakley et al., 2019; Darrall-Jones et al., 2015) and isoinertial strength tests (Darrall-Jones et al., 2015; Weakley et al., 2017; Jones et al., 2018; Weakley et al., 2019; Weakley et al., 2019). Till et al. (2020) showed that strength and power are greater in older age categories (Darrall-Jones et al., 2015; Howard et al., 2016) (which supports data in rugby league (Till et al., 2017)), and can distinguish between playing standard (Jones et al., 2018) and resistance training experience (Weakley et al., 2017). One study within rugby league by Till et al. (2016) suggested that strength measures acquired from the squat, bench press and prone row, together with 10 m sprint momentum and height may be the best discriminating factors in 16–19 year-old players in contributing to acquiring professional status, and suggested the consideration of such qualities in the identification and development of the players, and for their subsequent success within the adult professional game (Till et al., 2016).

1.6.6 Potential role of genetic factors

It has long been established that elite athlete status is a complex heritable trait (De Moor et al., 2007), as are the many anthropometric, physical, physiological and psychological traits

underpinning elite sports performance (Puthucheary et al., 2011; Zempo et al., 2017; Miyamoto-Mikami et al., 2018). In recent years, our understanding of the specific genetic variants that contribute to these sport- and exercise-related traits has grown (Barh and Ahmetov, 2019). This is reflected, in part, in considerable interest in utilising genetic information as a tool to aid talent identification processes and assess potential for future sports performance; and a corresponding emerging market of direct-to-consumer genetic testing (Webborn et al., 2015; Vlahovich et al., 2017; Pickering et al., 2019).

A group of world experts (e.g. fields of genomics, sport performance, disease and injury) highlighted a key concern regarding the misuse of research evidence and the misinformation about direct-to-consumer genetic testing, in particular; when marketed directly to the public, coaches or parents for the purpose of talent identification and to assess potential for future sports performance (Webborn et al., 2015). The authors added that there is concern among the scientific community, in that the level of knowledge is being misrepresented for commercial purposes, highlighting the lack of clarity of information over which specific genetic variants are being tested (Webborn et al., 2015). This raises obvious questions among the scientific community – firstly, on expert opinion on the current state of knowledge of sport genetic research in relation to talent identification since the report by Webborn et al. (2015), and secondly; on whether sports genetics research can, in future, be utilised to identify talent or aid talent identification and development processes. More recently, a position statement by the Australian institute of sport stated; “there are, currently, no scientific grounds for the use of genetic testing for athletic performance improvement, sport selection or talent identification” (Vlahovich et al., 2017). Other recent reports have attempted to gauge the current state of knowledge with respect to the potential utility of genetic testing in identifying talent (Pickering et al., 2019; Varillas-Delgado et al., 2022) –

clearly expressing a common view that currently, genetic testing is of limited use for talent identification or to assess potential athletic status.

Importantly, in view of the current state of knowledge in sports genetics and its potential utility in talent identification, although described as 'still in its infancy' by experts in the area (Bouchard, 2015; Pitsiladis et al., 2016), it should be acknowledged that recent years have witnessed substantial progress in sports genetics (Ahmetov et al., 2021). Athletic ability is highly complex – with intermediate traits (e.g. muscle strength; aerobic endurance) also accepted to be highly complex. Each of these traits depends, in turn, on other intermediate traits (e.g. strength depends on muscle size; fibre-type proportion – Section 1.3) – all known to be complex. Undoubtedly, many DNA polymorphisms need to be identified to elucidate the genetic contribution to these traits, to genuinely attempt to translate the acquired knowledge to aid talent identification (Pitsiladis et al., 2016). The concept that strength performance is likely to be determined by the simultaneous presence of many advantageous genetic variants has been addressed in principle by Hughes et al. (2011), with only a single recent research effort confirming this concept in elite Russian weightlifters and powerlifters (Moreland et al., 2022). The work in this thesis aims to extend this concept by investigating the simultaneous presence of genetic variants deemed advantageous for strength/power in an elite rugby union cohort.

In the eventuality of substantial progress in sports genetics, a thorough multidisciplinary approach would need to be conducted to analyse the usefulness and efficacy of genetic testing and its applicability in talent identification (Williams et al., 2016). This will serve to determine whether genetic data can provide information not already captured within other

traditional non-genetic tests that are already routinely used in rugby – to aid talent identification and development in rugby.

1.7 Assessment of Muscular Strength and Power

Muscular strength and power assessments are implemented on a regular basis in rugby to determine the athletes' strength/power status (Baker, 2013; Oliver et al., 2015; Redman et al., 2021), to evaluate the effectiveness of strength/power training programmes (Dobbin et al., 2018), and to determine the necessity and timing of modifications to a periodised strength/power training programme (Harries et al., 2016). In addition, strength and power assessments are used frequently, but arguably less regularly to monitor the athletes' readiness to train and return to play after injury (Ashworth et al., 2018; McBride and Oxford, 2020; Breen et al., 2021); and to monitor the effects of concurrent training and competition load on the athletes' overall fatigue and recovery (McLellan et al., 2011; Twist and Highton, 2013; Aben et al., 2020). In addressing the requirements for strength and power assessments in light of the physical and physiological demands in rugby, numerous strength/power tests have been developed and implemented to assess both upper and lower body strength and power qualities that are pertinent to rugby performance (reviewed by Chiwaridzo et al. (2017)). In a more recent review that included 42 studies with a focus on testing methods and physical qualities in rugby, Owen et al. (2020) identified 23 strength tests (e.g. back squat 1 RM (repetition maximum); bench press 1 RM; chin-up 3 RM) and 11 power tests (e.g. squat jump; countermovement jump; medicine ball throw) that were used in male age grade rugby union players ranging from 11–21 years of age. This sheds light on the emphasis placed on the development of strength and power from a young age, and the potential use of strength/power assessments as part of a talent identification programme in rugby (Section 1.6).

1.7.1 The force platform method for assessing strength and power

Often, strength and power assessments require that the force generated by the performer is applied against the ground by the lower limbs, creating reaction forces from the ground that reflect the magnitude of the generated force, and in some occasions (e.g. in jumping) the kinematics of the body (to some extent) above the ground (Hamill and Knutzen, 2006). This ground reaction force (GRF) can be measured by an instrument known as a force platform which is a standard tool in biomechanics research, most often used in gait analysis and sports related skills and manoeuvres (Maud and Foster, 2006). This technique was used by researchers in the 1930's (Elftman, 1938; Fenn, 1930; Manter, 1938), although the idea has been proposed some time earlier (Marey, 1874; Amar, 1920). The principle of the GRF is derived from Newton's law of 'action and reaction' and closely represents the reaction of the ground to the accelerations of all the body segments (Enoka, 2008). The forces exerted on the force platform in the vertical (up-down), forward-backward (anteroposterior), and side to side (mediolateral) directions generate analogue voltage signals outputs from the force platform (Maud and Foster, 2006). The generated voltages (which are proportional to the forces exerted on the force platform) are ultimately translated to force units (Newtons), representing the three dimensional temporal resolution of the ground reaction force (GRF). A modern and sophisticated force platform, combined with advanced data processing techniques can provide high-resolution records of three dimensional force application versus time, from which human mechanical power output and other neuromuscular parameters can be derived. This extends the suitability of force platforms for quantifying forces associated with many movements critical to sport, often characterised by high force exertion over short time intervals (Maud and Foster, 2006; Winter et al., 2016).

1.7.2 Assessing maximal isometric strength using the Isometric mid-thigh pull (IMTP)

The isometric mid-thigh pull (IMTP) involves a maximum upward effort of ~5 s against an immovable steel bar. The performer is required to grab the bar with both hands (distance between the hands is generally wider than shoulder width, but this varies between participants and protocols used) while adopting a position that replicates the body position at the start of the second pull phase of the clean (Haff et al., 1997) (Figure 1.10a), and to pull as hard and as fast as possible (when prompted to start), sustaining the effort for pulling maximally upwards for the required predetermined duration; generally of ~5 s.

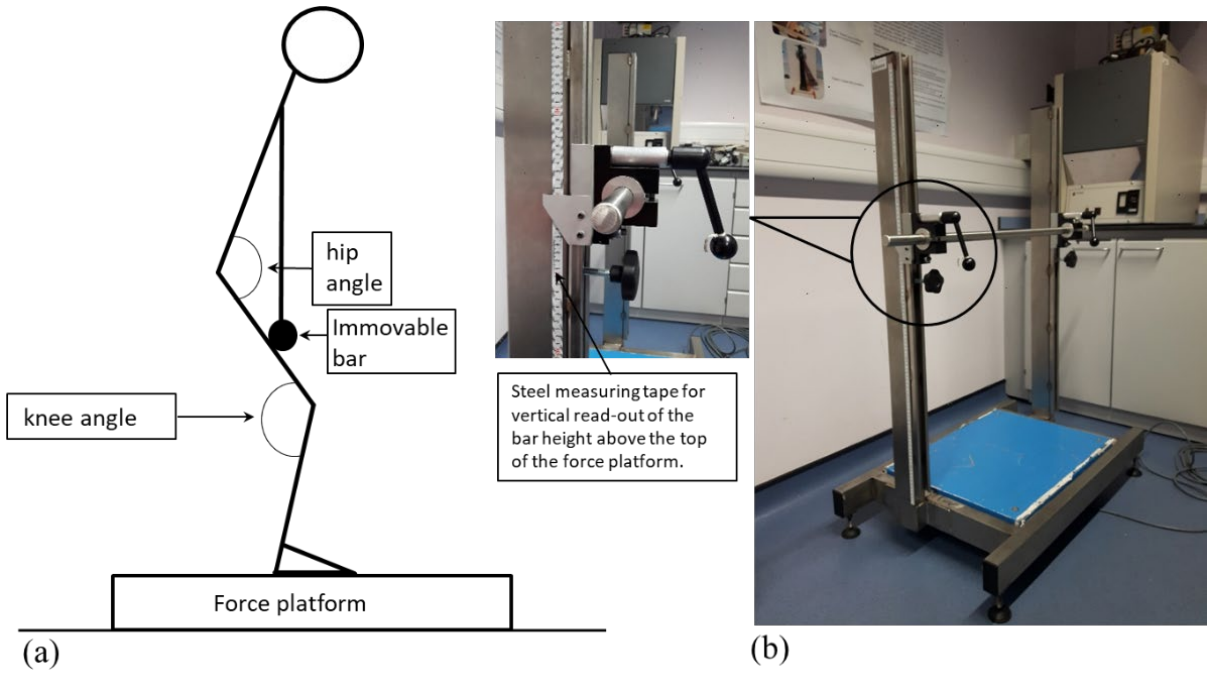


Figure 1.10 (a) Performance of the Isometric mid-thigh pull (IMPT) test using the force platform method. The diagram refers to the hip angle (measured between torso and front of thigh) and knee angle (measured between shank and back of thigh). **(b)** Bespoke IMTP rig with force platform that was used for testing the athletes and non-athlete control participants for this thesis.

For testing purposes, the performer is required to stand on a force platform that is most often positioned inside an IMTP rig. The bespoke rig that was used for the IMPT test in this thesis

(with both athletes and non-athlete controls) is shown in Figure 1.10b. The insert picture shows the right side (when the rig is viewed from the rear) mechanism for adjusting the bar height according to the performer's physical characteristics. A similar mechanism is fitted on the other (left) side of the rig to ensure the bar is securely locked horizontally, and longitudinally parallel with the surface of the force platform.

The relevance of isometric strength testing in monitoring the strength of athletes has caused some conflicts in the literature, mainly due to contradicting findings in terms of the strength of the relationship between neuromuscular parameters acquired from isometric strength tests, and parameters acquired from dynamic tests. Lum et al. (2020) reviewed the data on the relationship between the force-time characteristics of multi-joint isometric strength tests (peak force; rate of force development; impulse) and dynamic performance (Lum et al., 2020). The authors selected 47 studies that showed significant small to large relationships between isometric bench press force-time variables and upper body dynamic performances (r^2 = 0.221 to 0.608, $p < 0.05$) and significant small to very large relationship between isometric squat (r^2 = 0.085 to 0.746, $p < 0.05$) and IMTP (r^2 = 0.120 to 0.941, $p < 0.05$) force-time variables with lower body dynamic performances. All the studies reviewed by Lum et al. (2020) investigated athletes or physically active individuals, however, only five of those studies investigated elite athletes, with the study by (West et al., 2011) involving rugby players that were described as 'professional' in that study. Table 1.1 shows the correlation coefficients (*r)* between the peak force acquired from the IMTP force-time histories, and parameters of dynamic performance for three studies that involved elite athletes. The criterion method developed by (West et al., 2011) was used to analyse the IMTP force-time histories that were recorded for participants in this thesis. Details for processing the IMTP force-time histories are provided in Section 2.2.

Table 1.2 Correlation between Isometric strength and dynamic performance in elite athletes.

Authors	Participants	Isometric test	Dynamic test	Correlation coefficient (*r*)
Haff et al. (2005)	Six female elite Weightlifters	IMPT peak force	CMJ peak power	0.88
			Squat jump peak power	0.92
			DMTP 30% IPF: peak force	0.96
			DMTP 100kg: peak force	0.99
			DMTP 100kg: velocity	0.80
			DMTP 100kg: peak power	0.93
			Maximum snatch	0.93
			Maximum total Snatch and Clean and Jerk	0.80
		IMTP peak RFD	CMJ peak power	0.81
			Squat jump peak power	0.84
			Maximum total Snatch and Clean and Jerk	0.80
Spiteri et al. (2014)	12 female elite Basketball athletes	IMTP relative peak force	*505 protocol for COD	-0.792
			**T-test protocol for COD	0.854
(West et al., 2011)	39 male professional Rugby athletes	IMTP peak force	10 m sprint time	-0.23
			CMJ concentric power	0.52
		IMTP relative peak force	10 m sprint time	-0.37
			CMJ height	0.45
		IMTP peak rate of force development	10 m sprint time	-0.66
			CMJ height	0.39
		IMTP force at 100ms	10 m sprint time	-0.54
			CMJ concentric power	0.55
		IMTP relative force at 100ms	10 m sprint time	-0.68
			CMJ height	0.43
			CMJ concentric power	0.38

NB. IMTP = Isometric mid-thigh pull; DMPT = dynamic mid-thigh pull; CMJ = countermovement jump; RFD = rate of force development; IPF = Isometric peak force; *505 protocol for COD (change of direction) = tests for high velocity 180º directional change; **T-test protocol for COD = tests how fast the athlete can change direction (side-shuffle; forward run; backward run).

1.7.3 Assessing lower body power output using the countermovement jump (CMJ)

When an individual is asked to jump in an attempt to reach as high as possible with the head or arms, it generally becomes autonomous to the person to adopt the technique of countermovement jumping (Enoka, 2008). From a standing position, the individual performs a small-amplitude downward movement (countermovement) that involves flexion at the hip, knee, and ankle. This is followed by a rapid extension of the legs, and a forward upward rotation (flexion) of the arms about the shoulders (Bobbert, 2001). Therefore, jumping variations that involve a countermovement in an attempt to maximise jump height are somewhat autonomous and are performed by athletes during their respective infield performance (e.g. to reach as high as possible in a rebound in basketball; to maximise the height of the centre of mass in the high jump in athletics; in assisted jumping during a line-out throw in rugby; (Hamill and Knutzen, 2006)). Consequently, jumping performance has been previously extensively investigated to determine parameters that reflect neuromuscular function (e.g. the effect of training on the power-, force-, and velocity-time curves throughout the countermovement jump (CMJ); (Cormie et al., 2009); electromyography of the vastus medialis during different phases of the jump (Bobbert and van Ingen Schenau, 1988); joint moment and mechanical power flow in the lower limb during the CMJ, squat jump and repetitive submaximal hopping (Fukashiro and Komi, 1987)).

Often, studies investigating jumping in relation to some aspect of athletic performance focus on determining the height of the centre of mass of the performer, and the mechanical power output (peak instantaneous power and average power) throughout the CMJ (Haugen et al., 2020; Linthorne, 2021) and the squat jump (Valenzuela et al., 2020). Of note is that the major difference between the CMJ and the squat jump is that the former is performed with an almost instantaneous transition between the squat and the jump, thereby utilising the stretch-

shortening cycle that generally results in a higher jump (Bobbert et al., 1996; Maud and Foster, 2006; Van Hooren and Zolotarjova, 2017). In contrast to the CMJ, the squat jump has no initial downward displacement, and the jump starts from a crouched position (Hasson et al., 2004). Sayers et al. (1999) developed estimates of peak mechanical power output for the countermovement and squat jumps that involve simple arithmetic, and measurements of jumping height and bodyweight of the performer (Sayers et al., 1999). The estimates have shown to predict peak power output with reasonable accuracy (in athletes and non-athletes) for both the countermovement (R^2 = 0.88, standard error of estimate = 373 W; and squat jumps R^2 = 0.78, standard error of estimate = 562 W; (Maud and Foster, 2006)).

It is well-established that the most valid and reliable method for determining jump height and mechanical power output during jumping involves the use of the force platform. The force platform has been widely used with variations of the vertical jump (Davies and Rennie, 1968; Harman et al., 1990; Newton et al., 1999; Cormie et al., 2009), and as a reference method to validate estimates of peak instantaneous lower body mechanical power (LBPP) output during jumping (Hatze, 1998; Hori et al., 2006). The force platform method of determining both the instantaneous mechanical power production throughout the jump, and jump height involves the application of the impulse-momentum relationship to the temporal history of the force using fundamental principles derived from Newton's laws of motion. Of note is that the impulse can be defined graphically as the area under a force-time curve, numerically as the product of the average force (N) and time (s), and mathematically (as shown below) as the integral of force with respect to time (Enoka, 2008).

$$\text{Impulse} = \int_{t_1}^{t_2} \boldsymbol{F}\, dt$$

Where t_1 and t_2 define the beginning and end of the force-time period measured, **F** = force, and t = time. For the purposes of measuring mechanical power output in the CMJ using the force platform method, t_1 and t_2 are the starting and ending time points for each sampling interval respectively (e.g. sampling interval = 1ms [1 $\times 10^{-3}$ s] for a sampling frequency of 1000 Hz), and **F** = vertical component of the ground reaction force (VGRF) minus bodyweight (BW). The impulse momentum relationship can be derived from Newton's law of acceleration.

$$\sum \boldsymbol{F} = ma$$
$$\sum \boldsymbol{F} = m\left(v_f - v_i\right) \cdot t^{-1}$$
$$\sum \boldsymbol{F}t = m\left(v_f - v_i\right)$$
$$\sum \boldsymbol{F}t = \Delta \mathrm{m}v$$

Impulse = change in momentum

Where **m** = mass; **a** = acceleration; v_f = final velocity; v_i = initial velocity. For the purposes of measuring mechanical power in the CMJ, **m** = body mass of performer; **a** = acceleration of centre of mass (CM) of performer; v_f = final velocity of CM at the end of time interval; v_i = initial velocity of CM at the start of time interval; t = sampling time (e.g. sampling interval = 1ms for a sampling frequency of 1000 Hz); $\sum F$ = vertical component of the GRF minus bodyweight (BW).

Therefore, the **Impulse-momentum** relationship is given by:

$$\int_{t_1}^{t_2} \boldsymbol{F}\, dt = \Delta \boldsymbol{G}$$

Where Δ**G** = change in momentum. For our purposes of measuring power output in the CMJ ΔG represents the change in vertical momentum of the CM of the performer during the sampling interval (from t_1 to t_2). The change in velocity during a sampling interval is

calculated by dividing the impulse (during the sampling interval) by the mass of the performer. The vertical velocity of the CM at the end of a sampling interval is calculated by adding the change in velocity during the same sampling interval, to the velocity at the start of that sampling interval (v_i). Instantaneous power (P) in watts (W) at the end of each sampling interval is given by:

$$P\ (W) = (VGRF\ (N) - BW\ (N)) \times \text{vertical velocity}\ (m \cdot s^{-1})$$

Figure 1.11 shows a typical force-time record of the vertical component of the GRF, the external power produced throughout a CMJ, and histories of the vertical components of the velocity, and displacement of the CM of the performer. Important to note is that the CMJ in the study by Cormie et al. (2009) was performed with an unloaded plastic barbell across the shoulders (not specified whether in front or behind the neck), held in place by the performer during the jump.

Owen et al. (2014) established a criterion method to determine maximum lower body mechanical power output, and jump height during a CMJ using the force platform (Owen et al., 2014). The investigators used the key variables; vertical force range and resolution (using a 16 bit analogue to digital convertor), force sampling frequency and corresponding force integration frequency, method of integration, determination of BW, and determination of the initiation of the jump, to develop this criterion method (Owen et al., 2014). The investigators found that measurements of LBPP were most sensitive to the determination of BW, and the assessment of the initial conditions influencing the determination of the start of the movement.

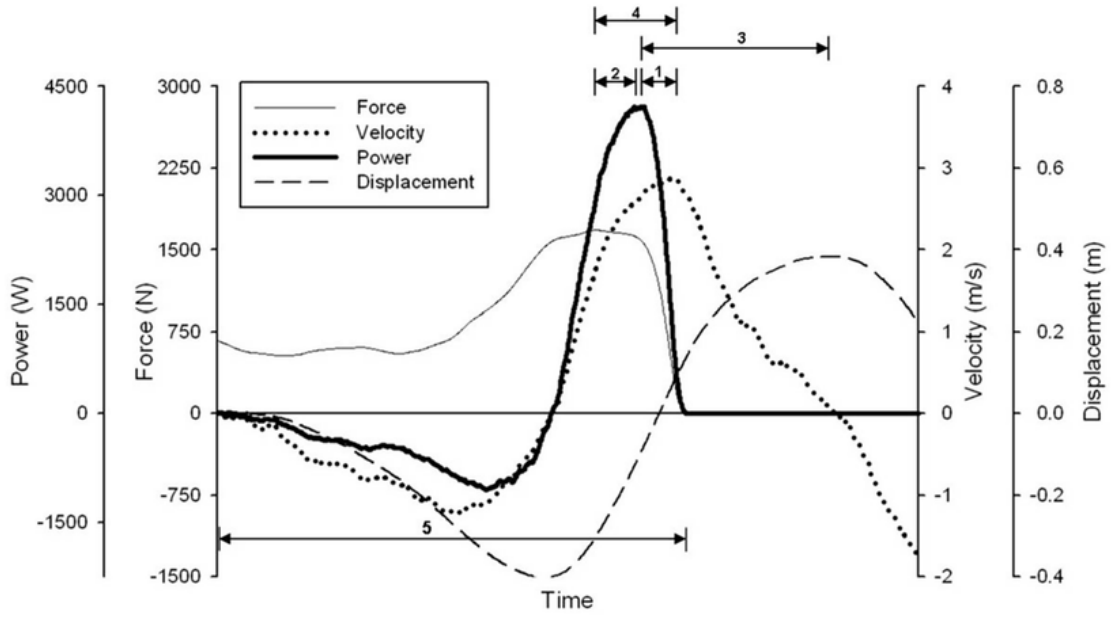


Figure 1.11 Power, force, velocity, and displacement curves plotted against time during a representative trial of CMJ performance. Critical time differences are indicated by the numbered arrows: 1. Time between peak power and peak velocity; 2. Time between peak power and peak force; 3. Time between peak power and peak displacement; 4. Time between peak force and peak velocity; 5. Time from beginning of movement to take-off. Adapted from (Cormie et al., 2009).

They have reported errors of <1% ($P = \leq 0.05$) in the measurement of LBPP output using these specifications; (a) Six times BW for vertical force range, and preferably 16-bit analogue to digital convertor (ADC) over the 12 bit resolution, (b) 1000 Hz for force sampling and integration frequency (c) Simpson's rule or the trapezoidal rule for method of integration (d) mean VGRF for 1 second of quiet standing immediately before the signal to jump for determining body weight, and (e) 30 ms before the instant body weight ± 5 standard deviations is exceeded after the signal to jump – for determining the initiation of the jump. This was in agreement with the findings of Vanrenterghem et al. (2001), that focussed more on the determination of jumping height in that study, and also included the determination of the instant of take-off as an important parameter in determining jumping performance. The criterion method developed by Owen et al. (2014) was used to analyse the force-time

histories of CMJs that were recorded for participants in this thesis. Details for processing the CMJ force-time histories and for acquiring both maximum concentric power and jump height are provided in Section 2.2.

1.8 Genetics of Muscular Strength and Power

Muscular strength is a quantitative multi-factorial phenotype that shows a continuous, often Gaussian variation in the population – with a portion of this variation known to have a genetic origin. Traditionally, top-down or unmeasured genotype approach has been utilized to estimate the heritability of muscular strength/power phenotypes (Beunen and Thomis, 2004), and to some extent, to elucidate the environmental contribution to the observed phenotypic variation (Section 1.3). Intermediate phenotypes of muscular strength such as skeletal muscle fibre type composition (Simoneau and Bouchard, 1995), muscle enzyme activity (Bouchard et al., 1986), leg strength (Zhai et al., 2005), and handgrip strength (Schutte et al., 2016) have estimated heritability values congregating around the 50% value, with some strength phenotypes above this typical 50% estimated heritability such as mesomorphy (characterised by the predominance of muscle, bone and connective tissue) at 86% and 82% for men and women respectively (Peeters et al., 2007). Zempo et al. (2017) identified 24 studies (dating from 1971 to 2016) that have reported the heritability of 58 muscle strength-related traits in healthy subjects in a sedentary state. The authors found that the weighted mean for the 58 heritability estimates was 0.52.

Whilst the unmeasured genotype approach is useful to identify phenotypes under strong genetic control, and to estimate the genetic contribution to a phenotype, it does not provide evidence of the specific genes or combination of genes that influence the observed phenotypic variation. However, for almost two decades, sport and exercise science has entered the ‘molecular exercise physiology’ era (Spurway and Wackerhage, 2006), that allowed a shift from a descriptive to a mechanistic paradigm, by examining the genomic basis of inter-individual variability in performance, and the intracellular signalling pathways that explain some of the effects of environmental factors influencing a phenotype via their

interaction with the genome. Consequently, a main focus of sports and exercise genomics research in recent years has been to identify the specific variations at the DNA level that contribute to the observed heritability and ultimately, to the total phenotypic variation in complex sport- and exercise-related traits (Pitsiladis et al., 2013; Williams et al., 2014); and explore the mechanisms by which these genetic variations exert their effect on these complex traits.

1.8.1 Identifying candidate genes

Most often, genetic research in muscular strength phenotypes has focussed on identifying phenotypic associations with a single candidate gene, via cross-sectional, case-control and longitudinal association studies (MacArthur and North, 2005; Tanaka et al., 2016). A preliminary step in these studies is to identify candidate genes and the polymorphisms in those genes, thought to contribute to the phenotype of interest. Candidate genes can influence a phenotype by encoding a protein important in the structure or function of the cells underlying the phenotype. Polymorphisms in these candidate genes are examined, with the idea that different alleles could affect the structure of the encoded protein or the regulation of the gene, thus impacting cell function and the phenotype itself. Often, priority is given to non-synonymous (missense) single nucleotide polymorphisms (SNPs) that change an amino acid in a protein, or a nonsense variation that creates a premature stop codon, as these are most likely to have substantial biological function that effects the observed phenotype (Roth and Wackerhage, 2014). However, many polymorphisms in genomic regions that regulate gene expression have recently become more appreciated for their functional roles on complex sport- and exercise- related traits (Bouchard, 2019).

The first attempts at identifying genetic markers associated with human exercise-related phenotypes started in the late 1960s with studies based on polymorphisms in red blood cell proteins and enzymes as well as variations at the human leukocyte antigen loci (genes important for immune function), with no studies yielding significant genetic predictors of fitness or performance traits. The more recent high-throughput transcriptomic and genotyping technologies have impacted the domain of human exercise genomics and more candidate genes have been derived from GWAS – based on large numbers of measured and imputed SNPs (Visscher et al., 2017). There are about 10 million of these common polymorphisms in the human genome and each individual carries about 3–4 million of these common variants (Bouchard, 2015). Common alleles have been exposed to selection pressures for thousands of generations since the advent of early human Australopithecus populations, with their alleles (that can exert either a positive or negative extreme effect) becoming fixed or eliminated throughout this evolutionary journey. Common polymorphisms are unlikely to exert dramatic effect size taken individually, however, they often associate or predict with low effect sizes exercise-related traits (Bouchard, 2015). Of note is the identification of two candidate gene mutations that, in contrast to the majority of candidate gene variations, had a major effect on athletic performance. de La Chapelle et al. (1993) identified an activity-enhancing mutation of the erythropoietin receptor in a Finnish family and showed that it has a large effect on the haematocrit of heterozygous carriers. With regards to strength, Schuelke et al. (2004) found that a knockout mutation in the myostatin gene was correlated with greatly increased muscle mass and strength of a toddler (Schuelke et al., 2004). In general, however, genetic variations with a large effect on performance are rare; and it is believed that most sport and exercise-related traits depend on DNA variations of many genes which all contribute with a small percentage to phenotype variation.

Linkage analysis studies were successfully used to identify genome regions that harbour candidate genes relevant to muscular strength (Huygens et al., 2004; De Mars et al., 2008; Thomis et al., 2011). Linkage studies aim to associate different regions of the genome with the phenotype of interest by recruiting families with known (predicted) DNA sequence similarities. A linkage map is produced for each chromosome, with the logarithm of the odds (LOD) score shown on the y-axis, and charted for each of the linkage markers genotyped on that chromosome. When a marker allele is correlated with the phenotype of interest, a higher LOD score will be calculated, resulting in a peak in that region of a particular chromosome. For example, De Mars et al. (2008) conducted a genome-wide linkage scan using 6008 SNP markers that aimed to identify chromosomal regions linked to muscle and bone cross-sectional area, isometric knee flexion and extension torque, and torque–length relationship for knee flexors and extensors, in 283 male siblings (17–36 years of age). The strongest evidence for linkage was found for the torque–length relationship of the knee flexors at 14q24.3 (LOD = 4.09; $p = <10^{-5}$) (Figure 1.12), with suggestive evidence for linkage for bone and muscle cross-sectional are (CSA) at 14q32.2 (LOD = 3.0; $p = <0.005$; (De Mars et al., 2008)). Generally, an LOD score value between 2.0 and 3.0 is considered significant, with LOD scores of 3.0 and higher generating considerable interest, especially when a similarly high score is replicated in other studies (Lander and Kruglyak, 1995). Linkage analysis has been successful in identifying causative genes via genetic mapping for both monogenetic (Mendelian) and polygenic traits with familial aggregation. However, these studies pose some limitations in identifying candidate genes for highly complex phenotypes, that are thought to depend on innumerable genetic variants or quantitative trait loci (QTLs) (Wackerhage, 2014).

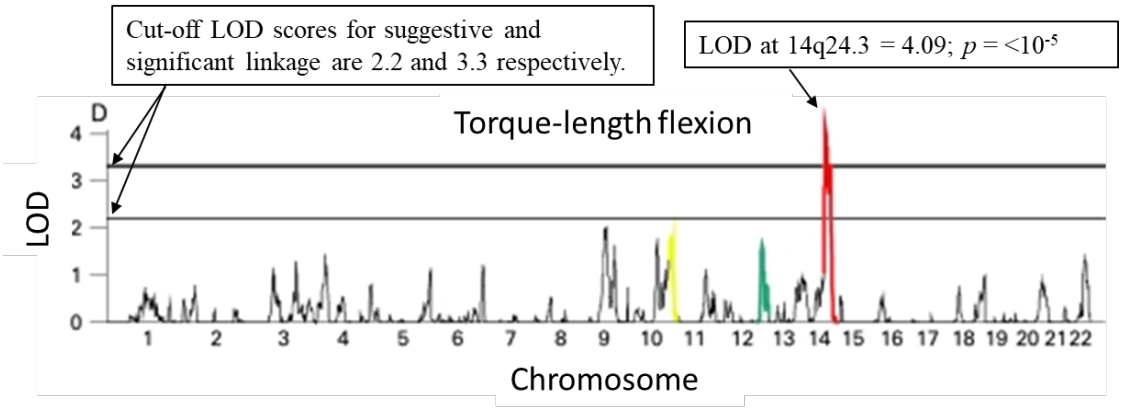


Figure 1.12 One of five LOD scores for autosomal linkage analysis reported by De Mars and colleagues for a knee strength phenotype (torque-length flexion). Adapted from De Mars et al. (2008).

In recent years, linkage analysis was largely superseded by the adoption of high-throughput genome-wide association studies (GWAS) and whole-genome sequencing (WGS). GWAS are an increasingly popular alternative approach in identifying candidate genes now possible as a result of technological advances and decreasing costs. Claude Bouchard and his team pioneered the first genome-wide DNA sequence analysis in a sport and exercise context as part of the HERITAGE family study (Timmons et al., 2010; Bouchard et al., 2011). The study involved 324,611 SNPs to identify those associated with the adaptation to maximal oxygen uptake in response to a 20-week exercise training programme. When combined with informatics and statistics, GWAS allows for the analysis of polymorphic sites of the whole genome to link genetic markers, usually SNPs, to physiological phenotypes (Eynon et al., 2011; Kim et al., 2011; Pitsiladis et al., 2013). GWAS uses SNP chips (microarrays) that measure hundreds of thousands (up to 5 million) of SNPs in the whole human genome, aiming to identify candidate genes and polymorphisms that contribute to a trait (hypothesis-free) rather than hypothesise candidate genes in advance. The usefulness of GWAS depends on large sample sizes (ideally tens of thousands of individuals), a dense microarray of SNPs, and reliably measured phenotypes; requiring international collaborative efforts to be met (Wang et al., 2016) – this poses some great challenges for conducting GWAS. A limitation of

GWAS is that they are able to quantify the variation of traits that are due to common SNPs, but do not identify the variation due to rare alleles, copy-number variations (CNVs) and other polymorphisms that are not included on the SNP microarray. It is very important to note that each DNA locus can probably explain a very small proportion of the phenotypic variance (e.g. ~0.1–1%) of complex traits (Ahmetov and Fedotovskaya, 2015). Additionally, an emerging view is that rare variants, which are not well interrogated by GWAS, could be responsible for a substantial proportion of complex human traits (Bouchard, 2015). Therefore, the question is whether the 'missing heritability' in many GWAS studies is mainly because the data analysis underestimates the importance of common alleles, or whether rare alleles play a major role on the phenotypes of interest.

At present, population-based case-control studies are, arguably, still the most common research designs in sports genomics. They usually involve determining whether the allele or genotype of a particular DNA sequence (gene or non-coding DNA region) is more common in a group of examined athletes than it is in the control group, thus implying that the allele or the genotype improves physical performance. Another type of commonly adopted research are cross-sectional association studies. They examine whether individuals with one allele or genotype of a chosen DNA sequence show different measures of a particular trait compared to the rest of the sample. A general consensus in sports genomics is that case-control and cross-sectional association studies must be replicated in independent samples, while emphasising the issues with respect to appropriate study designs, sample size, population stratification, and quality of the genotype/ phenotype measurement (Bouchard, 2015; Barh and Ahmetov, 2019). Candidate gene association studies can be utilised to further study specific variants that may have had strong associations at GWAS level to provide further evidence that the associations are not false-positive findings. In contrast to GWAS, candidate

gene association studies are hypothesis driven and aim to determine an association between genetic variations and a particular phenotype.

Pioneer researchers in sports genomics suggest that with further advances in genotyping technology, genetics will move from being a primarily methodological problem to being a bioinformatics and ethical problem. At that point the challenge will no longer be the analysis of the DNA sequence but instead the computational analysis of the DNA sequence in order to link variations in the DNA to the phenotype of interest. For example, next-generation sequencing, commonly termed 'second generation sequencing' (Slatko et al., 2018), has the potential of changing sport and exercise genetics fundamentally. The key challenge in applying next generation sequencing to sports genomics will be to obtain information useful for exercise physiologists from the wealth of information that is contained in sequenced human genomes. At that point ethical questions must be considered, because a whole-genome analysis with a main intention to investigate sport- and exercise-related traits may, for example, discover DNA sequence variations that are associated with an increased disease risk.

1.8.2 Candidate genes and variants of interest

The most common type of genetic variations tested for association with strength and/or power in athletes are single nucleotide polymorphisms (SNPs). Millions of SNPs exist across the human population, and these vary in how they affect protein levels and regulatory mechanisms and ultimately, biological phenotypes. Most of the SNPs investigated for association with muscle strength fall into three general categories: muscle structure, growth factors, and inflammatory factors. Whilst the majority of strength related loci studied to date fit into one of these categories, some gene associations with muscle phenotypes have been

found in genes outside of the normal hypertrophy pathways (Bray et al., 2009; Ahmetov et al., 2016). Many of the identified polymorphisms associated with muscle strength have not been investigated in athletes and therefore; although previous studies have associated some variants with a physiological or performance strength phenotype in non-athletic populations, the influence of these variants on phenotypes that are vital for strength- and power-oriented sports remains poorly understood.

As discusses in Section 1.3, variations in muscle strength in the untrained state and in response to training depend on genetic and environmental factors, with heritability of muscle strength/power related traits estimated at around 0.52. Strength is a complex phenotype that is highly polygenic in nature, and many of its sub-phenotypes are also complex and polygenic. Consequently, it is believed that strength phenotypes depend on innumerable common and rare variants with each contributing a proportion to the estimated heritability of strength and power phenotypes. A decade of research on GWAS of diseases and other complex physiological phenotypes have shown that the full genotype accounting for the heritability of such traits is composed of very large numbers of DNA variants (Manolio, 2017; Visscher et al., 2017). In addition, it is a challenge to identify the DNA sequence variants accounting for the genetic component of the variance (heritability) in complex human traits, primarily because the underlying biology of such traits is more complex than once anticipated (Bouchard, 2019). The scientific literature has confirmed the existence of some of the polymorphisms that influence strength, while acknowledging that these polymorphisms typically only explain a small proportion of the variation in strength phenotypes. The general agreement is that most variants that contribute to complex sport- and exercise-related traits have not been discovered as of yet (Puthucheary et al., 2011; Pitsiladis et al., 2016; Bouchard, 2019).

Hughes et al. (2011) identified 22 common genetic polymorphisms in the literature that have been associated with a muscular strength and/or power phenotype (performance variable: one-repetition maximum, or; physiological attribute: fibre-type distribution) in athletes or non-athletes (Hughes et al., 2011). More recently, Ahmetov et al. (2016) reviewed a total of 155 genetic markers that showed an association with elite athlete status, with 62 of these related to power athlete status (Ahmetov et al., 2016). Fourteen (23%) of these genetic markers have shown a positive association with power athlete status in at least two studies, whilst seven markers (11%) were replicated in two or more studies (Ahmetov et al., 2016). All of these strength-related genetic markers are of great interest for the work presented herein and thus, in an ideal world the inclusion of all polymorphisms would have been an obvious choice. However, due to the main limitations of costs and time that are required to perform such investigation, a more realistic and viable approach for this thesis was to select 10 polymorphisms that have been previously associated with strength and/or power phenotypes. It may seem logical that the selected variants should have been those that, in addition to having a known biological role in augmenting strength, have shown the strongest associations with strength/power in an athletic population; thus increasing the chances to discover novel genetic associations with established measures of strength/power and rugby in-game performance – investigated in this thesis. Using this approach favoured, in part, the selection of some of the SNPs (*ACTN3* rs1815739; *AMPD1* rs17602729; *HIF1A* rs11549465; *FTO* rs9939609; *KDR* rs1870377). Although all the selected polymorphisms have been linked to strength/power in previous studies, the variants warranted further investigation due to either producing contrasting findings when studied in power-oriented athletes (*NOS3* rs2070744; *ACE* rs4341), or due to the need for investigation in athletic cohorts for the first time (*TRHR* rs7832552; *PTK2* rs7460 and rs7843014). Two exceptions for the approach just mentioned were the *ACE* rs4341 (although this variant also fits with the selection based on

controversial results) and *ACTN3* rs1815739 polymorphisms which (in the opinion of the author), deserved a somewhat automatic inclusion in the panel of strength/power SNPs for this work. It is very important to note that none of the selected SNPs were previously investigated for genotype-phenotype associations with established measures of strength/power in elite rugby (with an exception for the *FTO* rs9939609 on its own in a sub-sample of elite rugby players by Heffernan and colleagues as part of the RugbyGene project). Furthermore, none of the SNPs was studied for any association with in-game performance in an elite rugby context.

Further investigation of these polymorphisms in an elite rugby cohort will add to the current body of literature of genetic variation in sport, extend our understanding of the genetic contribution to muscular strength and power in elite rugby athletes, and provide new knowledge on the potential role of strength and power associated variants for elite rugby athlete status and in-game performance. Furthermore, in combination with physiological markers already used in talent identification in rugby, the findings from this work can be utilised to aid the processes of talent identification in rugby, discussed in Section 1.6.6.

Table 1.3 lists the 10 polymorphisms (found within nine genes) investigated in this thesis (in alphabetical order of the official HGNC symbols) and provides the main findings of some previous studies (referenced in Table 1.3) that have found an association between the SNPs and a strength/power performance or physiological phenotype. The preferential allele (underlined in Table 1.3) is thought to be advantageous in terms of muscular strength and power – in accordance with the findings of the referenced studies. The following Section (Section 1.9) provides a review for each polymorphism; with a main emphasis on the mechanisms by which the variants are thought to exert their effect on the muscular strength

and power phenotypes and ultimately, in-game performance. In addition, a justification for the selection of each SNP is included in the reviews.

Table 2.3. Genes and polymorphisms investigated in this thesis. Included are the main finding of previous studies (referenced) that have found an association between the SNPs and a strength/power performance or physiological phenotype. The preferential allele (underlined) is thought to be advantageous in terms of muscular strength and power – in accordance with the findings of the referenced studies. Minor allele frequencies (MAF) are also provided.

Gene symbol (HGNC) and rs number	Candidate gene (HGNC)	Candidate protein (UniProt)	Alleles-preferential Underlined	MAF	Co-dominant, dominant	Findings supporting preferential allele (underlined) for the generation of muscular strength and power.
ACE rs4341	Angiotensin I converting enzyme	Angiotensin converting enzyme	I/D	I: 0.43	Co-dominant	DD genotype in *ACE* associated with left ventricular hypertrophy in military recruits after basic training (Montgomery et al., 1997); I allele overrepresented in high altitude climbers (Montgomery et al., 1998). D allele considered preferential for strength/power in Hughes et al. (2011). D allele shown to be advantageous for strength- and power-oriented events in many studies as shown in the review by Puthucheary et al. (2011).
ACTN3 rs1815739	Actinin Alpha 3	Alpha-actinin-3	C/T	T: 0.43	Dominant	*ACTN*3 XX (TT) genotype underrepresented in elite rugby union backs compared with controls. R (C) allele more common in back three than controls and forwards (Heffernan et al., 2016). R allele appears to provide advantage for high velocity muscle action.
AMPD1 rs17602729	Adenosine Monophosphate Deaminase 1	AMP deaminase 1	G/A*	A: 0.12	Co-dominant	Cięszczyk et al. (2011) demonstrated that Polish power oriented athletes (n = 158; short-distance runners, short-distance swimmers, and weightlifters) have lower frequency of the ***AMPD1*** mutant (A) allele than controls.
FTO rs9939609	FTO alpha-ketoglutarate dependent deoxygenase	Alpha-ketoglutarate-dependent dioxygenase FTO	T/A	A: 0.41	Dominant	Heffernan et al. (2017) demonstrated that the T allele is more common (94%) in the back three and centre players who are most reliant on lean mass rather than total body mass for success, compared to other rugby athletes (82%) and controls (84%). Accordingly, these athletes had greater peak power relative to body mass than other rugby athletes (14%).
HIF1A rs11549465	Hypoxia Inducible Factor 1 subunit alpha	Hypoxia-inducible factor 1-alpha	C/T*	T: 0.10	Co-dominant	Ahmetov et al. (2008) demonstrated that the frequency of the ***HIF1A*** 582Ser allele was significantly higher in weightlifters (n = 53) than in 920 controls (17.9% vs. 8.5%; P = 0.001), and increased with the athletes' levels of achievement (sub-elite (14.7%); elite (18.8%); highly elite (25.0%)).
KDR rs1870377	Kinase insert domain receptor	Vascular endothelial growth factor receptor 2	T/A	A: 0.24	Co-dominant	Ahmetov et al. (2009) reported that individuals who are Gln (A allele) carriers (Gln/His and Gln/Gln genotypes) are associated with an increased proportion of type I muscle fibres of the vastus lateralis muscle compared to individuals with the His/His genotype in both Russian speed skaters and controls. This polymorphism is a potential candidate for determining muscle fibre-type in European populations (Ahmetov et al., 2009; Yvert et al., 2016).
NOS3 rs2070744	Nitric Oxide Synthase 3	Nitric Oxide Synthase 3	C/T	C: 0.44	Co-dominant	Gómez-Gallego et al. (2009) have shown that the frequency of the TT genotype was significantly higher in power athletes (57%) than in the endurance (33%) or control group (34%). The same authors demonstrated that the frequency of the T allele was also higher in power sportsmen (71%) than in their endurance (55%) and control referents (56%).
PTK2 rs7460	Protein tyrosine kinase 2	Focal adhesion kinase 1	A/T	T: 0.48	Co-dominant	Baseline specific force of male non-athletes (before a resistance training intervention) higher in AA homozygotes (Erskine et al., 2012). Specific force (no training intervention) higher in AA homozygotes (Stebbings et al., 2017).
PTK2 rs7843014	Protein tyrosine kinase 2	Focal adhesion kinase 1	A/C	C: 0.46	Co-dominant	AA genotype associated with higher specific force in 120 Caucasian males non-athletes (Stebbings et al., 2017).
TRHR rs7832552	Thyrotropin-releasing hormone receptor	Thyrotropin-releasing hormone receptor	C/T	T: 0.27	Dominant	Liu et al. (2009) demonstrated that lean body mass of individuals homozygous for the rs7832552 T allele was, on average, 2.55 kg higher than heterozygotes and C allele homozygotes. The researchers replicated the significant associations in three independent samples (1488 unrelated US whites, 2955 Chinese unrelated subjects, and 593 nuclear families comprising 1972 US whites).

*Indicates homozygotes for the minor allele frequency (MAF; on the side of the asterisks) that are very low in number to allow an additive comparison using chi-square. In that case, a recessive/dominant model that includes the homozygotes for the MAF is used.

1.9 Genes and polymorphisms investigated

This Section provides specific reviews for 10 polymorphisms (found within nine genes) investigated in this thesis, while emphasizing the mechanisms by which each polymorphism is thought to exert its effect on strength- and power- related phenotypes.

1.9.1 Angiotensin I converting enzyme (*ACE*)

One gene of particular interest for sport performance that has been studied more extensively than any other is the angiotensin I converting enzyme gene (*ACE*), that codes for the angiotensin converting enzyme (ACE); an enzyme critical to the renin angiotensin system (RAS) (Rigat et al., 1990) also known as renin angiotensin aldosterone system (RAAS). The RAS combines elements of renal physiology (control of renal hemodynamics and sodium excretion), cardiovascular physiology (blood pressure regulation), endocrinology (secretion of aldosterone and other hormones), and neurophysiology (actions of angiotensin on the brain and autonomic nervous system) (Reid, 1998). Renin, a 37kDa aspartyl protease which is produced in the kidney, cleaves hepatically derived angiotensinogen to yield decapeptide angiotensin I. Acted upon by ACE, angiotensin I is converted into angiotensin II, a potent vasoconstrictor whose effects are mediated predominantly through two specific human receptors, angiotensin type-1 receptor (AT_1R) and angiotensin type-2 receptor (AT_2R) (Payne and Montgomery, 2003). It is the agonist action of angiotensin II at the AT_1R that causes an elevation in arterial blood pressure through direct arterial vasoconstriction, and through salt and water retention provoked by adrenal aldosterone release. ACE is also involved in the degradation of bradykinin, a potent vasodilator due to its actions on the bradykinin type-1 receptor (BK_1R) and bradykinin type-2 receptor (BK_2R) (Regoli et al., 1998; Dendorfer et al., 2001). Therefore, bradykinin levels are inversely related to ACE activity (Brown et al., 1998; Murphey et al., 2000). In addition to endocrine RAS, local RAS are known to exist in skeletal muscle (Dragović et al., 1996), cardiac muscle (Dzau, 1988) and adipose tissue (Jonsson et

al., 1994); and are involved in the regulation of tissue growth and muscle hypertrophy (Dzau, 1988; Ishigai et al., 1997; Nazarov et al., 2001). Functional genetic polymorphisms have been identified for most components of RAS including; renin, angiotensinogen, and the Ang II and bradykinin receptors. By far the best known and studied is a 287-nucleotide insertion/deletion (I/D) polymorphism of the *ACE* gene – identified within intron 16 of the gene on chromosome 17 (Rigat et al., 1990). The absence (deletion, D) rather than presence (insertion, I) of this 287 amino acid base pair Alu repeat sequence is associated with increased concentrations of tissue (Costerousse et al., 1993; Danser et al., 1995) and serum (Rigat et al., 1990) ACE activity. Homozygous for the D allele demonstrate cardiac and monocyte ACE activity almost >75% than that found in I/I or I/D genotypes (Costerousse et al., 1993; Danser et al., 1995).

The *ACE* I/D polymorphism became the first genetic element that have shown to impact substantially on human physical performance (Montgomery et al., 1998). The first of two parallel studies by Montgomery et al. (1998) involved 25 elite male mountaineers and 1,906 male non-athletes. The researchers found that both genotype distribution and allele frequency differed significantly between the mountaineers and non-athletes, with the I allele overrepresented in mountaineers (Montgomery et al., 1998). In the second study, the maximum duration of performing a standardised repetitive elbow flexion exercise (utilising a 15 kg barbell) was recorded for 78 Caucasian military recruits before and after 10 weeks of military training. Baseline performance was independent of *ACE* genotype however, improvements in exercise duration capacity after training were strongly genotype-dependent (increase in duration (s): I/I, 79.4 ± 25.2; I/D, 24.7 ± 8.8; DD, 7.1 ± 14.9) (Montgomery et al., 1998). Notably, homozygotes for the I allele showed an 11-fold greater improvement than homozygotes for the D allele (Montgomery et al., 1998). Since the publication of these two

studies, numerous other studies have supported an association of the *ACE* genotype with sporting performance with the I allele, in general, associated with endurance-orientated events and the D allele with strength- and power- oriented events (Ma et al., 2013). In a study of 91 British Olympic-standard runners (79 Caucasian) I allele frequency (in the sub-analysis of the 79 Caucasian) increased from 0.35 to 0.53 and 0.62 for the ≤200 m, 400–3000m and ≥5000 m distance groups, respectively (Myerson et al., 1999). Tsianos et al. (2004) found that genotype frequencies and I allele carriage of 35 elite ultra-distance swimmers differed for those who showed superiority at 1–10 km distances (I/I, 6% vs. I/D, 47% vs. D/D, 47%; I allele frequency, 0.29) when compared with those who showed superiority at 25 km races (I/I, 18.8% vs. I/D, 75% vs. D/D, 6.2%; I allele frequency, 0.56) (Tsianos et al., 2004). In swimming, muscular power rather than endurance is likely to be the determinant of success with most events undertaken in <120 s (Nazarov et al., 2001). In Olympic level swimmers an excess of D allele (0.60 vs. 0.51) was observed compared with controls (Myerson et al., 1999); a finding replicated in elite Caucasian swimmers from European and Commonwealth championships, where a significant overrepresentation of D allele was observed, in particular in swimmers competing over shorter distances of ≤400m (Woods et al., 2001). This association was supported by other studies, but restricted for elite swimmers competing in distances of ≤200 m (Tsianos et al., 2004; Costa et al., 2009).

With regards to the response to strength training, it appears that Ang II is involved in the transduction of mechanical load to yield growth responses which in turn, may translate to greater strength gains (Folland et al., 2000). Folland et al. (2000) examined the response to a 9-week leg training regime (isometric and dynamic leg extensions) in 33 healthy males and found a significant interaction between *ACE* genotype and isometric training, with greater strength gains shown by D allele carriers. In addition, a consistent genotype-training

interaction was observed across strength measures and both types of training (Folland et al., 2000). Williams et al. (2005) examined the relationship between circulating ACE activity, strength, and the response to training in 81 untrained men; from whom 44 performed an 8-week program of dynamic strength training of the quadriceps. Although the *ACE* genotype appeared to be related to pre-training muscle strength, it showed no association with the training-induced change in strength (Williams et al., 2005). These findings may be, in part, mediated by genotype-dependent differences in skeletal muscle growth however, there is also evidence that the genotype-dependent mediator is the difference in muscle fibre type distribution (Zhang et al., 2003). Zhang et al. examined the association between the *ACE* genotype and skeletal muscle fibre types in 41 untrained healthy young volunteer subjects, and found that *ACE* I/I homozygotes had significantly higher percentages of type-I fibres (50.1 ± 13.9% vs. 30.5 ± 13.3%) and lower percentages of type-II fibres (16.2 ± 6.6% vs. 32.9 ± 7.4%) than homozygotes for the D allele (Zhang et al., 2003). Conflicting data do exist (Rankinen et al., 2000; Amir et al., 2007; Kim et al., 2010) but such findings seem explained, generally, by the inclusion of heterogeneous participant groups in terms of mixed race and sex, and sporting discipline. A notable exception was the study by Amir et al. (2007) who reported a positive association between D/D genotype and elite endurance performance in 121 Israeli runners, although this atypical finding may occur due to the heterogeneous nature of the Israeli Caucasian Jewish population (Zoossmann-Diskin, 2008).

It can be theorised that the association of *ACE* genotype with physical performance might be due to linkage of this variant with a functional variation in an adjacent locus, such as the nearby growth hormone (GH) (Bennani-Baiti et al., 1995). The T/A polymorphism in intron 4 of the *GH1* gene has been associated with lower levels of GH and insulin-like growth factor-I (Hasegawa et al., 2000) however, no association was found between this variant and

performance in South African Ironman (Walpole et al., 2006). Alternatively, the effects of *ACE* I/D genotype may be mediated through changes in Ang II activity that in turn, acts on the AT_1R gene. Notably, AT_1R has two polymorphisms (Berge et al., 1997; Poirier et al., 1998) that appear functional (Karjalainen et al., 1999; Diet et al., 2001; Delmonico et al., 2005) however, again here, these polymorphisms do not appear to be associated with variations in performance (Alvarez et al., 2000; Fatini et al., 2000). As already mentioned above, *ACE* genotype does influence bradykinin levels; with the ACE I allele associated with higher kinin activity. A common gene variant in the BK_2R exists: the absence (-9) rather than the presence (+9) of a 9-bp fragment is associated with greater receptor response and gene transcription (Lung et al., 1997; Houle et al., 2000). A study that have examined running distance in 81 Olympic-standard track athletes have shown that the 'high-kinin receptor activity' haplotype (*ACE* I/BK_2R -9) is associated with running endurance (Williams et al., 2004); suggesting that a fraction of the association of *ACE* genotype with performance traits is mediated through changes in kinin activity at its BK_2R. Similarly, in South African Ironman triathletes the BK_2R -9/-9 genotype was overrepresented amongst the triathletes (27.0%) when compared with controls (19.3%); while the -9 allele was associated with shorter finishing times (Saunders et al., 2006).

Although the role of *ACE* genotype in sports performance have been investigated for over two decades (Puthucheary et al., 2011), the first decade of research was, arguably the most exciting due to the *ACE* gene (quite recent during that time period) being the first to be associated with physical performance (Montgomery et al., 1998). There is a wealth of research that addressed the role of *ACE* genotype in relation to both endurance-oriented and strength- and power-oriented sports disciplines (Ma et al., 2013) that in general, have demonstrated the importance of *ACE* gene, RAS components and polymorphisms within

them for sports performance as seen above. Numerous valuable investigations in relation to *ACE* genotype could not be covered here, but it is worth mentioning in brief the importance of *ACE* genotype in the hypertrophic response of cardiac muscle to exercise, for example; as this could also be involved in skeletal muscle hypertrophy an thus, contribute to strength and power. Montgomery et al. (1997) found an association between the increase in left ventricular mass in response to 10 weeks of physical training in male Caucasian military recruits, with a strong association between the magnitude of the response and *ACE* genotype (Montgomery et al., 1997). Subsequently, this association has been confirmed in elite wrestlers (Kasikcioglu et al., 2004), elite football players (Fatini et al., 2000) and endurance athletes (Hernández et al., 2003). Furthermore, *ACE* genotype have been associated with differences in the metabolic efficiency of skeletal muscle. In a study of Caucasian male military recruits undergoing an 11-week physical training programme, baseline delta efficiency (defined as the change in work performed per min to the change in energy expended per min) was independent of genotype; however, recruits of I/I genotype showed a significant increase in delta efficiency after training that was not seen in recruits of D/D genotype (Williams et al., 2000). Such effects may, in part, be mediated through *ACE* genotype-dependent modulations in kinin activity. Williams et al. (2004) examined 115 healthy men and women and found that delta efficiency was strongly associated with *BK_2R* genotype (Williams et al., 2004). There is also evidence of interaction between *BK_2R* genotype and *ACE* genotype – those who were of *ACE* I/I and *BK_2R* -9/-9 genotype have shown the highest baseline delta efficiency (Williams et al., 2004). Despite the relatively large body of research that examined the *ACE* genotype and its role in sport performance (as compared to research that examined other genes in sport), there is a scarcity of research that have studied *ACE* genotype in strength- and power-oriented team sports such as rugby. As part of the RugbyGene project, Heffernan et al. (2016) examined the *ACE* I/D and *ACTN3* R577X polymorphisms (the two most studied SNPs in

sports (Barh and Ahmetov, 2019)) in elite rugby athletes (rugby league and rugby union) to explore whether these variants are associated with elite rugby athlete status and/or playing position (only in rugby union for the latter). Quite surprisingly, no associations were discovered between any variant and elite athlete status, but *ACTN3* R577X was associated with playing position (Heffernan et al., 2016). Considering the important role of *ACE* genotype for physical performance, together with the lack of association in the study by Heffernan et al. (2016), elicits the need for a study-design in which the phenotype of interest is 'purified' by categorising the athletes by their ability in established measures of the phenotype (in contrast to categorisation by playing position). This approach attempts to reveal any genotype-phenotype associations that might exist between *ACE* genotype and vital performance traits (such as strength and power) that are known to be influenced by this variant and that ultimately, underpin performance in rugby. With this in mind, the inclusion of *ACE* gene as a candidate in the panel of strength/power SNPs for the work presented herein seems inevitable. In addition, it is important to mention that no studies has attempted to explore whether associations exist between any of the SNPs selected for this work (previously associated with strength/power in the literature) and in-game performance in elite rugby – as accomplished in this thesis.

1.9.2. Alpha-actinin 3 (*ACTN3*)

One of the most (arguably the most) studied polymorphisms in relation to sports performance is the *ACTN3* R577X genotype. Alpha-actinins constitute a family of actin-binding proteins located in the Z discs of sarcomeres necessary to anchor actin filaments to the sarcomeric Z-line (Blanchard et al., 1989; MacArthur and North, 2004). There are two actinin isoforms of which alpha-actinin 2 (ACTN2) is expressed in skeletal and cardiac muscle fibres; whereas ACTN3 is a structural component expressed almost exclusively in fast glycolytic type II

muscle fibres, and binds the actin thin filament to the Z line by its distinct N terminal actin binding domain and (Beggs et al., 1992; Mills et al., 2001) (Figure 1.13).

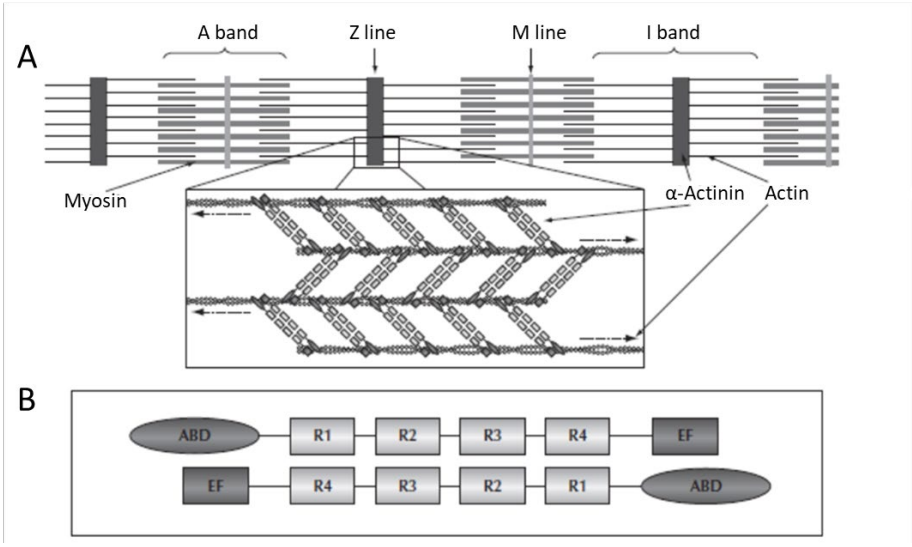


Figure 1.13 Localization and structure of the sarcomeric alpha-actinins. A: the sarcomeric alpha-actinins localize to the sarcomeric Z-line, where they form head-to-tail dimers cross-linking actin filaments from adjacent sarcomeres. B: each alpha-actinin molecule contains an N-terminal actin-binding domain (ABD), a C-terminal hand domain (EF), and four spectrin-like repeats (R1-R4). In muscle, alpha-actinin molecules are always present in the form of head-to-tail dimers. Adapted from Bouchard and Hoffman (2011).

North et al. (1999) identified a functional polymorphism in *ACTN3* gene in humans located in exon 16 of the gene on chromosome 11, where a C > T transition results in the conversion of an arginine (R) to a premature stop codon (X) at amino acid 577 (R577X) (North and Beggs, 1996; North et al., 1999). The consequence of this premature stop codon is that a shortened non-functional version of ACTN3 is produced which is degraded and thus, the *ACTN3* 577X allele is comparable to a gene knockout (Wackerhage, 2014). Individuals homozygotes for the 577R allele have the fully functioning gene variant, whereas those homozygous for the 577X allele are unable to produce the ACTN3 protein in muscle (Mills et al., 2001; Clarkson et al., 2005). However, complete absence of ACTN3 in muscle fibres is not associated with

an obvious disease phenotype, raising the possibility that ACTN3 is functionally redundant in humans, and that ACTN3 deficiency is compensated by ACTN2 (Mills et al., 2001).

Yang et al. (2003) were the first to demonstrate a highly significant association between *ACTN3* genotype and athletic performance by investigating this polymorphism in 107 sprint/power athletes (46 track athletes of events ≤800 m; 42 swimmers of events ≤200 m; 9 judo athletes; 7 short-distance track cyclists; 3 speed skaters), 194 endurance athletes (77 long-distance cyclists; 77 rowers, 18 swimmers of events ≥400 m; 15 track athletes of events ≥5,000 m; 7 cross-country skiers) and 436 controls. Thirty-two of the sprint/power and 18 endurance athletes were of Olympic-standard. Each athlete group and also the control group was composed of white Australian men and women, and the analyses were performed for these three groups (as whole groups) and for sub-groups by gender. Given the localization of ACTN3 in fast skeletal muscle fibres, the authors hypothesized that deficiency of ACTN3 would reduce performance in sprint/power events and would therefore, be less frequent in elite sprint/power athletes compared with either endurance athletes or controls. Although there were no significant allele or genotype frequency differences between the elite athlete group as a whole and controls, a strong evidence of allele frequency variation was observed when athletes were categorised into sprint/power and endurance athlete groups. Significant differences in allele frequency were observed between sprint athletes and controls for both males and females. Specifically, sprint/power athletes had a lower frequency of the XX (ACTN3 null) genotype (6% vs. 18%) than controls; and no female sprint/power athlete (whether Olympian or not) or sprint/power Olympian (whether a man or a woman) were homozygotes for the null allele (Yang et al., 2003), meaning that a few non-Olympian male athletes were homozygotes for the 577X allele (XX). Notably, sprint/power athletes also had a higher frequency of the RR genotype (50% vs. 30%) and a lower frequency of the

heterozygous RX genotype (45% vs. 52%) compared with controls; while elite endurance athletes had a slightly higher frequency of the XX genotype (24% vs. 18%) than controls. Interestingly, allele frequencies in sprint/power and endurance athletes deviated in opposite directions and differed significantly from each other in both males and females (Yang et al., 2003).

In support of the trend toward an increased XX genotype frequency in endurance athletes found by Yang et al. (2003), replication studies with independent cohorts of elite endurance athletes have shown that the X allele or the XX genotype is associated with elite endurance performance (Niemi and Majamaa, 2005; Eynon et al., 2009) although these findings are not unequivocal (Saunders et al., 2007; Döring et al., 2010; Kikuchi et al., 2016). On the other hand, the influence of *ACTN3* R577X on elite power performance is much more consistent. The initial findings by Yang et al. (2003) that have evidenced, for the first time, the influence of *ACTN3* R577X on elite power performance have been replicated in many other independent cohorts of elite power-oriented athletes (reviewed by Ma et al. (2013)). There is substantial evidence indicating that the frequency distribution of *ACTN3* XX genotype is significantly lower in elite power track and field athletes (i.e. sprinters, jumpers and throwers) and elite weightlifters compared with either elite endurance athletes or controls (Niemi and Majamaa, 2005; Papadimitriou et al., 2008; Eynon et al., 2009; Muniesa et al., 2010; Ginevičienė et al., 2011); suggesting that the *ACTN3* XX genotype is detrimental to strength- and power-oriented performance, in particularly at the elite level of competition. In support of the mentioned previous investigations, more recent replication studies reported significantly higher frequencies of the 577R allele in strength- and power-oriented athletes compared with endurance athletes and/or controls (Kikuchi et al., 2016; Papadimitriou et al., 2016; Wagle et al., 2018), with consistent prominence on *ACTN3* gene attributed to the

robust relationship between the 577R allele and strength/power phenotype. This has generated interest amongst researchers that is reflected in a number of reviews (Eynon et al., 2013; Ma et al., 2013; Ahmetov and Fedotovskaya, 2015; McAuley et al., 2021) and meta-analysis (Ma et al., 2013; Tharabenjasin et al., 2019) that have further explored and strengthened the relationship between *ACTN3* R577X and strength/power sports.

Being the first group of researchers to demonstrate an association between a gene polymorphism and physical performance, Yang et al. (2003) hinted that the functional basis of the *ACTN3* 577R allele for power-oriented performance is likely related to the fact that ACTN3 is the predominant fast fibre isoform in both mouse and humans (Mills et al., 2001) and therefore, it may confer a greater capacity for the absorption or transmission of force at the Z line during high-velocity muscle contraction. The Z line in fast, glycolytic fibres is the structure most vulnerable to exercise-induced injury resulting in morphological damage and degradation of associated proteins, including the alpha-actinins (Friden and Lieber, 2001). Previous research have shown that sarcomeric alpha-actinins bind to the gluconeogenic enzyme fructose-1, 6-bisphosphatase (Gizak et al., 2003), to the glycogen phosphorylase amorphin (Chowrashi et al., 2002) and to the calsarcins (Frey et al., 2000; Frey and Olson, 2002), which interact with calcineurin. Calcineurin is a signaling factor that plays a role in the specification of muscle fibre type (Serrano et al., 2001) and thus, alpha-actinin 3 may promote the formation of fast-twitch fibres or alter glucose metabolism in response to training (Yang et al., 2003). Accordingly, carriers of the 577R allele have a greater proportion of type II and IIx fibres and larger relative surface area per IIx fibre than XX homozygotes (Vincent et al., 2007; Ahmetov et al., 2011; Broos et al., 2012). More recently, Seto et al. (2013) have shown that the likely mechanism for the association between the 577R allele and muscle fibre phenotype is via the calcineurin muscle fibre remodelling pathway (Seto et al.,

2013). The researchers found greater calcineurin activity (which induces slow myogenic programming and a shift toward oxidative phenotype) in *actn3* knockout mice and humans (homozygotes for *ACTN3* 577XX genotype) due to preferential binding of alpha-actinin 2 (upregulated in alpha-actinin 3 deficient muscle) to the fast fibre-specific calsarcin-2, with the latter being a key inhibitor of calcineurin activation (Seto et al., 2013). This mechanism could explain the advantage of R allele carriers over ACTN3 deficient individuals (XX genotype) for high-velocity contractions, particularly important for strength- and power-oriented sport.

Emerging evidence suggests that *ACTN3* genotype may impact a number of other traits such as the adaptation to strength training (Pereira et al., 2013; Erskine et al., 2014) and injury risk (Massidda et al., 2019). For example, Norman et al. (2014) reported that mammalian target of rapamycin (mTOR) and p70s6k phosphorylation (Section 1.3) were greater in 577R allele carriers than in XX genotype following sprint exercise (Norman et al., 2014). As discussed in this work (Section 1.3) both mTOR and p70S6k are involved in the regulation of skeletal muscle hypertrophy, providing a mechanistic support for the belief that hypertrophying muscle, and hence positive adaptations with respect to strength/power, should be greater in 577R allele carriers in response to resistance training. Furthermore, Ahmetov et al. (2014) reported that testosterone levels were higher in male and female athletes with at least one 577R allele compared to XX homozygotes (Ahmetov et al., 2014). Although the association between *ACTN3* genotype and testosterone in elite athletes is not clear, it provides a possible mechanism explaining the advantageous role of the 577R allele for greater training-induced muscular strength/power improvements. When considering the collective research evidence for the role of *ACTN3* genotype in strength/power performance, including emerging evidence of the role of this structural gene in the response to strength training, the inclusion of *ACTN3*

R577X in the panel of polymorphisms for this thesis becomes inevitable. Notably, despite the large body of research evidence in relation to *ACTN3* genotype, no studies have attempted to explore the associations between *ACTN3* genotype and in-game performance in a well-defined cohort of elite rugby union athletes – hence its inclusion is also justified.

1.9.3. Adenosine Monophosphate Deaminase 1 (*AMPD1*)

Adenosine monophosphate (AMP) deaminase (AMPD) is an important regulator of muscle energy metabolism during exercise. AMPD is an enzyme that catalyses the deamination of AMP to inosine monophosphate (IMP) and ammonia (Sabina et al., 1990). The skeletal muscle-specific isoform of AMPD accounts for more than 95% of the total AMPD in muscle (Fishbein et al., 1993) and is mainly located in type II muscle fibres particularly at the neuromuscular junction, but also in capillaries (Van Kuppevelt et al., 1994). In skeletal muscle, AMPD is activated during exercise when the rate of adenosine triphosphate (ATP) utilisation exceeds the potential of the cell to resynthesize ATP (Norman et al., 1998). AMP deamination displaces the equilibrium of the myokinase reaction (two adenosine diphosphate (ADP) ↔ ATP + AMP) toward ATP resynthesis (Sabina et al., 1984)). Thus, a proposed role for AMPD is to alleviate the exercise-induced decrease in the ATP/ADP ratio and the inhibitory effect of this decrease on muscle contraction (Lowenstein, 1990). AMPD activities in skeletal muscle are quite variable across many neuromuscular disorders (Norman et al., 1998). Variability in AMPD activities is attributed to a combination of genetic and pathological factors that influence the expression of this enzyme (Kar and Pearson, 1973; Fishbein, 1985; Nagao et al., 1986; Sabina et al., 1991; Morisaki et al., 1992; Gross, 1997). It has been estimated that ~2% of human skeletal muscle biopsies submitted for pathological evaluation are deficient in AMPD (Gross, 1994; Gross, 1997). One-half of the individuals deficient for AMPD exhibit exercise-related muscle cramps, pain, and early fatigue but have

no other neuromuscular abnormalities (Norman et al., 1998) – reflecting the proposed role for AMPD in skeletal muscle energetics and function. On the other hand, deficiency of AMPD in other individuals is secondary to many well-defined neuromuscular disorders (Norman et al., 1998).

The skeletal muscle-specific isoform of AMPD is encoded by the *AMPD1* gene, which is located on the short arm of chromosome 1 (Sabina et al., 1990). In the majority of cases, AMPD deficiency has been attributed to a nonsense mutation (G→A transition) in exon 2 of *AMPD1* that converts a glutamine codon into a premature stop codon (Morisaki et al., 1992). The encoded polypeptide product of this mutant sequence would be severely truncated and catalytically inactive, thereby accounting for most cases of inherited skeletal muscle AMPD deficiency (Norman et al., 1998). Consequently, homozygotes for the mutant allele have extremely low skeletal muscle AMPD activity, whereas heterozygotes and normal homozygotes have intermediate and high enzyme activities, respectively (Figure 1.14) (Norman et al., 1998). The high prevalence of the identified *AMPD1* mutant allele predicts a relative large group of asymptomatic, AMPD deficient individuals in the Caucasian and African American populations (Gross, 1997; Morisaki et al., 1992). Specifically, Morisaki et al. (1992) reported that the *AMPDI* mutant allele is found in 12% of Caucasians and 19% of African-Americans, but none of the Japanese subjects surveyed had the mutant allele (Morisaki et al., 1992). In healthy Swedish individuals, AMPD deficiency was observed in 2% of subjects (40 men and 80 women), while the remainder exhibited a typical bimodal distribution (Figure 1.14) of intermediate and high AMPD activities (Norman et al., 1995). Norman et al. (1998) reported an *AMPD1* mutant allele frequency of 13.7% and a 1.7% incidence of AMPD deficiency across 175 Caucasian Swedish healthy students. The investigators examined the influence of genetic and fibre-type composition, training status,

and gender on the variability of AMPD activity, and found the *AMPD1* genotype had the greatest effect on enzyme activity compared to the other factors examined (Norman et al., 1998).

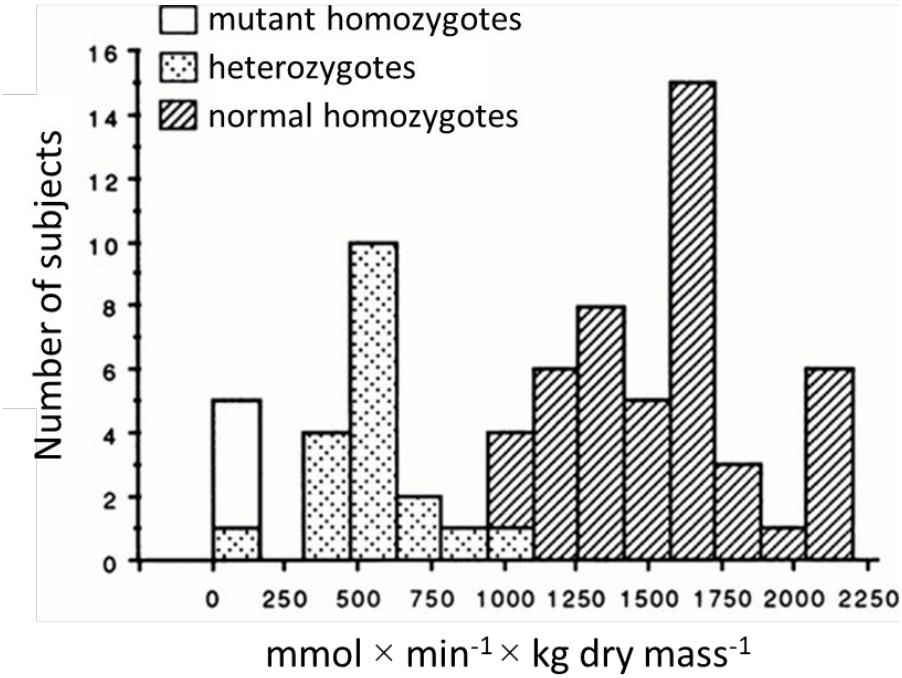


Figure 1.14 Skeletal muscle AMP deaminase activity in healthy subjects with different *AMPD1* genotypes. Histogram illustrates AMP deaminase activity (mmol × min^{-1} × kg dry $mass^{-1}$) and *AMPD1* genotype in 70 healthy subjects. Mutant homozygotes and atypical heterozygote all have <15 mmol × min^{-1} × kg dry $mass^{-1}$ of AMP deaminase activity. Adapted from (Norman et al., 1998).

The combination of high incidence of AMPD deficiency and associated clinical variability has prompted some investigators to refer to AMPD deficiency simply as a 'harmless genetic variant' (Verzijl et al., 1998). However, since the period during which early studies proposed that a deficiency of AMPD causes muscular weakness or cramping after exercise (Fishbein, 1985), several studies have tried to elucidate the mechanisms by which AMPD deficiency might regulate muscle metabolism and cause premature onset of fatigue. The rationale for the reduced exercise capacity in connection with AMPD deficiency could be based on the premise of accentuated ADP (and AMP) accumulation during fatiguing exercise (Sabina et

al., 1984). Normally, the AMPD reaction should minimize ADP accumulation in skeletal myocytes during short-term high-intensity exercise; by removing AMP and displacing equilibrium of the myokinase reaction (2 ADP ↔ ATP+AMP) in the direction of ATP formation as already mentioned above (see also Figure 1.15). It is thought that this process prevents the inhibitory effect exerted by a decrease in the ATP/ADP ratio during muscle contraction (Norman et al., 1998) to maintain 'contraction homoestasis' (Sabina et al., 1984). Notably, it has been shown that increases in ADP reduce maximal shortening velocity of contracting skeletal muscle (Cooke and Pate, 1985; Westerblad et al., 1998) – a fundamental characteristic of muscle fatigue. Thus, it appears that AMPD deficiency leads to an earlier decline in exercise performance during conditions of high ATP turnover rates.

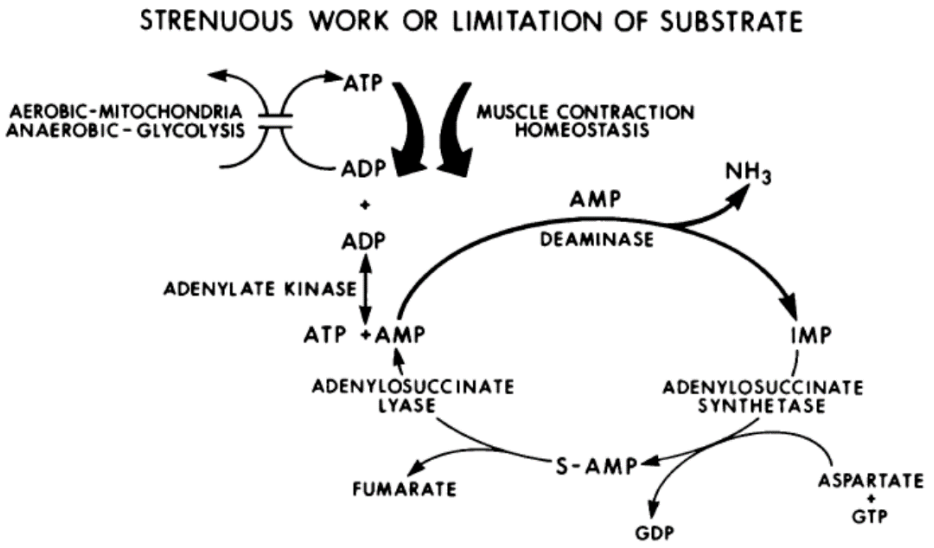


Figure 1.15 Simplified illustration of the reactions of adenylate metabolism in skeletal muscle. Adapted from (Sabina et al., 1984).

To investigate the effect of *AMPD1* genotype on adenylate catabolism and physical performance Norman et al. (2001) used the 30 s Wingate test as a means of 'short-term high-intensity exercise' based on evidence that the test leads to a pronounced activation of AMPD

(Bogdanis et al., 1995; Esbjornsson-Liljedahl et al., 1999; Hargreaves et al., 1998). Quite surprisingly, exercise performances were similar across *AMPD1* genotypes. However, normal homozygotes exhibited the highest levels of AMPD activities, net ATP catabolism, and IMP accumulation; whereas intermediate values were observed in heterozygotes. Conversely, mutant homozygotes had very low AMPD activities and showed no significant net catabolism of ATP or IMP accumulation (Norman et al., 2001). The authors concluded that deficiency of AMPD might cause higher oxidative metabolism during exercise, that may result from an increased adenosine levels enhancing blood flow, and increased ADP levels stimulating oxidative phosphorylation and thereby; compensating for the decreased purine nucleotide cycling (Norman et al., 2001). In support of the findings by Norman et al. (2001) but only with respect to power output; Tarnopolsky et al. (2001) did not observe significant differences in power produced on a cycle ergometer between the mutant and the normal homozygotes, or heterozygotes individuals (Tarnopolsky et al., 2001). However, De Ruiter et al. (2002) observed a reduction in force generating capacity during repetitive submaximal isometric contraction in AMPD deficient subjects, but not in sedentary controls (De Ruiter et al., 2002). Further, Rico-Sanz et al. (2003) examined the *AMPD1* genotype for associations with cardiorespiratory phenotypes (using cycling exercise) as part of the HERITAGE family study. The authors found that homozygotes for the mutant A allele had diminished exercise capacity and cardiorespiratory responses to exercise (Rico-Sanz et al., 2003). Furthermore, Fischer et al. (2007) found a higher rate of power decrement during the 30 s Wingate cycling test in those who were deficient in AMPD, within 139 healthy and physically active men and women (Fischer et al., 2007).

For the first time in athletes, Rubio et al. (2005) examined *AMPD1* genotype in 104 Caucasian (Spanish) elite male endurance cyclists and runners and 100 healthy non-athletes.

The authors reported a low frequency (8.5 vs. 4.3%) of the mutant A allele in the athletes compared to non-athletes (Rubio et al., 2005), indicating the possibility of a detrimental effect of the A (mutant) allele for endurance-oriented performance at the elite level (Rubio et al., 2005). More recently, Cieszczyk et al. (2012) have shown that Polish power-oriented athletes (n = 158; short-distance runners and swimmers, and weightlifters) had significantly lower frequency (5.4% vs. 13.1%) of the *AMPD1* mutant allele than controls (Cieszczyk et al., 2012). These results were replicated in cohorts of Russian power-oriented athletes (n = 305; boxing, wrestling, speed skating, powerlifting, swimming, and weightlifting; frequency of the mutant (A) allele was 8.4% and 15.0% for athletes and controls, respectively) (Fedotovskaya et al., 2013) and 47 Lithuanian sprint and power athletes (frequency of the mutant allele was 4.3% and 16.0% for athletes and controls, respectively) (Ginevičienė et al., 2014). Collectively, the more recent findings that examined *AMPD1* genotype in athletes provide substantial evidence that the mutant allele of *AMPD1* is detrimental for both endurance- and strength/power-oriented performance. Since the study by Ginevičienė et al. (2014), there was a paucity of research that have examined *AMPD1* genotype in elite athletes, in particular in team sports where strength and power are vital for performance. Considering the strong evidence for the role of AMPD in physical performance as detailed here, combined with the proposed mechanisms by which *AMPD1* genotype might influence strength/power-oriented performance, it would be interesting to explore the *AMPD1* genotype within the elite rugby cohort in this thesis. For example, of particular interest is to discover whether our cohort contains any mutant homozygotes for *AMPD1* genotype. Secondly, in such presence of mutant homozygotes within these athletes, it would be interesting to explore whether these homozygotes differ in strength and power from alternative genotypes. In light of the collective evidence, and the opportunity to further our understanding on the role of *AMPD1*

genotype in elite rugby, the inclusion of *AMPD1* rs17602729 in the panel of SNPs for this thesis seems appropriate.

1.9.4. FTO alpha-ketoglutarate dependent deoxygenase (*FTO*)

FTO gene has shown the largest effect size and explained variance on body mass index (BMI) and obesity risk in different age groups and across diverse ancestries (Lu and Loos, 2013). Frayling et al. (2007) identified a cluster of highly correlated SNPs (linkage disequilibrium (LD) $r^2 > 0.80$) in the first intron of the gene on chromosome 16 that showed highly significant association with risk of type-2 diabetes, although the association was abolished after adjusting for BMI (Frayling et al., 2007). This suggested that the association between *FTO* and type-2 diabetes was mediated through the gene's effect on BMI, confirmed through a follow-up analyses in 38,759 individuals as part of the same study; although Fall et al. (2013) reported that *FTO* may increase the risk of type-2 diabetes independently of its effect on BMI (Fall et al., 2013). Subsequent GWAS studies in populations of European ancestries (Loos et al., 2008; Willer et al., 2009; Speliotes et al., 2010; Kilpeläinen et al., 2011; Bradfield et al., 2012; Berndt et al., 2013) and East-Asian ancestries (Cho et al., 2009; Wen et al., 2012; Okada et al., 2012) all confirmed the association between the *FTO* locus and obesity related traits.

Evidence of co-regulatory mechanisms between *FTO* and *RPGRIP1L* gene, points towards the possibility that the association between *FTO* SNPs and regulation of body weight is mediated via changes in the expression of both genes (Peters et al., 2013; Stratigopoulos et al., 2008). It appears that variants in the first intron of *FTO* might be sensitive to epigenetic effects, via their association with methylation capability (Bell et al., 2010; Toperoff et al., 2012; Almén et al., 2012), shedding light on another important mechanism by which *FTO*

can exert its effects on adiposity and other physiological mechanisms. Furthermore, there is evidence demonstrating that a part of the additional body mass attributable to *FTO* risk genotypes is lean mass (Tanofsky-Kraff et al., 2009; Sonestedt et al., 2011), and this observation is independent of fat intake and physical activity (Sonestedt et al., 2011). This *FTO* genotype association with muscle properties is supported by a large UK twin study that involved 4,523 female individuals (40 to 80 years of age) that were assessed for BMI, waist and hip circumferences, and total body fat mass and lean mass (Livshits et al., 2012). This study related *FTO* SNPs with body composition while controlling (separately and combined) for lean mass and fat mass. The authors reported that *FTO* is associated with body composition across a wide range of ages, and that *FTO* appears to affect primarily the amount of body soft tissue, influencing both fat and lean mass, but not adipose tissue alone (Livshits et al., 2012).

These findings generated interest in *FTO*'s effects in athletic populations. The *FTO T > A* (rs9939609) polymorphism was investigated by Eynon et al. (2013) in three European cohorts (Spanish, Polish and Russian) of power- (n = 285) and endurance-oriented (n = 266) elite-level and national-level athletes from a variety of sporting disciplines, but no associations were reported in that study. However, more recently Heffernan et al. (2017) identified an association between the *FTO* rs9939609 SNP and playing position in 530 elite rugby athletes. More specifically, athletes from the back three and centres playing positions who are more reliant on lean mass than total body mass compared to athletes of other playing positions, showed overrepresentation of the TT genotype and T allele at rs9939609. In the same study, genotype comparisons within 120 non-athletes showed that TT genotype and T allele carriers had greater total body (4.8% and 4.1%) and total appendicular lean mass (3.0% and 2.1%) compared to AA genotype counterparts (Heffernan et al., 2017).

Notably, despite the well-established association between *FTO* genotype and BMI and other phenotypes of body composition, relatively little is known on the underlying molecular mechanisms of these associations (Ran et al., 2020) due, in part, to the lack of associations between the SNPs and *FTO* protein expression (Grunnet et al., 2009; Wåhlén et al., 2008). However, there is evidence suggesting that *FTO* influences IRX3 protein (Iroquois-class homeodomain protein; in humans encoded by the *IRX3* gene) expression via evolutionarily conserved long range chromatin looping. Smemo et al. (2014) reported a negative association between *FTO* 'protective' genotype/allele (TT/T) and *IRX3* expression; and showed that wild type mice exhibited similar *FTO* SNP 'risk' (A) allele associated phenotypes such as higher body mass, BMI and percentage body fat (Smemo et al., 2014). Possibly due to a greater browning of white fatty tissue, *IRX3* knock-out mice expended more energy compared to wild type mice (Smemo et al., 2014). Interestingly, recent findings have shown a relationship between brown fat and muscle developmental precursor Myf5 (Schulz et al., 2011), providing some evidence for a possible mechanism that is responsible for the greater lean mass in non-athletes who were *FTO* T allele carriers, observed by Heffernan et al. (2017). Although the precise mechanisms of action of IRX3 in mammalian physiology are not fully understood, the primary role of IRX3 in embryonic development and actions in motor neuron development and neuronal programming shed light on the association between *FTO* and muscle properties (Bellefroid et al., 1998). A second mechanism that might explain the association between rs9939609 genotype and lean mass is that greater serum IGF-1 levels were found in T allele carriers, compared to alternative genotype (Rosskopf et al., 2011). This is in light of the known upregulation of IGF-1 as a consequence of mechanical loading, and this hormone's important role in cellular development of muscle hypertrophy (Folland and Williams, 2007; Sharples et al., 2015).

Recent studies have supported the findings by Heffernan et al. (2017), by strengthening the evidence for the associations between *FTO* SNPs and lean mass in various population groups (Wang et al., 2017; Zillikens et al., 2017; Ran et al., 2020; Hebbar et al., 2020), although few studies have examined this association in elite athletes. Guilherme et al. (2019) examined the rs9939609 in Brazilian and Russian athletes from endurance- and power- oriented sport and combat sport (mixed metabolic demands), and found that A/A genotype is underrepresented in athletes more reliant on a lean phenotype whilst overrepresented in power-oriented and combat sport athletes from heavier categories (Guilherme et al., 2019). To the author's knowledge, no studies have explored whether SNPs within *FTO* are associated with strength- and power-related phenotypes in elite athletes from strength- and power-oriented sport. Although it is acknowledged that the discovery of such genotype-phenotype associations do not confer a cause and effect by which *FTO* rs9939609 could exert its effect on lean mass and strength, they provide a rationale for further exploration of specific polymorphisms within *FTO* that could potentially play an important role in physiological mechanisms that influence strength phenotypes. Emerging evidence of the role of *FTO* is very promising with respect to its influence on body composition and importantly muscle mass and strength. For example, Wang et al. (2017) performed in vitro and in vivo experiments using cultural myoblasts and skeletal muscle-specific *FTO*-deficient mice to elucidate the mechanisms by which *FTO* may play a role in myogenesis. They found that *FTO* expression increases during myoblasts differentiation while a silenced *FTO* inhibited differentiation and thus, skeletal muscle development was impaired in *FTO*-deficient mice. Moreover, this study demonstrated that mTOR-PGC-1α (mammalian target of rapamyacin – peroxisome proliferator-activated receptor gamma coactivator-1 alpha) pathway is involved in the connection between *FTO* and muscle differentiation (Wang et al., 2017). In light of the strength of evidence for the

association between SNPs within *FTO* and body composition and *FTO* and lean mass, together with the emerging evidence that shows *FTO*'s crucial role in myogenic differentiation; the inclusion of the rs9939609 variant in the panel of strength-related SNPs for this work seems justified.

1.9.5. Hypoxia-inducible factor 1 subunit alpha (*HIF1A*)

Adaptation to hypoxia in cells and tissues leads to the transcriptional induction of a series of genes that participate in angiogenesis, iron metabolism, glucose metabolism, and cell proliferation/survival. The transcription factor hypoxia-inducible factor 1 (HIF-1) is a key regulator responsible for the induction of these genes that facilitate adaptation and survival of cells and the whole organism from normoxia (~21% O_2) to hypoxia (~1% O_2) (Wang et al., 1995; Semenza, 1998). HIF-1 was discovered by the identification of a hypoxia response element (HRE; 5′-RCGTG-3′) in the 3′ enhancer of the gene for erythropoietin (EPO), a hormone that stimulates erythrocyte proliferation and undergoes hypoxia induced transcription (Goldberg et al., 1988; Semenza et al., 1991). Semenza et al. (1991) revealed that HIF-1alpha is ubiquitously expressed in human and mouse tissues and has a general role in multiple physiological responses to hypoxia, such as erythropoiesis (Semenza et al., 1991), iron metabolism (Rolfs et al., 1997) and glycolysis (Semenza et al., 1994; Chen et al., 2001), which quickly counteract oxygen deficiency; and angiogenesis (Levy et al., 1995; LeCouter et al., 2001), the latter providing a long-term solution to hypoxia.

HIF-1 is a heterodimeric complex consisting of a hypoxically inducible subunit HIF-1alpha (encoded by *HIF1A* gene), and a constitutively expressed subunit HIF-1beta (encoded by Aryl hydrocarbon receptor nuclear translocator gene, *ARNT*); the former heterodimeric complex (HIF-1) binding to a 50-base pair *cis*-acting hormone response element (HRE;

located in the enhancer and promoter regions of many genes) under hypoxic conditions (Wang et al., 1995). HIF-1alpha and HIF-1beta proteins belong to the basic helix-loop-helix–Per-ARNT-Sim (bHLH–PAS) protein family (Wang et al., 1995). The bHLH and PAS motifs are essential to form a heterodimer between the HIF-1alpha and HIF-1beta subunits of HIF-1; their downstream basic region 'affording' specific binding to the HRE DNA sequence (Crews, 1998). The transcription and synthesis of HIF-1alpha are constitutive and seem not to be affected by oxygen (Wang et al., 1995; Kallio et al., 1997; Wiesener et al., 1998), however, in normal oxygen conditions, the HIF-1alpha proteins are rapidly degraded, resulting in essentially no detectable HIF-1alpha protein (Wang et al., 1995). In contrast, during hypoxia, HIF-1alpha becomes stabilized and translocates from the cytoplasm to the nucleus, where it dimerizes with HIF-1beta; forming the HIF-1complex that becomes transcriptionally active (Huang et al., 1996; Kallio et al., 1997). Subsequently, the HIF-1 complex formed associate with HREs in the regulatory regions of numerous target genes, and binds the transcriptional coactivators to induce gene expression (Lando et al., 2002; Lando et al., 2002).

By using DNA microarrays, Manalo et al. (2005) has shown that more than 2% of all human genes are regulated by HIF-1 in arterial endothelial cells, directly or indirectly (Manalo et al., 2005). Given the importance of HIF-1alpha in regulating the expression of a number of genes that are implicated in various cellular functions, including those occurring in response to hypoxia, one might suggest that a functional *HIF1A* gene variation is associated with human physical performance. One such missense polymorphism (Pro582Ser; rs11549465) was identified in exon 12 of *HIF1A* gene in chromosome 14, where a cytosine substitution with thymine (C → T transversion; T being the rare allele) results in the conversion of a proline (Pro) to serine (Ser) at amino acid sequence of HIF-1alpha. This substitution increases HIF-1

alpha protein stability and transcriptional activity and therefore, it is thought that may positively affect glucose metabolism under hypoxia stress. Ahmetov et al. (2008) investigated the hypothesis that *HIF1A* Pro582Ser genotype distribution may differ between controls and Russian strength athletes, due to the importance of anaerobic glycolysis as a major energy source for high force and/or high velocity muscle actions required for strength/power performance (Ahmetov et al., 2008). The authors found the frequency of the *HIF1A* 582Ser allele was significantly higher in weightlifters (n = 53) than in 920 controls (17.9% vs. 8.5%; P = 0.001), and increased with the athletes' levels of achievement (sub-elite (14.7%); elite (18.8%); highly elite (25.0%)). These results were replicated in three cohorts of 158 Polish power-oriented athletes (Cięszczyk et al., 2011), 208 Russian power-oriented athletes (122 weightlifters and 86 wrestlers) (Gabbasov et al., 2013), and 110 Ukrainian power-oriented athletes (Drozdovska et al., 2013). In contrast, Eynon et al. (2010) reported that there were no differences across the *HIF1A* (Pro582Ser polymorphism) genotype and allele frequencies among 74 long distance runners (60 male and 14 female), 81 sprinters (59 male and 22 female) and 240 non-athletes (167 men and 73 women). Furthermore, the study by Ahmetov et al. (2008) found a significant association between the *HIF1A* 582Ser allele and an increased proportion of fast-twitch muscle fibres in the vastus lateralis muscle of speed skaters (Pro/Ser 46.2% (13.8), Pro/Pro 31.4% (8.2); p = 0.007), demonstrating that the Pro582Ser polymorphism may be advantageous for sports that necessitate a predominance of fast-twitch muscle fibres.

Given the importance of HIF-1α in regulating the expression of a number of genes that are implicated in various cellular functions (Ke and Costa, 2006), it is reasonable to assume that the *HIF1A* Pro582Ser variant might be associated with elite athlete status and/or strength/power within our cohort of elite rugby athletes. Previous research suggests that the

association between 582Ser allele and athletic performance could be, in part, mediated via differences in muscle fibre type proportion (Ahmetov et al., 2008); shedding light on the potential advantageous role of the 582Ser allele for different playing positions in rugby. With regards to strength and power, it is important to note that the expression of HIF-1α can also be activated by oxygen independent mechanisms such as in response to insulin-like growth factor-1 (IGF-1). For example, the action of the IGF-1–HIF-1α–IGF-2/transforming growth factor-α axis may explain the hypertrophic effect of HIF-1α on skeletal muscle (Fukuda et al., 2002; Slomiany and Rosenzweig, 2006; Gariboldi et al., 2010; Hughes et al., 2011). Collectively, research evidence highlights the importance of the Pro582Ser polymorphism for athletic performance with the 582Ser allele appearing advantageous, in general, for strength- and power-oriented sport. It would be interesting to explore the *HIF1A* genotype in a cohort of elite rugby athletes by examining whether any associations exist between this variant and muscle strength/power, and in-game performance. The collective evidence indicates that *HIF1A* rs11549465 might belong to a growing panel of variants that influence elite athletic performance and thus, it deserves the inclusion with the panel of SNPs for the work presented herein.

1.9.6. Vascular endothelial growth factor receptor 2 gene (*VEGFR2*) – kinase insert domain receptor gene (*KDR*).

The increase in capillary action during prolonged exercise is associated with an increase of the maximal rate of oxygen consumption and is thought to be mediated by a number of angiogenic factors, including vascular endothelial growth factor (VEGF) (Ferrara, 1999). VEGF mRNA is upregulated in human vastus lateralis of sedentary/moderately active young males in response to 30–45 min of dynamic unilateral knee extension exercise (Gustafsson et al., 1999; Richardson et al., 1999). In rat gastrocnemius muscle VEGF mRNA expression (following one-hour of treadmill running or electrical stimulation) is stronger in deep regions

of the muscle with a high proportion of oxidative fibres (Brutsaert et al., 2002). VEGF is described as a growth factor for vascular endothelial cells (Ferrara et al., 1992) and plays essential roles in the regulation of angiogenesis, vascular development, vascular permeability, and embryonic haematopoiesis (Ferrara, 1999; Neufeld et al., 1999). The biological activity of VEGF is produced through ligand binding to its tyrosine-kinase receptors VEGFR1 (Flt-1) (De Vries et al., 1992) and VEGFR2 (Flk-1) (Terman et al., 1992); which are high-affinity VEGF receptors localized predominantly to the vascular endothelium of both proliferating endothelial cells as well as on quiescent cells (Ferrara, 1999). VEGF-induced activation of VEGFR2 stimulates endothelial cell to proliferate, migrate and differentiate (Waltenberger et al., 1994; Wu et al., 2000; Gille et al., 2001), and invade surrounding tissue to form capillary tubules capable of carrying blood during angiogenesis (Bernatchez et al., 1999). Shalaby et al. (1995) demonstrated that *VGFR2* gene knock-out mice die in utero between 8.5 and 9.5 days post-coitum due to a lack of vasculogenesis; indicating that VEGFR2 signalling is essential for the differentiation of VEGFR2-positive endothelial precursor cells into vascular endothelial cells and for their proliferation (Shalaby et al., 1995). Activation of VEGFR2 elicits a potent tyrosine kinase signalling cascade, which includes activation of phosphoinositol-3-kinase, phospholipase C-γ, and protein kinase C-ε (Wu et al., 2000); to stimulate gene expression for a variety of genes including *NOS3* (Shen et al., 1999). There is evidence indicating that VEGF activation of VEGFR2 elicits the complete angiogenic response (Conway et al., 2001). Milkiewicz et al. (2003) have shown in animals that during chronic muscle ischaemia (blood flow was reduced by iliac artery ligation of rat hindlimb muscle) VEGF receptors (Flt-1 and Flk-1) are regulated differentially and that electrical stimulation of ischaemic muscles can promote angiogenesis via up-regulation of VEGF receptor (Flk-1) (Milkiewicz et al., 2003). Genetic variants within *VEGFR2* gene have been

associated with several clinical phenotypes such as coronary heart disease (Wang et al., 2007), risk of stroke occurrence (Zhang et al., 2007) and breast cancer (Försti et al., 2007).

With regards to athletic performance, previous research revealed associations between polymorphisms within *VEGF* gene and aerobic capacity (Prior et al., 2006), and between *VEGF* gene and endurance athlete status (Ahmetov et al., 2008). In addition, Timmons et al. (2010) have shown that VEGFR2 mRNA expression increased only in a high responder group in response to six weeks of high-intensity aerobic cycle training (significant increase in peak rate of oxygen consumption) compared to a low responder group to the same training regime; illustrating the interindividual variability in VEGFR2 mRNA expression (Timmons et al., 2010). A functional polymorphism (rs1870377) in exon 11 of the *VEGFR2* gene (located on 4q11–q12 and consisting of 26 exons) is known to substitute histidine (His) to glutamine (Gln) at position 472 of the coding region (His472Gln) by a T→A transversion. Due to the importance of VEGFR2 in inducing the full spectrum of VEGF influences on the response to physical exercise (Conway et al., 2001), and also due to the involvement of VEGFR2 in this response; then one might anticipate genetic variation in the *VEGFR2* gene to be associated with elite endurance athlete status and/or with intermediate endurance performance phenotypes. This hypotheses was tested by Ahmetov et al. (2009) who examined His472Gln variant in 471 Russian Caucasian athletes (men and women: 52 elite, 154 sub-elite and 265 non-elite from 23 different sport) and 603 non-athlete controls. Athletes were stratified into four groups (and sub-groups by sport) according to event duration, distance and type of activity, (endurance-oriented; mixed endurance/power; sprint/power; strength-oriented). Ahmetov et al. (2009) found significant differences in genotype and allele frequency distribution of His472Gln variant between all athletes compared with controls, and between both endurance-oriented and sprint/power-oriented

athletes compared with controls (Ahmetov et al., 2009). Specifically, significant associations between 472Gln allele and athlete status were found within road-cycling athletes (endurance-oriented), and sub-groups of swimmers who specialise in long (endurance-oriented), medium (mixed endurance/power) and short (sprint/power) distances. No associations were found in any of the strength-oriented athletes. Furthermore the 472Gln allele was associated with higher proportion of slow-twitch type I fibres (in *m. vastus lateralis*, determined by immunohistochemistry) within the sub-group of all-round speed skaters (mixed power/endurance capacity), and also within a sub-group of healthy non-athlete males (Ahmetov et al., 2009).

It is well established that swimming performance in all distances is accompanied by exercise-induced hypoxia (Spanoudaki et al., 2004). In addition, local factors such as peripheral circulation, capillary density, perfusion pressure and metabolic capacity of working muscles are important determinants of the muscular power production capacity and contribute to training movements specific for swimming (Holmer, 1992). Thus, since angiogenic pathways (VEGF-VEGFR1/VEGFR2) are activated by hypoxia-inducible factors (HIFs), Ahmetov et al. (2009) speculated that the overrepresentation of the 472Gln allele in swimmers who specialise in all distances examined in their study reflects a potential advantageous role of His/Gln and Gln/Gln genotypes for a spectrum of sport performance; from the most endurance-oriented to sprint/power-oriented athletic performance. Indeed, athletes from events requiring significant levels of sprint/power performance have been shown to possess high numbers of capillaries per unit muscle area comparable to those of endurance athletes (Zoladz et al., 2005). Thus, the potential role of the His472Gln variant and specifically, the apparent beneficial role of the 472Gln allele could also manifest itself in sprint/power-oriented athletes. Important to note is that in the study by Ahmetov et al. (2009) swimmers

who specialise in the shortest distances (50–100 m sprint/power swimming) had the greatest proportion of the 472Gln allele from all 23 sports examined. Eider et al. (2013) investigated the *VEGFR2* His472Gln polymorphism in 176 male Polish long distance endurance athletes (runners, cyclists, rowers and swimmers) of nationally competitive standard. In support of one of the findings by Ahmetov et al. (2009), the authors reported that the frequency of the Gln/Gln genotype and 472Gln allele were overrepresented in the athletes compared to the controls (Eider et al., 2013). More recently, Yvert et al. (2016) examined *VGFR2* His472Gln variant and 20 other genetic endurance-related polymorphisms in 175 Japanese middle- and long-distance runners (60 international level; 115 national and regional level) and 649 controls. Surprisingly, only two polymorphisms (*ACTN3* rs1815739 and *GNB3* rs5443) have shown an association with athlete status. While acknowledging that differences exist in genotype frequencies and haplotype networks between ethnic groups, the authors concluded that the lack of associations was due to the fact that most polymorphisms they studied were found to be associated with endurance performance in Caucasian populations (Yvert et al., 2016). More recently, Kumagai et al. (2018) examined the effects of genetic polymorphisms (*ACTN3* rs1815739, *ACE* rs4341, *HIF1A* rs11549465 and *VEGFR2* rs1870377) on muscle fibre composition with respect to sex differences in 211 (men and women) healthy Japanese individuals. Interestingly, the authors reported that men with the *ACTN3* RR + RX genotype had a higher proportion of myosin heavy chain (MHC) IIx than homozygotes for the *ACTN3* null (577X) allele, whilst men with the *ACE* ID + DD genotype had a higher proportion of MHC I than those with the *ACE* II genotype. Of note is that no associations were observed between muscle fibre-type composition and either *HIF1A* rs11549465 or *VEGFR2* rs1870377 (His472Gln) genotypes.

Collectively, the findings of studies that examined *VEGFR2* His472Gln in relation to athletic performance indicate that 472Gln allele is associated with both endurance-oriented and sprint-power oriented athlete status. However, relatively few research efforts have attempted to examine this variant in elite strength- and power-oriented team sports athletes to substantiate the findings by Ahmetov et al. (2009) with respect to the apparent beneficial role of the 472Gln allele on sprint/power performance; specifically demonstrated by the sub-group of sprint/power swimmers (50–100 m) in that study. To the author's knowledge, no studies have examined the *VEGFR2* His472Gln variant in elite rugby athletes and thus, the inclusion of the *VEGFR2* His472Gln polymorphism in the work presented here seems justified.

1.9.7 Nitric oxide synthase 3 (*NOS3*)

Nitric oxide has emerged as a chemical messenger in a multitude of biologic systems having homeostatic activity in the maintenance of cardiovascular tone, platelet regulation, and central nervous system signalling; as well as a role in gastrointestinal smooth muscle relaxation and immune regulation (Schroeder and Kuo, 1995). Biological nitric oxide is mostly produced enzymatically from L-arginine by a family of three nitric oxide synthases (NOS) (Marletta, 1994; Cooke and Dzau, 1997): neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) (Wang et al., 2000). In the endothelium of both humans and animals the synthesis of nitric oxide is catalysed by the enzyme endothelial nitric oxide synthase (eNOS), which is encoded by the *NOS3* gene (Moncada, 1991; Robinson et al., 1994). The continuous generation of nitric oxide plays a key role in the regulation of basal vascular tone (Cooke et al., 1991; Quyyumi et al., 1995). Nitric oxide directly regulates vasorelaxation and blood supply to tissues, possibly including exercising muscles (Hickner et al., 1997; Heydemann and McNally, 2009); and plays important roles in both myocardial

repair and regeneration (Otani, 2009). It has been shown that endurance exercise training stimulates nitric production in the endothelial cells (thus, reactive hyperaemia – a transient increase in organ blood flow that occurs following a brief period of ischemia (Doshi et al., 2001)) in hypertensive (Higashi et al., 1999) and cardiac (Hambrecht et al., 1998) patients. There is evidence in humans that nitric oxide is important for the regulation of skeletal muscle glucose uptake during exercise (McConell and Kingwell, 2006). Furthermore, nitric oxide represents an important limitation to the acceleration of oxidative metabolism following the onset of supra-maximal exercise in skeletal muscle (Wilkerson et al., 2004), and is involved in the modulation of oxygen consumption in the myocardium (Loke et al., 1999; Loke et al., 1999). Gouill et al. (2007) demonstrated that eNOS knockout mice had significantly lower oxygen consumption and defective mitochondria compared with non-transgenic controls (Gouill et al., 2007).

Previous research has shown that a missense Glu298Asp (G894 → T) polymorphism (rs1799983) within *NOS3* gene coding region is associated with reduced eNOS activity and basal nitric oxide production, and vascular disease in several populations (Yoshimura et al., 1998; Wang et al., 2000). The Glu298Asp polymorphism is also associated with cardiovascular responses to exertion in non-elite athletic populations (Rankinen et al., 2000) and with elite endurance performance status in Ironman triathletes (Saunders et al., 2006); although controversy also exists (Wolfarth et al., 2008). Another candidate within the *NOS3* gene that can explain human variations in health- and exercise-related traits is the -786 T/C polymorphism (rs2070744) in the 5`-flanking region of *NOS3* (Nakayama et al., 1999; Nakayama et al., 2000). In contrast to other linked polymorphic mutations (A-922 → G and T-1468 → A) that are not associated with gene transcription (Nakayama et al., 1999), the T-786 → C mutation at rs2070744 results in significantly reduced gene promoter activity and

the associated reduced endothelial nitric oxide synthesis (Nakayama et al., 1999). The T-786 → C mutation is associated with coronary vasospasm (Nakayama et al., 1999) and myocardial infarction in Japanese patients (Nakayama et al., 2000). Furthermore, the T-786 → C mutation is associated with increased risk of essential hypertension in Caucasians (Hyndman et al., 2002) and with decreased cardiac function in African Americans (McNamara et al., 2009).

Based on the pivotal role of nitric oxide in the regulation of important body systems and functions (Moncada, 1991), and the catalytic role of the enzyme eNOS in the synthesis of nitric oxide (Marletta, 1994), the *NOS3* gene was proposed as a candidate for explaining individual variations in health- and exercise-related traits (Wolfarth et al., 2008; Bray et al., 2009). More specifically, given that endurance performance is limited by local blood flow, and the fact that the C allele at rs2070744 is associated with reduced endothelial nitric oxide synthesis (Nakayama et al., 1999) (that can result in reduced oxygenated blood flow to the active musculature), the *NOS3* -786 T/C polymorphism was proposed as a potential variant that might influence endurance performance. Hand et al. (2006) found no association between *NOS3* T-786 → C mutation and cardiovascular hemodynamic (heart rate; cardiac output; systolic and diastolic blood pressure) responses to sub-maximal and maximal exercise in sedentary, physically active and endurance-trained postmenopausal women (Hand et al., 2006). Gómez-Gallego et al. (2009) were the first to examine the *NOS3* T-786 → C mutation in elite athletes. The study involved two groups of elite Spanish Caucasian male athletes (100 endurance-oriented road cyclists and runners and 53 power-oriented throwers, jumpers and sprinters) and 100 non-athlete controls. The authors hypothesised that the T allele at rs2070744 would be overrepresented in endurance compared with either power athletes or non-athletes. In contrast, the authors found the T allele and TT genotype were

overrepresented in power athletes compared to either endurance athletes or non-athletes (Gómez-Gallego et al., 2009). In support of this, Sessa et al. (2011) reported an overrepresentation of the T allele at rs2070744 in national level Italian male power-oriented athletes (sprinters, short distance swimmers) compared to athletes from intermittent sport (football, basketball and hockey) and non-athletes (Sessa et al., 2011). Another study by Drozdovska et al. (2013) examined the *NOS3* T-786 → C mutation in a cohort of 210 (male and female) Ukrainian athletes from power-oriented (short-distance running and swimming, jumping, and throwing) and endurance-oriented (cross-country skiing and rowing) sport, and 326 non-athlete controls. The authors found an overrepresentation of the T allele in power athletes compared with either endurance athletes or non-athletes (Drozdovska et al., 2013). More recently, Weyerstraß et al. (2018) conducted a series of meta-analysis (35 articles published between 2008 and 2016 that involved 5834 power athletes and 14,018 controls) for polymorphisms associated with power athlete status. The authors found significant associations for nine polymorphisms within the genes: *NOS3* (rs1799983, rs2070744), *ACE* (rs4363, rs1799752), *ACTN3* (rs1815739), *AGT* (rs699), *IL6*-174 (rs1800795), *MnSOD* (rs1799725) and *SOD2* (rs4880) and power athlete status (Weyerstraß et al., 2018). With regards to *NOS3* genotype at rs2070744, the authors' conclusion supported the findings of previous studies mentioned above, in that athletes carrying the C allele of the T-786 → C mutation were significantly less likely to a become a power athlete compared to athletes carrying the T allele (Weyerstraß et al., 2018). Recently, Murtagh et al. (2020) investigated the association of 10 polymorphisms with athlete status and speed/power performance in 583 elite male youth soccer players (aged 8–23 years) and control participants at different stages of maturity. After being grouped according to years from predicted peak-height-velocity (PHV) (i.e. pre- or post-PHV to determine maturity status), athletes were assessed for muscular power (bilateral vertical countermovement jumps, bilateral horizontal-forward

countermovement jumps, 20m sprints, and modified agility tests). Post-PHV players had a higher frequency of the *NOS3* (rs2070744) T allele compared to controls, whereas TT homozygotes at rs2070744 sprinted quicker than CC homozygotes. This study have shown that pre- and post-PHV elite soccer players have distinct polygenic profiles; with post-PHV players displaying a genetic profile that favours superior power/speed characteristics, while pre-PHV players showing a more endurance-associated polygenic profile (Murtagh et al., 2020).

Accumulating evidence suggests that *NOS3* T-786 → C mutation has a role in exercise performance with the T allele and TT genotype, in general, suggested to be preferential for power-oriented athletic performance. This might be attributed, in part, to the role that nitric oxide might play on muscle hypertrophy. The T allele at rs2070744 is associated with increased nitric oxide activity compared with the C allele (Nakayama et al., 1999). In addition, nitric oxide is involved in myoblast proliferation and fusion – the mechanism by which supplementation with the semi-essential amino acid L-arginine (which is the substrate for endogenous synthesis of nitric oxide) can stimulate muscle hypertrophy (Long et al., 2006). Research in animals has shown that pharmacological inhibition of nitric oxide synthase activity interferes with the normal fibre hypertrophy of the rats' plantaris in response to overload induced by surgical removal of the synergists muscles (Smith et al., 2002). Inhibition of nitric oxide synthase also interferes with the up-regulation of contractile protein gene expression in response to chronic skeletal muscle overload in rats; with NOS activity possibly providing negative feedback control of IGF-1/p70 s6 kinase signaling during muscle growth (Sellman et al., 2006) (Section 1.3.3). Moreover, maintenance of exercise-stimulated nitric oxide signalling mechanisms and protein turnover by exercise countermeasure may be crucial steps to attenuate human skeletal muscle atrophy (Salanova et

al., 2008). In light of the considerable body of evidence indicating a potential role of *NOS3* on strength/power-oriented performance and on muscle hypertrophy, and the opportunity of extending this association to measures of strength/power of elite rugby athletes, the selection of this polymorphism for the work presented here seems appropriate. No studies have examined the *NOS3* T -786 → C mutation at rs2070744 in elite rugby and thus, whilst further research that examines the *NOS3* rs2070744 polymorphism in strength- and power-oriented athletes is warranted, the inclusion of this variant in the panel of strength/power SNPs for this thesis seems justified.

1.9.8. Protein tyrosine kinase 2 (*PTK2*)

Focal adhesion kinase (FAK), encoded by protein tyrosine kinase 2 gene (PTK2), is an integrin-associated protein tyrosine kinase localised at focal adhesion complexes via interactions between its focal adhesion targeting domain and other integrin-associated proteins (Hildebrand et al., 1993; Schaller, 2001). Focal adhesion complexes are fundamental components of cell costameres (Pardo et al., 1983). Costameres were originally described as electron-dense plaques rich in the focal adhesion protein vinculin and located between the plasma membrane (or sarcolemma) and myofibrils (Koteliansky and Gneushev, 1983; Shear and Bloch, 1985; Bloch et al., 2002); and are present in a rectilinear array that parallels the organization of the underlying contractile apparatus (Bloch et al., 2002). Costameres overlie the sarcomeric Z- and M-lines of muscle fibres in a way that forms regular connections with the sarcolemma (Pardo et al., 1983). This provides a pathway of force transmission – force can be transmitted laterally across adjacent muscle fibres and the sarcolemma to the extracellular matrix, via these regular costamere connections (Patel and Lieber, 1997; Flück et al., 1999; Bloch and Gonzalez-Serratos, 2003). It has been speculated that due to their structure and location, costameres could effectively split the serial sarcomeres of a muscle

fibre into multiple force-generating units to enable this pathway of force transmission to the muscle fascia (Jones et al., 1989; Huijing, 1999). Costamere formation and turnover is regulated by FAK activity, which is known to increase in hypertrophying muscle (Flück et al., 1999). It has been shown that the force per unit cross-sectional area of muscle (muscle specific force) also increases following hypertrophy of skeletal muscle (Reeves et al., 2004). Thus, it is thought that increases in muscle specific force result from an increase in FAK activity and costamere density, and a corresponding increase in later force transmission (Erskine et al., 2012).

Erskine et al. (2012) investigated two polymorphisms within *PTK2* (intronic rs7843014 (A/C) and 3`-untranslated region rs7460 (A/T)) for associations with indexes of muscle strength (unilateral knee extension one-repetition maximum (1 RM); maximum isometric voluntary contraction (MVC) knee joint torque; quadriceps femoris muscle specific force), and the interindividual variability in the strength responses to resistance training; in 51 non-athlete Caucasian healthy young males (Erskine et al., 2012). No genotype associations were found with baseline measures or post-training changes in unilateral knee extension 1 RM or knee joint torque MVC. The training-induced increase in muscle specific force was also similar for all *PTK2* genotypes however; baseline specific force was higher in rs7843014 A/A homozygotes (compared to C allele carriers) and rs7460 A/A homozygotes (compared to T allele carriers). This favourable homozygosity for both SNPs (combined) was found to explain ~10% of the interindividual variability in muscle specific force in the untrained state (Erskine et al., 2012). These first associations between polymorphisms within *PTK2* and muscle specific force indicated that the interindividual differences which exist in the way force is transmitted from the muscle fibres to the tendon could be attributed, in part, to genetic variation. The authors speculated that the improved muscle specific force

demonstrated by A/A homozygotes (for both rs7843014 and rs7460) could have reflected an increase in lateral force transmission and costamere density (Erskine et al., 2012). A study by Garatachea et al. (2014) investigated *PTK2* rs7843014 and rs7460 polymorphisms in three cohorts (Spanish, Italian and Japanese) of centenarians for associations with exceptional longevity. Interestingly, the authors reported that rs7843014 C/C and rs7460 T/T genotypes are possibly associated with lower gene expression and might favour the likelihood of reaching exceptional longevity (Garatachea et al., 2014). More recently, an independent replication study by Stebbings et al. (2017) examined *PTK2* rs7843014 and rs7460 polymorphisms for associations with muscle strength phenotypes (maximal isometric voluntary knee extension torque; net isometric voluntary knee extension torque; vastus lateralis specific force), in 120 Caucasian healthy men. The researchers found that vastus lateralis specific force was 8.3% higher in rs7843014 AA homozygotes than C allele carriers, and 5.4% higher in rs7460 AA homozygotes than T allele carriers (Stebbings et al., 2017). However, no associations were observed between any of the SNPs and maximum/net isometric knee extension torque. Notably, the inter-individual variability in vastus lateralis specific force observed in this study was 16%, and together, the two *PTK2* SNPs were found to explain a substantial proportion (~25%) of this variability (Stebbings et al., 2017).

Although scarce, the available evidence indicates that *PTK2* genotype, specifically homozygosity for the A allele in both rs7843014 and rs7460, might enhance muscle specific force in untrained young muscle (Erskine et al., 2012; Stebbings et al., 2017). *PTK2* encodes FAK, a protein integral to the formation and turnover of muscle costameres (Quach and Rando, 2006), which are involved in transmitting force laterally from the muscle contractile elements to the extra-cellular matrix (Bloch and Gonzalez-Serratos, 2003). An increased ability of a muscle to transmit force laterally may translate into enhanced muscle specific

force and/or muscle strength. The enhanced muscle specific force shown by some individuals in the studies by Erskine et al. (2012) and Stebbings et al. (2017) may be explained by an altered *PTK2* expression – experienced by A/A homozygotes (rs7843014 and rs7460), compared to individuals carrying the C allele (rs7843014) and T allele (rs7460). Garatachea et al. (2014) reported a possible association between C/C (rs7843014) and T/T (rs7460) genotypes, and lower gene expression. In addition, it has been shown that in vitro FAK-null cells show a diminished integrin activation and a reduced adhesion-strengthening rate, which results in the formation of fewer integrin-extra cellular matrix bonds, compared to cells expressing FAK (Michael et al., 2009). However, there is contrasting evidence that shows FAK-null cells form stronger adhesions, possess enhanced contractile properties and migrate slower than their wild-type counterparts (Furuta et al., 1995; Ren et al., 2000; Chen et al., 2002). These contrasting findings reflect the complexity surrounding FAK-regulated adhesion strength and the possible role of *PTK2* in the variation of muscle lateral force transmission and the production of muscle specific force. It would be fascinating to further our understanding with regards to the role of *PTK2* on properties of muscle function (e.g. as those examined in relation to *PTK2* in untrained individuals and mentioned above), but in trained muscle of elite strength/power athletes; although the logistics surrounding time (for both researchers and athletes) and athlete availability and willingness would probably be very challenging in that case. Nevertheless, in light of the evidence provided to date on the role of *PTK2* genotype on muscle specific force, and the possibility of genotypes in polymorphisms within *PTK2* being associated with strength and power in elite rugby athletes, the rs7843014 and rs7460 within *PTK2* were included in the panel of SNPs for this thesis.

1.9.9. Thyrotropin releasing hormone receptor (*TRHR*)

The *TRHR* gene (located in the region 8q.23) encodes the thyrotropin-releasing hormone receptor (TRHR), which belongs to the superfamily of G protein-coupled seven-transmembrane domain receptors (Straub et al., 1990; Strader et al., 1995). Thyrotropin releasing hormone (TRH) was the first hypothalamic releasing factor to be identified, and its multifaceted action was unknown until Bøler et al. (1969) determined its final structure. Thyrotropin-releasing hormone (TRH) is a tripeptide (Glu-His-Pro) hormone secreted by the hypothalamus, and exerts its effects by binding to TRHR on the surface of pituitary thyrotrophins (Matre et al., 1999). TRH and its receptor are widely distributed throughout the central and peripheral nervous systems as well as in extraneural tissues (Sharif, 1989; Satoh et al., 1993; Fukusumi et al., 1995). The binding of TRH with its receptor TRHR activates the inositol phospholipid-calcium-protein kinase C transduction pathway which, in turn, stimulates secretion of thyroid-stimulating hormone (TSH, or thyrotropin) and prolactin from the anterior pituitary gland (Gershengorn, 1989). The TSH response to TRHR is the first step in the hormonal cascade of hypothalamic-pituitary-thyroid axis (HPTA) that eventually leads to the release of tetraidothyronine (T_4, thyroxin) and triiodothyronine (T_3, the active form of thyroid hormone), which are important in the development of vertebrate skeletal muscle (Larsson et al., 1994) and as major metabolic hormones.

One case of central hypothyroidism related to *THRH* gene alteration was reported by Collu et al. (1997). The 8.9 year old boy born to nonconsanguineous Caucasian parents was referred for evaluation of short stature, and initially diagnosed for central hypothyroidism. The identification of different mutations in two of the child's alleles (each mutation inherited from each parent) indicated that he is a compound heterozygote for two mutations located in the *TRHR* gene; that resulted in a greatly reduced or absence of both TRH binding (to its

receptor TRHR), and TRH receptor-mediated stimulation of the synthesis and release of TSH and prolactin (Gershengorn, 1989). In relation to thyroid function, Vadaszova et al. (2006) demonstrated that chronic hypothyroid and hyperthyroid status in female inbred rats affects the expression of myosin heavy-chain (MHC) isoforms both at the mRNA and protein levels (Vadaszova et al., 2006). Montgomery have used rat thyroidectomy to discover differences in a number of muscle parameters that characterise the mechanical and histochemical status of skeletal muscle (Montgomery, 1992); with a main finding that hypothyroidism in rats increases the percentage of type I muscle fibres, with a concomitant decrease in percentage of type II muscle fibres. Administration of triiodothyronine (T_3) to the hypothyroid rats have shown to exert an opposite effect on type I and type II muscle fibres, with such reversal of changes detected after two days of triiodothyronine administration (Montgomery, 1992). In support of these findings, Norenberg et al. (1996) have observed significant increases in the number of type I muscle fibres (11%) in the soleus of rats with a concomitant decrease in absolute tension (16%) of the same fibres, by inducing hypothyroidism in the rats via thyroidectomy and a 6-week injection period of the antithyroid drug 6-n-propyl-2-thiouracilto (Norenberg et al., 1996). Although the majority of studies have used rats, these studies shed light on the potential link between hypothyroidism, muscle fibre-types and muscle force characteristics in humans. In addition, taking also into account the important role of TRH for thyroid function, one might expect an association between specific alleles and genotypes in polymorphisms in the *TRHR* gene and exercise performance in humans. This hypothesis was tested by Liu and colleagues who conducted a GWAS study (that contained a GWA scan set of 379, 319 SNPs) on lean body mass variation in 492 and 481 unrelated US men and women respectively (Liu et al., 2009). The authors identified two genome-level significant SNPs (*p* values of 7.55×10^{-8} and 7.58×10^{-8} for rs16892496 and rs7832552, respectively) within the single intron of *TRHR* gene, that are in strong linkage disequilibrium (LD; $r^2 = 0.98$).

Interestingly, an additional 146 SNPs were suggestive of an association with lean body mass, with 15 of these located in the *TRHR* gene. To ensure robustness of associations, the authors applied further stringent methods of analyses as suggested by Price et al. (2006) to correct for potential population admixture and differences in laboratory treatment amongst samples; with the strongest associations with lean body mass being observed for the same two SNPs. The main finding of the study by Liu et al. (2009) was that the GG homozygotes for the rs16892496 had an average of 2.70 kg higher lean body mass when compared to subjects carrying the GT or TT genotypes; whilst TT homozygotes for the rs7832552 had 2.55 kg higher lean body mass compared to subjects with CC or CT genotypes (Liu et al., 2009). The researchers replicated the associations in three independent samples (1488 unrelated US whites, 2955 Chinese unrelated subjects, and 593 nuclear families comprising 1972 US whites), largely confirming the significant associations in the GWA scan (Liu et al., 2009). Based on these findings, a more recent study by Miyamoto-Mikami et al. (2017) included *TRHR* rs7832552 in a panel of 21 SNPs for examination in 211 Japanese sprint/power track and field athletes (62 interational level, 72 national, and 77 regional) and 649 Japanese non-athletes used as controls. The authors reported that the T allele at rs7832552 was one of three SNPs (together with *AGT* rs699, and *CNTFR* rs41274853) that tended to show an association with sprint/power athlete status (Miyamoto-Mikami et al., 2017).

Thus, the collective evidence indicates that *TRHR* might have an important role for lean body mass. Studies in rats have shown an association between thyroid function, muscle fibre-type and muscle force characteristics. TRH-TRHR binding is important for thyroid function and thus, functional variants within *TRHR* gene might affect the binding affinity of TRH with TRHR which, in turn, affects thyroid function and lean mass. This might explain the robust association between *TRHR* rs7832552 and lean mass reported by Liu et al. (2009) in non-

athlete populations. Interestingly, Fuku et al. (2015) studied the functionality of the rs7832552 and rs16892496 (both associated with lean mass by Liu et al. (2009)) polymorphisms in vitro using mice skeletal muscle cell lines. The authors reported that the T allele at rs7832552 increased gene expression compared to the C allele (Fuku et al., 2015). Although the method used by Fuku et al. (2015) was considered a main limitation of that study by the same authors, their findings support the observations by Liu et al. (2009) on the apparent advantageous role of the T allele at rs7832552 in relation to lean body mass. In light of the collective evidence that supports the role of *TRHR* for lean mass and the well-established notion that lean mass is a major determinant of strength, it is reasonable to assume the *TRHR* polymorphism at rs7832552 may explain some of the variability in strength within our cohort of elite rugby athletes and thus; its inclusion with the panel of strength- and power- related SNPs for this thesis seems justified.

1.10 Aims and Objectives

The overall aim of the current thesis was to investigate some of the genetic contribution to the variability in muscular strength/power and in-game performance in an elite rugby context. More specifically the objectives were:

1. To characterise muscular strength/power of elite rugby athletes using specific measures acquired from the force-time histories of the IMTP and CMJ, comparing between positional groups and with non-athletes.

2. To determine the genetic characteristics of muscular strength/power in elite rugby athletes using ten polymorphisms (previously associated with strength/power in the

literature) found in nine genes (***ACE***, ***ACTN3***, ***AMPD1***, ***HIF1A***, ***FTO***, ***KDR***, ***NOS3***, ***PTK2***, and ***TRHR***), comparing between positional groups and with non-athletes.

3. To investigate whether these ten polymorphisms are associated (individually, and/or collectively using two polygenic profile approaches) with the measures acquired from the force-time histories of the IMTP and CMJ of the athletes.

4. To investigate whether these ten polymorphisms are associated (individually, and/or collectively using two polygenic profile approaches) with in-game performance variables of the athletes.

1.11 Thesis Overview

The current Chapter aims to lay the foundation on fundamental aspects of muscular strength and power with particular emphasis on the relationship between strength and power, the heritability and genetic-environmental interaction influencing the inter-individual variability observed in strength/power phenotypes, and methods used for testing strength/power using the CMJ and IMTP. Additionally, the current Chapter provides reviews of specific genes and their polymorphisms that were investigated in this thesis.

The second Chapter (General Methods) describes the procedures used in gathering the phenotype and genotype data that were subsequently utilised in four investigative Chapters (Chapters 3–6). The force platform method was used to produce the force-time histories in the CMJ and IMTP, from which measures of muscular strength and power were gathered. The procedures for setting-up the force platform and interface equipment, and the principles and concepts used in acquiring the strength/power measures from the force-time histories are

detailed in Chapter 2. Additionally, Chapter 2 provides details on the procedures used for blood and saliva collection, DNA extraction, and genotyping.

Chapter 3 is the first of four investigative chapters. The main aim of Chapter 3 was to characterise the strength/power of elite rugby athletes using the CMJ and IMTP. Measures acquired from the CMJ force-time histories were maximum concentric power, relative maximum concentric power (deduced from the former) and jumping height; whilst measures acquired from the IMPT force-time histories were peak force and relative peak force (deduced from peak force). The strength/power measures were compared between athletes and non-athletes, and between athletes of different playing positions. One hypothesis was that the heavier forwards could generate greater CMJ maximum concentric power and IMTP peak force, whilst the backs would dominate when the measures are expressed relative to body mass, and in jumping performance.

The second investigative Chapter (Chapter 4) aimed to characterise the genetics of muscular strength and power in elite rugby athletes, using ten gene polymorphisms that were previously associated with strength/power in the literature. Genotype data for individual polymorphisms were compared between athletes and non-athletes, and between forwards and backs. Data from Chapter 4 were combined with CMJ and IMTP data (Chapter 3), and in-game performance data (Chapter 6), and subsequently used in Chapters 5 and 6, respectively.

Chapter 5 explored any associations that could exist between the ten individual polymorphisms and measures of strength and power obtained from the CMJ and IMTP. In addition, a polygenic profile that comprises seven polymorphisms was produced to investigate any associations with measures in CMJ and IMTP. It was hypothesised that some

of the individual polymorphisms and polygenic profiles would be associated with measures of strength/power.

The investigation in Chapter 6 aimed to explore any associations that could exist between the 10 polymorphisms and in-game performance variables in forwards and backs. The variables comprised; i) number of tries; ii) number of carries; iii) metres gained iv) clean breaks; vi) defenders beaten, and vi) tackles. In-game data were obtained from a minimum of 320 hours of tier 1 competition for each athlete. A polygenic profile comprising seven polymorphisms was also produced for the forwards and backs. It was hypothesised that associations would be found between some of the individual polymorphisms and in-game performance variables, and between polygenic profiles and in-game variables.

Chapter 7 took a retrospective view of the results observed within each investigative Chapter and aimed to combine the findings to explain how the polymorphisms could have associated with the athletes' strength/power and performance during match play. Chapter 7 also considered the strengths and limitations of this thesis, in addition to outlining possible future directions.

CHAPTER 2
GENERAL METHODS

GENERAL METHODS

This Chapter briefly describes the RugbyGene project, and provides details for the methods and procedures used to collect phenotype and/or genotype data for a total of 1892 participants. These data were subsequently used in Chapters 3–6.

2.1. RugbyGene Project

This thesis forms part of the ongoing RugbyGene project (Heffernan et al., 2015), which is a major sub-component of GENESIS. The GENESIS project is a multi-institutional UK project led by Manchester Metropolitan University, which aims to identify the molecular genetic characteristics associated with elite sports performance. GENESIS forms part of the larger, worldwide collaborative Athlome Project which aims to study the phenotype and genotype data presently available on elite athletes (www.athlomeconsortium.org) (Pitsiladis et al., 2016).

RugbyGene incorporates a large cohort of elite rugby athletes (the majority recruited from UK rugby league and rugby union professional clubs) from whom phenotypic and genetic data is gathered, and used to get insight into the genetic contribution to complex phenotypes that contribute to elite rugby performance and elite athlete status in rugby. RugbyGene aims to attenuate two common limitations of sport genetic research, being i) small sample sizes, and ii) recruitment of athletes from different sporting disciplines and levels of competition – often (but erroneously) treated as one athletic group in the study design and associated analyses (Heffernan et al., 2015). Investigations concerning the genetic contribution to complex performance related phenotypes should merge data from hundreds or preferably thousands of athletes, all of whom possessing a distinctive high level of the phenotype of interest if solid conclusions are to be drawn from the results of such studies (Bouchard, 2015;

Tanaka et al., 2016). In addition, investigations must include athletes who pertain to the same sport in an attempt to 'purify' the phenotype of interest as much as practically possible, further increasing the usefulness of the observations and findings from sport genetic research.

RugbyGene has already produced interesting results on the genetic contribution to elite status (Heffernan et al., 2016; Heffernan et al., 2017; Heffernan et al., 2017) in an elite rugby union context. More recently, three published reviews from RugbyGene focussed on the anthropometric and physiological characteristics (Brazier et al., 2020), tendon and ligament injury incidence and severity (Brazier et al., 2019) and concussion risk (Antrobus et al., 2021) in elite rugby athletes. A main long term goal of the RugbyGene project is to continue to increase the number of rugby athletes from all geographic ancestries as this can serve a very powerful scientific approach towards progress in sports genetics and genomics research (Heffernan et al., 2015).

2.1.1 Participants for each investigation

This thesis involved a total of 1892 participants. Of these, 707 were Caucasian elite male rugby union athletes and 1185 were male and female Caucasian non-athletes. Table 2.1 shows counts of participants (athletes and non-athletes) for this thesis, whilst Figure 2.1 shows the participants for each study (Chapters 3–6). Basic anthropometric characteristics of participants for each investigation are provided in the respective Chapter.

Table 2.1 All participants that were included in one or more studies in this thesis.

GROUP		Group (n)
ELITE MALE RUGBY UNION ATHLETES	In GENESIS	567
	NOT in GENESIS*	140*
	Total athletes	**707**
NON ATHLETES	All in GENESIS	1185
	Total participants	**1892**

*Participated only in Chapter 3.

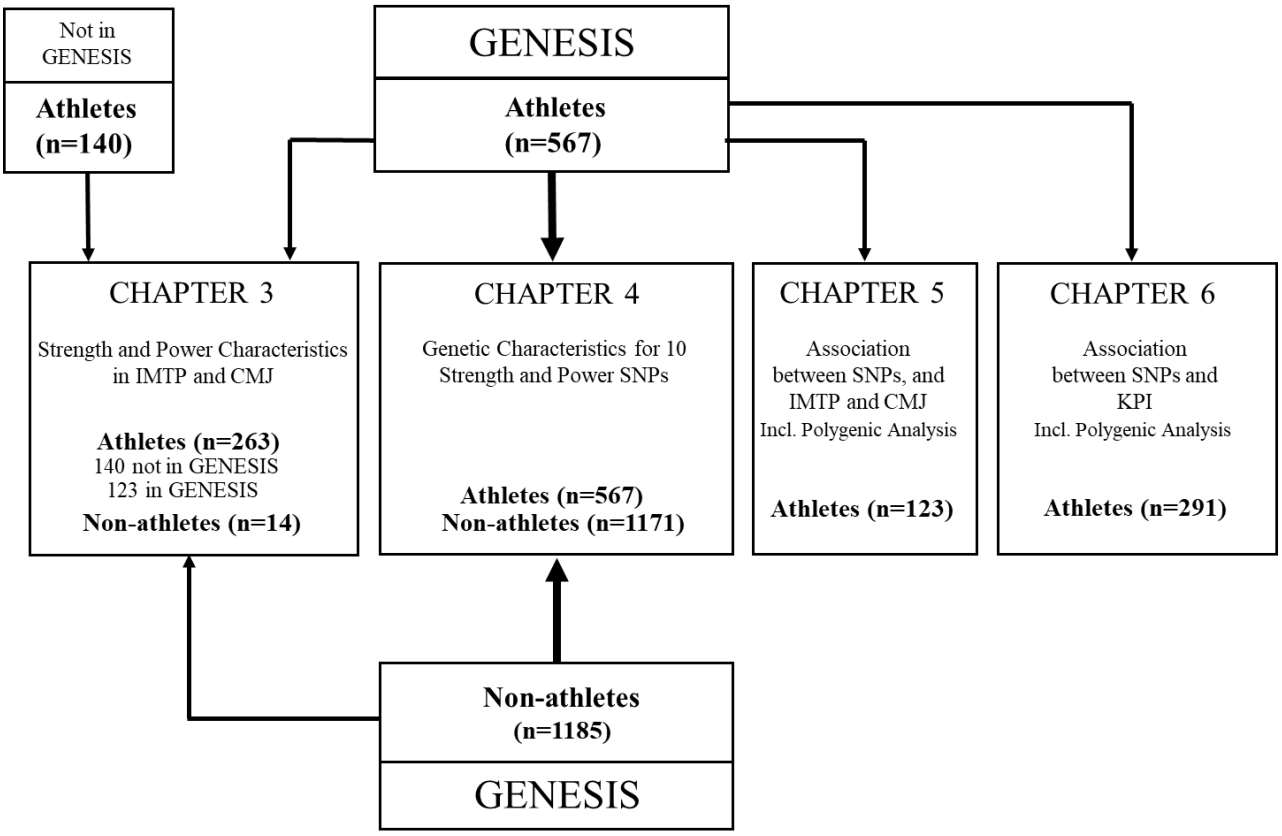


Figure 2.1 Participants for each of the four investigations, Chapters 3 to 6. Note that the 140 athletes that have participated only in Chapter 3 do not form part of GENESIS.
IMTP: Isometric mid-thigh pull. CMJ: Countermovement jump. KPI: Key performance indicators.

2.1.2 Eliteness and era of inclusion

The relevance of sport genomics research that can potentially serve the management and preparation of elite athletes depends on the athletic status of athletes from whom the initial data is obtained. In the context of rugby union as relevant for this thesis, the definition of 'elite' refers to rugby athletes competing in the highest competitive league of a 'Tier 1' rugby nation (Regulation 16, worldrugby.org) (Heffernan et al., 2015). Furthermore, given the evolving nature of elite rugby, the era in which athletes competed at an elite level also needs to be defined and considered in the study design. Rugby union has progressed dramatically during the over 100 years of its existence; and that change has certainly continued significantly since the sport turned professional in 1995. Therefore, it was proposed that 1995 onwards is a playing era inclusion criterion that can sensibly be justified (Heffernan et al., 2015). This thesis comprises four investigations in which only athletes that met the criteria for 'elite' and 'playing era' were included in the investigative chapters.

2.1.3 Geographical ancestry considerations

The underlying issue that substantiates the consideration for geographic ancestry in subject recruitment in genetic studies is that populations with different geographic ancestries (e.g. Asian, African, Americans, Northern European/Caucasian) carry different profiles of rare and common variants (Consortium, 2012), implying that all individuals carry a signature of the history of their geographic ancestry in their DNA sequence. The rationale for considering geographic ancestry as a variable in genetic studies stems from the fact that various diseases and disease risk factors occur more frequently in some geographic ancestries compared to others (Roth, 2007). Similarly, it is well-established that individuals from certain geographic ancestries display superior abilities in particular sports and exercise related traits (Epstein, 2014), although the disparities in these traits could be related to both genetic factors and

environmental factors such as socioeconomic status and cultural diet preferences, for example.

We have not restricted subject recruitment according to geographic ancestry, but most rugby athletes in RugbyGene were recruited in the UK, and white Caucasian is the most common (>90%) geographic ancestry in rugby athletes in the UK. Therefore, to avoid population stratification (where differences between groups, e.g. between athletes and non-athletes, could be wrongly attributed to athlete status when in fact they could be due to differences in geographic ancestry between groups), we have restricted all statistical analyses and publications to the single most common geographic ancestry in our athletes. Therefore, the work presented in this thesis followed the approach taken to date within RugbyGene. It would be fascinating to evaluate genetic associations with performance and injury risk in rugby athletes of other geographic ancestries, and this is a longer-term aim of RugbyGene.

2.1.4 Rugby playing positions and sample sizes for athletes and non-athletes

Investigations in this thesis involved comparisons of genotype and phenotype data between rugby athletes of different playing positions (and between athletes or athlete sub-groups, and non-athletes for Chapters 3 and 4) and therefore, athletes needed to be categorised according to the study design of the respective Chapter. In Chapters 4 and 6, athletes were categorised into two major groups; forwards (props, hookers, locks, flankers, number eights) and backs (scrum halves, fly halves, centres, wings, full backs) (Figure 2.2). This was due to diverse game demands for these two major playing positions (Roberts et al., 2008; Quarrie et al., 2013; Cahill et al., 2013; Jones et al., 2015), and the physiological and anthropometric quantities that reflect these demands for each group within rugby union (Appleby et al., 2012; Sedeaud et al., 2012; Brazier et al., 2020) (Figure 2.2). In addition, sample sizes of athletes

for Chapter 6 were limited by the existence (and provision) of in-game performance data for athletes that form part of RugbyGene, to allow for analyses of relationships between performance data and genotype in athletes. With regards to non-athletes for Chapter 4, sample size was only limited by geographic ancestry and thus, all Caucasian non-athletes that form part of RugbyGene were recruited for this investigation. Non-athletes were not required for Chapter 6 (Figure 2.1). Statistical power estimates for Chapters 4 and 6 are included in each respective Chapter.

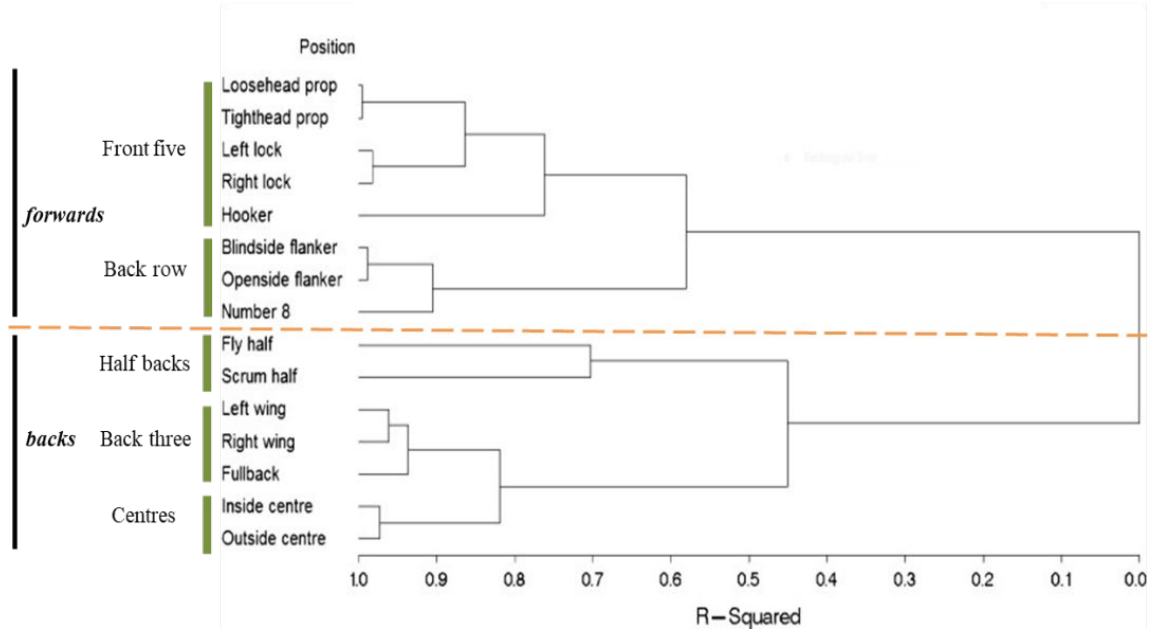


Figure 2.2 Cluster analysis of positions from activities and time-motion data. Adapted from Quarrie et al. (2013).

Chapter 5 required athletes that form part of RugbyGene and that have been assessed for strength and power in the isometric mid-thigh pull (IMTP) and countermovement jump (CMJ) by collaborators of RugbyGene at Swansea University. In Chapter 5, strength and power were compared between athletes of different genotypes and thus, categorisation by

playing position was not required – allowing for the preservation of sample size and thus, statistical power. Non-athletes were not required for Chapter 5 (Figure 2.1).

In Chapter 3, comparisons of strength and power (in the IMTP and CMJ) were made between five different playing positions. Due to diverse game demands for the five playing positions (Roberts et al., 2008; Quarrie et al., 2013; Cahill et al., 2013; Jones et al., 2015), and physiological and anthropometric quantities that reflect these demands within rugby union (Appleby et al., 2012; Sedeaud et al., 2012; Brazier et al., 2020), athletes were divided into these five positional groups according to similarities in their movement patterns (Cahill et al., 2013) and corresponding physiological differences (Brazier et al., 2020); front five (props, hookers, locks), back row (flankers, number eights), half backs (scrum halves, fly halves), centres (inside centres and outside centres), and back three (wings and full backs) (Figure 2.2).

In addition, in Chapter 3 comparisons of strength and power were made between all athletes and non-athletes, and between each of the major athlete sub-groups (forwards and backs) and non-athletes. Although subsequent Chapters (with exception of Chapter 4, which included all Caucasian non-athletes that form part of RugbyGene, who provide genetic data for some or all of the ten SNPs) did not require the inclusion of non-athletes, it was proposed that Chapter 3 includes a sample of non-athletes that form part of RugbyGene (that could provide strength and power data in the IMTP and CMJ using the same equipment as for the athletes) that could be used as controls. The author of this thesis (with the assistance of supervisors) was responsible for the collection of strength and power data from non-athletes (Section 2.2: 2.2.1–2.2.7). In brief, the author of this thesis was required to visit Swansea University to familiarise with the set-up of the equipment and the protocols used to administer the IMTP

and CMJ. Subsequently, the author was responsible for setting up the equipment at Manchester Metropolitan University and for the administration of the IMTP and CMJ for the non-athletes. The familiarisation and actual testing sessions were conducted on two separate occasions of 3 days each, separated by 4 weeks (see details in Section 2.2.2). Details of the rationale for the selection of IMTP and CMJ data for this thesis, and of the author's contribution for the collection of strength and power data are provided in Sections 2.2.1 and 2.2.7, respectively. Due to the logistics surrounding time and costs (mainly due to availability of testing equipment and its set-up at Manchester Metropolitan University, participant recruitment methods (posters and word of mouth), and the author of this thesis residing outside of the UK), the number of non-athletes recruited and assessed for Chapter 3 was 14. In considering that: i) a sample of non-athletes was required to provide a reference with which the elite athletes' could be compared (in addition to the main comparisons between athlete sub-groups (forwards and backs, or the five playing positions)); ii) the strength and power data of non-athletes was required only for Chapter 3 of this thesis; and iii) the relatively small sample of 14 non-athletes and the 263 athletes (147 forwards and 116 backs) can provide a statistical power of 80% with an effect size of 0.68 when alpha (α) is set at 0.05; the author felt that the inclusion of the non-athlete group in Chapter 3 could be sensibly justified. Statistical power calculations for Chapters 3 and 5 are included in each respective Chapter.

2.2 Laboratory-based Strength/Power measurements

Measures of muscular strength and power were obtained for 263 elite rugby union athletes and 14 non-athletes. The measures were extracted from the recorded force-time histories produced by countermovement jumps (CMJ) and isometric mid-thigh pulls (IMTP), performed on a force platform. These data were used in Chapters 3 and 5 (see Section 2.1.1 in this Chapter). Details of testing equipment and procedures are provided in Sections 2.2.1 – 2.2.5. Further details on the operation of the portable Kistler Force Plate used to administer the IMTP and CMJ tests are provided in Appendix B.

2.2.1 Rationale for the selection of IMTP and CMJ data for this thesis

It is reasonable to assume that a detailed profile of strength and power of rugby athletes is obtained from as many as practically possible physical exercises from which various parameters of neuromuscular function are acquired. The importance of muscular strength and power in rugby is well-established (Section 1.4.1) – strength and power could distinguish between competitive standard (Section 1.4.2), and have been associated with performance indicators such as superior tackling proficiency (Section 1.4.3) and sprinting ability (Section 1.4.4) – all addressed in detail in the Sections indicated. An ideal strength/power profile of the 'rugby athlete' that can potentially indicate superior rugby performance should, therefore, include parameters of neuromuscular function that are highly related with performance indicators in rugby. Such athlete characterization in relation to strength and power can serve to determine strength/power athlete status and predict future performance to some extent, the latter most valuable in talent identification and development, for example (Section 1.6).

Genomic information can complement existing phenotypic data that is collected and used by sports coaches and supporting exercise scientists (Williams et al., 2014), and potentially be

used in combination with such phenotypic data to aid talent identification processes in elite rugby (Section 1.6). A main aim of this thesis was to elucidate the genetic contribution to strength and power in elite rugby by examining whether 10 genetic variants (previously associated with strength and power) are associated with: i) elite rugby athlete status (Chapter 4); ii) measures of strength/power within elite rugby athletes (Chapter 5), and iii) performance indicators in elite rugby (Chapter 6). Thus, a primary necessity was to characterize strength/power in elite rugby athletes of different playing positions (Chapter 3) and genotype the same athletes for variants that are thought to contribute to strength/power (Chapter 4); in an attempt to explore any associations between the variants and the measures used to characterize strength/power of the elite athletes (Chapter 5). Although it seems quite logical that the best strength/power profile of elite athletes is composed from as many as practically possible neuromuscular parameters that are associated with performance indicators, as already mentioned above; a primary requirement for this thesis was that strength/power data are obtained for a substantial portion (ideally all) of the elite rugby athletes that form part of the RugbyGene project (i.e. elite athletes for whom genotype data could be acquired). Two exercises that are widely used to assess strength and power in elite rugby are the isometric mid-thigh pull (IMTP) and countermovement jump (CMJ) (Section 1.7), and data in both exercises could be provided for 263 athletes (123 of whom form part of RugbyGene) by Prof. Liam Kilduff who is a collaborator in the RugbyGene project. Although IMTP data were limited to peak force and relative peak force (the latter achieved by dividing peak force by the athletes' body mass), whilst CMJ data comprised peak power, relative peak power and jump height; the combination of data (from both exercises) provided measures for both strength and power using well-established assessments of strength/power in elite rugby. Also, important to note here is that all data were extracted from the force-time histories produced by the force platform – considered the 'gold standard' method for both

assessments. In considering the above, whilst acknowledging the novelty of the study presented in Chapter 5 (i.e. the first study to explore any associations between ten strength/power related variants and established measures of strength/power in elite rugby athletes), the author of the current thesis felt that the use of the strength/power data used in this thesis are justified.

2.2.2 Testing location and familiarisation sessions

The CMJ and IMTP tests for the rugby athletes were conducted at the School of Sports and Exercise Sciences laboratory of Swansea University, and at the indoor training facility of the athletes' respective club, under the supervision of Professor Liam Kilduff. Non-athletes were tested at Manchester Metropolitan University (Crewe) by the author of this thesis under the supervision of Dr Alun Williams.

Athletes undertook their strength/power tests during their preseason training period. Although familiar with both tests (as they form an element of their training and periodic testing regimes), athletes were required to visit the University's laboratory or their club's training facility 48 hr before testing, to become familiar with the testing procedures. Non-athletes undertook their strength/power tests during May and June 2017. They were required to attend for a familiarisation session 48 hr prior to their actual testing session, during which emphasis was placed on learning proper body positioning in both tests; primarily to maximise performance during actual testing and reduce the chance of injury. For the IMTP test, the height of the bar needed to be determined for each participant according to the individual's physical characteristics. This was accomplished during the familiarisation sessions.

2.2.3 Testing equipment and procedures

The testing equipment comprised a portable force platform (model 9286AA, Kistler Instruments Ltd., Farnborough, UK) with built in charge amplifier, a 16 bit analogue to digital convertor, lap top computer with Kistler's Bioware (Version 4.0.0.0; Kistler Instruments Ltd., Farnborough, United Kingdom) installed, interface cabling, and a custom IMTP rig (see details in Appendix B). The force platform was factory calibrated and underwent regular satisfactory calibration checks using masses that were traceable to national standards. This equipment was used by Professor Liam Kilduff and his research team to test the elite rugby athletes participants in this thesis (West et al., 2011; Owen et al., 2014; Cunningham et al., 2016; Cunningham et al., 2018). The same equipment was loaned by Professor Liam Kilduff from Swansea University to test the non-athletes at Manchester Metropolitan University by the author of this thesis.

All participants were asked to refrain from consuming foods and drinks containing caffeine or alcohol, and to avoid strenuous exercise during the 48 hr preceding testing. Participants' measurements for height were provided on testing day. Athletes completed their standardised warm-up before testing. Non-athletes were required to warm-up using a Monark cycle ergometer, pedalling at 60 rpm (120 W) for 5 min. Consumption of water was permitted during testing. Room ambient temperature was maintained at 20-24°C at all testing sites. Verbal encouragement was given to maximise performance.

CMJ test

For the CMJ test, participants were required to assume a standing position while centred on the force platform with arms akimbo (hands on hips) (Aragón-Vargas and Gross, 1997; Hatze, 1998), jump as high as possible (when prompted) by dipping to a self-selected depth

and immediately accelerating upwards in an attempt to gain maximum jump height, and land on the force platform, keeping arms akimbo throughout. A data sample length of 10 s was used for each trial, with the signal to jump given after 5 s of starting the 10 s sampling period. The signal to jump was given after the subject remained stationary in the standing position for ~2 s, ensuring that the 1 s immediately before the signal to jump could be used to accurately determine body weight. Each participant completed three CMJ, with 2 min rest between jumps.

IMTP test

For the IMTP the bar height was adjusted to a pre-determined height (determined during the familiarisation session) for each participant. Participants were required to stand on the force platform (placed inside a custom IMTP rig) and assume a body position similar to that of athletes completing the second pull of a power clean, with the shoulders in line with the IMTP rig's bar and flat trunk position (Section 1.7.2). This position allowed participants to maintain a knee angle of approximately 120-130° (Haff et al., 2005; Stone et al., 2004). Hands were strapped evenly to the bar and slightly wider than shoulder width using weightlifting straps. From this position, participants were required to pull the bar (when prompted by the tester) as hard and as fast as possible for 5 s. These commands were based on previous research indicating these instructions produce optimal results for peak force and rate of force development (Bemben et al., 1990; Halperin et al., 2016). A sample length of 16 s was used for all trials. This consisted of a stationary phase of 4 s, followed by a 3 s countdown and the signal to start, after which a maximal effort IMTP was initiated and completed during the remaining sampling time of 9 s. Each participant completed three maximal effort IMTP of 5 s each, with 4 min rest between trials. Trials were rejected if

excessive pretension or unloading was present during the stationary phase or the 3 s countdown, or if shoulders deviated from the bar's line during the maximal effort phase.

2.2.4 Acquiring parameters from the GRF force-time histories

Force-time histories of the vertical component of the ground reaction force (VGRF) were recorded for three CMJs and three IMTPs for each participant. The analogue signals from the force platform were sampled at a frequency of 1000 Hz through the 16-bit analogue to digital convertor, and recorded on the computer using Kistler's Bioware software. A sample rate of 1000 Hz has been shown to be optimal to maximise the reliability and validity of the force-time histories produced during the execution of both CMJ (Owen et al., 2014) and IMTP (West et al., 2011; Dos'Santos et al., 2016). The force platform's vertical range was set to its maximum, 0–20 kN (0–5 kN per corner transducer) as suggested by Owen et al. (2014). Body weight and strength/power measures for both CMJ and IMTP were extracted from the recorded VGRF-time histories by means of a custom software developed at Swansea University.

2.2.5 Parameters for the Isometric Mid-Thigh Pull (IMTP)

Body mass, peak force and relative peak force were determined from the VGRF-time history of the maximal effort IMTP. IMTP peak force was defined as the highest value of the VGRF produced during the ~5 s of maximal effort minus the subject's body weight. IMTP relative peak force was determined by dividing IMTP peak force by body mass. The mean and standard deviation of VGRF were calculated for the 1 s period of quiet standing time immediately before the signal to start. Body weight was taken as the mean of VGRF during the 1 s period. Body mass was determined from body weight (BW) and taken as $BW \cdot g^{-1}$ (kg), with g = acceleration due to gravity.

A reliable initiation time of the IMTP was needed as a reference point for calculation of subsequent variables. The VGRF-time history was not suitable to define a start time because it lacked stability, due the subject holding onto the bar of the IMPT rig thus causing a considerable variation in the force being transmitted through the force platform during the 1 s stationary phase. However, the rate of change of force with respect to time (i.e. rate of force development (RFD)) did show stability during this phase. Therefore, this variable was suitable to determine a quiet stationary baseline value and threshold, beyond which the IMTP could be defined as having started. The instantaneous rate of change of force with respect to time was calculated for the VGRF–time history during the 1 s period before the signal to start. Firstly, the VGRF–time history was filtered using a dual-pass Butterworth filter (low pass, 20-Hz cut-off), and then numerically differentiated using the central difference method. Filter settings were determined from a pilot study based on Fourier analysis and inspection. The first second of the first derivative-time history was then discarded to avoid the edge effects associated with digital filtering, and a mean and standard deviation were calculated for the remaining 1 s of quiet standing immediately before the signal to jump. The initiation time of the IMTP was defined as the instant, after the signal to start, that the first derivative of the VGRF-time history exceeded the mean plus 5 standard deviations of the first derivative values calculated during the 1 s period immediately before the signal to start the IMTP. Figure 2.2 shows an IMTP force-time history produced by an athlete participant in this thesis.

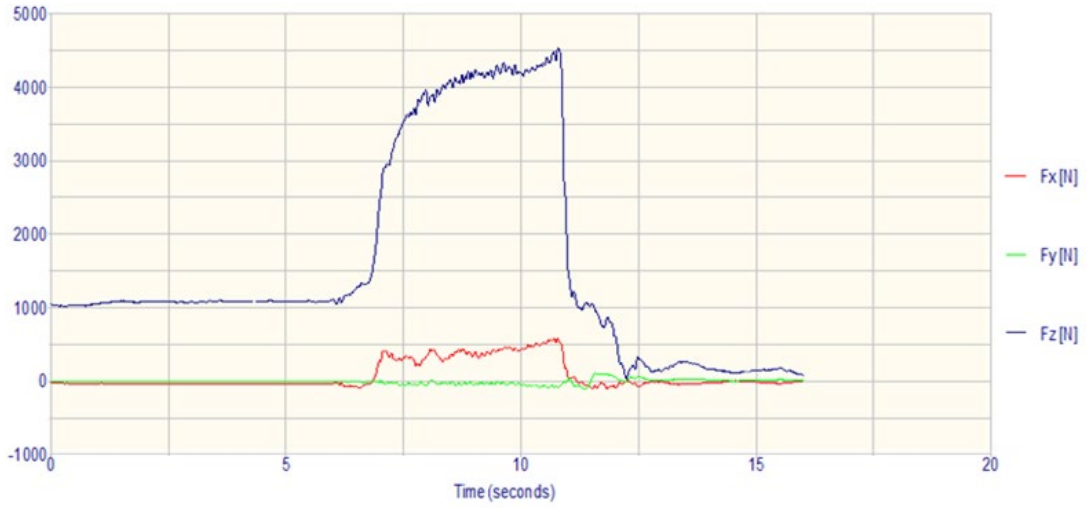


Figure 2.2 Force-time curve produced by a rugby athlete, participant in this thesis. Note the high peak force (4532 N) achieved at the end of the isometric effort, reflected by the force-time history of the vertical component of the GRF, shown by the blue line.

2.2.6 Parameters for the Countermovement Jump (CMJ)

Body mass, maximum concentric power, and jump height were determined from the VGRF-time histories of the CMJs. The highest measures for CMJ maximum concentric power and CMJ jump height were chosen for subsequent analyses. CMJ relative maximum concentric power was achieved by dividing CMJ maximum concentric power by body mass.

Force-time histories were recorded from the time before the start of the jump until after take-off. For the reasons explained in 2.2.3 above, force-time histories with a sampling frequency of 1000 Hz were processed. Specifically for the CMJ, this is also because 1000 Hz is the highest sampling frequency normally used to measure peak lower body mechanical power output in a CMJ (Kibele, 1998; Hanson et al., 2007; Bevan et al., 2009), and provides the most accurate lower body mechanical power measurement (Owen et al., 2014). A sample length of 5 s (including the 1 s phase before the signal to jump) was used (from the 10 s data

sampling period) to derive the required measures. CMJ force-time histories produced by an elite rugby athlete participant in this thesis are shown in figures 2.1a and 2.1b.

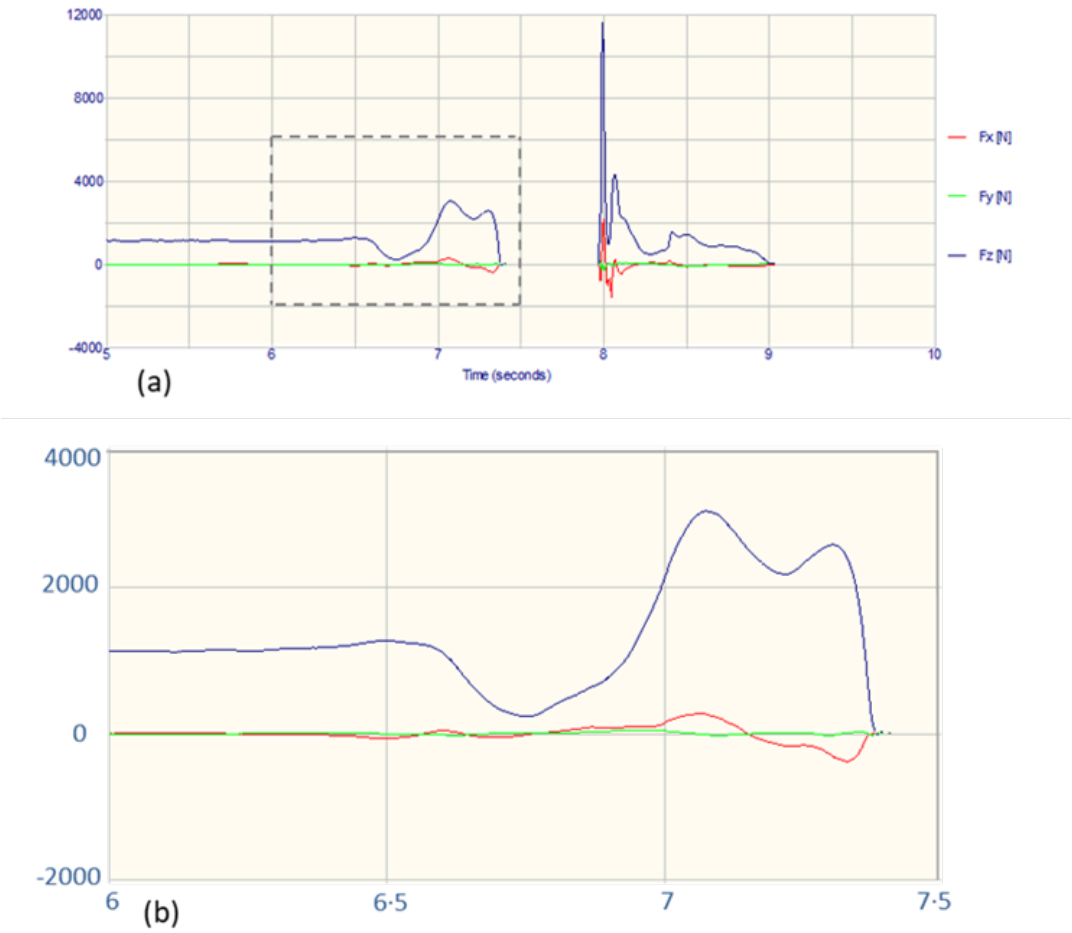


Figure 2.3a Force-time histories of a CMJ produced by an elite rugby athlete participant in this thesis, including the initiation of the jump, take-off and landing phases. **Figure 2.3b** Magnified force-time histories (shown in (a) enclosed in rectangle) for the period from the initiation of the countermovement to take-off (contact phase). VGRF is shown with blue line.

Body weight was determined from the 1 s period of the stationary phase before the signal to jump, by first calculating the mean of the VGRF during the 1 s period. Body mass was determined from body weight (BW) and taken to be BW $\cdot$ g^{-1} (kg), with g = acceleration due to gravity.

Maximum concentric power was taken as the maximum value for instantaneous power after the time that the velocity of the performer's centre of gravity (CG) was zero after the signal to jump and during the contact phase. The velocity of the CG was determined by the integration of 1 ms intervals (a period equal to the sample width). Before the calculation of the 1 ms 'strip area', the subject's body weight was subtracted from the VGRF values of the integrated 'strip area'. The 'strip area', then represented the impulse for that 1 s time interval. Using the principle of the impulse-momentum relationship, the 'strip area' was divided by the subject's body mass to produce a value representing the change in velocity of the subject's CG during the 1 ms interval. This change in CG velocity was added to the CG's previous instantaneous velocity (at the start of the 'strip area') to produce a new instantaneous velocity at the end of the 'strip area.' This method can only determine the change in velocity during a given time period, so a reference velocity of zero (for CG) was taken at the time point identified as the initiation of the jump (Owen et al., 2014). The initiation of the jump (starting point for integration) was defined as the time when the VGRF exceeded the mean body weight ± 5 standard deviations (calculated during the 1 s stationary phase for body weight measurement) after the signal to jump (Vanrenterghem et al., 2001). Instantaneous power was determined by the fundamental relationship: Power (W) = VGRF (N) × vertical velocity of CG ($m \cdot s^{-1}$).

Vertical displacement of the subject's CG was determined from a second integration of the instantaneous velocity-time history. The vertical displacement of the CG at the initiation of the jump was defined as zero. Jump height was defined as the difference in the vertical displacement of the CG between take-off (toes leave the force plate) and maximum vertical displacement achieved.

2.2.7 Author contribution to obtaining IMTP and CMJ data

The force plate and all associated equipment (see Section 2.2.2 and Appendix B) were used by Professor Liam Kilduff and his research team at Swansea University (collaborators of RugbyGene) to test the elite rugby athlete participants in this thesis (West et al., 2011; Owen et al., 2014; Cunningham et al., 2016; Cunningham et al., 2018). IMTP and CMJ data for the athletes were subsequently provided to the current author, and used in Chapters 3 and 5 of this thesis.

The same equipment was loaned to test the non-athletes at Manchester Metropolitan University. The author of this thesis visited Swansea University to familiarise with the set-up of the equipment and the protocols used to administer the IMTP and CMJ. Subsequently, the author was responsible for setting up the equipment at Manchester Metropolitan University and administering the IMTP and CMJ for the non-athletes. The familiarisation and actual testing sessions for the non-athletes were conducted on two separate occasions of 3 days each, separated by 4 weeks (see Sections 2.2.1 and 2.2.2).

IMTP and CMJ data for all athletes and non-athletes were extracted from the recorded force-time histories using bespoke software developed at Swansea University, using the principles described in Sections 2.2.3 – 2.2.5.

2.3 In-Game Performance Data

In-game performance data were obtained from Opta Sports (London, UK) for eight seasons (2012-13 to 2019-20) of rugby union competition in the highest professional competitive leagues in England (Premiership) and Wales / Ireland / Scotland / Italy / South Africa (Celtic/PRO12/PRO14). In-game performance data were used in Chapter 6 of this thesis. Athletes for whom in-game performance data were gathered had to form part of GENESIS (i.e. DNA was available for analysis), and have a minimum of 320 competitive minutes analysed by Opta Sports during the competitive seasons 2012-13 to 2019-20. Performance indicators were used to represent in-game performance for individual players. Table 2.2 shows the operational definitions of six performance indicators used in Chapter 6.

Table 3.2 Operational definitions of performance indicators used in Chapter 6. Adopted from Cunningham et al. (2018).

Performance indicator	Definition
tries scored	Count of tries the player has scored.
carries made	Count of times a player carried the ball into forward contact.
metres gained	Total number of metres a player has carried the ball in a forward direction.
clean breaks	Count of times a player in possession of the ball breaks through the line of defense.
defenders beaten	Number of defenders a player has successfully eluded.
tackles	Count of tackles made by the player.

2.3.1 Rationale for the selection of performance indicators for this thesis

A main aim of this thesis was to examine some of the genetic contribution to strength and power in elite rugby union athletes. This required that strength and power of athletes is quantified to examine whether associations exist between the strength/power measures and the genetic characteristics of the athletes. Chapter 3 quantified measures of strength and power in the isometric mid-thigh pull (IMTP) and countermovement jump (CMJ) of the athletes, and Chapter 5 investigated whether associations exist between the genetic characteristics of the athletes (such characteristics investigated in Chapter 4, using ten strength/power related SNPs) and these strength/power measures. As already mentioned in Section 2.2.1, it is reasonable to assume that a detailed profile of strength and power of rugby athletes is obtained from as many as practically possible physical exercises and movements from which various parameters of neuromuscular function are acquired. Thus, Chapter 6 used in-game performance data of elite rugby athletes and aimed to explore whether associations exist between the genetic characteristics of the athletes (examined in Chapter 4) and in-game performance of the same athletes. Thus, in-game performance data used in Chapter 6 needed to reflect the strength and power abilities of the athletes.

In-game performance data were available for eight seasons (2012-13 to 2019-20) of rugby union competition in the highest professional competitive leagues (see 2.3 above). Chapter 6 required in-game performance data for individual players for whom genetic data were available (i.e. those players who form part of GENESIS and RugbyGene, and are Caucasian). In-game performance data were available as counts of discrete variables for each performance indicator and for individual players of teams that participated in the competitions mentioned above (Section 2.3, introduction). Thus, counts of each performance indicator were the number of times the individual player performed that specific performance

during the total time played by that same player (minimum of 320 minutes) during some or all of the eight seasons. For the analyses, each of the selected performance indicator variables was normalized to 80 min of playing time. Eighty-seven performance indicators were available for three seasons (2017-18 to 2019-20). These were categorized as scoring, attack, defence, turnovers/discipline, lineout, handling, turnovers won types, kick outcomes, tackle outcomes, missed tackle outcomes, carry outcomes, offload outcomes, pass outcomes, penalty types and turnover types. For the previous five seasons (2012-13 to 2016-17), 32 of the 87 performance indicators were available and thus, the selection of performance indicators for Chapter 6 was made from those 32 available for all eight seasons (2012-13 to 2019-20). This allowed for maximising the number of athletes for whom we had both genetic and in-game performance data (i.e. those athletes that could be included in Chapter 6). The 32 performance indicators and their categorization are shown in Figure 2.5.

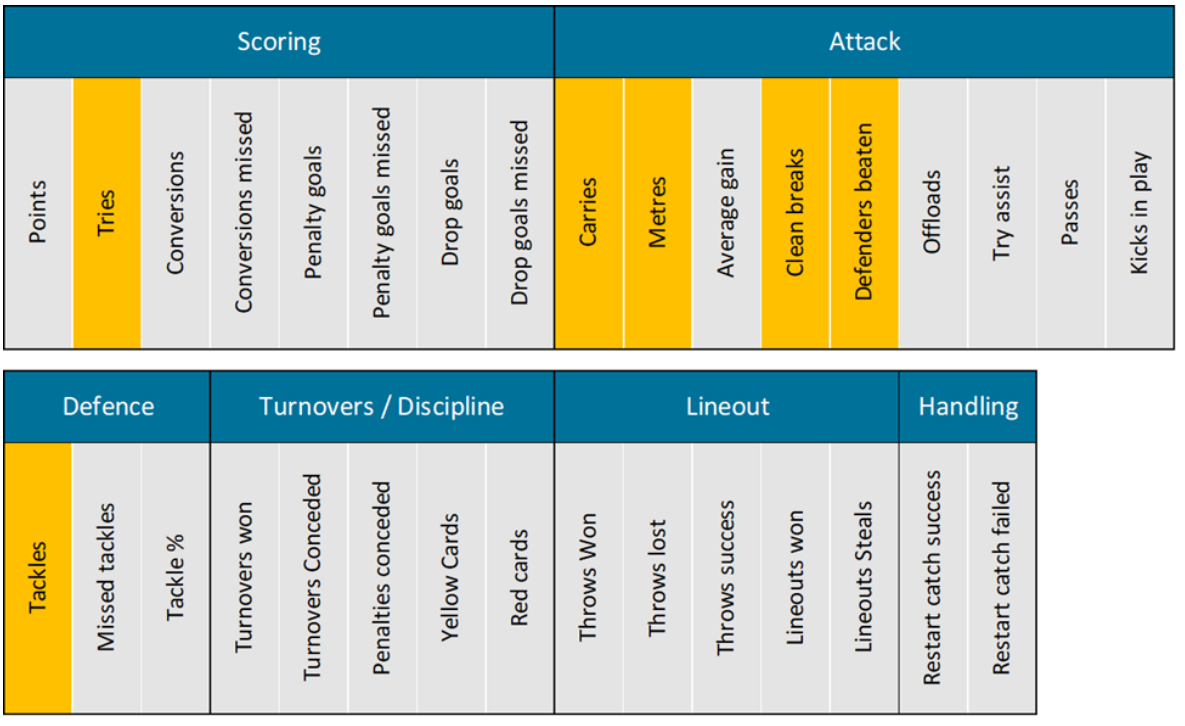


Figure 2.5 Thirty two performance indicators that were available for eight seasons (2012-13 to 2019-20). The six selected performance indicators used for Chapter 6 are highlighted in yellow.

Performance analyses in rugby has become an integral part of the coaching process and talent identification and development processes, and allows coaches to objectively assess team performance while identifying oppositions' strengths and weaknesses, and opportunities to exploit these during competition (Jones et al., 2008). The majority of performance analysis studies in rugby union measure performance through the collection and analysis of performance indicators. Important to note here is that the direction and scope of performance analyses research in rugby union has primarily explored a combination of performance indicators deemed relevant to successful outcomes (Colomer et al., 2020). However, for the purposes of this thesis the selected performance indicators were those that best reflected the strength and power abilities of individual athletes. For example, we have assumed that all else being equal, an outside back player who performed 5 clean breaks and 2 tries in 3 matches (average of 58 min per appearance) was stronger and more powerful than another outside back who made fewer clean breaks and scored fewer tries during the same playing time. All 32 performance indicators shown in Figure 2.5 could reflect, at least to some extent, performance that is underpinned by strength/power abilities. However, certain performance indicators better reflect superior strength/power of individual athletes, and those most informative were identified via reasoning informed by the input of an expert practitioner of sport science in rugby (Mr Mark Bennett). Thus, these performance indicators were selected and termed key performance indicators (KPI's) for use in Chapter 6. The primary justification for selection/deselection of the performance indicators for each category (shown in Figure 2.5) is provided in Table 2.3.

Table 2.3 The primary justification for selection/deselection of the performance indicators for each category.

Category for performance indicators	Main reason for selection/deselection of performance indicators for each category
Scoring (tries)	Tries were selected because it was assumed that this performance indicator reflects superior strength/power of the player in key moments of a match, and more than other performance indicator in this category (conversions, penalty goals and drop goals).
Attack (carries; metres; clean breaks; defenders beaten)	Four of the six KPI's were selected from this category. It was assumed that counts of carries and total metres gained (while carrying the ball) could reflect more involvement in the game via increased exertions that require strength and power. With regards to number of clean breaks and defenders beaten, it was assumed that these reflect increased sprint acceleration, sprinting speed and agility/change of direction – all underpinned by muscular strength/power abilities (Section 1.4.4).
Defence (tackles)	Tackling proficiency has been positively associated with match outcomes (Colomer et al., 2020). For the purposes of Chapter 6 we have assumed that increased efforts made to accomplish more successful tackles can reflect superior strength/power abilities (Section 1.4.3).
Turnover / discipline (none selected)	Although the performance indicators in this category (for a whole team) have been positively associated with match outcomes (Colomer et al., 2020), they probably do not reflect superior strength/power of the player as much as the selected KPI's.
Lineout (none selected)	Can reflect strength/power, for example, in timed and rapid displacement and positioning of body, in elevating teammates and jumping. However, performance indicators in this category probably do not reflect superior strength/power of the player as much as the selected KPI's.
Handling (none selected)	Can reflect timed and rapid displacement and positioning of body, combined with catching ability (for restart catch success). But the performance indicators in this category probably do not reflect superior strength/power of the player as much as the selected KPI's.

2.3.2 Author's contribution to obtaining in-game performance data

All in-game performance data (the six KPI's) for 291 elite rugby athletes were cross-referenced, extracted, and converted to a format amenable to analysis, by the author of this thesis. Selecting and extracting data for the 291 rugby athlete participants required ~100 hours of work.

2.4 Genetic Analyses

2.4.1 DNA sample collection

The majority of participants' deoxyribonucleic acid (DNA) was obtained via blood, saliva, and buccal samples from researchers of the sports genomics team at Manchester Metropolitan University. Whole blood sampling is generally preferable for collection of large amounts of genomic DNA due to being cheaper and potentially quicker; however, given that ~24% of the general population have a phobia of needles (Taddio et al., 2012), saliva or buccal swabs were the preferred less invasive sample collection method by some participants (Feigelson et al., 2001), and/or a more practical method of sampling at remote sites.

For ~70% of participants, a 5 mL blood sample was collected by a trained phlebotomist from a superficial forearm vein into ethylenediamine tetra acetic acid (EDTA) anticoagulant treated tubes (BD Vacutainer Systems, Plymouth, UK) before being aliquoted into 2 mL microcentrifuge tubes (Eppendorf AG, Hamburg, Germany) and stored at -20°C. Saliva samples were collected following a minimum of 30 min of abstinence from food and drink into Oragene DNA OG-500 collection tubes (DNA Genotek Inc., Ontario, Canada) in accordance with the manufacturer's guidelines before being stored at room temperature, followed by long term storage at -20°C. Buccal cell samples were collected following a minimum of 60 min abstinence from food and drink. Sterile buccal swabs (Omni swab, Whatman, Springfield Mill, UK) were rubbed against the inside of one cheek for ~30 s. The same procedure was used to collect a second sample from the opposite cheek of the same participant. Each collection tip was ejected into a 2 mL microcentrifuge tube and stored at -20°C.

All sample collection tubes were stored anonymously through coding and labelled in accordance with the Human Tissue Act (2004). Only members of the research team had access to the samples and any other information and/or data that could have been extracted from them.

2.4.2 DNA extraction

DNA extraction and genotyping were performed in the laboratories of Manchester Metropolitan University, the University of Glasgow, University of Cape Town (extraction only) and University of Northampton. A large proportion of the samples were processed and genotyped in the Sports Genomics laboratory at Manchester Metropolitan University by researchers of the sports genomics team. There was 100% agreement among reference samples genotyped in the three laboratories, i.e. Glasgow, Northampton and Manchester.
The protocols used differed between the laboratories, as summarised below.

DNA extraction at Manchester and Glasgow

At Manchester and Glasgow, genomic DNA was extracted using the Qiagen DNA Blood Mini kit (Qiagen, Crawley, UK) and standard spin column protocol, in accordance with the manufacturer's guidelines. DNA isolation from 200 µL whole blood or saliva, or one buccal swab required cell lysing with protease and AL buffer during incubation at 56°C for 10 min. Following centrifugation and the addition of ethanol, the resultant lysate was centrifuged at 8000 rpm for 60 s to allow silica gel membrane binding to occur. Additional buffer centrifugation cycles were used for the removal of proteins, nucleases and other impurities before elution of the remaining solution with 200 µL of AE buffer into a 1.5 mL microcentrifuge tube. The extracted DNA was then stored at 4°C until further analysis.

The extraction of DNA from Buccal swabs followed the same protocol as for the extraction from whole blood and saliva samples, however, an additional stage of transferring the lysate into sterile 2 mL microcentrifuge tubes prior to the DNA purification phase was required. At Manchester, the automated Qiagen QIAcube was used with most samples, to standardise these procedures and process up to 12 samples at a time.

DNA extraction at Cape Town University

At the University of Cape Town, DNA was isolated from whole blood using the protocol suggested by Lahiri and Nurnberger (Lahiri and Nurnberger, 1991). In brief, each sample was combined with lysis buffer, nuclei were pelleted by centrifugation and re-suspended in a high salt buffer. DNA was further precipitated following protein digestion, 100% ethanol was added, and the sample was centrifuged, washed with 70% ethanol and dried. DNA hydration buffer was added, and samples were stored at -20ºC until subsequent analysis.

DNA extraction at the University of Northampton

At the university of Northampton, DNA was isolated from whole blood using Flexigene kits (Qiagen). In brief, each sample was combined with lysis buffer, nuclei were pelleted by centrifugation and resuspended in protease-containing denaturation buffer. DNA was further precipitated following protein digestion, isopropanol was added, and the sample was centrifuged, washed with 70% ethanol and then dried. DNA hydration buffer was added, and samples were stored at -20ºC until subsequent analysis.

2.4.3 Genotyping

Genotyping of selected SNPs for this thesis were performed using different machines, depending on the genotyping site, with a large portion of the genotyping work performed at

Manchester Metropolitan University. An average of 1200 participants (athletes and non-athletes) were each genotyped for 10 SNPs.

Genotyping was performed in duplicate for all samples and SNPs, and on all machines. There was 100% agreement within duplicates for all samples, in all genotyping laboratories. All genotyping machines used the fluorophore-based detection technique of TaqMan real-time polymerase chain reaction (PCR), which requires amplification of a section of DNA overlapping the specific SNP being genotyped. To achieve amplification, forward primers were used to identify the starting point of the DNA segment and reverse primers were used to identify the end point of the DNA segment of interest. Allele specific probes, identified by either VIC or FAM attached to their respective complementary sequences, emitted a different fluorescent dye that could be distinguished by the respective PCR machine used. End point fluorescence measurement of VIC or FAM determined the different genotypes. The results were analysed using the software supplied by the respective manufacturers of each PCR machine. Importantly, precise descriptions for protocols used for genotyping at different sites were communicated between researchers, and are briefly described in this Section below.

Genotyping at the University of Glasgow

At the University of Glasgow, genotyping was performed by combining 10 μL Genotyping Master Mix (Applied Biosystems, UK), 1 μL SNP-specific TaqMan assay (Applied Biosystems), 6 μL nuclease free H_2O and 3 μL DNA solution (~9 ng DNA) into each well. Genotyping of DNA extracted in Cape Town was also performed in Glasgow. PCR was performed using a StepOnePlus Real-Time detector (Applied Biosystems). In brief, denaturation began at 95°C for 10 min, with 40 cycles of incubation at 92°C for 15 s, then

annealing and extension at 60°C for 1 min. Genotyping was performed in duplicate for all samples, and there was 100% agreement within duplicates for all samples.

Genotyping at the University of Northampton

At the University of Northampton, genotyping was performed by combining 10 µL of Genotyping Master Mix, 1 µL assay mix, 8 µL nuclease free H_2O and 1 µL of purified DNA (~10 ng DNA) into each well. PCR was performed using a StepOnePlus Real-Time detector (Applied Biosystems). In brief, denaturation began at 95°C for 10 min, with 40 cycles of incubation at 92°C for 15 s, then annealing and extension at 60°C for 1 min. Genotyping was performed in duplicate for all samples, and there was 100% agreement within duplicates for all samples.

Genotyping at Manchester Metropolitan University

At Manchester Metropolitan University, the majority of genotyping was performed using the StepOnePlus Real-Time detector (Applied Biosystems) and the Fluidigm EP1 (Fluidigm Corp., South San Francisco, California, US). The Chromo4 Real-Time system (Bio-Rad, Hertfordshire, UK) and the LightCycler 96 Real-Time PCR System (Roche Diagnostics Ltd, UK) were also used for some samples (Table 2.2).

For the StepOnePlus, Chromo4 and LightCycler, genotyping of DNA extracted from blood or saliva was performed by combining 5 µL Genotyping Master Mix, 0.5 µL assay mix, 4.3 µL nuclease free H_2O and 0.2 µL of purified DNA (~9 ng DNA) into each well. Genotyping for DNA extracted from buccal swabs was performed by combining 5 µL Genotyping Master Mix, 0.5 µL assay mix, 3.5 µL nuclease free H_2O and 1 µL DNA solution (~9 ng DNA) into each well. In brief (for the three machines), denaturation began at 95°C for 10 min, with 40

cycles of incubation at 92°C for 15 s, then annealing and extension at 60°C for 1 min. Again here, genotyping was performed in duplicate for all samples, and there was 100% agreement within duplicates for all samples. Figure 2.6 shows an allelic discrimination plot for *PTK2* rs7460 (A/T polymorphism) obtained using the StepOnePlus Real-Time PCR system.

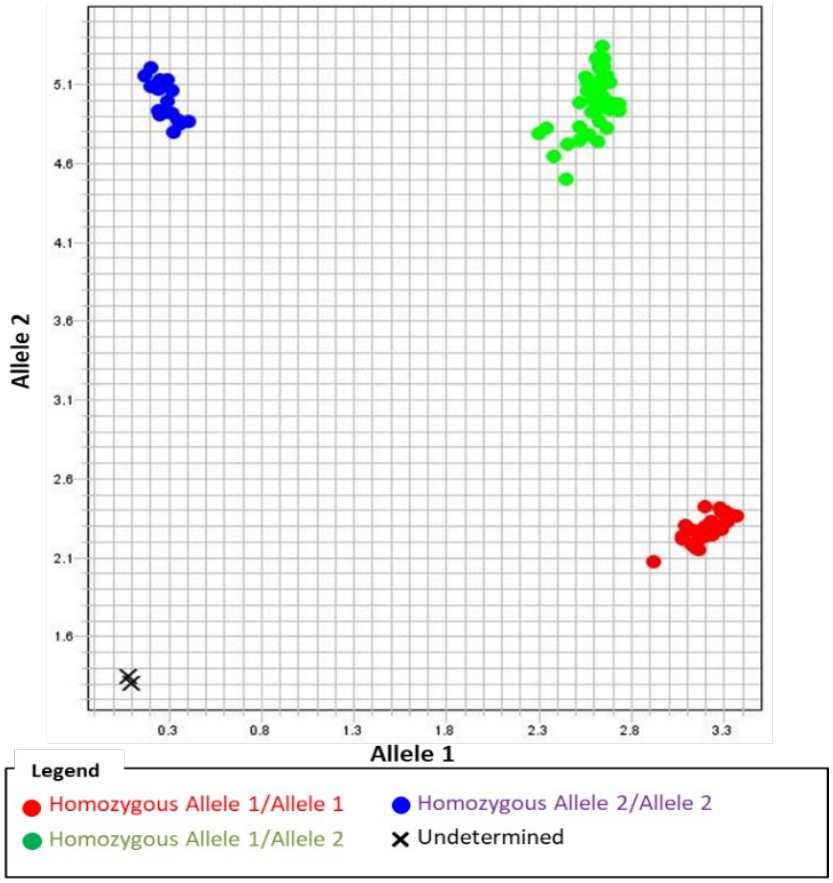


Figure 2.6 Example allelic discrimination plot for *PTK2* rs7460 (A/T polymorphism) obtained using the StepOnePlus Real-Time PCR system. Endpoint FAM represents the rs7460 T allele, whilst endpoint VIC represents the rs7460 A allele.

For the Fluidigm EP1, genotyping was performed using a 192.24 Dynamic Array® IFC (Fluidigm Corp., South San Francisco, California, US) and TaqMan SNP genotyping assays (Applied Biosystems, Paisley, UK) following the manufacturer's instructions. Briefly, a 4 µL genotyping mix consisted of 2 µL assay loading reagent [2X] (Fluidigm), 1 µL SNP genotyping Assay Mix [40X] (Applied Biosystems), 0.2 µL ROX [50X] (Invitrogen,

Carlsbad, California, US), and 0.8 µL DNA-free water (Qiagen). The sample mix of 4 µL consisted of 1.6 µL DNA sample, 2.0 µL GTXpress master mix [2X] (Applied Biosystems, PN 4401892), 0.2 µL Fast GT Sample Loading Reagent [20X] (Fluidigm, PN 100–3,065), and 0.2 µL DNA-free water. All reaction mixes were of 7.75 µL (3.75 µL genotyping mix and 4 µL sample mix), and were loaded onto the Dynamic Array IFC following the manufacturer's instructions. Amount of DNA for each reaction volume of 7.75 µL was of ~0.35 ng. The array was subsequently placed into a thermal cycler (FC1 Fluidigm, PN 100–1279 D1), and the GT 192.24 Fast v1.pcl protocol was performed. The thermal cycling protocol included an amplification at 95°C for 120 s followed by 45 cycles of denaturation for 2 s at 95°C and extension for 20 s at 60°C. Reporter dyes VIC and FAM were used for genotyping based on fluorescence detection. Also for the Fluidigm EP1 system, genotyping was performed in duplicate for all samples, and there was ~99% agreement within duplicates for all samples. ~1% of SNP-sample data points showed unsuccessful detection or inconsistent genotype results between duplicates using the Fluidigm system. These SNP sample were reassessed in duplicates using the StepOnePlus Real-Time PCR system with TaqMan SNP genotyping assays and analysed using StepOnePlus analysis software (Applied Biosystems, version 2.3). Figure 2.7 shows an example allelic discrimination plot for the *NOS3* rs2070744 polymorphisms (C→T transversion) obtained using the Fluidigm EP1 system.

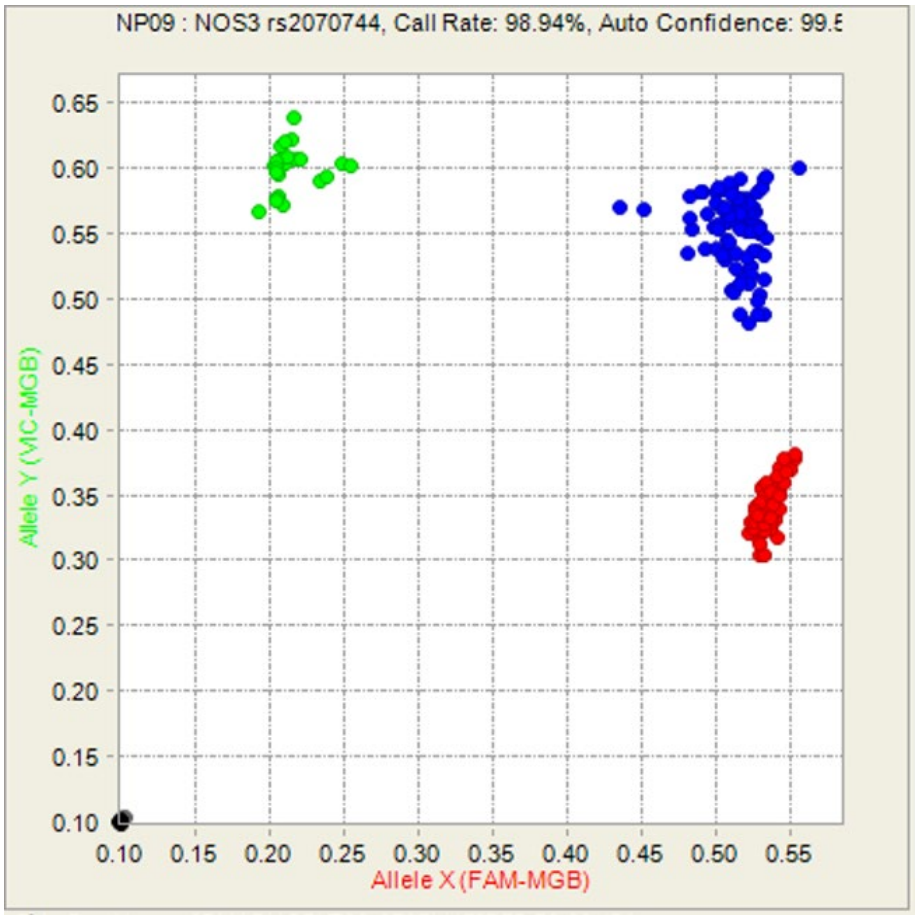


Figure 2.7 Example allelic discrimination plot for *NOS3* rs2070744 (C/T polymorphism) obtained using Fluidigm EP1. Endpoint FAM represents the rs2070744 T allele, whilst endpoint VIC represents the *NOS3* rs2070744 C allele.

Reproducibility between the StepOnePlus real-time PCR and the Fluidigm EP1 systems was evident when ~10% of samples from all polymorphisms were genotyped on both the StepOnePlus and Fluidigm PCR systems and were in 100% agreement.

ACE I/D *variant*

The majority (~90%) of samples were genotyped for the *ACE* I/D polymorphism by genotyping for the tag SNP rs4341 (C→G transversion) which is in perfect linkage disequilibrium with the *ACE* I/D variant in Caucasians (Glenn et al., 2009) and Asian (Tanaka et al., 2003). The remaining (~10%) of the samples were genotyped directly for the

ACE I/D variant by using three primers (150 nM each) and probes (VIC, 150 nM and FAM, 75 nM) (Koch et al., 2005). For this method, the reaction volume using DNA obtained from whole blood and saliva samples contained 5 µL of Genotyping master mix, 0.9 µL of I and D allele specific probes, 0.38 µL of *ACE* primer (111, 112 and 113) (Koch et al., 2005), 1.55 µL of nuclease-free H_2O and 0.5 µL of participant DNA (~23 ng DNA) into each well. For DNA samples obtained from buccal cells, volumes of the Genotyping master mix, probes and primers remained the same, instead 0.05 µL of nuclease-free H_2O and 2 µL of participant DNA (~18 ng DNA) were used. In brief, DNA amplification protocol for *ACE* I/D was 50 cycles of denaturation at 92°C for 15 s, primer annealing and extension at 57°C for 1 min and plate read.

2.4.4 Author contribution to obtaining genetic data

Table 2.2 shows the proportion of the genotyping performed at each University, and the contribution of the author of this thesis for DNA sample collection and extraction, and genetic analyses.

Table 2.4 Proportion of genetic analyses performed at each University, genotyping machines used, and author's contribution to the genetic analyses for the 10 strength and power associated SNPs.

Genotyping site	Genotyping machine/s used	Proportion of total genotyping work (%)	**Author's contribution to all genetic analyses work**	Estimated duration of work **by author**
Manchester Metropolitan University	StepOnePlus*	15%	~20% of the DNA isolation	25 hr
			~25% genotyping using the StepOnePlus.	120 hr
			For **all data** gathered by StepOnePlus:	
			Checked quality of data and consistency between duplicates.	20 hr
			Extracted all raw data into a form amenable to statistical analyses, assisted by the Principal Investigator.	70 hr
	Fluidigm EP1**	75%	Sample collection from 92 (majority Italian) elite rugby athletes from two teams in Italy, together with two other members of the Genetics research team and the Principal Investigator.	2 full days collecting samples from 2 clubs, with Genetics team
			For **all data** gathered by Fluidigm (not including laboratory work):	
			Checked quality of data and consistency between duplicates (~19,000 data points).	80 hr
			Extracted **all** raw data into a form amenable to statistical analyses assisted by the Principal Investigator. Data were all double checked to reduce error rate to a negligible amounts (~9,500 data points processed).	260 hr
	Chromo4 # LightCycler §	~5% for the two machines combined	Performed prior to 2012 – author not involved in this work	
University of Glasgow	StepOnePlus*	~5% of genotyping for both sites combined	Performed prior to 2012 – author not involved in this work	
University of Northampton	StepOnePlus*			

*StepOnePlus Real-Time detector (Applied Biosystems). **Fluidigm EP1 (South San Francisco, California US). #Chromo4 Real-Time system (Bio-Rad, Hertfordshire, UK). §LightCycler 96 Real-Time PCR System (Roche Diagnostics Ltd, UK).

CHAPTER 3

MUSCULAR STRENGTH AND POWER CHARACTERISTICS OF ELITE MALE RUGBY ATHLETES OF DIFFERENT PLAYING POSITIONS

3.1 Introduction

Rugby is a field-based invasion sport that requires highly developed physical and physiological capabilities (Duthie et al., 2003; Brazier et al., 2020). The game is characterised as a high intensity intermittent collision sport, requiring athletes to perform repeated running actions, collisions, wrestling, tackling, and *quasi* static efforts of differing work-to-rest periods (Duthie et al., 2003; Cunniffe et al., 2009). The nature of rugby match-play places different performance demands on athletes from different playing positions (Quarrie et al., 2013; Pollard et al., 2018) and these are, in part, reflected in considerable large variations in the anthropometric and physiological characteristics between athletes of a single team (Quarrie et al., 1996; Sedeaud et al., 2013; Brazier et al., 2020). Highly developed strength and power are central physical aspects in rugby (Duthie, 2006; Argus et al., 2009; Comfort et al., 2011), specifically in tasks such as scrummaging, mauling, tackling and sprinting (McMaster et al., 2014). Thus, strength and power require special attention with regards to both development (Duthie, 2006; McMaster et al., 2014; Gannon et al., 2016) and regular assessment (Gannon, 2015; Breen et al., 2021); as an integral part of the athletes' preparation and monitoring programmes.

Performance analyses demonstrated that success in rugby is largely attributable to the athletes' abilities in repeating high intensity efforts (Roberts et al., 2008; Austin et al., 2011) that necessitate maximal forces (strength) and mechanical power outputs (Duthie et al., 2003; McMaster et al., 2014). Indeed, the significance of enhanced physiological abilities, such as strength/power, is demonstrated when a physiological parameter transfers to superior performance (Gabbett et al., 2011). For example, it is well-established that sprinting ability is a fundamental component of performance and success in both rugby league (Gabbett, 2002; Gabbett, 2002) and rugby union (Wheeler et al., 2010; Austin et al., 2011). Players need to

accelerate over short distances or accelerate and sprint to make position (Cunniffe et al., 2009), to achieve the required ball carriage and opponent avoidance outcomes (Duthie et al., 2006). Lower body strength is often considered of utmost importance for sprinting ability in rugby (Barr et al., 2014), and improving lower body relative strength and decreasing ground contact time have been suggested to improve sprinting speed (Stone et al., 2007; Weyand et al., 2010; Mann, 2015). More specifically, decreasing ground contact time at maximal sprint speed is considered the most important kinematic change for improving sprinting (Weyand et al., 2010; Barr et al., 2014), and this can be achieved by increasing the foot-to-ground force (Weyand et al., 2010; Mann, 2015) and thus, by increasing lower body strength. The ability to control and dominate the tackle contest is one of the most important components for success in rugby (Gabbett and Kelly, 2007; Van Rooyen et al., 2014; Hendricks et al., 2018); and accordingly, successful teams are involved in fewer ineffective tackles than unsuccessful teams (Van Rooyen et al., 2014). Gabbett et al. (2010) have shown that lower body power is required to provide leg drive in tackles, which is a key tackler characteristic associated with positive tackle outcome (Hendricks et al., 2014). More recently, Speranza et al. (2016) found that training-induced improvements in lower-body strength are associated with enhanced tackling ability, whilst upper- and lower-body strength are significantly related to success in the tackle contest (Speranza et al., 2017). Collectively, these findings strengthen the large body of evidence on the importance of strength and power in rugby.

The extant literature has shown that muscular strength/power requirements in rugby, tend to increase in concert with competition levels (Baker, 2002; Argus et al., 2012; Quarrie et al., 2013). The evolving nature of the game, in particular since rugby turned professional in 1995, has also contributed to the increase in the strength/power abilities required in competition (Smart, 2011; Appleby et al., 2012; Brazier et al., 2020). Concordant with this is the

phenomenon that elite rugby athletes are becoming increasingly heavier, taller, and leaner (Sedeaud et al., 2013; Fuller et al., 2013; Brazier et al., 2020), providing further evidence of the increasing strength/power requirements of contemporary elite rugby athletes. Research into the performance requirements of rugby players has suggested anthropometric and physical characteristics associated with players in each position, which reflect the demands of the sport (Rigg and Reilly, 1988; Quarrie et al., 1996; Quarrie et al., 2013; Cunningham et al., 2018). Traditional rugby is distinguished by the physical commitment of forwards and the game-specific agility of backs (Sedeaud et al., 2013). Similarly, in the modern game the primary role of the forwards is to contest possession in scrums, lineouts, rucks, and tackles (Quarrie et al., 2013; Smart et al., 2013), which requires greater mass, height, strength and power to be successful (Casagrande and Viviani, 1993; Duthie, 2006). On the other hand, the backs use possession to gain territory and/or score points (Quarrie et al., 2013); and when not in possession they attempt to prevent the opposition from gaining territory or scoring via defensive actions such as tackling (Quarrie et al., 2013). Thus, back positions require a combination of speed, acceleration, and agility (Quarrie et al., 1995; Duthie et al., 2003; Quarrie et al., 2013). These large differences in movement patterns, contact loads and activities between playing positions (Quarrie et al., 2013; Cunningham et al., 2016; Cunningham et al., 2018) imply the need for more specific physiological characteristics, conditioning programmes and recovery strategies for each playing position.

The plethora of literature that highlighted the importance of strength/power in rugby has fuelled interest in the development of training methods that target the athletes' force generation and power production capabilities required at the elite level of competition (Duthie, 2006; Kilduff et al., 2008; Bevan et al., 2010; Cook et al., 2014; Johnston et al., 2016). Thus, athletes' monitoring with regards to neuromuscular function have been

implemented (Gannon, 2015; Chiwaridzo et al., 2017; Thornton et al., 2019), with the main aims of guiding training interventions and managing athletes' load (e.g. matches, training, injury/illness management) (Quarrie et al., 2017). With regards to lower body mechanical power output assessment, the countermovement jump (CMJ) has been used for many decades and can be performed with a number of variations (Sargent, 1924; Maud and Shultz, 1984; Newton and Dugan, 2002; Canavan and Vescovi, 2004). The general agreement in the literature is that the most valid and reliable method of measuring lower body peak mechanical power output in a CMJ is by using a force platform, although there seems to be no solid consensus on the protocol used for administering the test and analysing the force-time data. On the other hand, for the assessment of maximal multi-joint strength that involves the upper body musculature, the isometric mid-thigh pull (IMTP) using the force platform appears to be one of the most widely used, relatively safe, and efficient methods in monitoring athletic populations (Haff et al., 1997; Comfort et al., 2019; Lum et al., 2020). Scientific evidence demonstrated that the IMTP allows for the expression of maximum strength, that is largely attributable to the favourable position (in terms of maximal generated force) adopted during the entire isometric pulling phase (Garhammer, 1993) of ~5 s duration. This is based on research that investigated the kinetics and kinematics of the Olympic clean and snatch lifts, with both lifts involving a second pull phase, and during which the force that is transmitted to the bar is the highest throughout the respective lift (Garhammer and Takano, 1992; Garhammer, 1993; Beckham, 2015). Inconsistencies in the methodologies used in assessing strength/power of elite athletes have been a major limitation in this area of research. For example, the lack of agreement between research groups on the role that isometric tests such as the IMTP (using the force platform) play in sports performance diagnosis is mainly attributable to methodological differences related to the mode of isometric data collection and subsequent analyses of the force-time histories. Similarly, for the CMJ using a force platform,

methodological differences concern data collection and analyses, in particular in the determination of the time at which the CMJ can be assumed to have started. An additional limitation of numerous investigations is the recruitment of athletes from different competition levels, making comparisons between studies and overall conclusions difficult. Therefore, further research that employs the 'gold standard' method for the assessment of the CMJ and IMTP in elite rugby athletes is warranted. The gathered information can serve as a reference set of normative data of established measures of strength/power, that can aid the evaluation process of assessments of neuromuscular function, and subsequent training prescription and preparation of elite rugby athletes.

Thus, the aim of the present study was to characterise muscular strength/power in a cohort of elite Caucasian male rugby athletes and a group of non-athletes using the CMJ and IMTP tests, and the 'gold standard' method of assessment using the force platform. Comparisons of strength- and power-derived variables from the CMJ and IMTP were made between athletes and non-athletes (that served as controls), and between athletes of different playing positions. It was hypothesised that athletes would demonstrate greater abilities in all strength- and power-derived variables, compared to non-athletes. A second hypothesis was that differences in all variables would be observed between the forwards and backs, and these would be reflected in some of the comparisons between the five playing positions (i.e. front five, back row, halves, centres, and back three).

3.2 Method

Detailed descriptions of participants are provided in Section 2.1 (Chapter 2: General Methods). Procedures for conducting the CMJ and IMTP tests, and for obtaining the five measures from the force-time histories produced in both tests are included in Section 2.2.

Therefore, only brief descriptions of participants, and statistical analyses used in this investigation are detailed in this Section. The author's contribution for all procedures involved in the collection and processing of IMTP and CMJ data is provided in Section 2.2. Procedures for the athletes were approved by the Ethics Committee of Swansea University. Procedures for the non-athletes were approved by the Ethics Committee of Manchester Metropolitan University. All procedures were conducted in accordance with the guidelines in the Declaration of Helsinki (World Medical Association, 2013).

3.2.1 Participants

Participants were 263 Caucasian male elite rugby union athletes [mean (standard deviation): height 1.86 (0.08) m, body mass 102.4 (12.9) kg, BMI 29.6 (3.1) $kg \cdot m^{-2}$], and 14 Caucasian male students and staff [height 1.81 (0.07) m, mass 85.7 (12.0) kg, BMI 26.3 (3.7) $kg \cdot m^{-2}$], all British and recruited from Manchester Metropolitan University, representing the non-athletes. Non-athletes were apparently healthy and asymptomatic, unrelated and recreationally active.

Of the athletes, 123 form part of GENESIS and RugbyGene, and were also recruited for subsequent investigations in this thesis. The remaining 140 athletes were participants in research at Swansea University, and recruited only for this investigation. All athletes were considered 'elite', having competed regularly (>5 matches) since 1995 in the highest professional league in the UK, Ireland, or South Africa. From all athletes, 53% had competed at an international level, and 99% of those international athletes represented a 'High Performance Union' (Regulation 16, worldrugby.org). Data for international status were confirmed as of 1st July 2019.

3.2.2 Experimental procedure

Participants were required to attend for a familiarisation session 48 h before testing. During this 48-h period participants refrained from alcohol, caffeine, and strenuous exercise. On testing day, participants were required to perform three CMJs and three IMTPs on a force platform. Room temperature was maintained at 20–24˚C. Verbal encouragement was given to maximise performance. Participants were informed of the rationale and potential applications of the investigation, and procedures and risks associated with their participation; after which they provided written consent to participate.

3.2.3 Statistical analysis

Comparisons of strength/power in IMTP and CMJ variables were made between athletes (combined) and athlete subgroups (by playing position), and non-athletes. Therefore, athletes were either considered as one group, categorised in two major groups (forwards and backs), or five subgroups (forwards: front five and back row; backs: half backs, centres and back three). Independent samples *t*-tests were used to compare athletes (combined) with non-athletes. One-way ANOVA was used for comparisons between the major groups (forwards and backs) and non-athletes, and between subgroups of athletes from the five playing positions. The Hochberg's post hoc test was used to account for unequal group sizes. Welch (1951) and Brown and Forsythe (1974) statistics were used to provide an alternative *F* ratio in the case where the assumption of homogeneity of variance was not met. The Games-Howell post hoc test was used in such case. Benjamini-Hochberg (B-H) adjustment was applied to control false discovery rate (FDR) (Benjamini and Hochberg, 1995). FDR was set at 10% and the reported *p* values are those significant after B-H adjustment. Effect size was calculated for all comparisons of strength and power using Cohen's *d* (Cohen, 1988). This allowed for the determination of meaningful differences (for all comparisons) where

differences in variables reached statistical significance. For comparisons involving the non-athletes the standard deviation of non-athletes was used in the denominator for the calculation of Cohen's *d*. For comparisons between any two groups of athletes the pooled standard deviation (calculated from the standard deviations of the two groups) was used in the denominator for the calculation of Cohen's *d*. Cohen's *d* was interpreted as follows: 0.2 or less is a small effect size, about 0.5 is a moderate effect size, and 0.8 or more is a large effect size (Cohen, 1988). A sensitivity analysis was conducted in G*Power (Faul et al., 2007) to determine, given the limited sample size of non-athletes, the minimum detectable effect size in the comparisons involving non-athletes. Using the samples of 263 athletes (147 forwards and 116 backs) and 14 non-athletes and alpha (α) set at 0.05, it was determined that a minimum effect size of 0.68 (Cohen's *d*) is required to achieve 80% power. Similarly, to retain 80% power in the comparisons between either the forwards or backs, and non-athletes and α set at 0.05, effect sizes of 0.69 (*d*) and 0.71 (*d*) were required, respectively. For the comparisons between the five positional groups (α set at 0.05; power of 80%) and total sample size ranging from 66 (halves and centres) to 147 (front five and back row); the sensitivity analysis indicated that the lowest detectable effect sizes were in the range of 0.48–0.72 (*d*), depending on the groups compared. The Statistical Package for Social Sciences (version 26.0, SPSS Inc., Chicago, IL, USA) was used for the analyses. Statistical significance was accepted when $p \leq 0.05$.

3.3 Results

Athletes (combined) were heavier (mean difference 16.7 kg, $p < 0.001$; Cohen's $d = 1.40$), taller (mean difference 0.05 m, $p = 0.009$; Cohen's $d = 0.86$) and had a higher BMI (mean difference 3.3 $kg \cdot m^{-2}$, $p < 0.001$; Cohen's $d = 0.89$) than non-athletes. Forwards were heavier (mean difference 20.6 kg, $p < 0.001$; Cohen's $d = 2.65$), taller (mean difference 0.07 m, $p <$

0.001; Cohen's d = 0.91) and had a higher BMI (mean difference 4.0 $kg \cdot m^{-2}$, $p < 0.001$; Cohen's d = 2.08) than backs. Additionally, forwards were heavier (mean difference 25.8 kg, $p < 0.001$; Cohen's d = 2.17), taller (mean difference 0.08 m, $p < 0.001$; Cohen's d = 1.28) and had a higher BMI (mean difference 5.0 $kg \cdot m^{-2}$, $p < 0.001$; Cohen's d = 1.35) than non-athletes. The backs appeared somewhat heavier, taller and had a higher BMI than non-athletes, but those differences did not reach statistical significance (Table 3.1).

Muscular strength and power comparisons between athletes (combined) and non-athletes

Athletes achieved 34.4% higher IMTP peak force (mean difference 741 N; $p < 0.001$), 12.7% higher IMTP relative peak force (mean difference 3.2 $N \cdot kg^{-1}$; $p = 0.028$) and 23.4% higher CMJ maximum concentric power (mean difference 1041 W; $p = 0.002$) than non-athletes. No other significant differences were observed between athletes and non-athletes (Table 3.1 and Figure 3.1). Effect sizes are provided in Table 3.2.

Muscular Strength and power comparisons between forwards, backs, and non-athletes.

Forwards achieved 10.5% higher IMTP peak force and 9.1% higher CMJ maximum concentric power than backs (both $p < 0.001$) and non-athletes (40.4% and 28.1%, both $p < 0.001$, respectively). Backs achieved 10.7% higher IMTP relative peak force and 12.3% higher CMJ relative maximum concentric power than forwards (both $p < 0.001$) and non-athletes (19.2%, $p = 0.003$ and 11.5%, $p = 0.002$, respectively). In addition, backs achieved 27.1% higher IMTP peak force ($p < 0.001$) and 17.4 % higher CMJ maximum concentric power ($p = 0.036$) than non-athletes. Further, backs were able to jump 13.4% higher than forwards ($p < 0.001$) (Table 3.1 and Figure 3.1). Effect sizes are provided in Table 3.2.

Table 3.1 Physical and muscular strength/power characteristics of athletes (combined), athlete sub-groups, and non-athletes. Data are presented as **mean** (SD).

				forwards			**backs**			
		athletes (combined)	**non-athletes**	**forwards**	front five	back row	**backs**	half backs	centres	back three
		(*n* = 263)	(*n* = 14)	(*n* = 147)	(*n* = 92)	(*n* = 55)	(*n* = 116)	(*n* = 40)	(*n* = 26)	(*n* = 50)
		mean (SD)	**mean** (SD)	**mean** (SD)	**mean** (SD)	**mean** (SD)	**mean** (SD)	**mean** (SD)	**mean** (SD)	**mean** (SD)
Physical Characteristics										
body mass (kg)	*Valid cases*	*260*	*14*	*146*	*91*	*55*	*114*	*39*	*26*	*49*
		102.4 (12.9)	**85.7** (11.9)	**111.5** (8.4)	**113.6** (8.1)	**107.9** (7.8)	**90.9** (6.9)	**86.5** (5.8)	**94.6** (5.8)	**92.3** (6.6)
height (m)	*Valid cases*	*263*	*14*	*147*	*92*	*55*	*116*	*40*	*26*	*50*
		1.86 (0.07)	**1.80** (0.07)	**1.89** (0.07)	**1.88** (0.08)	**1.90** (0.05)	**1.82** (0.06)	**1.79** (0.05)	**1.86** (0.03)	**1.83** (0.07)
BMI ($kg \cdot m^{-2}$)	*Valid cases*	*260*	*14*	*146*	*91*	*55*	*114*	*39*	*26*	*49*
		29.6 (3.1)	**26.3** (3.7)	**31.3** (2.7)	**32.2** (2.9)	**29.8** (1.6)	**27.3** (1.8)	**27.0** (1.5)	**27.4** (1.7)	**27.6** (1.9)
IMTP										
peak force (N)	*Valid cases*	*255*	*14*	*141*	*89*	*52*	*114*	*39*	*26*	*49*
		2889 (546)	**2149** (418)	**3018** (528)	**3027** (535)	**3003** (521)	**2730** (528)	**2633** (441)	**2668** (591)	**2841** (547)
relative peak force ($N \cdot kg^{-1}$)	*Valid cases*	*253*	*14*	*140*	*88*	*52*	*113*	*39*	*26*	*48*
		28.4 (5.4)	**25.2** (4.5)	**27.1** (4.8)	**26.7** (4.8)	**27.8** (4.7)	**30.0** (5.6)	**30.5** (5.3)	**28.1** (5.3)	**30.7** (5.9)
CMJ										
maximum concentric power (W)	*Valid cases*	*257*	*14*	*144*	*90*	*54*	*113*	*40*	*26*	*47*
		5486 (682)	**4445** (1020)	**5695** (689)	**5651** (730)	**5769** (615)	**5219** (573)	**4917** (500)	**5328** (523)	**5417** (559)
relative maximum concentric power ($W \cdot kg^{-1}$)	*Valid cases*	*256*	*14*	*144*	*90*	*54*	*112*	*39*	*26*	*47*
		54.0 (6.8)	**51.6** (7.5)	**51.2** (6.4)	**49.9** (6.3)	**53.5** (5.9)	**57.5** (5.5)	**56.8** (6.3)	**56.3** (4.3)	**58.8** (5.2)
jump height (cm)	*Valid cases*	*253*	*14*	*142*	*89*	*53*	*111*	*39*	*26*	*46*
		35.5 (7.0)	**35.3** (6.3)	**33.5** (6.8)	**32.7** (7.3)	**34.9** (5.5)	**38.0** (6.5)	**38.1** (5.6)	**34.7** (5.9)	**39.8** (6.9)

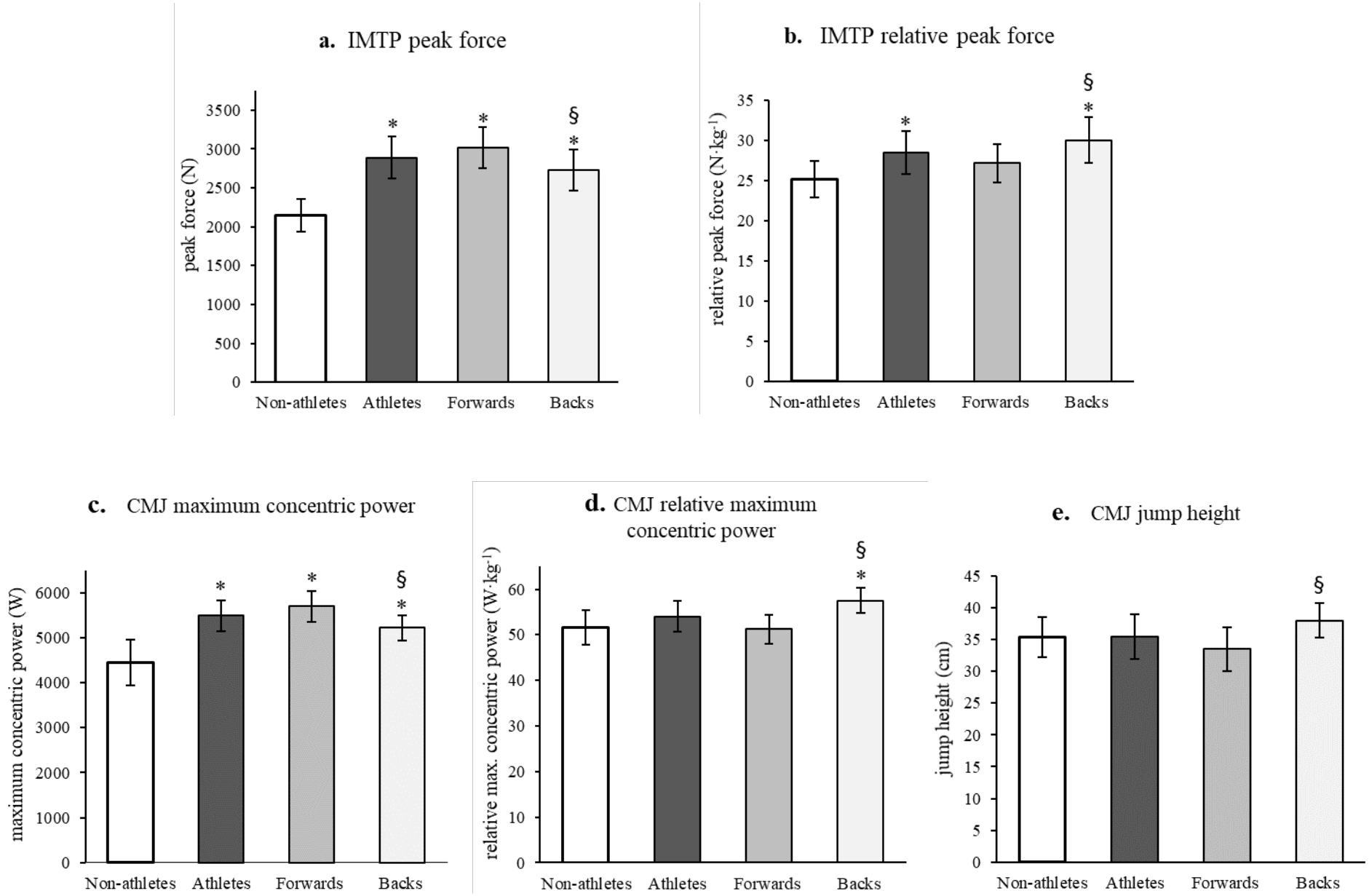


Figure 3.1 Muscular strength/power of non-athletes, major athlete groups (forwards and backs), and all athletes (forwards and backs combined) in IMTP (graphs a and b) and CMJ (graphs c, d and e). * different from non-athletes ($p < 0.05$). § different from forwards ($p < 0.01$).

Table 3.2 Statistical significance and effect size for the comparisons between athletes and non-athletes; between forwards, backs and non-athletes; and between forwards and backs.

	comparison		mean difference	*p* value	effect size (Cohen's *d*)	
IMTP peak force (N)	non-athletes vs	athletes	-741	<0.001	1.77	***
		forwards	-869	<0.001	2.08	***
		backs	-582	<0.001	1.39	***
	forwards vs backs		288	<0.001	0.55	**
IMTP relative peak force ($N \cdot kg^{-1}$)	non-athletes vs	athletes	-3.2	0.028	0.71	**
		forwards	-1.9	0.450	0.42	
		backs	-4.8	0.003	1.06	***
	forwards vs backs		-2.9	<0.001	0.56	**
CMJ maximum concentric power (W)	non-athletes vs	athletes	-1041	0.002	1.02	***
		forwards	-1250	0.001	1.23	***
		backs	-774	0.036	0.76	**
	forwards vs backs		476	<0.001	0.74	**
CMJ relative maximum concentric power ($W \cdot kg^{-1}$)	non-athletes vs	athletes	-2.4	0.205	0.32	
		forwards	0.4	0.995	0.05	
		backs	-5.9	0.002	0.79	**
	forwards vs backs		-6.3	<0.001	1.05	***
CMJ jump height (cm)	non-athletes vs	athletes	-0.1	0.950	0.02	
		forwards	1.8	0.684	0.29	
		backs	-2.7	0.402	0.43	
	forwards vs backs		-4.5	<0.001	0.67	**

** medium effect size; *** large effect size.

Comparisons between athlete subgroups by playing position in IMTP variables

Both forwards subgroups (front five and back row) achieved higher IMTP peak force than the half backs (14.9%, $p = 0.001$ and 14.1%, $p = 0.01$, respectively). In addition, the front five achieved 13.5% higher IMTP peak force than the centres ($p = 0.025$). Two backs subgroups (half backs and the back three) achieved higher IMTP relative peak force than the front five (14.1%, $p = 0.002$ and 14.7%, $p < 0.001$, respectively) (Table 3.1 and Figure 3.2). Effect sizes are provided in Table 3.3.

Comparisons between athletes subgroups by playing position in CMJ variables

Both forwards subgroups (front five and back row) achieved higher CMJ maximum concentric power than the half backs (14.9% and 17.3% respectively, both $p < 0.001$). In addition, the back row achieved higher CMJ maximum concentric power than the centres and back three (8.3%, $p = 0.012$ and 6.5%, $p = 0.027$, respectively). Further, the back three achieved 10.2% higher CMJ maximum concentric power when compared to the half backs ($p < 0.001$), and centres achieved 8.4% higher CMJ maximum concentric power than the half backs ($p = 0.020$). Effect sizes are provided in Table 3.3.

The three backs subgroups (half backs, centres and back three) achieved higher CMJ relative maximum concentric power than the front five (13.8%, 12.8% and 17.8% respectively, all $p < 0.001$). In addition, the back three achieved 9.9% higher CMJ relative maximum concentric power than the back row ($p < 0.001$). Lastly, the back row achieved 7.2% higher CMJ relative maximum concentric power than the front five ($p < 0.004$). Effect sizes are provided in Table 3.3.

The best jumping performance was achieved by the back three, by jumping 21.7% higher than front five (p = 0.002), 14% higher than back row (p = 0.002) and 14.4% higher than centres (p = 0.018). In addition, the half backs jumped 16.5% higher than front five (p = 0.002) (Table 3.1 and Figure 3.2). Effect sizes are provided in Table 3.3.

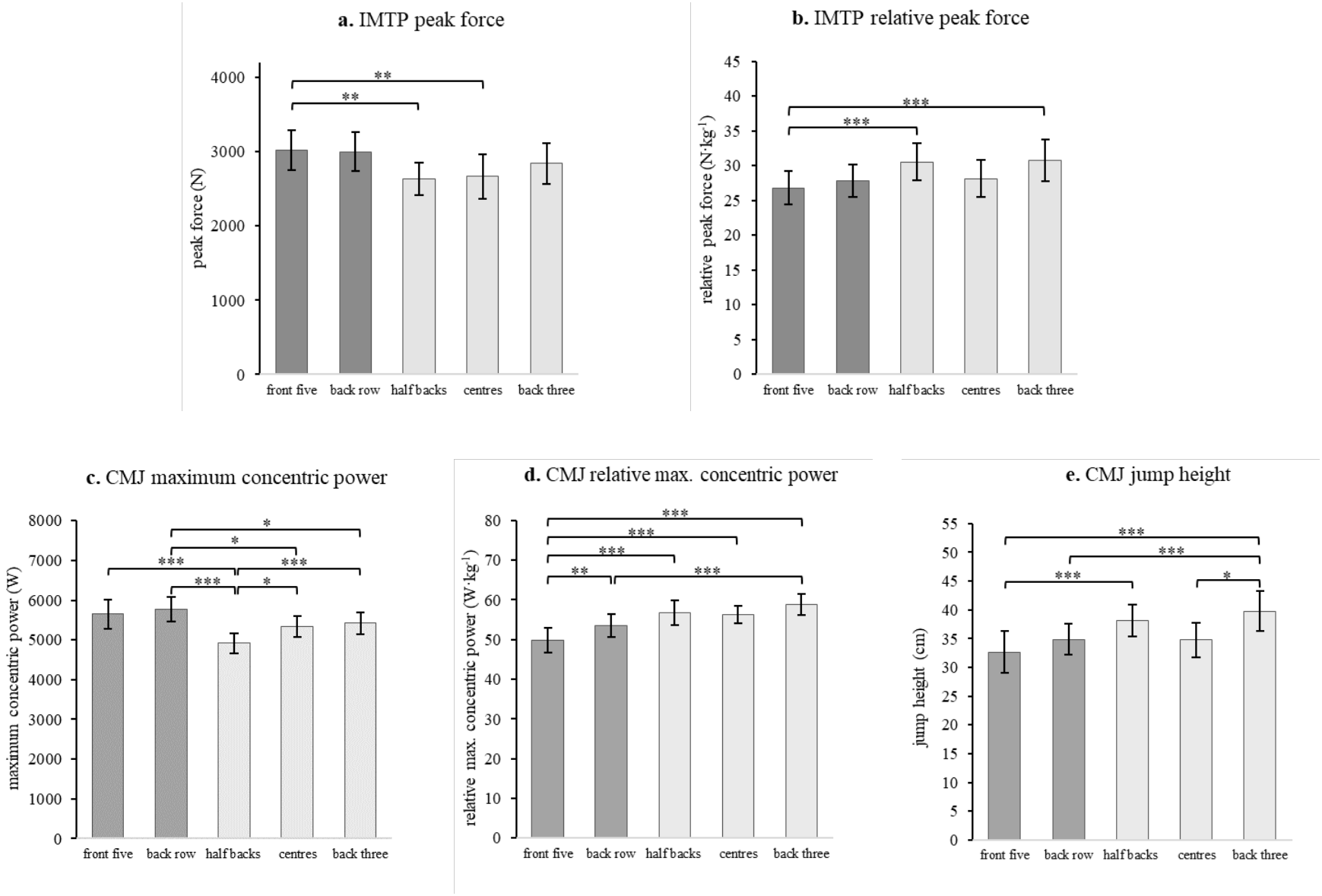


Figure. 3.2 (a – e) Muscular strength/power characteristics of athlete subgroups (forwards: front five and back row, in dark grey bars; backs: half backs, centres and back three, in light grey bars) in IMTP (graphs a and b) and CMJ (graphs c, d and e). * $p < 0.05$. ** $p < 0.01$. *** $p <$ 0.001. max. (graph d) = maximum.

Table 3.3 Statistical significance and effect size for comparisons between athletes from different playing positions.

	comparison		mean difference (N)	*p* value	effect size (Cohens *d*)	
IMTP peak force (N)	front five vs	back row	23	0.999	0.04	
		halves	394	0.001	0.77	**
		centres	359	0.025	0.66	**
		back three	185	0.393	0.34	
	back row vs	halves	371	0.010	0.76	**
		centres	335	0.083	0.61	**
		back three	162	0.729	0.30	
	halves vs	centres	-36	0.999	0.07	
		back three	-209	0.493	0.42	
	centres vs back three		-173	0.855	0.31	
IMTP relative peak force ($N \cdot kg^{-1}$)	front five vs	back row	-1.1	0.930	0.23	
		halves	-3.8	0.002	0.76	**
		centres	-1.4	0.934	0.29	
		back three	-3.9	<0.001	0.75	**
	back row vs	halves	-2.7	0.129	0.54	
		centres	-0.3	0.999	0.06	
		back three	-2.9	0.057	0.55	
	halves vs	centres	2.4	0.482	0.45	
		back three	-0.2	0.999	0.04	
	centres vs back three		-2.6	0.337	0.46	

** medium effect size; *** large effect size.

Table 3.3 Continued.

	comparison		mean difference (N)	*p* value	effect size (Cohen's *d*)	
CMJ maximum concentric power (W)	front five vs	back row	-118	0.837	0.17	
		halves	734	<0.001	1.09	***
		centres	323	0.100	0.47	
		back three	234	0.232	0.35	
	back row vs	halves	853	<0.001	1.49	***
		centres	441	0.013	0.75	**
		back three	352	0.027	0.59	**
	halves vs	centres	-411	0.020	0.81	***
		back three	-500	<0.001	0.94	***
	centres vs back three		-89	0.959	0.16	
CMJ relative maximum concentric power ($W \cdot kg^{-1}$)	front five vs	back row	-3.7	0.004	0.60	**
		halves	-6.9	<0.001	1.09	***
		centres	-6.5	<0.001	1.09	***
		back three	-8.9	<0.001	1.49	***
	back row vs	halves	-3.3	0.079	0.54	
		centres	-2.8	0.365	0.52	**
		back three	-5.3	<0.001	0.95	***
	halves vs	centres	0.5	0.999	0.09	
		back three	-2.0	0.699	0.35	
	centres vs back three		-2.5	0.586	0.51	

** medium effect size; *** large effect size.

Table 3.3 Continued.

	comparison		mean difference (N)	*p* value	effect size (Cohen's *d*)	
CMJ jump height (cm)	front five vs	back row	-2.2	0.399	0.33	
		halves	-5.4	<0.001	0.79	***
		centres	-2.1	0.801	0.29	
		back three	-7.1	<0.001	0.99	***
	back row vs	halves	-3.2	0.186	0.58	
		centres	0.1	0.999	0.02	
		back three	-4.9	0.002	0.79	**
	halves vs	centres	3.3	0.361	0.58	
		back three	-1.7	0.928	0.27	
	centres vs back three		-5.0	0.018	0.76	**

** medium effect size; *** large effect size.

3.4 Discussion

The main aims of this study were to characterise muscular strength/power using the IMTP and CMJ in a relatively large number of elite Caucasian male rugby union athletes of different playing positions; and compare the measures obtained with those of non-athletes and between playing positions. Athletes (combined) achieved higher IMTP peak force and relative peak force, and CMJ concentric power than non-athletes, but not CMJ relative concentric power and jump height, therefore, our first hypothesis was rejected. Athlete major groups, namely forwards and backs, achieved higher IMTP peak force and CMJ concentric power than non-athletes, with the backs performing better than non-athletes when both measures were taken relative to body mass, but not in jump height. In addition, there were

significant differences in all strength/power measures between the forwards and backs, and these were reflected in some of the comparisons involving the five positional groups. Thus, our second hypothesis was accepted.

The IMTP peak force achieved by the athletes (combined) was higher (difference of 740 N, 35%; p = <0.001; Cohen's d = 1.77) than that obtained by non-athletes. The athletes were heavier (difference of 16.7 kg, 19.5%; p = <0.001; Cohen's d = 1.40) than non-athletes, and the higher peak force achieved by the athletes may be attributed, in part, to this additional body mass. However, when peak force was expressed relative to body mass, athletes achieved higher (3.2 $N \cdot kg^{-1}$, 12.7%; p = 0.028; Cohen's d = 0.71) relative peak force than non-athletes, implying that the higher force generation capabilities of the athletes are not only attributable to the additional body mass, but also to other factors (environmental and genetic) that are advantageous to neuromuscular function in terms of isometric force generation. Similar to the comparison between the athletes and non-athletes, the comparison between the forwards and backs in the IMTP revealed that the heavier forwards (difference of 20.6 kg, 22.7%; p = <0.001; Cohen's d = 2.61) were able to generate the highest peak force (difference of 288 N, 10.5%; p = <0.001; Cohen's d = 0.55). However, in contrast to the observation in the athletes vs. non-athletes comparison, the group with the relatively lower body mass (i.e. backs) achieved higher relative peak force than forwards (difference of 2.9 $N \cdot kg^{-1}$, 10.7%; p = <0.001; Cohen's d = 0.56). There is substantial evidence in the literature on the relationship between relative peak force in the IMTP and dynamic performance, although the evidence in elite rugby is scarce. West et al. (2014) demonstrated this relationship in 39 professional rugby league athletes, with dynamic performance being sprint acceleration (r = 0.37; the time taken to cover a distance of 10 m from a stationary start) – considered a fundamental performance characteristic for all playing positions. A more recent

study by Cunningham et al. (2018) demonstrated that IMTP relative peak force is correlated with effective attacking ruck (r = 0.58; number of times players were effective at the breakdown as one of the first three support players to the ruck while their team is attacking), percentage tackle success (r = 0.60) and percentage carries over the gain line (r = 0.53) in elite rugby union backs (n = 14) but not forwards (n = 15), with the three variables considered as key performance based match activities for both positional groups (Cunningham et al., 2018). Further, but also exclusively for the backs, peak force in the IMTP was related to the number of possessions (r = 0.79), passes made (r = 0.79), effective attacking ruck percentage (r = 0.63), and the number of offloads (r = 0.62) (Cunningham et al., 2018). Collectively, the findings by West et al. (2014) and Cunningham et al. (2018), and those presented herein for the IMTP (that clearly show the superiority of the forwards and backs for peak force and relative peak force respectively), provide support to the well-established notion that the forwards' performance depends on absolute strength while that of the backs relies more on relative strength. In other words, it seems that elite forwards possess sufficient absolute strength that allows them to perform optimally in their specific roles; and strength increases for them are desirable via a concomitant increase in body mass. However, although the backs' performance would probably benefit from an increment in their capabilities for generating high forces, they cannot allow that strength increases occur at the cost of large deviations from their 'normal', ideal body mass, as the additional mass might impede them to perform at their best in all of their specific roles. When the five playing positions were considered for the IMTP variables, there were no differences in either peak force or relative peak force in any comparison concerning two subgroups pertaining to the same major athlete category [i.e. forwards or backs: Fig. 3.2 (a and b)]. This provides further evidence of the distinct strength characteristics of elite rugby union forwards and backs as measured by the IMTP. According to the authors' knowledge, there are no other studies that

have examined the IMTP in a large cohort of elite rugby athletes and, therefore, direct comparisons for this physical attribute are limited to those possible in the work herein.

With regards to lower body power production in the CMJ, athletes (combined) achieved higher maximum concentric power than non-athletes (difference of 1041 W, 23%; $p = 0.002$; Cohen's $d = 1.02$). Surprisingly, when this measure was expressed relative to body mass, the difference (2.4 $N \cdot kg^{-1}$, 4.6%; $p = 0.205$) did not reach statistical significance. However, when the athletes were categorised as forwards and backs, comparisons with non-athletes revealed that the large difference in absolute concentric power obtained in the athletes vs. non-athletes comparison are attributable to both the larger body mass of the forwards, and to the backs' capabilities of producing the highest relative maximum concentric power (backs vs. non-athletes: difference of 5.9 $N \cdot kg^{-1}$, 11.4%; $p = 0.002$; Cohen's $d = 0.79$. backs vs. forwards: 6.3 $N \cdot kg^{-1}$, 12.3%; $p = <0.001$; Cohen's $d = 1.05$). As was the case for the IMTP, the comparisons between the five positional groups reflected the superiority of the forwards and backs: this time in their capabilities of producing absolute and relative concentric power in the CMJ, respectively. However, in contrast to the case of the IMTP, differences in both CMJ variables were also observed between pairs of subgroups pertaining to the same major athlete category [Figure 3.2 (c and d)]. For the CMJ performance as measured by jump height, the backs showed superior performance when compared to forwards (difference of 4.5 cm, 13.4%; $p = <0.001$; Cohen's $d = 0.67$) however, the small difference in jump height obtained by the backs in their comparison with non-athletes did not reach significance (difference of 2.7 cm, 7.6%; $p = 0.402$). Unexpectedly, the athletes (combined) and even the backs did not prove superior in jump height when compared to non-athletes. The comparisons between the five playing positions revealed the superiority of the back three athletes in jumping, with differences in jump height reaching significance compared with both forwards subgroups and

with the centres (Figure 3.2 and Table 3.3). It seems plausible that the relatively lower body mass of the backs' subgroups, and their capability of producing high relative maximum concentric power, contributed to their superior jumping performance. Jump height varies in proportion with the square of take-off velocity; increasing the velocity during the propulsion phase of the jump and taking advantage of the stretch-shortening cycle by optimising the jump technique can result in considerably better jumping performance (Enoka, 2008). Undoubtedly, body mass played a major role in determining the measures achieved by athlete groups in this performance variable.

The study is not without limitations. The sample of elite athletes was limited by the existence and provision of strength/power data in IMTP and CMJ – specifically for elite rugby union athletes and acquired using the 'gold standard' force platform method. In addition, the limited sample size for non-athletes resulted in an estimation of greater effect sizes (using sensitivity analysis of G*Power) than those that could have been estimated in case the sample was larger and thus; it is most probable that for the comparisons between athletes and non-athletes the study is underpowered. Secondly, with respect to strength/power assessments in IMTP and CMJ, measures such as those related to rate of force development (RFD) (e.g. maximal RFD; RFD at predetermined time epochs) were not included, and this could have limited the ability of the study to detect novel findings related to strength/power as measured by the IMTP and CMJ in an elite rugby union context.

In conclusion, this study demonstrated that elite rugby union athletes of different playing positions possess distinct strength and power characteristics as measured by the IMTP and CMJ using the force platform. A positive aspect of this study was to recruit a large number of high calibre rugby athletes and characterise their strength/power using the 'gold standard'

method of data collection, and highly reliable protocols of data analyses. The findings of this study can be used by coaches and scientists as normative measures of strength/power in elite rugby union.

Practical applications

This study highlights differences in strength and power characteristics between rugby athletes of different playing positions, reflecting variations in athlete's performance requirements within an elite rugby union team. To the author's knowledge no study has investigated up to, or more than 263 elite rugby union athletes in either the CMJ or IMTP using the force platform method. Thus, we propose firstly, that these results are used as a normative set of data by sport scientists working in rugby. For example, we predict that future elite rugby union players might require greater strength/power in each respective playing position (Brazier et al., 2020) – in concert with the evolving nature of the game and thus, these results can be used as a reference to aid in quantifying these increased requirements via assessments of CMJ and IMTP in elite athletes throughout time. With regards to talent identification, differences in the physical qualities (e.g. maximal speed; acceleration; maximal strength; peak power) have been suggested as key discriminative functions between playing standards and age categories in rugby union (Jones et al., 2019) – addressed in detail in Section 1.6. Therefore, we propose, secondly, that these data (in combination with other physiological data) are used to gauge the strength/power of those athletes aspiring to be selected in senior teams. However, it is important to note that these data will serve their main purpose for this thesis (in combination with genotype data of the same athletes – Chapter 4) in an attempt to explore any associations between the variables investigated and ten genetic polymorphisms (Chapter 5) that were previously associated with strength/power.

CHAPTER 4

GENETIC CHARACTERISTICS OF MUSCULAR STRENGTH AND POWER IN ELITE MALE RUGBY ATHLETES OF DIFFERENT PLAYING POSITIONS

4.1 Introduction

Large inter-individual variability in muscular strength and power exists within diverse population groups (Silventoinen et al., 2008; Stebbings et al., 2014; Zempo et al., 2017), with athletic populations in particular those from strength/power oriented sport at the high end of the strength and power continuum (Hill, 1925; Garhammer, 1993; Cormie et al., 2011; Suchomel et al., 2018). It is well-established that strength and power are influenced by genetic factors (Beunen and Thomis, 2004; MacArthur and North, 2005; Williams et al., 2014). For example, heritability of grip strength and push/pull are in the range of 44 - 58% (Beunen and Thomis, 2004) and heritability for longitudinal changes in lower limb muscle strength is estimated at 64% (Zhai et al., 2005). The remaining variability in strength/power phenotypes is attributable to environmental factors and gene-environment interaction; the latter including epigenetic mechanisms that describe the integration of genes and environment (Ramani et al., 2016; McGee and Walder, 2017; Alyamani and Murgatroyd, 2018; He et al., 2020; Hall et al., 2020).

Performance demands in rugby union differ between playing positions (Duthie et al., 2003; Quarrie et al., 2013), and this is partly reflected in the athletes' anthropometric and physiological characteristics according to playing position (Brazier et al., 2020). Accordingly, elite rugby union athletes possess different strength and power levels (Smart et al., 2013; Smart et al., 2014; Brazier et al., 2020), although a high degree of muscular strength, power and speed is required regardless of playing position (Duthie et al., 2003; Kilduff et al., 2007; Cunningham et al., 2013). Despite the relative homogeneity in strength and power within groups of athletes of specific playing positions (as compared to the pool of athletes from all positions combined), where environmental factors such as training, nutrition and permitted ergogenic aids (often according to specific positional roles) are of highest quality and tightly

controlled, inter-individual variability in strength and power persists. Given the highly heritable nature of fundamental physical and physiological phenotypes, such as strength and power, that contribute to success for a given playing position (Heffernan et al., 2015), the phenotypic differences between groups of athletes from different playing positions can potentially be reflected in distinct genetic characteristics.

Over 62 genetic loci have been associated with strength and power via linkage analyses or genome-wide association studies (GWAS) thus far (Beunen and Thomis, 2004; Huygens et al., 2004; Hughes et al., 2011; Ahmetov et al., 2016), and this number will potentially continue to increase. Many of the previously discovered candidate genes have had little or no replication in an elite rugby context through further study, which means only a very small number can be confidently suggested to have an association with strength and power phenotypes or athlete status (Bouchard, 2015; Pitsiladis et al., 2016; Massidda et al., 2019) in sports that rely heavily on strength and power capabilities such as rugby. Furthermore, there is evidence suggesting that adaptation to resistance training in untrained muscle has a genetic component (Erskine et al., 2010). Although the heritability of adaptive responses to strength/power have not been extensively studied, some researchers have reported positive associations between polymorphisms in genes such as *ACE* (Erskine et al., 2014; Pescatello et al., 2006), *ACTN3* (Pereira et al., 2013) and *PTK2* (Erskine et al., 2012), and increased sensitivity to resistance exercise in training naive muscle. Substantial further study is needed to identify candidate genes associated with strength and power phenotypes and adaptation to strength training, as well as greater exploration of these genes in strength/power oriented athletic populations, and the implications this would have in its wider application in elite rugby.

Consequently, the primary aim of this study was to determine the genotype and allele frequency distribution of 10 polymorphisms previously associated with strength or power in the literature (*ACE* rs4341, *ACTN3* rs1815739, *AMPD1* rs17602729, *FTO* rs9939609, *HIF1A* rs11549465, *NOS3* rs2070744, *KDR* rs1870377, *PTK2* rs7460 and rs7843014, *TRHR* rs7832552) in elite Caucasian male rugby union athletes of different playing positions, and compare with non-athletes and between athlete groups. For *ACE* rs4341, *ACTN3* rs1815739 and *FTO* rs9939609 the aim was to expand on the previous work of Heffernan et al. (2016) and Heffernan et al. (2017) by investigating the variants in a larger sample of elite rugby union athletes (n=566, 544 and 486 for *ACE, ACTN3* and *FTO*, respectively). Of note is that Heffernan et al. also included a group of elite rugby league athletes, who were not included in the present study. It was hypothesised that we would further strengthen the associations for *ACTN3* rs1815739 and *FTO* rs9939609 previously found by Heffernan et al. and possibly detect an association between *ACE* rs4341 and elite athlete status/playing position by including an additional 135 athletes. Secondly, it was hypothesised that associations would exist between at least half of the eight SNPs (in addition to *ACTN3* rs1815739 and *FTO* rs9939609) and athlete status or playing position.

4.2 Method

Detailed descriptions of the 567 elite rugby athletes and 1171 non-athletes, all participants in this study are provided in Section 2.1 (Chapter 2: General Methods). Procedures for DNA sample collection, DNA extraction, and genotyping are included in Section 2.4. Therefore, only brief descriptions of participants, and statistical analyses used in this investigation are detailed in this Section.

The author's contribution to all procedures involved for sample collection, DNA extraction and genotyping for the 567 elite rugby athletes and 1171 non-athletes is provided in Section 2.4. Ethical approval was granted by Manchester Metropolitan University, University of Glasgow, University of Cape Town and Northampton University ethics committees. All procedures were conducted in accordance with the guidelines in the Declaration of Helsinki (World Medical Association, 2013).

4.2.1 Participants

Using G*Power (Faul et al., 2007), an a priori calculation for 80% power and alpha (α) set at 0.05, to detect a small effect size (w) of 0.10 indicated >964 participants were required. A total of 1738 individuals that form part of RugbyGene were recruited for the present study. The sample comprised 567 elite Caucasian male rugby union athletes [(mean (standard deviation); height 1.86 (0.07) m, mass 103 (12.5) kg, BMI 29.7 (3.1) $kg \cdot m^{-2}$] including 57% British, 16% South African, 13% Irish, 11% Italian and 3% from other nationalities; and 1171 non-athlete Caucasians (male and female) including 84% British, 14% South African, and 2% from other nationalities. Further details of nationality and physical characteristics are provided in Appendix C (Table AppC4.1).

Athletes were considered elite if they had competed regularly (> 5 matches) since 1995 in the highest professional league in the UK, Ireland or South Africa. Of the athletes, 52.7% had competed at an international level for a "High Performance Union" (Regulation 16, worldrugby.org). Athletes' international status were confirmed as of 1st July 2019. Non-athletes were apparently healthy and asymptomatic, unrelated, and did not take part in competitive sport above recreational level.

4.2.2 Participant recruitment

A main aim of this Chapter was to recruit the largest possible samples of elite rugby union athletes and non-athletes and genotype all participants for polymorphisms that were previously associated with a muscular strength and/or power phenotype in the literature. This served to maximise the recruitment of athletes for subsequent Chapters of the thesis, that aimed to associate the genotypes of athletes in the polymorphisms investigated with established measures of strength and power and in-game performance variables in Chapters 5 and 6, respectively. It was somewhat autonomous that as part of the selection process with regards to athlete recruitment and studied polymorphisms the author of this thesis considered the inclusion of those elite Caucasian athletes who were already genotyped for the maximum number of relevant polymorphisms that could potentially be studied. In addition, 135 elite rugby union athletes were recruited with a main aim of increasing statistical power of the investigations of this thesis. With regards to this Chapter, this served also to attempt an exploration of associations for SNPs (e.g. *ACE* rs4341) that were not found in previous papers (Heffernan et al., 2016) using the existing samples of athletes and non-athletes. Analyses conducted using G*Power (Faul et al., 2007) indicated that the addition of 135 athletes to the existing cohort of rugby union athletes served to retain a statistical power of 80% with a capacity of detecting a smaller effect size ($w = 0.74$; $\alpha = 0.05$) than that possible (0.77, $\alpha = 0.05$) without the additional athletes. It can also be said that the capacity of detecting an effect size of 0.74 could have been retained, however, with an increase in statistical power from 80.0 % to 83.3 % and thus, a concomitant increased ability of rejecting a false null-hypothesis. Furthermore, the addition of 135 elite rugby union athletes served to increase statistical power and the ability to detect relatively smaller effect sizes also for the comparisons between athlete groups for all SNPs. Taking again the example of the *ACE* rs4341 for the comparison between forwards and backs, the additional participants served to

increase statistical power from 80.0 % to 88.6 %, whilst retaining the ability to detect an effect size (w) of 0.12 (α set at 0.05). Thus, the addition of 'new' participants for this thesis as part of RugbyGene, and the inclusion of existing participants and related data to this thesis can sensibly be justified. The author has been involved in athlete recruitment and genotyping that has served for this thesis and other contemporary (in relation to this thesis) research work as part of RugbyGene (Section 2.4.4).

4.2.3 Procedures for DNA sample collection, DNA extraction and genotyping

All participants provided a whole blood, saliva or buccal swab sample, from which DNA was subsequently extracted and analysed to obtain genotype data for ten SNPs. Detailed descriptions of all procedures for obtaining genetic data are provided in Section 2.4 (General Methods).

4.2.4 Statistical analysis

Athletes were considered either as one group or two major groups (forwards and backs). The backs' subgroups (sometimes combined according to the analyses; half backs, centres, and back three) were used only for analyses of *ACE* rs4341, *ACTN3* rs1815739 and *FTO* rs9939609 to compare directly with Heffernan et al. (2016) and Heffernan et al. (2017). Genotype and allele frequencies for athletes (combined), athlete groups and subgroups, and non-athletes (for ten SNPs) were assessed for compliance with Hardy-Weinberg equilibrium using Pearson's chi-square (χ^2) goodness-of-fit test. Three analysis models [additive (AA / Aa / aa), recessive (AA / Aa+aa), and dominant (AA+Aa / aa)] were used for genotype comparisons. Pearson's chi-square goodness-of-fit test was used to compare genotype and allele frequency distributions between athlete groups/subgroups, and non-athletes. Chi-square test of independence was used to compare athlete groups/subgroups. Odds ratio was used to

estimate effect size. The Statistical Package for Social Sciences (version 26.0, SPSS Inc., Chicago, IL, USA) was used for the analyses, and alpha was set at 0.05. Probability values were subjected to Benjamini-Hochberg (B-H) correction (Benjamini and Hochberg, 1995) to control false discovery rate (FDR). FDR was set at 20% and the reported *p* values are those significant after B-H correction.

4.3 Results

Genotype frequencies were in Hardy-Weinberg equilibrium for athletes (combined), athlete groups (forwards and backs), and non-athletes, for the ten SNPs. Exceptions were for *ACTN3* rs1815739 (all athletes and backs; χ^2 = 5.204, p = 0.022 and χ^2 = 3.988, p = 0.046, respectively), *FTO* rs9939609 (centres; χ^2 = 3.857, p = 0·049), and *HIF1A* rs11549465 (non-athletes; χ^2 = 4.062, p = 0.044). Detailed test results for Hardy-Weinberg equilibrium are provided in Appendix C (Table AppC4.2a and b). Table 4.1 shows genotype/allele frequency distributions and chi-squared (χ^2) statistics for all comparisons and for ten SNPs.

<u>All Athletes vs. non-athletes</u>

For *FTO* rs9939609, athletes had a greater proportion of A allele carriers than non-athletes (67.3% vs. 63.3%, p = 0.065; odds ratio (OR) = 1.19 [confidence interval (CI) = 0.95–1.51]; Table 4.1 and Figure 4.1), with TT genotype more common in non-athletes. For *TRHR* rs7832552, the additive model revealed genotype differences between athletes and non-athletes (p = 0.083, significant after B-H correction). Athletes included more T allele carriers (58.5% vs. 53.3%, p = 0.032; OR = 1.23 [CI = 0.96–1.58]), with CC genotype more common in non-athletes. Additionally, athletes had a higher T allele frequency (35.3% vs. 31.8%, p = 0.027; OR = 1.17 [CI = 0.97–1.41]), with the C allele more common in non-athletes (Table 4.1 and Figure 4.2).

Table 4.1 Genotype/allele frequency distribution (left), and group comparisons (right). Genotype/ allele frequency data are percentages. Chi-square results include *p* values in parentheses.

SNP		GROUPS				COMPARISONS				
		non-athletes	athletes (comb)	forwards	backs		athletes **vs.** non-athletes	forwards **vs.** non-athletes	backs **vs.** non-athletes	forwards **vs.** backs
		Genotype frequency (%) Allele frequency (%)				Model	Chi-square (*p* value)			
ACE	**DD**	30.5	28.3	27.4	29.5					
	ID	49.6	50.7	51.4	49.8	DD vs. ID vs. II	1.413 (0.493)	1.522 (0.467)	0.173 (0.917)	0.296 (0.863)
rs4341	**II**	19.9	21.0	21.2	20.7	DD vs. I carriers	1.314 (0.252)	1.476 (0.224)	0.120 (0.729)	0.294 (0.588)
	Group	**925**	**566**	**325**	**241**	D carriers vs. II	0.456 (0.500)	0.366 (0.545)	0.111 (0.740)	0.020 (0.889)
	D	55.3	53.6	53.1	54.4	D allele vs. I allele	1.285 (0.257)	1.296 (0.255)	0.172 (0.678)	0.182 (0.669)
	I	44.7	46.4	46.9	45.6					
ACTN3	**CC**	34.1	33.6	29.8	38.8					
	CT	46.4	44.5	46.5	41.8	CC vs. CT vs. TT	2.079 (0.354)	4.589 (0.101)	2.538 (0.281)	4.980 (0.083)
rs1815739	**TT**	19.5	21.9	23.7	19.4	CC vs. T carriers	0.056 (0.812)	2.584 (0.108)	2.251 (0.134)	4.812 (**0.028**)*
	Group	**1140**	**544**	**312**	**232**	C carriers vs. TT	2.000 (0.157)	3.584 (0.058)	0.001 (0.976)	1.454 (0.228)
	C	57.3	55.9	53.0	59.7	C allele vs. T allele	0.925 (0.336)	4.672 (0.031)	1.069 (0.301)	4.778 (**0.029**)*
	T	42.7	44.1	47.0	40.3					
AMPD1	**GG**	79.1	75.9	78.1	73.0					
	GA	19.4	22.6	19.7	26.4	GG vs. GA vs. AA	2.757 (0.252)	0.632 (0.729)		
rs17602729	**AA**	1.5	1.5	2.1	0.6	GG vs. A carriers	2.501 (0.114)	0.133 (0.715)	3.941 (0.047)	1.423 (0.233)
	Group	**459**	**411**	**233**	**178**	G carriers vs. AA	0.012 (0.914)			
	G	88.8	87.2	88.0	86.2	G allele vs. I allele	1.992 (0.158)	0.297 (0.586)	2.313 (0.128)	0.553 (0.457)
	A	11.2	12.8	12.0	13.8					
FTO	**TT**	36.7	32.7	29.7	36.7					
	TA	47.7	51.0	50.2	52.0	TT vs. TA vs. AA	3.444 (0.179)	7.685 (**0.021**)*	3.548 (0.170)	7.725 (**0.021**)*
rs9939609	**AA**	15.6	16.3	20.1	11.1	TT vs. A carriers	3.400 (**0.065**)*	5.880 (**0.015**)*	0.000 (0.992)	2.619 (**0.106**)*
	Group	**898**	**486**	**279**	**207**	T carriers vs. AA	0.163 (0.686)	4.258 (**0.039**)*	3.156 (**0.076**)*	7.009 (**0.008**)*
	T	60.6	58.2	54.8	62.8	T allele vs. A allele	2.245 (0.134)	7.699 (**0.006**)*	0.857 (0.355)	6.196 (**0.013**)*
	A	39.4	41.8	45.2	37.2					
HIF1A	**CC**	79.4	78.1	73.3	83.9					
	CT	20.3	19.4	23.0	15.2	CC vs. CT vs. TT				
rs11549465	**TT**	0.3	2.4	3.7	0.9	CC vs. T carriers	0.230 (0.631)	3.008 (0.083)	1.420 (0.233)	4.023 (**0.045**)*
	Group	**606**	**247**	**135**	**112**	C carriers vs. TT				
	C	89.5	87.9	84.8	91.5	C allele vs. T allele	1.464 (0.226)	6.376 (**0.012**)*	0.950 (0.329)	5.155 (**0.023**)*
	T	10.5	12.1	15.2	8.5					
KDR	**TT**	55.0	58.0	59.5	56.0					
	TA	39.0	36.4	33.8	40.0	TT vs. TA vs. AA	2.035 (0.361)	3.578 (0.167)	1.681 (0.432)	3.437 (0.179)
rs1870377	**AA**	6.1	5.6	6.8	4.0	TT vs. A carriers	2.032 (0.154)	2.575 (0.109)	0.099 (0.754)	0.651 (0.420)
	Group	**726**	**536**	**311**	**225**	T carriers vs. AA	0.202 (0.653)	0.261 (0.609)	1.678 (0.195)	1.872 (0.171)
	T	74.4	76.2	76.4	76.0	T allele vs. A allele	1.753 (0.186)	1.202 (0.273)	0.569 (0.451)	0.019 (0.889)
	A	25.6	23.8	23.6	24.0					
NOS3	**TT**	38.7	38.0	36.7	39.9					
	CT	44.2	47.1	47.6	46.5	TT vs. CT vs. CC	2.608 (0.271)	1.438 (0.487)	1.926 (0.382)	0.814 (0.666)
rs2070744	**CC**	17.0	14.8	15.8	13.6	TT vs. C carriers	0.112 (0.738)	0.567 (0.451)	0.133 (0.715)	0.592 (0.442)
	Group	**728**	**539**	**311**	**228**	T carriers vs. CC	1.830 (0.176)	0.359 (0.549)	1.905 (0.167)	0.485 (0.486)
	T	60.9	61.6	60.5	63.2	T allele vs. C allele	0.250 (0.617)	0.042 (0.837)	1.018 (0.313)	0.815 (0.367)
	C	39.1	38.4	39.5	36.8					
PTK2	**AA**	26.6	25.3	25.1	25.5					
	AT	48.7	53.4	53.4	53.4	AA vs. AT vs. TT	3.785 (0.151)	2.121 (0.346)	1.675 (0.433)	0.012 (0.994)
rs7460	**TT**	24.8	21.4	21.5	21.1	AA vs. T carriers	0.336 (0.562)	0.242 (0.623)	0.100 (0.752)	0.006 (0.937)
	Group	**606**	**384**	**223**	**161**	A carriers vs. TT	2.381 (0.123)	1.247 (0.264)	1.142 (0.285)	0.009 (0.924)
	A	50.9	52.0	51.8	52.2	A allele vs. T allele	0.336 (0.562)	0.140 (0.708)	0.207 (0.649)	0.011 (0.917)
	T	49.1	48.0	48.2	47.8					
PTK2	**AA**	31.9	27.1	26.3	28.1					
	AC	47.7	53.3	55.8	50.0	AA vs. AC vs. CC	5.398 (0.067)	5.776 (0.056)	1.049 (0.592)	1.405 (0.495)
rs7843014	**CC**	20.5	19.6	18.0	21.9	AA vs. C carriers	4.040 (0.044)	3.148 (0.076)	1.039 (0.308)	0.161 (0.688)
	Group	**665**	**377**	**217**	**160**	A carriers vs. CC	0.157 (0.692)	0.820 (0.365)	0.199 (0.655)	0.889 (0.346)
	A	55.7	53.7	54.1	53.1	A allele vs. C allele	1.223 (0.269)	0.432 (0.511)	0.870 (0.351)	0.077 (0.781)
	C	44.3	46.3	45.9	46.9					
TRHR	**TT**	10.3	12.2	12.5	11.8					
	CT	43.0	46.2	47.1	45.2	TT vs. CT vs. CC	4.971 (**0.083**)*	4.092 (0.129)	1.165 (0.559)	0.292 (0.864)
rs7832552	**CC**	46.7	41.5	40.4	43.0	TT vs. C carriers	1.624 (0.203)	1.223 (0.269)	0.452 (0.501)	0.044 (0.834)
	Group	**610**	**426**	**240**	**186**	T carriers vs. CC	4.578 (**0.032**)*	3.832 (**0.050**)*	1.029 (0.310)	0.290 (0.590)
	T	31.8	35.3	36.0	34.4	T allele vs. C allele	4.882 (**0.027**)*	3.976 (**0.046**)*	1.164 (0.281)	0.245 (0.621)
	C	68.2	64.7	64.0	65.6					

*statistically significant after Benjamini-Hochberg correction. Genotype and allele frequency distribution (left) shaded in light grey have shown slight deviations from Hardy-Weinberg equilibrium (*ACTN3* rs1815739 [all athletes and backs; $\chi^2 = 5.204$, $p = 0.022$ and $\chi^2 = 3.988$, $p = 0.046$, respectively]; and *HIF1A* rs11549465 [non-athletes; $\chi^2 = 4.062$, $p = 0.044$]).

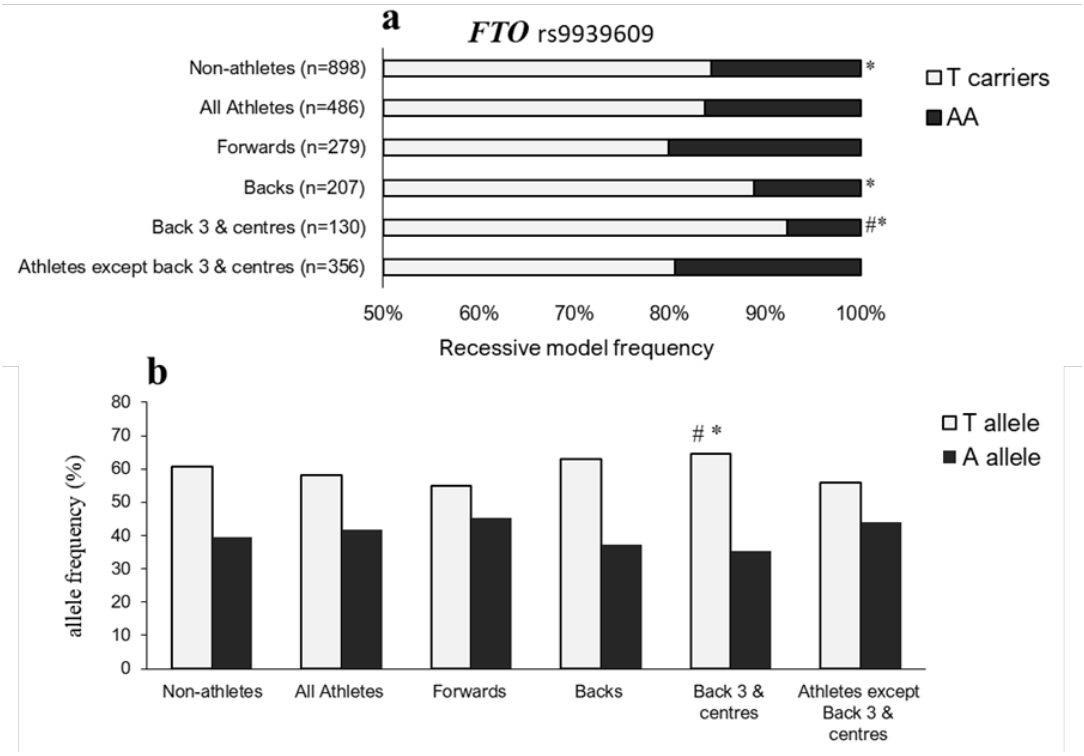


Figure 4.1a Recessive model for ***FTO*** rs9939609 showing proportion of T allele carriers and AA genotype, and (**b**) allele frequency for athlete groups, subgroups, and non-athletes. *different from forwards. #Different from 'Athletes except back 3 & centres'.

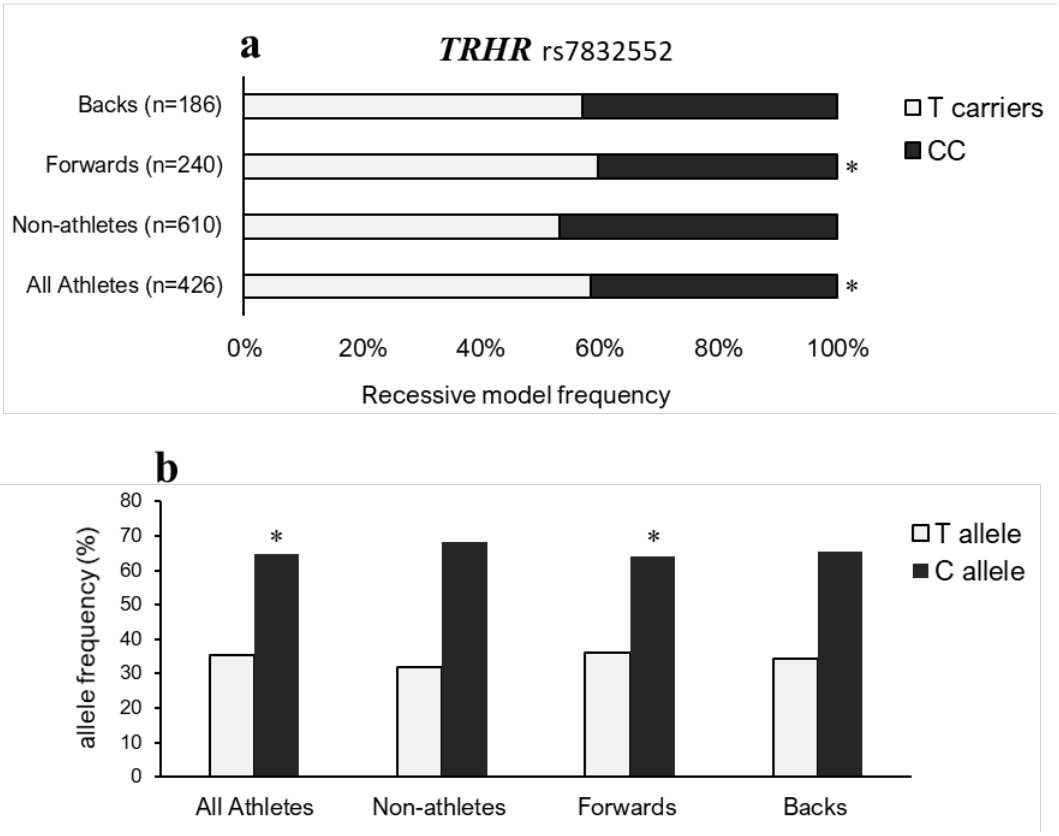


Figure 4.2a Recessive model for ***TRHR*** rs7832552 showing proportion of T allele carriers and CC genotype, and (**b**) allele frequency for athlete groups, subgroups, and non-athletes. *different from non-athletes.

Forwards **vs.** non-athletes

For *FTO* rs9939609, the additive model revealed genotype differences between the forwards and non-athletes (p = 0.021). Forwards had a higher frequency of AA genotype (20.1% vs. 15.6%, p = 0.039; odds ratio (OR) = 1.36 [confidence interval (CI) = 0.96–1.92]; Table 4.1 and Figure 4.1) and a greater proportion of A allele carriers (70.3% vs. 63.3%, p = 0.015; OR = 1.37 [CI = 1.03–1.83]) than non-athletes, with more than 1.3 times greater odds of being A allele carriers than non-athletes. In addition, forwards had a greater A allele frequency than non-athletes (45.2% vs. 39.4%, p = 0.006; OR = 1.27 [CI = 1.04–1.53]), with almost 1.3 times greater odds of possessing A alleles than non-athletes. For *HIF1A* rs11549465, forwards had a greater T allele frequency (15.2% vs. 10.5%, p = 0.012; OR = 1.53 [CI = 1.05–2.24]; Table 4.1 and Figure 4.3) than non-athletes, and showed more than 1.5 times greater odds of possessing T alleles than non-athletes. For *TRHR* rs7832552, forwards included more T allele carriers (59.6% vs. 53.3%, p = 0.05; OR = 1.29 [CI = 0.95–1.75]; Table 4.1 and Figure 4.2), and fewer CC homozygotes than non-athletes (40.4% vs. 46.7%). Additionally, forwards had a greater T allele frequency than non-athletes (36.0% vs. 31.8%, p = 0.046; OR = 1.21 [CI = 0.97–1.51]).

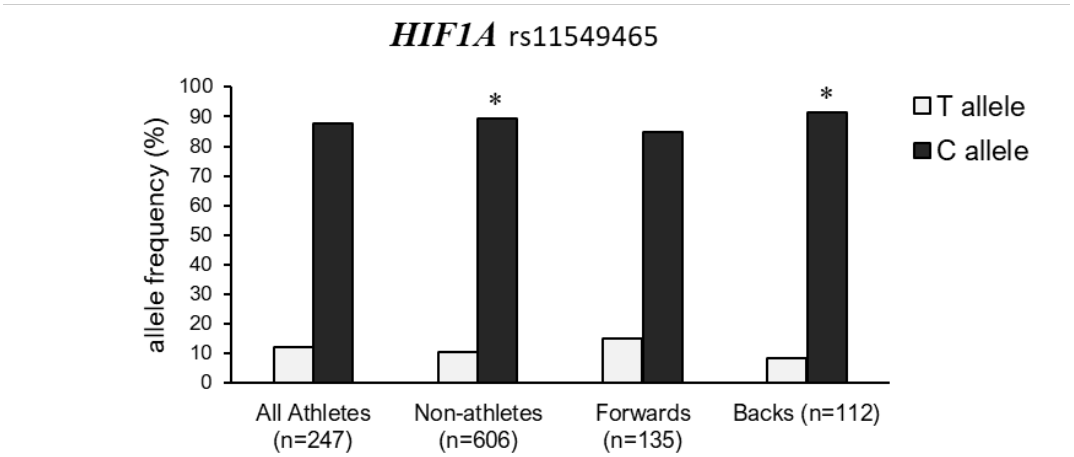


Figure 4.3 ***HIF1A*** rs11549465 allele frequency for all athletes, athlete groups, and non-athletes. *different from forwards. Deviation from Hardy-Weinberg equilibrium for non-athletes; χ^2 = 4.062, p = 0.044.

Backs vs. non-athletes

For *FTO* rs9939609, the backs included fewer AA homozygotes (11.1% vs. 15.6%) and more T allele carriers (88.9% vs. 84.4%, $p = 0.076$ (significant after B-H correction); odds ratio (OR) = 1.48 [confidence interval (CI) = 0.92–2.36]; Table 4.1 and Figure 4.1) than non-athletes.

Forwards **vs.** backs

For *ACTN3* rs1815739, backs included more CC homozygotes than forwards (39.8% vs. 29.8%, $p = 0.028$; odds ratio (OR) = 1.49 [confidence interval (CI) = 1.04–2.14]; Table 4.1 and Figure 4.4) and showed almost 1.5 times greater odds of being CC homozygotes than T allele carriers. In addition, backs had a greater C allele frequency than forwards (59.7% vs. 53.0%, $p = 0.029$; OR = 1.31 [CI = 1.03–1.67]), and showed more than 1.3 times greater odds of possessing C alleles. For *FTO* rs9939609, the additive model revealed genotype differences between forwards and backs ($p = 0.021$). Backs had a greater frequency of TT genotype (36.7% vs. 29.7%, $p = 0.106$; OR = 1.37 [CI = 0.93–2.00]; Table 4.1 and Figure 4.1) and contained more T alleles than forwards (62.8% vs. 54.8%, $p = 0.013$; OR = 1.39 [CI = 1.07–1.80]). Forwards had more AA homozygotes (20.1% vs. 11.1%, $p = 0.008$; OR = 2.00 [CI = 1.19–3.39]) and less T allele carriers (79.9% vs. 88.9%) than backs. In addition, forwards showed twice the odds of being AA genotype than T allele carriers, compared to backs. For *HIF1A* rs11549465, forwards included more T allele carriers (26.7% vs. 16.1%, $p = 0.045$; OR = 1.90 [CI = 1.01–3.57]; Table 4.1 and Figure 4.3) and T alleles (15.2% vs. 8.5%, $p = 0.023$; OR = 1.93 [CI = 1.09–3.44]) than backs. In addition, forwards had almost twice the odds of being T allele carriers than CC homozygotes, compared to backs.

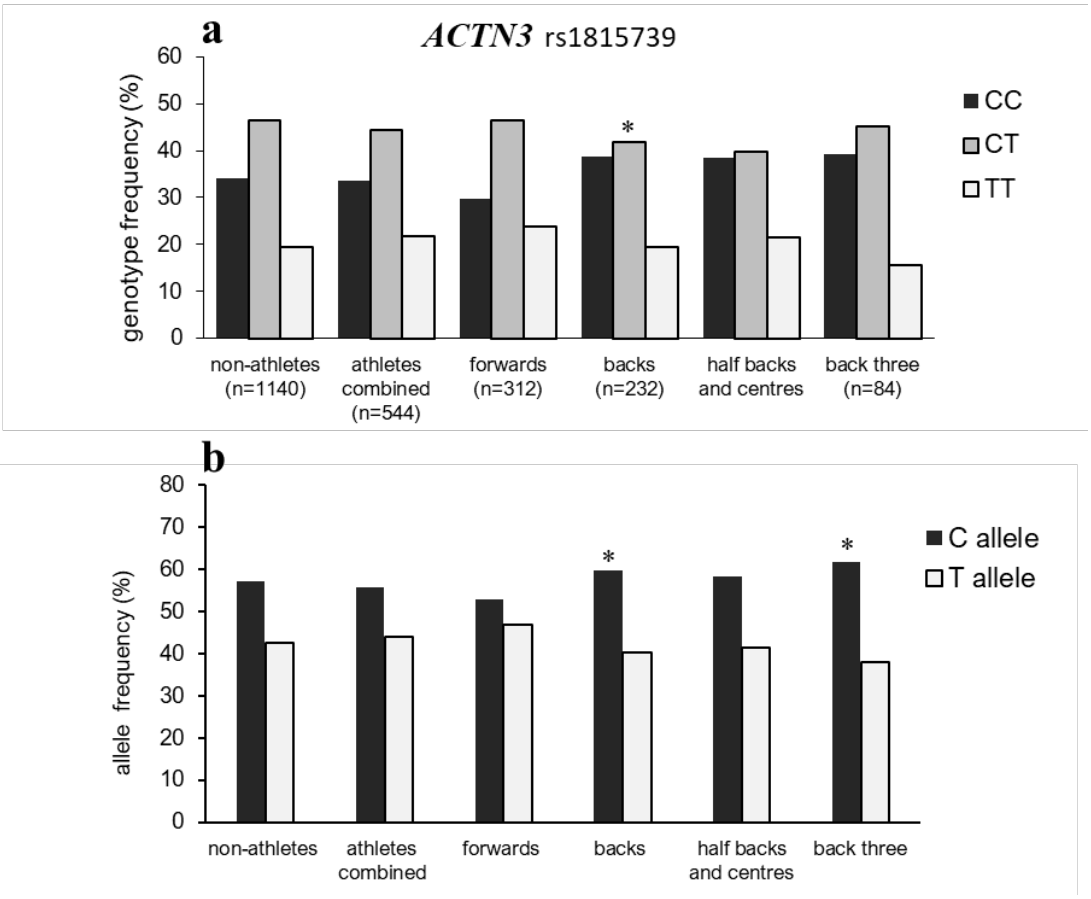


Figure 4.4a Genotype frequency for ***ACTN3*** rs1815739, and (**b**) allele frequency for athlete groups, subgroups and non-athletes. *different from forwards. Deviation from Hardy-Weinberg equilibrium for all athletes and backs; $\chi^2 = 5.204$, $p = 0.022$ and $\chi^2 = 3.988$, $p = 0.046$, respectively),

Back three vs. non-athletes/forwards/half backs and centres for ***ACTN3*** rs1815739

The back three subgroup contained more C alleles than forwards (61.9% vs. 53%, $p = 0.041$; odds ratio (OR) = 1.44 [confidence interval (CI) = 1.01–2.04]; Table 4.3 and Figure 4.4b), with almost 1.5 times greater odds of possessing C alleles than T alleles, compared to forwards.

Back three and centres vs. non-athletes/forwards/all athletes (except back three and centres), for ***FTO*** rs9939609

The additive model revealed genotype differences in the three comparisons of the back three and centres [back three and centres vs. non-athletes ($p = 0.033$), forwards ($p = 0.006$), and

forwards and half backs combined (p = 0.008; Table 4.4 and Figure 4.1a)]. The back three and centres contained more T allele carriers (92.3% vs. 84.4%, p = 0.013; odds ratio (OR) = 2.22 [confidence interval (CI) = 1.13–4.33]) and less AA homozygotes than non-athletes, with more than twice the odds of being T allele carriers than non-athletes. Compared to forwards, the back three and centres contained more TT homozygotes (36.9% vs. 29.7%, p = 0.148 (significant after B-H correction); OR = 1.38 [CI = 0.89–2.14]), included more T allele carriers (92.3% vs. 79.9%, p = 0.002; OR = 3.01 [CI = 1.48–6.12]), and showed three times greater odds of being T allele carriers than forwards. In addition, the back three and centres had a greater T allele frequency than forwards (64.6% vs. 54.8%, p = 0.008; OR = 1.50 [CI = 1.11–2.04)], with 1.5 times greater odds of possessing T alleles. Compared with other rugby athletes, the back three and centres contained more T allele carriers (92.3% vs. 80.6%, p = 0.002; OR = 2.89 [CI = 1.44–5.79]) and T alleles (64.6% vs. 55.9%, p = 0.015; OR = 1.44 [CI = 1.07–1.93]). Furthermore, the back three and centres showed almost three times greater odds of being T allele carriers, and almost 1.5 times greater odds of containing T alleles, when compared with all other athletes. Table AppC4.3 (Appendix C) shows genotype and allele distribution percentages in athlete groups and non-athletes. Table. AppC4.4 shows chi-square and odds ratio (OR) with 95% confidence intervals (CI) for the comparisons presented in table 4.4.

Table 4.3 Chi-square comparisons of backs subgroups for ***ACTN3*** rs1815739. Genotype and allele frequencies are presented as percentages (bottom right).

		Back three **vs** Non-athletes		Forwards **vs** Back three	
SNP	Model	$\chi 2$	*p* value	$\chi 2$	*p* value
ACTN3 rs1815739	CC vs. CT vs. TT	1.370	0.504	3.937	0.140
	CC vs. T carriers	0.996	0.318	2.741	0.098
	C carriers vs. TT	0.856	0.355	2.622	0.105
	C allele vs. T allele	1.441	0.230	4.197	**0.041***

		Half backs & Centres **vs** Back three		Back three	Half backs & centres
SNP	Model	$\chi 2$	*p* value	Genotype frequency (%) Allele frequency (%)	
ACTN3 rs1815739	CC vs. CT vs. TT	1.422	0.491	**CC** 39.3	38.5
	CC vs. T carriers	0.013	0.908	**CT** 45.2	39.9
	C carriers vs.TT	1.294	0.255	**TT** 15.5	21.6
	C allele vs. T allele	0.533	0.465	Group (n) **84**	**148**
				C 61.9	58.4
				T 38.1	41.6

Table 4.4 Chi-square comparisons involving the backs' subgroups for ***FTO*** rs9939609. Genotype and allele frequencies are presented as percentages (bottom right).

		Back three & Centres **vs** Non-athletes		Back three & Centres **vs** Forwards	
SNP	Model	χ^2	*p* value	χ^2	*p* value
FTO rs9939609	TT vs. TA. vs AA	6.829	**0.033***	10.310	**0.006***
	TT vs. A carriers	0.002	0.967	2.096	**0.148***
	T carriers vs. AA	6.162	**0.013***	10.042	**0.002***
	T allele vs. A allele	1.774	0.183	6.957	**0.008***

		Back three & Centres vs All other athletes		Back three & centres	All athletes (except back three &centres)
SNP	Model	χ^2	*p* value	Genotype frequency (%) Allele frequency (%)	
FTO rs9939609	TT vs. TA vs. AA	9.625	0.008*	**TT** 36.9	31.2
	TT vs. A carriers	1.427	0.232	**TA** 55.4	49.4
	T carriers vs. AA	9.559	0.002*	**AA** 7.7	19.4
	T allele vs. A allele	5.949	0.015*	Group (n) **130**	**356**
				T 64.6	55.9
				A 35.4	44.1

4.4 Discussion

This is the first study that examined whether ten polymorphisms (previously associated with strength and power in the literature) are associated with elite rugby status and/or playing position. Our results demonstrate that *TRHR* rs7832552 is associated with athlete status, while *HIF1A* rs11549465 is associated with both athlete status and playing position. In addition, consistent with Heffernan et al. (2016) and Heffernan et al. (2017), our data show that *ACTN3* rs1815739 is associated with playing position while *FTO* rs9939609 is associated with both athlete status and playing position. Of note is that for *ACE* rs4341, *ACTN3* rs1815739 and *FTO* rs9939609 we aimed to expand on Heffernan et al.'s work by studying the variants in a larger cohort of elite rugby union athletes. In relation to this, we hypothesised that we would further strengthen the findings of Heffernan et al.'s work. Although not entirely consistent, our results led us to accept this hypothesis. Secondly, we hypothesised that we would detect associations for at least four SNPs (that could include *ACE* rs4341), apart from those already established by Heffernan et al. for *ACTN3* and *FTO*. Despite our novel associations in relation to *TRHR* rs7832552 and *HIF1A* rs11549465, we reject our second hypothesis.

There were no differences in *ACTN3* rs1815739 genotype and allele distribution between athletes and non-athletes. When playing position was considered, we found that the C allele (59.7% vs. 53.0%, $p = 0.029$; OR = 1.31 [CI = 1.03–1.67]) and CC genotype (39.8% vs. 29.8%, $p = 0.028$; OR = 1.49 [CI = 1.04–2.14]) were more frequent in the backs than forwards, while the forwards had a greater proportion of T allele carriers and contained more T alleles. A main difference between our results and those of Heffernan et al. involved the back three subgroup. We did not observe a higher C allele frequency in the back three when compared with the half backs and centres (combined) or controls, nor the higher frequency of

CC homozygotes in the back three when compared to forwards – all remarkable findings in Heffernan et al.'s study. Undoubtedly, these inconsistencies are attributable, in large part, to the higher frequency of TT homozygotes introduced to the back three subgroup in the present study [n = 84 (15.5% TT) vs. n = 69 (8.7% TT)]. It appears that the additional 13 athletes (19%) in the back three deviated the genotype/allele distribution from that of Heffernan et al.'s for the same subgroup. However, when taken together our results are concordant with the novel findings of Heffernan et al.'s work, in that they support the notion that the C allele of *ACTN3* rs1815739 may be advantageous for the back playing positions, where strength and power relative to body mass are the predominant capabilities for performance, while the T allele appears advantageous for the heavier forwards who are reliant on maximal strength/power and increased capacity to recover after fatiguing activity. The TT genotype (present in ~18% of Caucasians; 19.5% in our non-athlete population, Table 4.1) indicates absence of the actin binding protein α-actinin-3 that is almost exclusively expressed in fast twitch myofibers (Beggs et al., 1992). As the major components of the Z line, the sarcomere α-actinins crosslink actin thin filaments and likely perform a static function in maintaining the ordered myofibrillar array, as well as a regulatory function in coordinating myofiber contraction (Blanchard et al., 1989; Mills et al., 2001). However, α-actinin-3 is the predominant fast-twitch myofiber isoform (Mills et al., 2001) that may confer an increased capacity for the absorption and transmission of force at the Z line during high velocity muscle action (Yang et al., 2003), with a concomitant advantage for rapid force generation and high mechanical power output relative to body mass. *In vivo* data demonstrated that compared to TT homozygotes, carriers of the C allele have a greater proportion of fast twitch myofibers and larger relative surface area per fibre (Ahmetov et al., 2011; Broos et al., 2012; Vincent et al., 2007), that is suggestive of an additional inherited advantage for the backs playing

positions in producing high power relative to body mass; the latter supported by data from Chapter 3.

As hypothesised, our findings for *FTO* rs9939609 with a slightly increased cohort size are concordant with those of Heffernan et al. (2017). We found that athletes (combined) had more A allele carriers (67.3% vs. 63.3%, $p = 0.065$; OR = 1.19 [CI = 0.95–1.51]) and less TT homozygotes that non-athletes – an observation not reported by Heffernan et al. (2017), because the authors only considered the additive model for this comparison while focusing on playing position. Similarly, we found that forwards contained more A allele carriers (70.3% vs. 63.3%, $p = 0.015$; OR = 1.37 [CI = 1.03–1.83]) and less TT homozygotes than non-athletes, notably in the opposite direction to the comparison between backs and non-athletes – backs had more T allele carriers (88.9% vs. 84.4%, $p = 0.076$; OR = 1.48 [CI = 0.92–2.36]). Furthermore, we found a greater frequency of TT genotype (36.7% vs. 29.7%, $p = 0.106$ (significant after B-H correction); OR = 1.37 [CI = 0.93–2.00]), proportion of T allele carriers (88.9% vs. 79.9%, $p = 0.008$; OR = 2.01 [CI = 1.19–3.39]) and T allele (62.8% vs. 54.8%, $p = 0.013$; OR = 1.39 [CI = 1.07–1.80]) in backs than forwards; and a greater proportion of T allele carriers (92.3% vs. 80.6%, $p = 0.002$; OR = 2.89 [CI = 1.44–5.79]) and T alleles (64.6% vs. 55.9%, $p = 0.015$; OR = 1.44 [CI = 1.07–1.93]) in backs' subgroup (centres and back three) than all other athletes – in agreement with Heffernan et al. (2017). Our data show that the T allele and TT genotype are advantageous for backs who are more reliant on a lean phenotype (Smart et al., 2013; Brazier et al., 2020), and on their ability to produce high levels of lower body mechanical power relative to body mass. This is in agreement with previously published data in elite rugby union (Crewther et al., 2012), and supported by Chapter 3. On the other hand, the A allele and AA genotype appear advantageous for forwards who depend on greater body mass (Sedeaud et al., 2012; Sedeaud

et al., 2013), and on their ability to generate the highest absolute force and power output (Brazier et al., 2020). The *FTO* rs9939609 polymorphism represents a cluster of 10 SNPs in high linkage disequilibrium (r = > 0.8) previously associated with BMI and obesity risk (Frayling et al., 2007), and type-2 diabetes (Fall et al., 2013; Lu and Loos, 2013). Chapters 5 and 6 further explore the association between *FTO* rs9939609 and rugby performance.

The present study is the first to identify an association between *HIF1A* rs11549465 and elite athlete status/playing position in rugby. We found a greater T allele frequency in forwards than non-athletes (15.2% vs. 10.5%, p = 0.012; OR = 1.53 [CI = 1.05–2.24]) and backs (15.2% vs. 8.5%, p = 0.023; OR = 1.93 [CI = 1.09–3.44]). Hypoxia inducible factor-1alpha (HIF-1α) is ubiquitously expressed in human and mouse tissues and has a general role in multiple physiological responses to hypoxia, such as erythropoiesis (Semenza et al., 1991), iron metabolism (Rolfs et al., 1997) and glycolysis (Semenza et al., 1994; Chen et al., 2001). During hypoxia, HIF-1α becomes stabilised and translocates from the cytoplasm to the nucleus, where it dimerises with HIF-1beta; forming the HIF-1complex that becomes transcriptionally active (Huang et al., 1996; Kallio et al., 1997). Subsequently, the HIF-1 complex formed associates with hormone response elements in the regulatory regions of numerous target genes, and binds the transcriptional coactivators to induce gene expression (Lando et al., 2002; Lando et al., 2002). Of particular importance is that the expression of HIF-1α can also be activated by oxygen independent mechanisms such as in response to insulin-like growth factor-1 (IGF-1). For example, the action of the IGF-1–HIF-1α–IGF-2/transforming growth factor-α axis may explain the hypertrophic effect of HIF-1α on skeletal muscle (Fukuda et al., 2002; Slomiany and Rosenzweig, 2006; Gariboldi et al., 2010; Hughes et al., 2011). In addition, evidence suggests an association between rs11549465 and skeletal muscle fibre type composition. Ahmetov et al. (2008) associated the *HIF1A* T allele

with an increased proportion of fast-twitch fibres (C/T, 46.2% fast-twitch; C/C, 31.4% fast-twitch) in the vastus lateralis muscle of Russian speed skaters. Furthermore, Cięszczyk et al. (2011) reported C/T genotype was overrepresented in 48 short distance runners (29.2%), 54 short distance swimmers (40.7%), and 56 weightlifters (32.1%), compared to non-athletes (18.1%). In support, Gabbasov et al. (2013) found the frequency of the T allele at rs11549465 variant was higher in weightlifters (26.2%) and wrestlers (29.1%) than non-athletes (14.1%). In agreement with the evidence presented (although some contradictory findings exist), our data shows that the *HIF1A* Pro582Ser amino acid substitution (T allele) is associated with elite athlete status and playing position in rugby. It may be plausible to attribute this to both the increases in HIF-1α protein stability and transcriptional activity associated with the T allele (and the associated positive affect on anaerobic glycolysis), and to the involvement of HIF-1α in skeletal muscle hypertrophy (Tesch, 1988; Lunde et al., 2011).

The present study is the first to investigate *TRHR* rs7825532 polymorphism in elite rugby athletes. We found that all athletes (58.5% vs. 53.3%, $p = 0.032$; OR = 1.23 [CI = 0.96–1.58]) and forwards (59.6% vs. 53.3%, $p = 0.05$; OR = 1.29 [CI = 0.95–1.75]) had a greater proportion of T allele carriers compared to non-athletes. Thyrotropin-releasing hormone (TRH) is secreted by the hypothalamus, and exerts its effects by binding to thyrotropin-releasing hormone receptor (TRHR; encoded by the *TRHR* gene) on the surface of pituitary thyrotrophins (Matre et al., 1999). The binding of TRH with its receptor activates the inositol phospholipid-calcium-protein kinase C transduction pathway, which, in turn, stimulates secretion of thyroid-stimulating hormone (TSH, or thyrotropin) and prolactin from the anterior pituitary gland (Gershengorn, 1989). The TSH response to TRHR is the first step in the hormonal cascade of hypothalamic-pituitary-thyroid axis that leads to the release of tetraidothyronine (T_4, thyroxin) and triiodothyronine (T_3, the active form of thyroid

hormone), which are important in the development of vertebrate skeletal muscle (Larsson et al., 1994; Norenberg et al., 1996). Considering the important role of TRH-*TRHR* binding for thyroid function, one might expect an association between specific alleles and genotypes of polymorphisms in the *TRHR* gene (that can affect TRH-TRHR binding capacity) and exercise performance in humans. This hypothesis was tested by Liu et al. who found rs7832552 TT homozygotes had 2.5 kg more lean body mass than CC or CT genotypes ($p = 7.58 \times 10^{-8}$) (Liu et al., 2009). Accordingly, our results suggest elite rugby athletes may have some inherited advantage of increased muscle mass by carrying more T alleles at *TRHR* rs7832552.

This study does have limitations. Firstly, the focus was specifically on Caucasian elite rugby union athletes, so the results of this study might not be replicated in other populations. Therefore, extension to, and replication within groups of differing geographic ancestry and equivalent sport-specific investigations are needed to translate these findings more broadly. Although expected to some extent, a potential limitation of the present study is that three of the 40 analysis groups have shown a slight deviation from Hardy-Weinberg Equilibrium (HWE). However, due to our 100% duplication process adopted during genotyping, we are confident in the internal validity of the data used in this study. Secondly, this study is limited to ten common polymorphisms that were primarily selected because of previously reported associations with various aspects of strength performance in athletes and/or non-athletes. We strongly believe that many additional common polymorphisms, and most probably rare mutations as well, will be shown to be associated with strength performance in future and therefore; we suspect that the three SNPs associated in this study constitute only a small fraction of the genetic factors that could influence the strength of elite rugby athletes.

In conclusion, this chapter demonstrates that elite rugby athletes possess favourable genotypes and alleles at *HIF1A* rs11549465 and *TRHR* rs7832552 that may predispose them to increased strength and power capabilities when compared to the general population. In addition, it appears the inherited advantage provided by *HIF1A* rs11549465 is reflected in playing position. Furthermore, we have extended the main findings of Heffernan et al.'s work in relation to *FTO* rs9939609 and *ACTN3* rs1815739 using a larger athlete cohort, with differences in findings for *ACTN3* (discussed herein). It would be most useful to assess strength and power characteristics of rugby athletes that are known to be relevant to rugby performance, and that is addressed in Chapter 5.

Practical applications

These results highlight the relationships between *ACTN3* rs1815739, *HIF1A* rs11549465 and *FTO* rs9939609 and elite athlete status in rugby union forwards and backs. Whilst we acknowledge that the mechanisms by which these variants exert their effect to influence strength/power in rugby athletes needs more elucidation (e.g. by addressing the need for more functional data via transcriptomic, histological, and physiological studies (Wang et al., 2016; Bouchard, 2019)); we propose that elite rugby athletes might possess an inherited predisposition for achieving the strength/power characteristics required in their respective playing position, assisting them to achieve elite athletes status. Although many more genetic factors that influence strength/power (both in the general population and athletes) undoubtedly remain undiscovered, these findings provide a basis on which future, more comprehensive, genetic assessments may augment systems of identifying and nurturing talent in elite rugby union. Thus, we suggest that further research employs genotype-phenotype designs to identify those genetic markers that influence physical performance (Kikuchi et al., 2018; Guilherme et al., 2019; Homma et al., 2020) and strength/power (e.g. using the variants

studied in the present study in combination with novel variants associated with strength/power) in cohorts of elite rugby athletes of diverse geographic ancestries. The identification of many more genetic variants that influence strength/power, and those genetic markers identified via genotype-phenotype association designs in elite rugby, could be used for the development of polygenic profiles with the appropriate predictive potential (Charlier et al., 2017) and suitability for use in talent identification and athlete selection. However, it should be acknowledge that presently, there is not sufficient evidence to be used in talent identification or selection of athletes (Webborn et al., 2015; Varley et al., 2018; Pickering et al., 2019). Currently, performance assessments (such as vertical jumping and weightlifting performance) or traditional laboratory tests (such as isokinetic dynamometry and handgrip strength) are used to help identify young athletes with appropriate physiological potential (Section 1.6), and to guide them into suitable training and competition. Such assessments may, in the future, be augmented by assessment of polygenic profiles that comprise markers known to influence strength/power abilities of elite rugby athletes. Chapters 5 and 6 investigate whether the polymorphisms used in the present study are associated (individually or in combination using the 'total genotype score' (Williams and Folland, 2008)) with the CMJ and IMTP, and in-game performance, respectively – in an elite rugby union context.

CHAPTER 5

GENETIC ASSOCIATIONS OF STRENGTH- AND POWER-RELATED POLYMORPHISMS WITH COUNTERMOVEMENT JUMP AND ISOMETRIC MID-THIGH PULL IN ELITE MALE RUGBY ATHLETES

5.1 Introduction

Given the multifaceted demands for strength and power in elite rugby union, and the sport's increasing dependence on these demands, the development of muscular strength (Duthie, 2006) and the ability to impress and sustain force to produce high impulses (Zatsiorsky, 2003) and peak mechanical power outputs (Cormie et al., 2011) are considered fundamental to success in this sport of high physicality. Individual differences in strength/power between elite rugby athletes (Brazier et al., 2020), where environmental factors such as training (Bevan et al., 2010) and nutrition (Kelly et al., 2020) are of high quality and tightly controlled, highlights the contribution of heritable factors to the complexity of strength and power phenotypes in sport. Investigating genes and their common genetic variants (previously associated with strength and power phenotypes) in elite rugby athletes can, therefore, serve to elucidate some of the genetic contribution to the inter-individual variability in strength and power observed within this athletic population.

The anthropometric and physiological characteristics of elite rugby athletes vary according to playing position (Nicholas, 1997; Brazier et al., 2020), and reflect differences in rare and complex convergences of genetic and environmental factors (Costa et al., 2012; Guth and Roth, 2013). Accordingly, variations in many strength and power measures between elite athletes of different playing positions have been widely documented (Argus et al., 2009; Baker, 2013; Baker, 2017) and are supported by findings of Chapter 3 relating to the countermovement jump (CMJ) and isometric mid-thigh pull (IMTP), where differences in all variables investigated were observed between and within the forwards and backs. Established physical and physiological assessments such as those investigated in Chapter 3 play a large part of the selection processes in rugby, initially from an early age for talent identification (Smart, 2011), and throughout the athletes' careers for physical preparation (Gamble, 2004;

Cook et al., 2014), selection and monitoring purposes (Gannon, 2015). Undoubtedly, physiological assessments are, and most probably will remain, invaluable tools for the purposes just mentioned (Williams et al., 2014). However, considering the highly heritable nature of many intermediate phenotypes that affect neuromuscular function and other important sport-related traits that are considered imperative in elite sport (e.g. cognitive ability; motivation), athlete selection and monitoring can potentially be complemented by genetic information (Williams et al., 2014). Important to note here is that further progress in the field of sports genomics is required to sufficiently inform athletes' selection and preparation, based on the individuals' genetic predisposition to fundamental physiological traits for the sport concerned (Williams et al., 2014). Nevertheless, the inter-individual variability in strength and power within elite rugby athletes offers a great opportunity to study the genetic influence on these phenotypes.

Hughes et al. (2011) identified 22 common genetic polymorphisms associated with either a strength/power performance phenotype such as hand grip strength or one repetition maximum (1RM), or a related physiological attribute such as skeletal muscle fibre type composition Hughes et al. (2011). More recently, Ahmetov et al. (2016) demonstrated that the total number of genetic markers identified which have been reported to contribute in some way to elite athlete status is ~155, with ~62 of these related to strength/power athlete status (Ahmetov et al., 2016) (in fact the number has probably increased in the 6 years since publication). Williams and Folland (2008) introduced the concept of 'total genotype score' (TGS: expressed 0 – 100) that results from the accumulated combination of polymorphisms (and favourable alleles) that are candidates for explaining individual variations in a trait important for performance (Williams and Folland, 2008). TGS can be used to indicate the extent of any given individual's genetic predisposition for a trait using genetic

polymorphisms that genuinely contribute to the trait investigated (Hughes et al., 2011) and thus; confidence in each discrete genotype-phenotype association (that establishes the influence of specific genes and their polymorphisms to the trait of interest) is essential for the development of an effective polygenic analysis. Polygenic analysis is based on the notion that strength and power phenotypes are highly polygenic in nature – that is, multiple genetic factors influence the observed phenotype. For example, a polygenic profile of polymorphisms in six genes (including *ACE, ACTN3* and *NOS3* genes) was able to distinguish elite power athletes (Spanish jumpers and sprinters) from endurance athletes and non-athletes (Ruiz et al., 2010). More recently, Murtagh et al. (2020) have demonstrated that power, acceleration and sprint performance were associated with five strength/power SNPs, both individually and in combination (using TGS for polygenic analysis), in elite youth soccer players (Murtagh et al., 2020). Further, a study by Moreland et al. (2022) demonstrated that the likelihood of becoming an elite strength athlete depends on the carriage of a high number of strength-related alleles. The authors identified 28 SNPs associated with strength/power athlete status in elite Russian weightlifters and powerlifters; located in or near genes that have multiple functions including growth and development, metabolism, cell motility, neurogenesis, and intracellular transport (Moreland et al., 2022). The authors found that strength athletes possessed at least 22 'strength alleles', whereas 27.8% of Russian and 17.9% of European non-athletes had less than 22 'strength alleles'. In addition, 84% of the most highly elite strength athletes were carriers of at least 26 'strength alleles', whereas it was estimated that only 26.3% and 37.8% of the Russian and European populations are carriers of such a polygenic profile (Moreland et al., 2022).

To this author's knowledge, no study has sought to investigate whether the distribution of genotypes in strength/power related genes and polymorphisms can explain some of the

variation in established measures of strength and power in an elite rugby context, where the inter-individual variability observed in strength and power could indicate an advantageous genetic predisposition of certain genotypes for candidate genes. Consequently, the primary aim of this study was to explore whether ten polymorphisms (*ACE* rs4341, *ACTN3* rs1815739, *AMPD1* rs17602729, *FTO* rs9939609, *HIF1A* rs11549465, *NOS3* rs2070744, *KDR* rs1870377, *PTK2* rs7460 and rs7843014, *TRHR* rs7832552) are associated with CMJ and IMTP variables in 123 elite rugby athletes (76 and 47 athletes from forwards and backs playing positions, respectively). During athlete recruitment we have considered competitive standard and era during which that competitive level of competition was achieved (Section 2.1.1), and geographic ancestry (Section 2.1.3) and thus; potential biases in study population have been accounted for as much as possible. Associations were explored for each variant individually, and collectively by using a total genotype score (TGS) and a favourable allele count (FAC). Athletes of different playing positions were analysed as a single group to maintain the largest sample sizes for all analyses, in particular when using the additive model for polymorphisms with a low minor allele frequency. It was hypothesised that athletes with the favourable genotype for each individual SNP would possess greater strength/power than those with an alternative genotype. A second hypothesis was that the polygenic influence of the variants (expressed as TGS and FAC) would be reflected in the athletes' strength and power as measured by the CMJ and IMTP, and anticipate those with higher TGS and FAC will demonstrate more strength and power.

5.2 Methods

Detailed descriptions of the 123 elite rugby athletes participants in this study are provided in Section 2.1 (Chapter 2: General Methods). Procedures for DNA sample collection, DNA extraction, and genotyping are included in Section 2.4. Procedures for conducting the CMJ

and IMTP tests, and for obtaining the five measures from the force-time histories produced in both tests are included in Section 2.2. Therefore, only brief descriptions of participants, and statistical analyses used in this investigation are detailed in this Section.

The author's contribution to all procedures involved for sample collection, DNA extraction and genotyping for the 123 elite rugby athletes is provided in Section 2.4 (General Methods). The author's contribution for all procedures involved in the collection and processing of IMTP and CMJ data for the 123 elite rugby athletes is provided in Section 2.2. Ethical approval for sample collection, DNA extraction and genotyping was granted by Manchester Metropolitan University, University of Glasgow, University of Cape Town and Northampton University ethics committees. Procedures for the collection of phenotype data were approved by the local Ethics Committee of Swansea University. All procedures were conducted in accordance with the guidelines in the Declaration of Helsinki (World Medical Association, 2013).

5.2.1 Participants

Participants were 123 elite Caucasian male rugby union athletes including 68% British, 28% Irish, 2% South African and 2% from other nationalities. Physical characteristics are presented in Table 5.1. Sensitivity analysis was conducted in G*Power (Faul et al., 2007), with alpha (α) set at 0.05, power of 80%, and a sample size ranging from 78 (for *PTK2* rs7843014) to 122 (for *ACE* rs4341 and *ACTN3* rs1815739) athletes. The results indicated that the lowest detectable effect size (Cohen's *d*) ranged from 0.45 to 0.57, depending on total sample size.

Table 5.4 Physical characteristics of all athletes, forwards and backs. Data presented are means (SD).

	Body mass (kg)	Height (m)	BMI ($kg \cdot m^{-2}$)	Age (yr)
Athletes (n=123)	103.0 (12.8)	1.86 (0.08)	29.7 (3.1)	27 (4)
Forwards (n=76)	110.9 (8.3)	1.88 (0.07)	31.3 (2.9)	27 (4)
Backs (n=47)	90.1 (7.1)	1.82 (0.06)	27.2 (1.4)	26 (4)

Athletes form part of GENESIS and RugbyGene, and were also included in Chapters 3 and 4 in this thesis. All athletes were considered 'elite', having competed regularly (>5 matches) since 1995 in the highest professional league in the UK, Ireland or South Africa. From all athletes, 59% had competed at an international level, and 99% of those international athletes represented a 'High Performance Union' (Regulation 16, worldrugby.org). Data for international status were confirmed as of 1st July 2019. Athletes were informed of the rationale and broad potential applications of the study, and procedures and risks associated with their participation; after which they provided written informed consent to participate.

5.2.2 Laboratory-based strength/power measurements

Measures of strength and power were obtained for CMJ (maximum concentric power, relative maximum concentric power, and jump height) and IMTP (peak force and relative peak force) performed on a force platform. The measures were acquired from the force-time histories produced using established procedures developed by Professor Liam Kilduff and his research team at Swansea University. Detailed descriptions of all procedures and equipment used are in Section 2.2.

5.2.3 Procedures for DNA sample collection, DNA extraction and genotyping

All participants provided a whole blood, saliva or buccal swab sample, from which DNA was subsequently extracted and analysed to obtain genotype data for 10 SNPs. Detailed descriptions of all procedures are in Section 2.4.

5.2.4 Statistical analysis

Genotype and allele frequency distributions for 10 SNPs were assessed for compliance with Hardy-Weinberg equilibrium using Pearson's chi-square (χ^2). One-way ANOVA was conducted to evaluate differences in variables derived from IMTP and CMJ between genotypes in the additive model (AA vs. Aa vs. aa). Independent samples *t*-test was used to identify differences in variables between genotype using the dominant (AA+Aa vs. aa) and recessive (AA vs. Aa+aa) models. Recessive and dominant models were taken with respect to the allele considered advantageous for strength and power. For example, for *TRHR* rs7832552, with the T allele considered advantageous, the recessive model was TT vs. CT+CC, whist the dominant model was TT+CT vs. CC. Table 5.2 shows the genes (and their candidate protein) and the ten polymorphisms investigated; the alleles (with the preferential allele underlined) and the minor allele frequency for each polymorphism. Table 1.3 in Chapter 1 includes key studies and findings supporting the preferential allele for the generation of muscular strength and power.

Table 5.5 Genes and polymorphisms investigated. The preferential allele (underlined in the penultimate column) is thought to be advantageous in terms of muscular strength and power – in accordance with the findings of previous literature, with some studies referenced in Table 1.3 (Chapter 1 – Literature Review).

Gene symbol (HGNC) and rs number	Candidate gene (HGNC)	Candidate protein (UniProt)	Alleles-preferential Underlined	MAF
ACE *rs4341*	Angiotensin I converting enzyme	Angiotensin converting enzyme	I/D	**I**: 0.43
ACTN3 rs1815739	Actinin Alpha 3	Alpha-actinin-3	C/T	**T**: 0.43
AMPD1 rs17602729	Adenosine Monophosphate Deaminase 1	AMP deaminase 1	G/A*	**A**: 0.12
FTO rs9939609	FTO alpha-ketoglutarate dependent deoxygenase	Alpha-ketoglutarate-dependent dioxygenase FTO	T/A	A: 0.41
HIF1A rs11549465	Hypoxia Inducible Factor 1 subunit alpha	Hypoxia-inducible factor 1-alpha	C/T*	**T**: 0.10
KDR rs1870377	Kinase insert domain receptor	Vascular endothelial growth factor receptor 2	T/A	**A**: 0.24
NOS3 rs2070744	Nitric Oxide Synthase 3	Nitric Oxide Synthase 3	C/T	**C**: 0.44
PTK2 rs7460	Protein tyrosine kinase 2	Focal adhesion kinase 1	A/T	**T**: 0.48
PTK2 rs7843014	Protein tyrosine kinase 2	Focal adhesion kinase 1	A/C	**C**: 0.46
TRHR rs7832552	Thyrotropin-releasing hormone receptor	Thyrotropin-releasing hormone receptor	C/T	**T**: 0.27

*Indicates homozygotes for the minor allele frequency (MAF; on the side of the asterisks) that are very low in number to allow an additive comparison. In that case, a recessive/dominant model that includes the homozygotes for the MAF is used. MAF for all SNPs is shown in the far right end column of Table.

The polygenic influence of 7 SNPs (*ACE* rs4341, *ACTN3* rs1815739*, AMPD1* rs17602729, *FTO* rs9939609, *KDR* rs1870377, *NOS3* rs2070740 and *TRHR* rs7832552) was assessed using TGS and FAC. Using those 7 SNPs provided the best compromise between including a high number of SNPs and maintaining a high number of athletes (n = 79), because not all athletes were genotyped for all SNPs. For each approach, the allele considered advantageous for strength and power (Table 5.2; Table 1.3 [Section 1.8 in introduction]) was identified from previous literature (Section 1.9). To determine TGS, each genotype within each SNP was allocated a genotype score (GS) of 0, 1 or 2. Accordingly, homozygotes for the favourable allele were given a score of 2, heterozygotes scored 1, and homozygotes for the other allele scored 0. TGS for each participant was thus given by:

$$\frac{100}{2\times 7} \times (GS_{ACE} + GS_{ACTN3} + GS_{AMPD1} + GS_{FTO} + GS_{KDR} + GS_{NOS3} + GS_{TRHR})$$

where (7) is the number of SNPs that constitute the TGS.

Pearson's correlation coefficient was calculated to investigate relationships between TGS and each of the five strength/power variables. FAC was determined by summing the number of favourable alleles possessed by each participant, for each of the seven SNPs. Thus, FAC for each participant could range from 0 to 14. Participants were categorised either into three groups (0-7, 8-9 or 10-14 favourable alleles) or two groups (0-8 or 9-14 favourable alleles) based on their FAC, with those numbers of favourable alleles providing approximately equal group sizes. One-way ANOVA and independent *t*-test were used to detect differences between FAC categories in each variable.

Effect size was calculated using the standardised mean difference (Cohen's *d*) with 95% confidence limits. This allowed for the determination of meaningful differences for those comparisons that have reached statistical significance. The pooled standard deviation (calculated from the standard deviations of the two groups being compared) was used in the denominator in the calculation of Cohen's *d*. Confidence intervals (CI) for Cohen's *d* were estimated using the formula provided by Hedges and Olkin (2014):

$$95\%\ \text{CI for Cohen's } d = [d - 1.96 \times \sigma(d);\ d + 1.96 \times \sigma(d)]$$

Where, $\sigma(d)$ = standard deviation of Cohen's *d* and calculated as shown below, and; N_1 and N_2 = number of cases in groups 1 and 2, respectively.

$$\sigma(d) = \sqrt{\frac{N_1 + N_2}{N_1 \times N_2} + \frac{d^2}{2(N_1 + N_2)}}$$

Cohen's *d* was interpreted as follows: 0.2 or less is a small effect size, about 0.5 is a moderate effect size, and 0.8 or more is a large effect size (Cohen, 1988). The Statistical Package for Social Sciences (version 26.0, SPSS Inc., Chicago, IL, USA) was used for the analyses, and alpha was set at 0.05. Probability values were subjected to Benjamini-Hochberg (B-H) correction (Benjamini and Hochberg, 1995) to control for false discovery rate (FDR). FDR was set at 20% and the reported *p* values are those significant after B-H correction.

5.3 Results

Genotype and allele frequency distributions were in Hardy-Weinberg equilibrium, except for *TRHR* rs7832552 (χ^2 = 3.851, *p* = 0.049). Strength/power measures for CMJ and IMTP according to genotype for all SNPs are presented in Table 5.3.

IMTP peak force and relative peak force – ***NOS3*** rs2070744

Carriers of the C allele at *NOS3* rs2070744 had 9.3% greater IMTP peak force than TT homozygotes (mean difference 250 N, *p* = 0.005; Cohen's *d* = 0.52 [CI = 0.15 – 0.89]; Figure 5.1a). In addition, CC homozygotes had 15.3% greater peak force than TT homozygotes (mean difference 413 N, *p* = 0.003; Cohen's *d* = 0.89 [CI = 0.30 – 1.47]), and CT heterozygotes had 7.5% greater peak force than TT homozygotes (mean difference 201 N, *p* = 0.035; Cohen's *d* = 0.44 [CI = 0.05 – 0.83]).

Furthermore, C allele carriers had 10.7% greater IMTP relative peak force than TT homozygotes (mean difference 2.8 $N \cdot kg^{-1}$, *p* = 0.003; Cohen's *d* = 0.58 [CI = 0.20 – 0.95];

Figure 5.1b). In addition, CC homozygotes had 13.0% greater relative peak force than TT homozygotes (mean difference 3.4 $N \cdot kg^{-1}$, $p = 0.018$; Cohen's $d = 0.73$ [CI = 0.15 – 1.31]), and CT heterozygotes had 9.9% greater relative peak force than TT homozygotes (mean difference 2.6 $N \cdot kg^{-1}$, $p = 0.009$; Cohen's $d = 0.55$ [CI = 0.15 – 0.95]).

Table 5.3 Strength and power measures according to genotype and allele carriage for 10 SNPs. Data are mean (SD).

		IMTP		CMJ		
		Peak force (N)	Relative peak force ($N \cdot kg^{-1}$)	Maximum concentric power (W)	Relative maximum concentric power ($W \cdot kg^{-1}$)	Jump height (cm)
ACE rs4341 n=122	DD	n=27 2936 (538)	n=27 28.8 (5.4)	n=25 5366 (780)	n=25 52.7 (7.2)	n=25 37.1 (7.0)
	ID	n=67 2797 (518)	n=67 27.8 (4.7)	n=69 5500 (728)	n=69 54.8 (7.3)	n=67 38.9 (7.1)
	II	n=26 2872 (393)	n=24 26.8 (5.2)	n=24 5511 (601)	n=24 51.8 (7.5)	n=24 34.5 (7.2)
	D carriers	2837 (524)	28.0 (4.9)	5464 (740)	54.2 (7.3)	38.4 (7.1)
	I carriers	2818 (485)	27.5 (4.8)	5502 (694)	54.0 (7.4)	37.7 (7.3)
ACTN3 rs1815739 n=122	CC	n=36 2875 (430)	n=35 28.0 (4.4)	n=34 5489 (669)	n=34 54.0 (7.6)	n=33 37.0 (8.2)
	CT	n=57 2840 (534)	n=57 27.6 (5.6)	n=58 5500 (746)	n=58 53.3 (7.5)	n=57 37.0 (7.2)
	TT	n=27 2801 (523)	n=26 27.9 (4.4)	n=26 5456 (762)	n=26 54.8 (7.5)	n=26 39.5 (5.9)
	C carriers	2853 (494)	27.7 (5.1)	5496 (715)	53.5 (7.5)	37.0 (7.5)
	T carriers	2827 (528)	27.7 (5.2)	5487 (747)	53.8 (7.5)	37.8 (6.9)
AMPD1 rs17602729 n=84	GG	n=58 2720 (454)	n=56 26.3 (4.7)	n=56 5465 (781)	n=56 53.4 (8.1)	n=55 36.5 (6.3)
	GA	n=23 2909 (556)	n=23 29.1 (5.1)	n=23 5397 (548)	n=23 54.4 (7.3)	n=22 37.7 (9.4)
	AA	n=1 2566 (n/a)	n=1 21.5 (n/a)	n=1 6065 (n/a)	n=1 50.9 (n/a)	n=1 27.0 (n/a)
	G carriers	2773 (489)	27.1 (4.9)	5445 (718)	53.7 (7.9)	36.8 (7.2)
	A carriers	2894 (548)	28.8 (5.2)	5425 (552)	54.3 (7.2)	37.2 (9.4)
FTO rs9939609 n=116	TT	n=38 2804 (516)	n=37 28.0 (4.7)	n=35 5234 (801)	n=35 53.1 (8.3)	n=35 39.1 (7.4)
	TA	n=51 2789 (455)	n=50 27.7 (5.0)	n=50 5550 (638)	n=50 55.3 (7.5)	n=49 37.3 (7.3)
	AA	n=25 2931 (507)	n=25 27.4 (5.6)	n=27 5712 (739)	n=27 53.3 (6.4)	n=26 36.7 (6.9)
	T carriers	2796 (479)	27.9 (4.9)	5420 (722)	54.4 (7.8)	38.0 (7.4)
	A carriers	2836 (474)	27.6 (5.2)	5606 (675)†	54.6 (7.1)	37.1 (7.1)
HIF1A rs11549465 n=29	CC	n=24 2810 (413)	n=23 27.6 (4.6)	n=23 5378 (593)	n=23 53.1 (8.0)	n=22 34.9 (6.8)
	CT	n=5 2646 (467)	n=5 25.2 (3.2)	n=5 5576 (593)	n=5 53.5 (7.1)	n=5 39.1 (9.1)
	TT	n=0 (n/a)	n=0 (n/a)	n=0 (n/a)	n=0 (n/a)	n=0 (n/a)
	C carriers	2782 (418)	27.2 (4.5)	5413 (586)	53.2 (7.7)	35.6 (7.3)
	T carriers	(n/a)	(n/a)	(n/a)	(n/a)	(n/a)

Table 5.3 (continued)

		IMTP				CMJ					
		Peak force (N)		Relative Peak force ($N \cdot kg^{-1}$)		Maximum Concentric Power (W)		Relative Maximum Concentric Power ($W \cdot kg^{-1}$)		Jump Height (cm)	
KDR rs1870377 n=116	TT	n=71	2811 (504)	n=70	27.7 (5.0)	n=71	5514 (738)	n=71	54.7 (8.1)	n=69	37.3 (7.5)
	TA	n=38	2840 (457)	n=37	27.7 (4.7)	n=37	5367 (755)	n=37	52.5 (6.4)	n=37	38.4 (7.2)
	AA	n=11	3048 (602)	n=11	27.9 (5.8)	n=10	5741 (372)	n=10	52.7 (6.6)	n=10	36.3 (6.0)
	T carriers		2821 (486)		27.7 (4.9)		5464 (743)		53.9 (7.6)		37.7 (7.4)
	A carriers		2887 (494)		28.8 (4.9)		5447 (705)		52.6 (6.3)		37.9 (6.9)
NOS3 rs2070744 n=120	TT	n=49	2691 (419)	n=49	26.1 (4.3)	n=49	5419 (742)	n=49	52.7 (7.2)	n=48	36.6 (6.2)
	CT	n=53	2892 (491)*	n=51	28.7 (5.1)*	n=50	5544 (749)	n=50	55.3 (7.5)	n=49	38.5 (7.9)
	CC	n=16	3104 (591)§	n=16	29.5 (5.7)§	n=17	5562 (627)	n=17	53.2 (8.0)	n=17	37.9 (8.2)
	T carriers		2795 (466)†		27.4 (4.9)		5482 (744)		54.0 (7.4)		37.5 (7.1)
	C carriers		2941 (519)‡		28.9 (5.2)‡		5549 (715)		54.8 (7.6)		38.3 (7.9)
PTK2 rs7460 n=78	AA	n=20	2796 (462)	n=20	28.6 (4.3)	n=21	5187 (595)	n=21	53.3 (7.7)	n=20	36.0 (7.1)
	AT	n=39	2995 (519)	n=38	29.5 (5.3)	n=38	5695 (661)§	n=38	56.1 (7.1)	n=38	39.8 (6.2)§*
	TT	n=18	3004 (467)	n=18	28.1 (5.2)	n=18	5581 (653)	n=18	52.0 (6.9)	n=18	34.3 (6.2)
	A carriers		2928 (505)		29.2 (4.9)		5514 (679)		55.1 (7.4)		38.5 (6.7)†
	T carriers		2998 (499)		29.0 (5.3)		5658 (655)‡		54.8 (7.2)		38.0 (6.7)
PTK2 rs7843014 n=78	AA	n=25	2961 (501)	n=25	28.0 (5.5)	n=25	5516 (637)	n=25	52.1 (7.6)	n=25	34.6 (6.3)
	AC	n=38	3017 (505)	n=37	29.5 (5.0)	n=37	5655 (714)	n=37	55.2 (6.7)	n=37	39.0 (6.5)*
	CC	n=14	2723 (414)	n=14	29.0 (3.8)	n=15	5244 (545)	n=15	56.0 (8.0)	n=14	38.6 (7.5)
	A carriers		2995 (500)#		28.9 (5.3)		5599 (682)#		54.0 (7.2)		37.2 (6.7)
	C carriers		2938 (496)		29.3 (4.7)		5536 (690)		55.4 (7.0)#		38.9 (6.7)‡
TRHR rs7832552 n=81	CC	n=28	2589 (408)	n=28	25.2 (4.3)	n=30	5391 (871)	n=30	52.5 (8.2)	n=30	36.9 (8.3)
	CT	n=45	2851 (520)	n=43	27.9 (5.2)	n=41	5534 (714)	n=41	54.8 (8.3)	n=39	37.0 (6.2)
	TT	n=6	2803 (445)	n=6	28.8 (5.8)	n=6	5407 (386)	n=6	55.6 (8.1)	n=6	37.8 (12.8)
	T carriers		2854 (508)†		28.0 (5.2)†		5517 (680)		54.9 (8.2)		37.1 (7.2)
	C carriers		2750 (494)		26.8 (5.0)		5473 (782)		53.8 (8.3)		36.9 (7.1)

* different from genotype with lowest value ($p < 0.05$). § different from genotype with lowest value ($p < 0.01$). *§ different from both genotypes. †different from homozygote counterparts ($p < 0.05$). ‡different from homozygote counterparts ($p < 0.01$). #different from homozygote counterparts ($0.060 \leq p \leq 0.065$, significant after B-H correction). Slight deviation in H-W equilibrium for *TRHR* rs7832552 ($\chi^2 = 3.851$, $p = 0.049$).

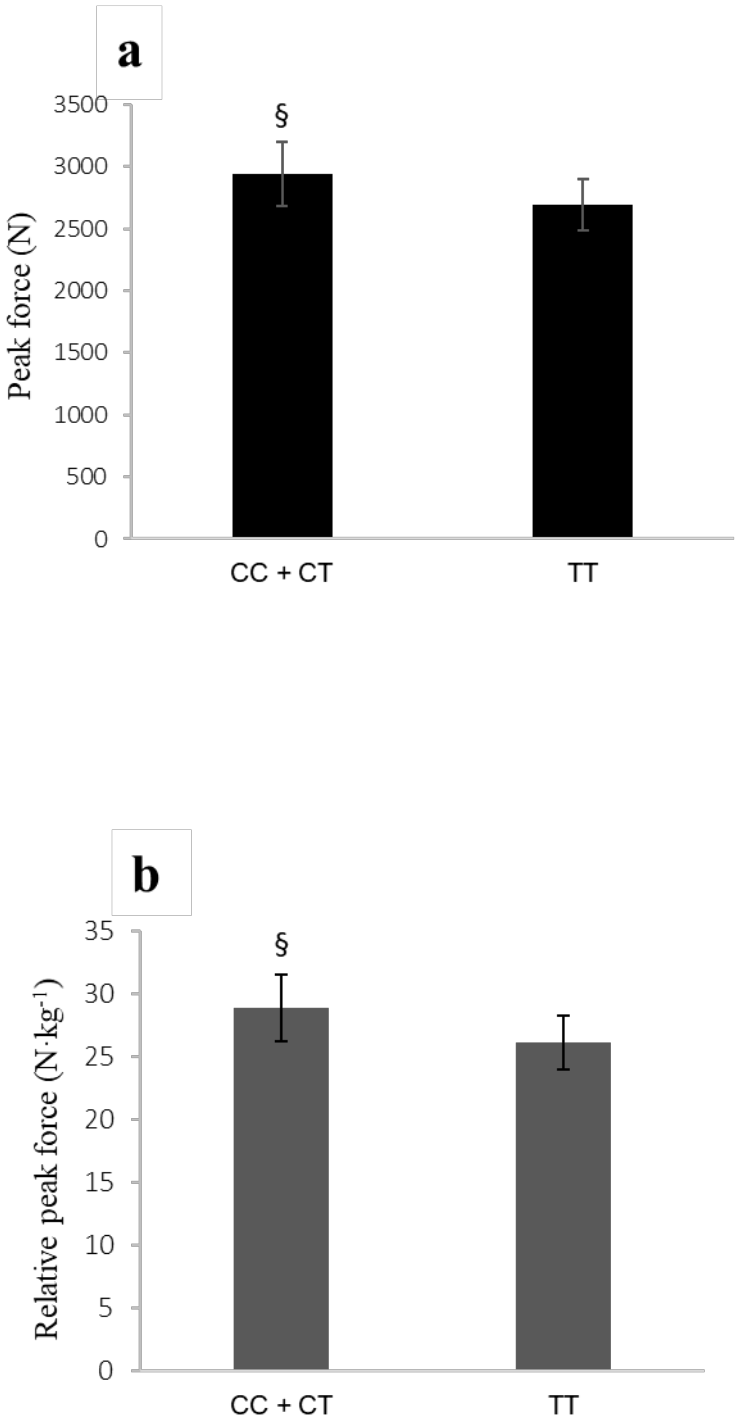


Figure 5.1 IMTP peak force (**a**) and IMTP relative peak force (**b**) for ***NOS3*** rs2070744, both in the recessive model. §Different from TT ($p < 0.01$)

IMTP peak force and relative peak force – ***TRHR*** rs7832552

T allele carriers at *TRHR* rs7832552 had 10.2% greater IMTP peak force (mean difference 265 N, $p = 0.024$; Cohen's $d = 0.56$ [CI = 0.09 – 1.03]; Figure 5.2a) and 11.1% greater IMTP relative peak force (mean difference 2.8 $N \cdot kg^{-1}$, $p = 0.017$; Cohen's $d = 0.14$ [CI = -0.33 – 0.60]; Figure 5.2b) than CC homozygotes.

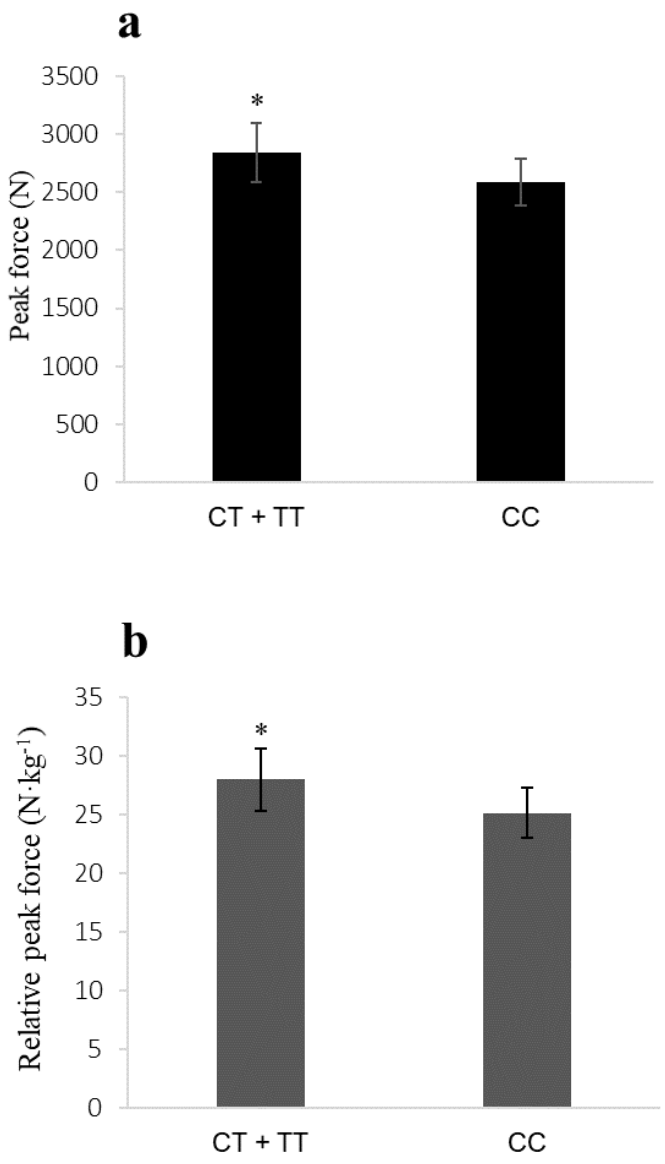


Figure 5.2 IMTP peak force (**a**) and IMTP relative peak force (**b**) for ***TRHR*** rs7832552, both in the dominant model. *Different from CC ($p < 0.05$). Deviation from Hardy-Weinberg equilibrium for all athletes; $\chi^2 = 3.851$, $p = 0.049$.

CMJ maximum concentric power and jump height – ***PTK2*** rs7460

T allele carriers at *PTK2* rs7460 had 9.1% greater CMJ maximum concentric power than AA homozygotes (mean difference 471 W, p = 0.005; Cohen's d = 0.74 [CI = 0.22 – 1.25]; Figure 5.3a). In addition, AT heterozygotes had 9.8% greater maximum concentric power than AA homozygotes (mean difference 508 W, p = 0.005; Cohen's d = 0.79 [CI = 0.24 – 1.35]).

On the other hand, A allele carriers achieved 12.2% greater CMJ height than TT homozygotes (mean difference 4.2 cm, p = 0.022; Cohen's d = 0.64 [CI = 0.10 – 1.18]; Figure 5.3b). Furthermore, AT heterozygotes achieved 15.3% (mean difference 5.5 cm, p = 0.004; Cohen's d = 0.89 [CI = 0.30 – 1.47]) and 10.6% (mean difference 3.8 cm, p = 0.039; Cohen's d = 0.58 [CI = 0.03 – 1.13]) greater CMJ height than TT homozygotes and AA homozygotes respectively.

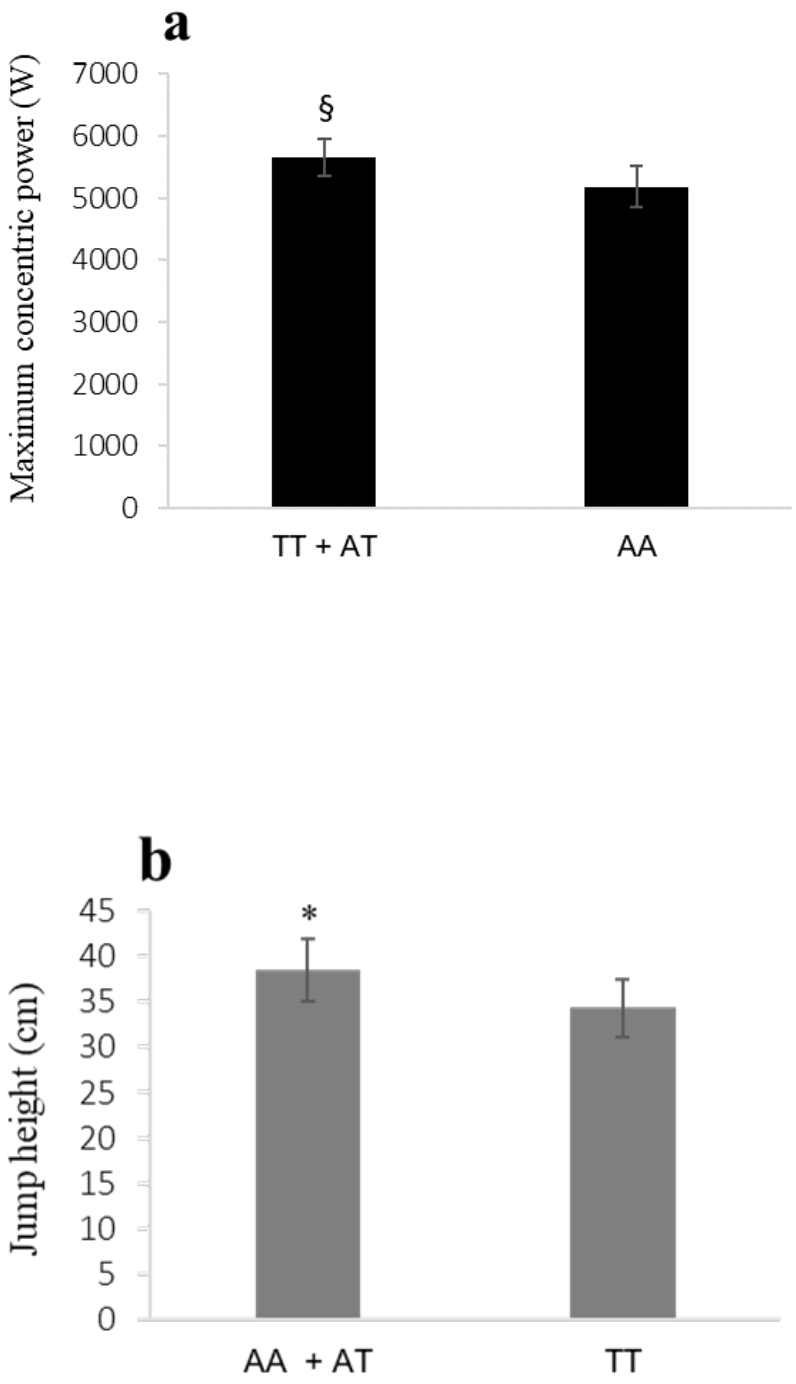


Figure 5.3 CMJ maximum concentric power (**a**) and jump height (**b**) for ***PTK2*** rs7460 in the recessive and dominant models, respectively. §different from AA ($p < 0.01$). *different from TT ($p < 0.05$).

IMTP peak force, and CMJ maximum concentric power, relative maximum concentric power and jump height – ***PTK2*** rs7843014.

A allele carriers at ***PTK2*** rs7843014 had 10.0% greater IMTP peak force than CC homozygotes (mean difference 272 N, $p = 0.062$ (significant after B-H correction); Cohen's $d = 0.56$ [CI = -0.03 – 1.15]). For the CMJ, A allele carriers at ***PTK2*** rs7843014 had 6.8% greater maximum concentric power than CC homozygotes (mean difference 355 W, $p = 0.065$ (significant after B-H correction); Cohen's $d = 0.54$ [CI = -0.03 – 1.11]). In addition, C allele carriers had 6.3% greater CMJ relative maximum concentric power than AA homozygotes (mean difference 3.3 $W \cdot kg^{-1}$, $p = 0.060$ (significant after B-H correction); Cohen's $d = 0.46$ [CI = -0.02 – 0.94]). Furthermore, C allele carriers achieved 12.4% greater CMJ height than AA homozygotes (mean difference 4.3 cm, $p = 0.009$; Cohen's $d = 0.65$ [CI = 0.16 – 1.21]; Figure 5.4); and AC heterozygotes achieved 12.7% greater CMJ height than AA homozygotes (mean difference 4.4 cm, $p = 0.013$; Cohen's $d = 0.68$ [CI = 0.16 – 1.21]).

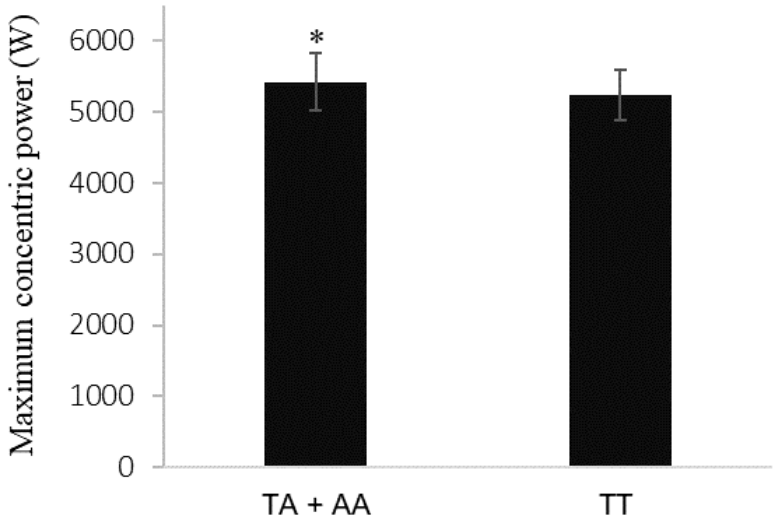


Figure 5.4 CMJ height for ***PTK2*** rs7843014 genotype in the recessive model. §Different from AA ($p < 0.01$).

CMJ variables – ***FTO*** rs9939609

Carriers of the A allele at ***FTO*** rs9939609 achieved 7.1% greater CMJ maximum concentric power than TT homozygotes (mean difference 372 W, p = 0.012; Cohen's d = 0.52 [CI = 0.11 – 0.92]; Figure 5.5).

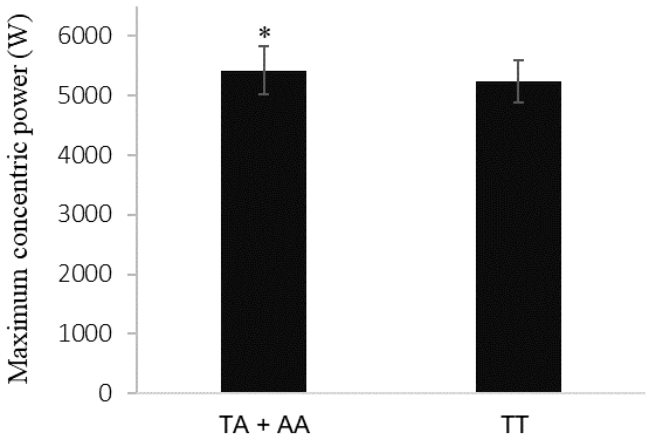


Figure 5.5 CMJ maximum concentric power for ***FTO*** rs9939609 in the recessive model. *different from TT (p < 0.05).

There were no differences between FAC categories when two groups (1-8 or 9-14 favourable alleles) or three groups (1-7, 8-9 or 10-14 favourable alleles) were considered (p value for two groups (three groups): IMTP peak force, p = 0.699 (p = 0.866); relative peak force, p = 0.278 (p = 0.891); CMJ maximum concentric power, p = 0.466 (p = 0.381); CMJ relative maximum concentric power, p = 0.456 (p = 0.708); CMJ height, p = 0.526 (p = 0.664).

In addition, no associations were observed between TGS and IMTP peak force (r = -0.038, p = 0.743), IMTP relative peak force (r = 0.079, p = 0.503), CMJ maximum concentric power (r = -0.126, p = 0.283), CMJ relative maximum concentric power (r = 0.061, p = 0.605) or CMJ height (r = 0.085, p =0.476). Figure 5.6 shows TGS and frequency (%) of athletes at different TGS; and IMTP peak force for clusters of athletes with different TGS.

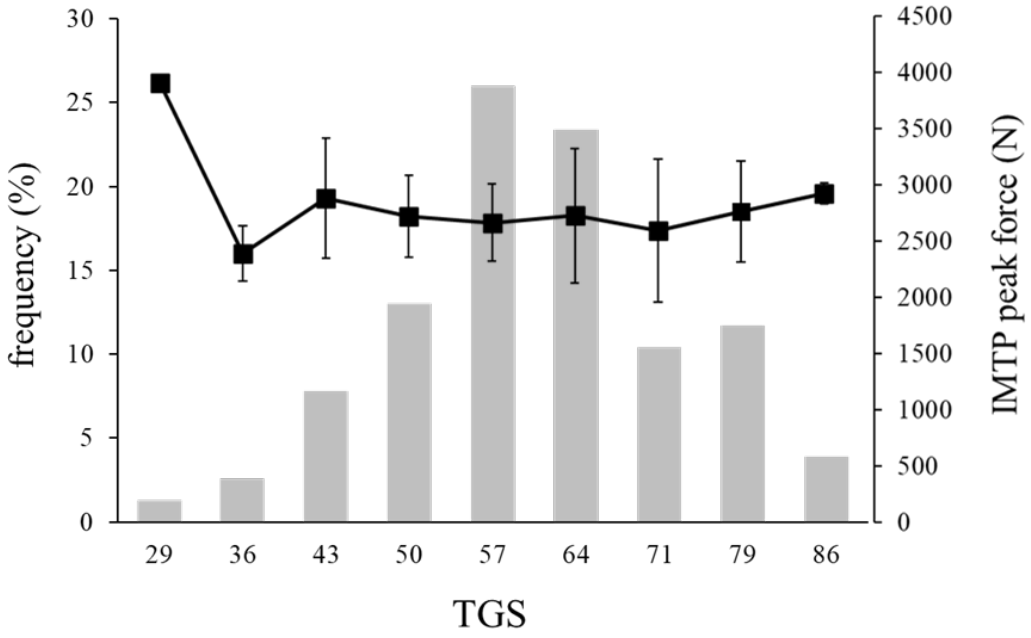


Figure 5.6 Total genotype score (TGS) and frequency (%) of athletes at different TGS (grey bars and left vertical axis). IMTP peak force for clusters of athletes with different TGS (black squares with solid line and right vertical axis). IMTP peak force data are means with error bars showing standard deviations.

5.4 Discussion

The present study examined whether 10 polymorphisms previously associated with strength and power in the literature were associated with IMTP and CMJ variables in elite rugby union athletes. Our results demonstrate that *TRHR* rs7832552, *NOS3* rs2070744 and *PTK2* rs7843014 were associated with IMTP, whilst *FTO* rs9939609 and *PTK2* (rs7843014 and rs7460) were associated with CMJ – all significant associations after applying false discovery rate. The direction of the association was not always as expected, thus, we reject our first hypothesis. Additionally, we have not observed associations between either TGS or FAC and any of the five variables. This could be attributed, in part, to the direction of some of the associations we report herein. Neverthless, we reject our second hypothesis. Of particular

interest was that individual SNPs were associated with either IMTP or CMJ variables but not with both, with an exception for *PTK2* rs7843014.

For *TRHR* rs7832552, T allele carriers achieved 10.2% greater IMTP peak force (mean difference 265 N, $p = 0.024$; Cohen's $d = 0.56$ [CI = 0.09 – 1.03]; Figure 5.2a) and 11.1% greater relative peak force (mean difference 2.8 $N \cdot kg^{-1}$, $p = 0.017$; Cohen's $d = 0.14$ [CI = -0.33 – 0.60]; Figure 5.2b) than CC homozygotes. Notably, there was a tendency for a significant difference regarding both peak force ($p = 0.079$) and relative peak force ($p = 0.054$) between genotypes in the additive model. Neverthless, our results indicate that carriers of the T allele at *TRHR* rs7832552 might possess an inherited advantage to develop maximum voluntary isometric force, with such advantage remaining consistent for force generation relative to body mass. In a GWA study, Liu et al. (2009) reported that rs7832552 and rs16892496 within *TRHR* were positively associated with lean body mass. Participants carrying unfavourable genotypes had, on average, 2.7 kg (rs7832552) and 2.6 kg (rs 16892496) lower lean body mass than those with alternative genotypes. Based on these observations, Miyamoto-Mikami et al. (2017) included rs7832552 in a panel of 21 SNPs for examination in 211 Japanese sprint/power track and field athletes and 649 Japanese controls. The authors reported that the T allele at rs7832552 was one of three SNPs (together with *AGT* rs699, and *CNTFR* rs41274853) that tended to show an association with sprint/power athlete status, but was not significant after correction for multiple testing. The present study extends the findings of Chapter 4 in this thesis, in which *TRHR* rs7832552 was associated with elite athlete status in rugby, by reporting here for the first time that the T allele at *TRHR* rs7832552 appears advantageous for elite rugby athlete – via an increased capability of generating maximal voluntary isometric strength.

For *NOS3* rs2070744, we found that C allele carriers achieved 9.3% greater IMTP peak force (mean difference 250 N, $p = 0.005$; Cohen's $d = 0.52$ [CI = 0.15 – 0.89]; Figure 5.1a) and 10.7% greater relative peak force (mean difference 2.8 $N \cdot kg^{-1}$, $p = 0.003$; Cohen's $d = 0.58$ [CI = 0.20 – 0.95]; Figure 5.1b) than TT homozygotes. In addition, CC homozygotes and CT heterozygotes achieved greater peak force (CC vs TT: mean difference 413 N (15.3%), $p = 0.003$; Cohen's $d = 0.89$ [CI = 0.30 – 1.47]. CT vs TT: mean difference 201 N (7.5%), $p = 0.035$; Cohen's $d = 0.44$ [CI = 0.05 – 0.83]) and relative peak force (CC vs TT: mean difference 3.4 $N \cdot kg^{-1}$ (13.0%), $p = 0.018$; Cohen's $d = 0.73$ [CI = 0.15 – 1.31]. CT vs TT: mean difference 2.6 $N \cdot kg^{-1}$ (9.9%), $p = 0.009$; Cohen's $d = 0.55$ [CI = 0.15 – 0.95]) than their TT counterparts. Interestingly, these results show a positive additive effect of the C allele on both IMTP variables, in that each C allele contributed to an additional ~200 N (7.5%) in peak force and an average of 1.7 $N \cdot kg^{-1}$ (6.5%) in relative peak force, although the differences between CT and CC homozygotes (for both variables) were not significant. Based on the multitude of biological systems in which nitric oxide is involved, it was suggested that variations within *NOS3* could explain some of the inter-individual variability of human health and exercise phenotypes (Bray et al., 2009; Wolfarth et al., 2008). The C allele at *NOS3* rs2070744 (T → C transition) results in significantly reduced gene promoter activity and reduced endothelial nitric oxide synthesis (Nakayama et al., 1999). Thus, given that endurance performance is limited by local blood flow, and the fact that the C allele at rs2070744 is associated with reduced endothelial nitric oxide synthesis (that could reduce oxygenated blood flow to working muscles), *NOS3* was proposed as a potential candidate for endurance performance. Based on these findings, Gómez-Gallego et al. (2009) hypothesised that the T allele would be overrepresented in endurance oriented compared with power oriented athletes and non-athletes. In contrast to their hypothesis, the authors found that the T allele and the TT genotype were overrepresented in power athletes compared to either

endurance athletes or controls. In support of this, Sessa et al. (2011) reported an overrepresentation of the T allele at rs2070744 in 29 national level Italian power oriented athletes compared to 38 controls, and Drozdovska et al. (2013) reported an overrepresentation of the T allele in 90 Ukrainian power oriented athletes (males and females, and mixed competitive level) compared separately with 84 endurance athletes and 283 controls. In considering these findings, we postulated that athletes carrying the T allele at rs2070744 could produce greater force and power in the IMTP and CMJ variables compared to CC genotype. In contrast, the present study showed a potential benefit of the C allele for maximal isometric force production, with the positive additive effect of each C allele reflected in both IMTP variables. Notably, in Chapter 4 of this thesis there were no differences in genotype and/or allele frequency distribution at rs2070744 between all athletes and non-athletes, or between either group (forwards or backs) and non-athletes and, therefore, no support for any of the highlighted studies (irrelevant of the direction of the association found) was provided. The body of evidence in relation to rs2070744 and other polymorphisms in *NOS3* [rs1799983 SNP (Glu298Asp); dinucleotide repeat D7S636 (Freedman et al., 2000)] seems contradictory, and this could be attributed, in part, to previous findings indicating that common *NOS3* alleles (T allele at rs2070744 and G allele at rs1799983; ~56% and ~66% in European populations, respectively) were advantageous for both power and endurance oriented performance (Ahmetov and Fedotovskaya, 2015). Important to note is that the present study explored genotype-phenotype associations exclusively in elite rugby union athletes, and therefore, a direct comparison with study designs such as those highlighted in relation to *NOS3* is not possible. Undoubtedly, there are many challenges in assessing elite athletes for strength/power, especially using the gold standard methods described and used herein. Unsurprisingly, study designs that incorporate elite athletes from a single sport such as the

present study are scarce and, therefore further research in relation to *NOS3* and its polymorphisms in similar and possibly larger elite athletic cohorts is warranted.

The present study examined *PTK2* rs7460 and rs7843014 in elite athletes for the first time. We report here novel genotype-phenotype associations for both rs7460 and rs7843014 within elite rugby union athletes, suggesting a role of *PTK2* for increased strength and power at the elite level of athletic performance. Muscle force is transmitted laterally across adjacent myofibrils and the sarcolemma to the extracellular matrix, and ultimately from the extracellular matrix to the tendon via costameres (Patel and Lieber, 1997; Flück et al., 1999; Bloch and Gonzalez-Serratos, 2003). Focal adhesion kinase has been shown to play a major role in costamere formation and turnover (Chanock et al., 2007; Quach and Rando, 2006), and its expression is controlled at the level of the protein at *PTK2* gene. Erskine et al. (2012) suggested that an increase in costameres in response to resistance training might result in enhanced muscle specific force (maximum force per unit physiological cross-sectional area) (Erskine et al., 2012), shedding light on the potential advantages provided by certain genotypes at *PTK2* polymorphisms for athletic performance via enhanced adaptability to resistance training. Our results indicate that AT genotype at rs7843014 and rs7460 could be advantageous for producing lower body maximal power and impulse (area under a force time graph); the latter determines take-off velocity (Enoka, 2002) and therefore could have been reflected in greater jump height for AT heterozygotes. Although it was expected that AA genotype at both rs7460 and rs7843014 would be advantageous for CMJ and IMTP performance, it appears that the potential advantage of a favourable *PTK2* genotype within elite rugby athletes could be met by one A allele at both polymorphisms; also demonstrated by the highest IMTP peak force achieved by AT heterozygotes at rs7843014. Stebbings et al. (2017) found that Caucasian healthy men who were AA homozygotes at rs7843014 had 8.3%

greater vastus lateralis specific force than their C allele counterparts, and AA homozygotes at rs7460 have shown 5.4% greater vastus lateralis specific force than T allele carriers, with both SNPs explaining ~25% of the variability in specific force (Stebbings et al., 2017). In partial support of this, Garatachea et al. (2014) found an association between CC (rs7843014) and TT (rs7460) homozygotes and lower gene expression, whilst both SNPs appeared optimal for exceptional longevity (Garatachea et al., 2014). The collective evidence shows that the role of focal adhesion kinase regulated strength and force transmission to the tendon, and the mechanism by which polymorphisms within *PTK2* affect variation in muscle specific force via focal adhesion kinase (Erskine et al., 2012; Stebbings et al., 2017) are complex. Undoubtedly, research in elite athletes on the functional relevance of *PTK2* in terms of muscle strength/power is inexistant and therefore, further research that supports and extends the novel findings of this work here and that further elucidates the role of *PTK2* and polymorphisms within this gene for increased strength/power is warranted.

In contrast to our hypothesis, we have not observed associations between either TGS or FAC and any of the five strength/power variables. This could be attributed, in part, to both the direction of the association we report for individual analyses (*NOS3* rs2070744 [C allele associated with IMTP variables], and *FTO* rs9939609 [A allele associated with CMJ]; but T allele [for both SNPs] considered as advantageous); and the inclusion (in the polygenic analyses) of polymorphisms that were not associated, individually, with any strength/power variable in this Chapter (*ACE* rs4341; *ACTN3* rs1815739; *AMPD1* rs17602729; *KDR* rs1870377) or athlete status in Chapter 4 (*ACE* rs4341, *AMPD1* rs17602729, *KDR* rs1870377, and *NOS3* rs2070744). Of note is that although some polymorphisms have shown an association with elite athlete status in Chapter 4 (comparisons with non-athletes), it cannot be implied that favourable alleles (in terms of strength/power) in these polymorphisms, when

included in a polygenic analyses, are able to contribute to an association between the polygenic score (TGS or FAC) and any of the strength/power variables within athletes. TGS and FAC approaches could be powerful in detecting the cumulative effect of SNPs that have been associated (or have shown tendencies for association), individually, with the phenotype of interest (Hughes et al., 2011); however, the power of both approaches depends, in part, on the similarity between the phenotype/population investigated in the association studies, and the phenotype/population analysed using the polygenic approaches. Our polygenic analyses included only seven of the ten strength/power SNPs investigated in this thesis, and from those seven only three were associated with elite athletes status (Chapter 4: *ACTN3* rs1815739, *FTO* rs9939609 and *TRHR* rs7832552) and three SNPs were associated with either the IMTP or CMJ in this Chapter (*FTO* rs9939609, *NOS3* rs2070744, and *TRHR* rs7832552) – this may have limited the ability of the polygenic analyses to detect an association between the SNPs and strength/power variables used in this Chapter.

The study is not without limitations. The sample of elite athletes was limited, in part, by the existence and provision of strength/power data in IMTP and CMJ, specifically for elite rugby union athletes that form part of RugbyGene; meaning that only those athletes who provided both genotype and strength/power data could be included. In addition, strength/power data were analysed for groups categorised by genotype; and this has resulted in unequal group sizes for all comparisons, and comparisons that included small groups of athletes who are homozygotes for those SNPs with a small minor allele frequency. To retain those sample sizes, all athletes were treated as one group for all analyses, however, with the cost of preventing the analyses and interpretation of results for specific playing positions. Furthermore, polygenic analyses included seven of the ten SNPs investigated and therefore, the ability to detect associations between polygenic data and strength/power variables was

somewhat reduced. Thus, considering the limitations mentioned above in relation to sample size, it is likely that the study was underpowered. Secondly, although assessments of strength/power have used the 'gold standard' force platform method, certain measures such as those related to rate of force development (RFD) (e.g. maximal RFD; RFD at predetermined time epochs) for both the IMTP and CMJ were not included. This could have hindered the ability of the study to explore novel associations between genotype and important neuromuscular parameters, using the same strength/power assessments and genotype data.

In conclusion, we have demonstrated that elite rugby athletes with certain genotypes and alleles at *TRHR* rs7832552, *NOS3* rs2070744 and *PTK2* rs7843014 may possess an inherited advantage to generate force isometrically when compared with athletes possessing alternative genotypes at these polymorphisms. In addition, we found that athletes who possess an A allele at *PTK2* rs7460 and rs7843014 appear to have an inherited advantage to generate lower body mechanical power and impulse. Furthermore, we have extended our previous findings in relation to the advantageous roles of the T allele and A allele for *TRHR* rs7832552 and *FTO* rs9939609 respectively, in that we have shown for the first time that these alleles (at their respective polymorphisms) are advantageous both for achieving elite rugby athlete status (Chapter 4), and for increased capability to generate isometric force or produce power.

Practical applications

These results highlight the relationship between five polymorphisms (*FTO* rs9939609, *PTK2* rs7460 and rs7843014, *NOS3* rs2070744 and *TRHR* rs7832552), and CMJ and IMTP variables within a cohort of elite rugby union athletes, indicating that these athletes might possess an inherited predisposition for achieving a level of strength/power that distinguishes

them from their elite peers, assisting them to achieve elite athlete status in their respective playing position. Whilst we acknowledge that these associations may reflect a very small portion of the genetic contribution of strength/power in elite rugby athletes, this evidence could mean there is a future valuable role for genetic testing to complement the physiological data currently employed to help identify talent in rugby. At present, however, there is a general agreement that the ability of genetic tests to aid talent identification is insufficient (Webborn et al., 2015; Vlahovich et al., 2017; Pickering et al., 2019), and the results of this chapter do not change that position. Although genetic testing has proven of value in the practice of clinical medicine in athletes (Castelletti et al., 2022), there are, however, currently insufficient scientific grounds for the use of genetic testing for talent identification, sport selection or athletic performance improvement. It should be acknowledged that achieving elite status in rugby is a multifactorial accomplishment – mediated by the complex interactions of multiple environmental factors and the polygenic nature of inherited characteristics and predispositions that compose each individual athlete. Furthermore, epigenetic regulation of genome function, in the context of those environmental stimuli typically experienced by athletes (mainly training and nutrition), might exert a substantial influence in modulating the response to resistance training (Hall et al., 2020), and thus the strength/power characteristics achieved by athletes. Nevertheless, a critical initial step in elucidating the role of genetic factors influencing strength/power in elite rugby athletes involves identifying as many genetic variations as possible that show an association with a strength/power phenotype or an intermediate phenotype of strength/power (e.g. number of muscle fibres; fibre-type proportions; number of motor neurons). Subsequently, those variations are further investigated using both case-control (Chapter 4) and genotype-phenotype (the present study) study designs, ideally in large cohorts of elite athletes of diverse geographic ancestries; to confirm and further strengthen the associations with specific

strength/power phenotypes most relevant to elite rugby. Therefore, highly collaborative research is required to achieve adequate sample sizes of elite rugby athletes, with a concomitant and sufficient increase in statistical power. Undoubtedly, future research that combines performance on the currently available physiological tests with detailed genomic information could aid talent identification processes, and increase the chances for each individual to compete at their most suitable level of competition. However, many more genetic factors that influence strength/power in elite rugby athletes have not been discovered; the variants used in the present study and the observed associations with established measures of strength/power provide a basis on which more comprehensive genetic assessments may augment systems for identifying and nurturing talent in rugby.

CHAPTER 6

GENETIC ASSOCIATIONS OF STRENGTH-AND POWER-RELATED POLYMORPHISMS WITH IN-GAME PERFORMANCE IN ELITE MALE RUGBY ATHLETES

6.1 Introduction

Rugby union is characterised as a high intensity intermittent collision sport, requiring athletes to perform repeated running actions, collisions, and *quasi*-static efforts of differing work to rest periods (Duthie et al., 2003; Roberts et al., 2008; Cunniffe et al., 2009). Performance analyses research has sought to define and quantify various performance variables that reflect these requirements for each playing position in rugby union (Quarrie et al., 2013; Jones et al., 2015; Cunningham et al., 2016). Collectively, the large body of research demonstrated substantial differences in the external workload (Jones et al., 2015; Cunningham et al., 2016), and various performance variables (Watson et al., 2017) between playing positions at the elite level. However, considerable inter-individual variation in performance is often observed also within athlete groups of the same playing positions, in that certain athletes are distinguished by their persistent superior performance in accomplishing specific roles and skills that influence match outcomes. A large body of research highlighted the importance of aerobic and anaerobic capacity/power, and muscular strength/power on indices of work rate, change of direction, speed, recovery and injury prevention in athletes (Sheppard and Young, 2006; Johnston et al., 2015; Swaby et al., 2016; Zouita et al., 2016; Cunningham et al., 2016). Given the high heritable nature of these phenotypes that have been shown to underpin successful performance in elite rugby, the distinguished performances of certain athletes can potentially be reflected in distinct genetic characteristics.

Performance analyses research in elite rugby focussed on various aspects of performance, with a main aim in benefiting the coaching process – by providing information that can aid the technical (Hughes et al., 2012; Bremner et al., 2013) and physical aspects of athletes' preparation (Cunningham et al., 2018; Crewther et al., 2019). The analysis of team and athlete performance has led to the identification of key performance indicators (KPIs) that

measure various aspects of performance (Hughes et al., 2012), and are often categorised as either scoring indicators (e.g. tries scored; points from conversions), or indicators of the quality of the performance (e.g. turnovers; tackles) (Hughes and Bartlett, 2002). By definition, a performance indicator is 'a selection, or combination, of action variables that aims to define some or all aspects of performance' (Hughes and Bartlett, 2002). KPIs differ from quantifications of certain in-game variables (e.g. distance covered at various speeds; movement patterns in offensive play), in that KPIs are linked with, and validated against athletes' performances and match outcomes (Bremner et al., 2013) that ultimately determine rugby success. Numerous studies in elite rugby have investigated the relation between various performance measures on physical assessments and movement capabilities in generic skills. For example, Cunningham et al. (2016) have reported strong negative correlations (*r* varied from -0.55 to -0.88) between indices of countermovement jump (CMJ) and drop jump performance (i.e. relative power output and jump height) and sprint times over both the initial accelerative (10 m) and maximum velocity phases (flying 10 m from a rolling 20 m start) in 20 professional rugby athletes (Cunningham et al., 2016). Another study by West et al. investigated the relation between indices of the isometric mid-thigh pull (IMTP: peak rate of force development; force at 100 ms; relative force at 100 ms) and 10 m sprint time in 39 professional rugby athletes; and reported moderate to strong correlations (*r* varied from 0.54 to 0.68) (West et al., 2011). A recent investigation by Cunningham et al. aimed to extend the link between movement capabilities and KPIs. The authors reported moderate to strong relationships between performance measures on physical assessments and various KPIs (Cunningham et al., 2018). The main impetus for the study by Cunningham et al. was that information of performance on physical assessments can be of higher practical value to aid the coaching process than information of the KPIs alone. Therefore, the authors emphasised on identifying specific variables of performance in physical assessments that contribute to

these KPIs. Adding a genetic association to these KPIs could serve as an additional practical tool in elite rugby. Combining genetic data that influences fundamental phenotypes that contribute to rugby performance with physiological data of athletes could be a powerful approach to meliorate athletes' preparation (Williams et al., 2014).

Therefore, the aim of the present study was to determine whether 10 SNPs that were previously associated with muscular strength and power in the literature (*ACE* rs4341, *ACTN3* rs1815739, *AMPD1* rs17602729, *FTO* rs9939609, *HIF1A* rs11549465, *NOS3* rs2070744, *KDR* rs1870377, *PTK2* rs7460 and rs7843014, *TRHR* rs7832552) are associated with in-game performance variables in 291 elite rugby athletes. The associations were explored for each SNP individually, and collectively as part of a total genotype score (TGS) and favourable allele count (FAC). It was hypothesised that associations would be found between individual SNPs and in-game performance variables. A second hypothesis was that the polygenic influence of the polymorphisms would be reflected in superior performance of athletes who possess favourable genotypes and a greater number of favourable alleles.

6.2 Methods

Detailed descriptions of the 291 elite rugby athletes, participants in this study are provided in Section 2.1 (Chapter 2: General Methods). Procedures for DNA sample collection, DNA extraction, and genotyping are included in Section 2.4. The rationale for the selection of the in-game performance indicators, and definitions of the selected performance indicators are provided in Section 2.3. Therefore, only brief descriptions of participants, and statistical analyses used in this investigation are detailed in this Section.

Details of the author's contribution to all procedures involved for sample collection, DNA extraction and genotyping for the 291 elite rugby athletes is provided in Section 2.4 (General Methods). Details of the author's contribution for all procedures involved in processing the in-game performance data for the 291 elite rugby athletes is provided in Section 2.3.

Ethical approval for sample collection, DNA extraction and genotyping was granted by Manchester Metropolitan University, University of Glasgow, University of Cape Town and Northampton University ethics committees. Procedures for the collection of in-game performance data were approved by the local Ethics Committee of Manchester Metropolitan University. All procedures were conducted in accordance with the guidelines in the Declaration of Helsinki (World Medical Association, 2013).

6.2.1 Participants

Participants were 291 elite Caucasian male rugby union athletes [mean (standard deviation): height 1.87 (0.07) m, mass 103.9 (12.1) kg, BMI 29.7 (2.9) $kg \cdot m^{-2}$] including 62% British, 16% Italian, 14% Irish, 4% South African, 2% New Zealanders, and 2% from other nationalities. Physical characteristics of all athletes, and forwards and backs separately, are presented in Table 6.1.

Table 6.1 Physical characteristics of all athletes, forwards and backs.
Data presented are means (SD).

	Body mass (kg)	Height (m)	BMI ($kg \cdot m^{-2}$)
Athletes (n=291)	103.9 (12.1)	1.87 (0.07)	29.7 (2.9)
Forwards (n=171)	112.1 (7.4)	1.89 (0.07)	31.3 (2.7)
Backs (n=120)	92.1 (6.6)	1.83 (0.05)	27.4 (1.3)

All athletes form part of GENESIS and were recruited as part of the ongoing RugbyGene project (Heffernan et al., 2015). Athletes were considered elite if they had competed regularly (>5 matches) since 1995 in the highest professional league in the UK, Ireland, South Africa. Of the athletes, 56% had competed at an international level, and 99% of those international athletes represented a "High Performance Union" (Regulation 16, worldrugby.org). Athletes' international status were confirmed as of 1st July 2019.

6.2.2 Procedures for DNA sample collection, DNA extraction and Genotyping

All participants provided a whole blood, saliva or buccal swab sample, from which DNA was subsequently extracted and analysed to obtain genotype data for 10 SNPs. Detailed descriptions of all procedures are provided in Section 2.4.

6.2.3 In-game performance data

In-game performance data for 291 rugby union athletes were obtained from Opta Sports (London, UK) during eight seasons (2012-13 to 2019-20) of rugby union competition in the highest professional competitive leagues in England (Premiership) and Wales / Ireland / Scotland / Italy / South Africa (Celtic/PRO12/PRO14). Athletes were included for analysis where performance data were available for a minimum of 320 competitive minutes [mean (standard deviation): appearances = 52 (31); min played = 2893 (1973); min played/appearance = 54 (13)]. The in-game performance variables selected for analyses (tries; carries; m gained in possession; clean breaks; defenders beaten; successful tackles) were expressed per 80 min of match play. Details of in-game performance variables are provided in Section 2.3.

6.2.4 Statistical analysis

Genotype and allele frequencies of each SNP were assessed for compliance with Hardy-Weinberg equilibrium using Pearson's chi-square (χ^2). To assess whether to employ parametric or non-parametric analyses, Kolmogorov-Smirnov test was used in conjunction with Q-Q plots to assess normality of distribution in the performance variables.

Due to differences in movement characteristics and performance demands between forwards and backs (Cahill et al., 2013) associations between each SNP and in-game performance variables were explored for the forwards and backs separately, using additive (AA vs. Aa vs. aa), recessive (AA vs. Aa+aa) and dominant (AA+Aa vs. aa) models. The Kruskal-Wallis ANOVA by ranks was used to compare the performance variables between different genotypes for each polymorphism, whilst the Mann-Whitney U-test was used to compare the performance variables between genotypes in the recessive and dominant models. A sensitivity analysis was conducted in G*Power (Faul et al., 2007), using alpha (α) set at 0.05, power of 80%, and sample sizes ranging from 71 (backs: *PTK2* rs7843014) to 171 (forwards: *ACE* rs4341). The results indicated that the lowest detectable effect size ranged from 0.39 to 0.61 (Cohen's *d*; equivalent to 0.19 to 0.29 (r) (Ruscio, 2008)), depending on total sample size. Effect size (*r*) was estimated when differences in performance variables between genotype groups reached statistical significance. Effect size was estimated using the equation $r = Z / \sqrt{N}$ (Rosenthal, 1991); in which Z is the 'standardised test statistic' (for both the Kruskal-Wallis ANOVA by ranks and Mann-Whitney U-test) and N is the total number of participants. Effect size (r) was interpreted as follows: 0.1 or less is a small effect size, about 0.3 is a moderate effect size, and 0.5 or more is a large effect size.

The polygenic influence of 7 SNPs (*ACE* rs4341, *ACTN3* rs1815739*, AMPD1* rs17602729, *FTO* rs9939609, *KDR* rs1870377, *NOS3* rs2070744 and *TRHR* rs7832552) was assessed using total genotype score (TGS) and favourable allele count (FAC). Using those 7 SNPs provided the best compromise between including a high number of SNPs and maintaining a high number of athletes (175), because not all athletes were genotyped for all SNPs. For both TGS and FAC, separate analyses were performed for forwards and backs. The allele considered advantageous for strength and power was identified from previous literature (Section 1.8). To determine TGS, each genotype within each SNP was allocated a genotype score (GS) of 0, 1 or 2. Accordingly, homozygotes for the favourable allele were given a score of 2, heterozygotes scored 1, and homozygotes for the other allele scored 0. TGS for each participant was thus given by:

$$\frac{100}{2\times7} \times (GS_{ACE} + GS_{ACTN3} + GS_{AMPD1} + GS_{FTO} + GS_{KDR} + GS_{NOS3} + GS_{TRHR})$$

where 7 is the number of SNPs that constitute the TGS.

FAC was determined by summing the number of favourable alleles possessed by each participant, for each of the 7 SNPs. Thus, FAC for each participant could range from 0 to 14. Participants were categorised either into three groups (0-7, 8-9 or 10-14 favourable alleles) or two groups (0-8 or 9-14 favourable alleles) based on their FAC, with those numbers of favourable alleles providing approximately equal group sizes. Kruskal-Wallis and Mann-Whitney tests were used to detect associations between performance variables and FAC categories, whilst Spearman rank difference correlation was used to detect associations between TGS and performance variables. For differences in performance variables between FAC categories, effect size was estimated using the equation $r = Z / \sqrt{N}$ (Rosenthal, 1991); in which Z is the 'standardised test statistic' and N is the total number of participants. For

correlation between TGS and performance variables, Spearman's r was used to estimate effect size (in addition to its primary use for indicating associations between TGS and performance variables).

The Statistical Package for Social Sciences (version 26.0, SPSS Inc., Chicago, IL, USA) was used for the analyses, and alpha was set at 0.05. Probability values were subjected to Benjamini-Hochberg (B-H) correction (Benjamini and Hochberg, 1995) to control for false discovery rate (FDR). FDR was set at 20% and the reported *p* values are those significant after B-H correction.

6.3 Results

Genotype frequencies were in Hardy-Weinberg equilibrium for the 10 SNPs, except *PTK2* rs7460 and rs7843014 ($\chi^2 = 6.06$, $p = 0.014$ and $\chi^2 = 5.64$, $p = 0.017$, respectively).

6.3.1 Individual SNPs

The additive model for *ACTN3* rs1815739 within the backs revealed that TT (XX) homozygotes performed 22.8% more successful tackles than CT (RX) heterozygotes ($p = 0.017$; effect size (r) = 0.33). CC (RR) homozygotes tended to perform more successful tackles than CT heterozygotes, but the 16.9% difference was not significant ($p = 0.067$). When the dominant model was considered (TT vs. C carriers), TT homozygotes tended to perform more tackles than C allele carriers but the difference (13.2%, $p = 0.048$) was not significant after B-H correction.

The dominant model for *TRHR* rs7832552 revealed that backs T allele carriers carried the ball forward (number of carries) 15.3% more often than CC homozygotes ($p = 0.017$; effect

size = (*r*) 0.26; Figure 6.1a). In addition, the additive model initially showed that TT homozygote backs carried the ball forward 9.2% and 22.8% more often than CT and CC genotypes, respectively (p = 0.037), although neither was significant after B-H correction. Furthermore, T allele carrier backs beat 31% more defenders than their CC homozygote counterparts (p = 0.021; effect size (r) = 0.25; Figure 6.1b). No other associations were observed between any of the ten individual polymorphisms and any of the six in-game performance variables (tries; carries; m gained; clean breaks; defenders beaten; tackles). *P*-values for all comparisons between genotypes for all performance variables and SNPs are provided in Appendix C (Tables AppC6.2a and AppC6.2b).

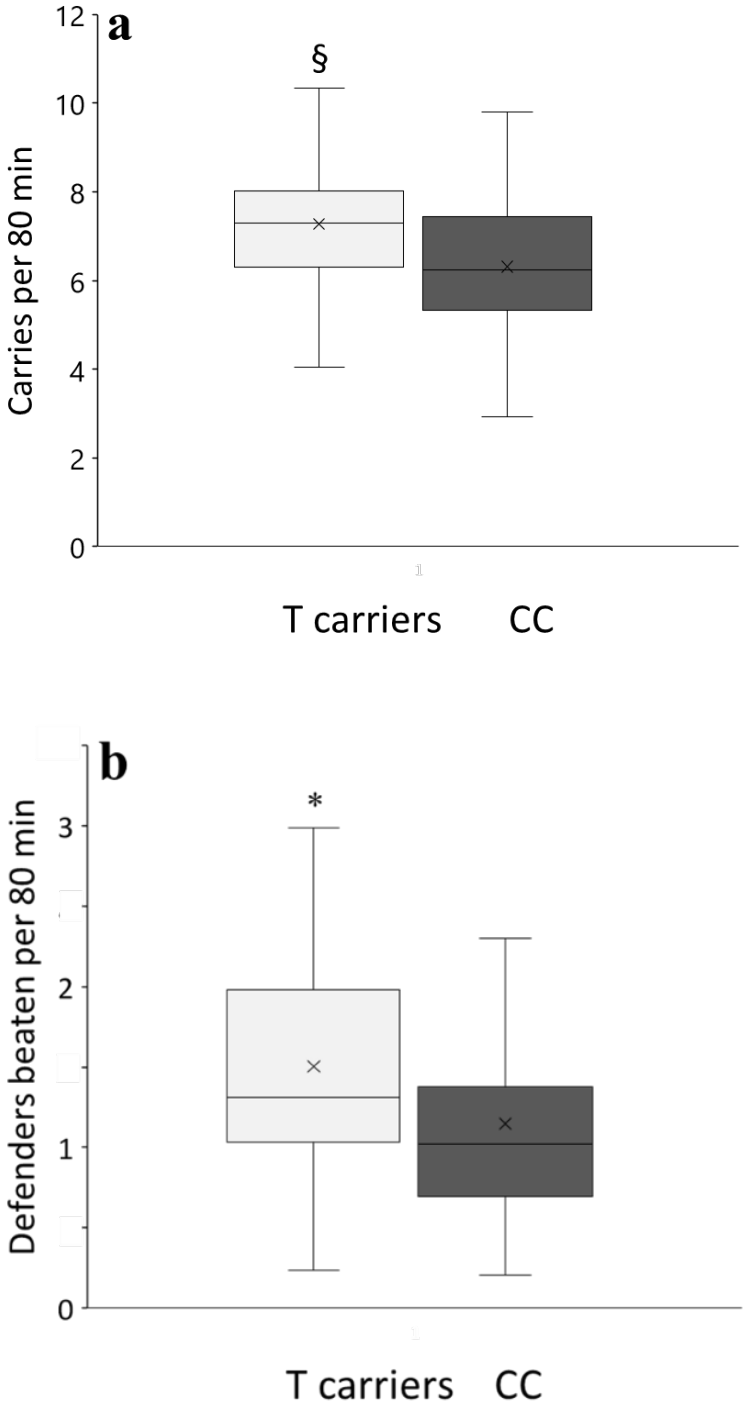


Figure 6.1 Number of carries (**a**) and defenders beaten (**b**) according to ***TRHR*** rs7832552 genotype. §different from CC (p = 0.017; effect size (r) = 0.26). *different from CC (p = 0.021; effect size (r) = 0.25). Horizontal line in box shows median, whilst × shows mean.

6.3.2 Total genotype score (TGS) and favourable allele count (FAC)

Forwards

An association was observed in forwards between TGS and number of tackles (r = 0.23, p = 0.012; effect size (r) = 0.23; Figure 6.2 A). When categorised by FAC (three categories), forwards pertaining to the highest FAC category (10-14 favourable alleles) performed 16.5% more successful tackles than the lowest FAC category (1-7 favourable alleles) (p = 0.019; effect size (r) = 0.30). In addition, the middle category (8-9 favourable alleles) performed 9.5% more successful tackles than the lowest FAC category (p = 0.054; significant after B-H correction; effect size (r) = 0.23; Figure 6.2 B). No other associations were observed between TGS (Spearman rank difference correlation r (p value): tries, r = -0.069 (0.249); carries, r = -0.149 (0.071); metres gained, r = -0.053 (0.302); clean breaks, r = -0.033 (0.372); defenders beaten, r = -0.107 (0.145)); or FAC (p value for FAC two categories, three categories): tries (0.303, 0.685); carries (0.234, 0.465); metres gained (0.796, 0.867); clean breaks (0.831, 0.977); defenders beaten (0.363, 0.350); tackles (0.110 [two categories]) and performance variables in forwards.

Backs

When backs were categorised by FAC (two categories), those in the higher FAC category (9-14 favourable alleles) gained 26.3% more territory (m gained) than the lower FAC category (1-8 favourable alleles) (p = 0.038; effect size (r) = 0.24). No other associations were observed between TGS (Spearman's correlation r (p value) tries, r = 0.093 (0.213); carries, r = 0.173 (0.068); metres gained, r = -0.163 (0.080); clean breaks, r = 0.119 (0.153); defenders beaten, r = 0.033 (0.388)); or FAC ((p value for FAC two categories, three categories): tries (0.354, 0.302); carries (0.071, 0.467); metres gained (three categories: 0.460); clean breaks

(0.071, 0.967); defenders beaten (0.189, 0.726); tackles (0.078, 0.078)) and performance variables in backs.

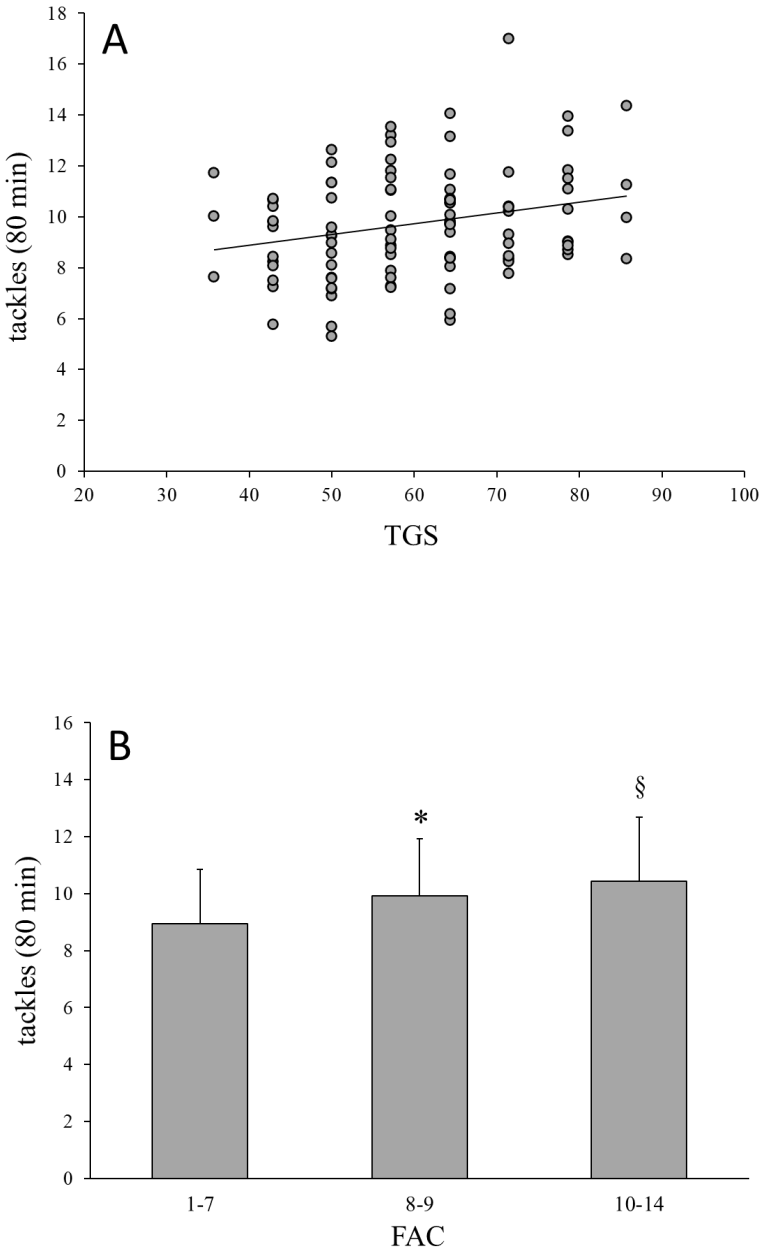


Figure 6.2 Total genotype score (TGS) distribution in forwards and number of tackles per 80 min (r = 0.23, p = 0.012) (**A**). Tackles per 80 min according to favourable allele count (FAC) for three categories (**B**). §different from category with least number of favourable alleles (p = 0.019; effect size (r) = 0.30); *different from category with least number of favourable alleles (p = 0.054, significant after B-H correction); effect size (r) = 0.23). Tackles (80 min) data are means with error bars showing standard deviations.

6.4 Discussion

The present study examined whether 10 polymorphisms (previously associated with strength and power in the literature) are associated with in-game performance variables in elite rugby union athletes. Our results demonstrate that *ACTN3* rs1815739 is associated with tackling frequency, whilst *TRHR* rs7832552 is associated with the ability to defeat defenders and in carrying the ball forward via a higher frequency of carries; with all associations observed within the backs. Due to these associations, we accept our first hypothesis. In addition, the present study showed that the polygenic influence of seven SNPs expressed as TGS and FAC is associated with the number of tackles within forwards. Furthermore, we found an association between FAC and the ability to gain territory (metres gained) within the backs and therefore, we accept our second hypothesis.

The association of *ACTN3* rs1815739 with rugby athletes status and playing position was first reported by Heffernan et al. (2016). The authors found a higher frequency of the T (X) allele in forwards compared with backs and non-athletes, with the TT genotype underrepresented in the back three athletes compared with forwards. Additionally, a higher frequency of the C (R) allele in the back three compared with forwards, the rest of the athletes, and non-athletes was reported in that study. Although not entirely in agreement, the findings in Chapter 4 of this thesis in relation to *ACTN3* rs1815739 were in line with those of Heffernan et al. (2016). In Chapter 5, no associations were reported between *ACTN3* rs1815739 genotype and variables in CMJ and IMTP, however, there was a tendency for association with CMJ height (p =0.119) that could reflect the advantageous role of the C allele for high velocity muscle contractions. The present study found that backs who are TT homozygotes in rs1815739 performed more tackles than CT heterozygotes (p =0.017; effect size (r) = 0.33), and tended to perform more tackles than CT and CC genotype combined (p = 0.048, not significant after

B-H correction); suggesting that TT genotype that was previously found to be overrepresented in, and advantageous for forwards, might be advantageous in terms of increased involvement in tackling within the backs playing positions. Contrastingly, neither TT nor CT genotype forwards have showed a distinct involvement in tackling compared to CC genotype forwards. The TT genotype is present in ~18% of Caucasians in the general population (Beggs et al., 1992) and found to represent 19.4% and 23.7% of elite rugby backs (n = 232) and forwards (n=312), respectively (Chapter 4). TT genotype indicates an absence of the α-actinin-3 protein (Mills et al., 2001) that is almost exclusively expressed in fast twitch skeletal muscle fibres. Accordingly, C allele carriers have a greater proportion of the fast twitch type II and IIx myofibres and larger relative surface area per IIx fibre than TT homozygotes (Ahmetov et al., 2011; Broos et al., 2012; Yang et al., 2003) and therefore, the C allele and CC genotype could benefit high velocity muscle contractions. On the other hand, TT genotype could hinder high velocity contractions and sprinting ability Heffernan et al. (2016) – a phenotype of utmost importance for the back three (wings and fullback) playing positions. Therefore, one plausible explanation for the observed association between the TT genotype and an increased involvement in tackles within the backs is that those backs' athletes who's sprint ability is not distinguishable, in part due to the unfavourable genotype at rs1815739, are more suited for the half backs and centres' roles where tackling involvement might be more frequent compared with the back three playing positions. Of note is that 19 of the 26 TT homozygotes in the present study were centres and half backs, revealing an underrepresentation of the back three athletes in our TT genotype sample within the backs, and this could have contributed to the obtained results in the present study. The proposed mechanisms for the advantageous role of the TT genotype at rs1815739 has been based on evidence that α-actinin-3-deficient mice have a higher propensity for aerobic enzyme activity and a greater force recovery after fatigue (Seto et al., 2013), indicating that TT homozygote

humans might have a greater capacity for recovery from fatiguing intermittent exercise. This phenotype is thought to benefit forwards due to their demands for sustained match play intensity and necessity for quick recovery and shorter rest periods compared with backs (Deutsch et al., 2007). However, it is unlikely that the observations of the present study in relation to *ACTN3* could be attributed to the probable beneficial molecular mechanisms provided by the TT genotype for the forwards. Thus, the findings in the present study may be taken as additional evidence that CC genotype at rs1815739 benefits exclusively the back three playing positions, but not half backs and centres, due to both the back three's reduced involvement in tackling, and increased necessity for sprinting ability.

The TT genotype at *TRHR* rs7832552 has been associated with increased lean mass at genome level significance in non-athletic populations (Liu et al., 2009). In Chapter 4 it was reported that athletes (combined) and forwards had a greater T allele frequency and a greater proportion of T allele carriers than non-athletes. Furthermore, in Chapter 5 it was shown that T allele carriers might possess an inherited advantage to develop force isometrically, with such advantage remaining consistent for force generation relative to body mass. The present study found that T allele carriers for rs7832552 within the backs were involved in 15.3% more carries (p = 0.017; effect size = (r) 0.26; Figure 6.1a) and showed an increased ability (31%) to defeat defenders (p = 0.021; effect size (r) = 0.25; Figure 6.1b), compared to their CC homozygote counterparts. Although the study by Liu et al. (2009) involved exclusively non-athletic populations, their findings in relation to the T allele's role for increased lean body mass and the collective results in this thesis in relation to rs7832552 shed light on the importance of *TRHR* in positively influencing the athletes' ability to produce force and their in-game performance via increased lean body mass. It is well established that lean body mass is under strong genetic determination, with heritability ranging from 52% to 84% (Hsu et al.,

2005; Arden and Spector, 1997; Nguyen et al., 1998). Liu et al. (2009) reported that TT homozygotes at *TRHR* rs7832552 had 2.5 kg higher lean body mass than CC or CT genotypes. Of note is that rs7832552 is in tight linkage disequilibrium with *TRHR* rs16892496 (r^2 = 0.98), at which GG homozygotes also showed 2.7 kg greater lean body mass than alternative genotypes (Liu et al., 2009). Interestingly, the authors found 15 other SNPs in *TRHR* also suggestive of an association with lean body mass. Thus, taking into consideration the novel findings by Liu et al. that involved large non-athletic populations, while also acknowledging the limitations of that study in terms of comparative relevance to the present work (mainly due to athletes being at different point of the performance continuum than non-athletes); the only plausible mechanism by which TT genotype at rs7832552 could have positively influenced athlete performance in the present study is via the variant's influence on lean body mass that, in turn, plays a major role in determining maximal force generation underpinning in-game performance. Thyrotropin releasing hormone (TRH) was the first hypothalamic releasing factor to be identified, and its multifaceted action was unknown until Bøler et al. determined its final structure (Bøler et al., 1969). The main role of TRH is the regulation of thyrotropin expression in the thyrotrophs of the anterior pituitary gland, which in turn induces the synthesis and release of thyroxine (T4) and triiodothyronine (T3) by the follicular cells of the thyroid gland (Fröhlich and Wahl, 2019). The cellular action of TRH is mediated by thyrotropin releasing hormone receptor (TRHR) ligation, with the receptor detected in the hypothalamus and pituitary gland, uterus, ovary and testis, intestinal epithelial cells, retina, lymphoid tissue and bone marrow (Fukusumi et al., 1995); although the overlapping pattern of TRH mRNA and TRHR expression in brain and other organs suggests a local (paracrine) action of TRH, the mechanisms are largely unknown (Fröhlich and Wahl, 2019). Fuku et al. (2015) investigated the functional significance of *TRHR* rs7832552 and rs16892496 at the muscle tissue level,

and found that the T allele at rs7832552 increased gene expression compared to the C allele (Fuku et al., 2015). Although the collective evidence provides considerable scientific rationale in postulating that higher *TRHR* expression might positively influence preservation of lean body mass, the underlying molecular mechanisms by which the T allele at rs7832552 might influence lean body mass in athletic populations are yet to be determined. Thus, further research that focuses on *TRHR* in athletic populations is warranted. Of note is that *TRHR* produced associations in Chapters 4, 5 and the present chapter, and the likelihood of all these associations being false positives is remote.

The present study reports that elite rugby forwards who possess the most favourable genotypes and alleles at seven of the studied strength and power related polymorphisms (*ACE* rs4341, *ACTN3* rs1815739, *AMPD1* rs17602729, *FTO* rs9939609, *NOS3* rs2070744, *KDR* rs1870377, *TRHR* rs7832552) were involved in a greater number of tackles than those with alternative genotypes (TGS: $r = 0.23$, $p = 0.012$; effect size (r) = 0.23; FAC: (highest number of favourable alleles of three categories) 16.5%, $p = 0.019$; effect size (r) = 0.30; FAC (medium number of favourable alleles of three categories) 9.5%, $p = 0.054$; effect size (r) = 0.23). In addition, backs possessing the highest number of favourable alleles showed an increased ability to gain territory (FAC (highest of two categories): 26.3%, $p = 0.038$; effect size (r) = 0.24), compared with those possessing fewer alleles. These results were obtained despite the fact that no associations were detected within the forwards for individual polymorphisms, or within either the forwards or backs in relation to the ability to gain territory; and thus, they demonstrate that both approaches could be powerful in detecting the cumulative effect of individual SNPs that show tendencies for associations and/or associations which were not statistically significant when subjected to FDR correction.

The study is not without limitations. Firstly, the sample of elite athletes was limited, in part, by the existence and provision of in-game performance data, specifically for athletes that form part of RugbyGene; meaning that only those athletes who provided both genotype and in-game performance data could be included. In addition, in-game performance data were analysed for forwards and backs separately, with each group categorised by genotype in all analyses; and this has resulted in unequal group sizes for all comparisons, and comparisons that included small groups of athletes who are homozygotes for SNPs with a small minor allele frequency. Furthermore, polygenic analyses included seven of the ten SNPs investigated and therefore, the ability to detect associations between polygenic data and performance variables was somewhat reduced. Thus, considering the limitations mentioned above with respect to sample sizes, we acknowledge that the study was underpowered. Secondly, although the selection of performance variables were based on the premise that they better reflect superior strength/power of individual athletes, with the most informative identified via reasoning informed by the input of an expert practitioner of sport science in rugby (Mr Mark Bennett); it is acknowledged that some important in-game variables (that were deliberately excluded from the study) could have produced additional interesting results.

The present study demonstrated, for the first time, the association of *ACTN3* rs1815739 and *TRHR* rs7832552 with in-game performance variables in elite rugby union. Athletes homozygous for the C allele at *ACTN3* rs1815739 performed more tackles, whilst T allele carriers at *TRHR* rs7832552 carried the ball forward more frequently and were better able to defeat defenders. Of note were the tendencies for association for *FTO* rs9939609 (number of tries in backs and forwards), *NOS3* rs2070744 (carries in forwards), and *ACE* rs4341 (number of tries in backs). Another novel finding was the relationship between the frequency of tackles in forwards and both TGS and FAC polygenic expression, with FAC also associated

with the backs' ability to gain territory. Collectively, these findings suggest that favourable genotypes in SNPs within strength- and power-related genes might exert a positive influence, individually and collectively, on in-game performance variables in elite rugby union.

Practical applications

These results highlight the relationship between *ACTN3* rs1815739 and *TRHR* rs7832552, and in-game performance variables in elite rugby union athletes. In addition, we have shown that a polygenic profile comprising seven strength/power polymorphisms is associated with two of the six in-game performance variables studied. Whilst we acknowledge that the mechanisms by which these variants exert their effect to influence in-game performance of elite rugby athletes need much more elucidation (e.g. by addressing the need for more functional data via transcriptomic, histological, and physiological studies), we propose that elite rugby athletes might possess an inherited predisposition for achieving distinguished competitive performance via, in part, superior strength/power abilities. In addition, it should be acknowledged that the number of variants studied is only likely to explain a small proportion of the genetic component of in-game performance inter-individual variability. Whilst there is substantial evidence for the role of genes in modulating the status of the determinants of athletic performance (Wackerhage, 2014; Barh and Ahmetov, 2019) such as strength/power (Zempo et al., 2017), it is acknowledged that an elite athlete needs to be an obligatory, highly responsive individual to regular concurrent training protocols and practice (Jones et al., 2016). Thus, the identification of potential talent should be based, in part, on individual responsiveness to diverse training protocols (Sarzynski et al., 2011). Currently, determination of potential talent and selection in young athletes involves the use of established assessments of anthropometric and physiological traits (e.g. maximal speed; acceleration; momentum; maximal strength; peak power; agility and change of direction; skill

speed – addressed in detail in Section 1.6). However, although not possible at present as accentuated throughout this thesis (Webborn et al., 2015), the potential for achieving elite athletic performance can, in future, be predicted via the use of genetic information. This predictive ability of genetic information, combined with the currently employed physiological assessments, may become a potent tool to aid talent identification processes and for nurturing young athletes (Pitsiladis et al., 2016; Bouchard, 2019). In that eventuality, large panels of genetic markers would be needed to determine which individuals are talented and more likely to be high responders to diverse training methods and thus, likely to exhibit greater athletic performance over time. Relatively few individuals achieve elite athlete status in rugby and therefore, highly collaborative research is required to achieve the sample sizes required for satisfactory statistical power, with a concomitant increased probability for detecting informative and valuable relationships.

CHAPTER 7
GENERAL DISCUSSION

GENERAL DISCUSSION

7.1 Overview

Muscular strength and power are known to have a genetic component. A complex interaction of genetic and environmental factors determine muscular strength/power phenotypes in diverse populations. The scientific literature has shown that certain genetic characteristics appear to provide an advantage for strength/power in the general population, with certain genotypes in polymorphisms associated with strength/power overrepresented in athletic populations. Most investigations involved small sample sizes of athletes and/or athletes of different sports. These studies have low statistical power due to both participant number and dilution of the strength/power phenotype, and thus most findings remain unclear. The RugbyGene project aims to attenuate such problems, by exploring the genetics that contribute to elite rugby performance using elite rugby athletes from both codes of rugby. Consequently, as part of the ongoing RugbyGene project, the overall aim of the current thesis was to characterise some of the genetic characteristics of elite rugby union athletes using polymorphisms that were previously associated with strength/power phenotypes. More specifically, the objectives were:

1. To characterise muscular strength and power of elite rugby athletes using specific measures acquired from the force-time histories of the IMTP and CMJ, comparing between positional groups and with non-athletes.

2. To determine the genetic characteristics of muscular strength and power in elite rugby athletes using 10 polymorphisms (previously associated with strength and/or power in the literature) found in nine genes (*ACE*, *ACTN3*, *AMPD1*, *HIF1A*, *FTO*, *KDR*, *NOS3*, *PTK2*, and *TRHR*), comparing between positional groups and with non-athletes.

3. To investigate whether these 10 polymorphisms are associated (individually, and/or collectively using two polygenic profile approaches) with strength/power measures acquired from the force-time histories of the IMTP and CMJ of the athletes.

4. To investigate whether these ten polymorphisms are associated (individually, and/or collectively using two polygenic profile approaches) with in-game performance variables of the athletes.

The objectives above were met in Chapters 3–6. Section 7.2 in the current chapter summarises the main findings of Chapters 3 and 4. Section 7.3 provides a retrospective view of the results observed within each investigation, and aims to combine these results for individual polymorphisms. Section 7.4 addresses the results of the polygenic analysis (chapters 5 and 6) and discusses these in relation to polygenic profiling in sport. Sections 7.5 and 7.7 consider important factors influencing rugby athletes' performance (such as environmental factors, and genetic variation in disease); whilst Section 7.6 considers the use of genetic testing for predicting talent. The current chapter also considers the strengths and limitations of this thesis and outlines the broader direction for future research.

7.2 Strength and power characteristics and genetic characteristics of elite rugby athletes

Chapter 3 characterised muscular strength/power of elite rugby union athletes, examining IMTP and CMJ variables in 263 athletes and 14 non-athletes using the force platform method of assessment (Bevan et al., 2010; Owen et al., 2014). A main observation was that athletes achieved higher IMTP peak force, IMTP relative peak force and CMJ maximum concentric power than non-athletes. In addition, significant differences in all IMTP and CMJ variables were observed between forwards and backs; with forwards dominating in absolute measures

of strength/power and backs dominating when both measures were expressed relative to body mass, and in CMJ jump height. Subsequently, when five playing positions were considered (front five, back row, half backs, centres, and back three) the differences in all variables between subgroups reflected those observed between forwards and backs. An interesting observation was that for both IMTP variables there were no differences between any two athlete subgroups within the forwards and backs. In contrast, differences were observed in CMJ variables between pairs of subgroups within both forwards and backs. Collectively, these findings exemplify the distinct strength/power requirements of elite rugby union athletes of different playing positions. Data in Chapter 3 were used in Chapter 5 to investigate whether ten polymorphisms (previously associated with strength/power in the literature) were associated with strength/power of elite rugby union athletes.

Chapter 4 involved 567 athletes and 1171 non-athletes, and is the first to investigate multiple strength/power related polymorphisms in elite rugby athletes. Genotype and allele frequency distributions were compared between athletes and non-athletes, and between forwards and backs. In addition, Chapter 4 aimed to expand on previous work of Heffernan et al. (2016) and Heffernan et al. (2017); by investigating *ACE* rs4341, *ACTN3* rs1815739 and *FTO* rs9939609 using a larger sample of athletes. In brief, Chapter 4 revealed that *TRHR* rs7832552 is associated with athlete status, while *HIF1A* rs11549465 is associated with athlete status and playing position. Furthermore, while consistent with the work of Heffernan et al., *ACTN3* rs1815739 was associated with playing position while *FTO* rs9939609 was associated with both athlete status and playing position. Genotype data from Chapter 4 were subsequently used in the genotype-phenotype association studies in Chapters 5 and 6.

7.3 Genotype associations

7.3.1 *ACTN3* rs1715739

ACTN3 rs1815739 was associated with playing position in Chapter 4. Specifically, the C (R) allele and CC genotype were overrepresented in backs compared with forwards. Backs showed 1.5 times greater odds of being CC homozygotes (than T (X) allele carriers) and 1.3 times greater odds of possessing C alleles, compared with forwards. In addition, the back three subgroup possessed more C alleles and showed 1.5 times greater odds of possessing C alleles than T alleles – also compared to forwards. *ACTN3* rs1815739 has been extensively studied for association with athletic ability and power performance. A meta-analysis by Ma et al. (2013) reported that the CC genotype and C allele showed a consistent association with elite power performance. In addition, it was postulated that the T allele and TT genotype could be advantageous for endurance performance (Ma et al., 2013). In relation to this, and while also consistent with Heffernan et al. (2016), Chapter 4 showed that rugby forwards had a greater proportion of T allele carriers and contained more T alleles than backs. Thus, although the potential benefit of the T allele for endurance performance is unclear, the T allele at rs1815739 appears beneficial in elite rugby forwards – evidenced by Heffernan et al. (2016) and in Chapter 4 using a larger cohort of rugby union athletes.

Chapters 5 and 6 combined data for *ACTN3* from Chapter 4 with strength/power (Chapter 3) and in-game performance data, respectively – in an attempt to further explore *ACTN3* in elite rugby via genotype-phenotype association studies. Unexpectedly, no associations were found between *ACTN3* rs1815739 genotype and any variable in CMJ and IMTP in Chapter 5. This lack of association may indicate that the advantageous role of the CC genotype and C allele (overrepresented in backs) for relative strength/power, and the greater absolute strength/power in forwards demonstrated in Chapter 3 (where the TT genotype and T allele

are more common as shown in Chapter 4) could have diluted the magnitudes for each phenotype measure in a way that obscured any existing differences in variables between genotypes. This suggests that larger samples of athletes may be necessary to capture genotype-phenotype associations investigating *ACTN3* in elite rugby, to allow the categorisation of athletes (e.g. forwards and backs) and further purify the phenotype being investigated. This was somewhat accomplished in Chapter 6, where *ACTN3* rs1815739 was associated with tackling frequency within the backs. Specifically, TT homozygotes performed 22.8% more tackles (effect size (r) = 0.33) than CT heterozygotes and tended to perform 16.9% more tackles than C allele carriers. This might indicate that whilst the TT genotype is more common in, and advantageous for forwards, it appears advantageous for increased involvement in effective multiple tackling by the backs. Although this may seem contradictory, due to the overrepresentation and most probable advantageous role of CC genotype and C allele in backs (where a requirement for high velocity muscle contractions prevails), it can be implied that this *ACTN3* genotype advantage for increased muscular power is exclusively reserved for the back three athletes. An alternative view is that the increased tackling frequency observed in TT genotype backs provides additional evidence that CC genotype at rs1815739 benefits exclusively the back three due to both their reduced involvement in tackling, and their increased necessity for sprint ability. This finding was explained in further detail in Chapter 6.

7.3.2 *TRHR* rs7832552

In Chapter 4, *TRHR* rs7832552 was associated with athlete status but not with playing position. Specifically, and as hypothesised, athletes had more T allele carriers and a greater T allele frequency than non-athletes; with similar genotype and allele frequency distributions observed between forwards and non-athletes. As hypothesised, in Chapter 5, *TRHR* was

associated with IMTP peak force and relative peak force, with the direction of association also as expected. Specifically, T allele carriers achieved 10.2% greater IMTP peak force (Cohen's d = 0.56) and 11.1% greater relative peak force (Cohen's d = 0.14) than CC homozygotes, indicating that T allele carriers at rs7832552 might possess an inherited advantage to develop force isometrically, with such advantage remaining consistent for force generation relative to body mass. Furthermore, Chapter 6 has shown that T allele carriers for rs7832552 within the backs were involved in 15.3% more carries (effect size (r) = 0.26) and showed 31.0% greater ability to defeat defenders (effect size (r) = 0.25), compared to their CC homozygote counterparts.

Notably, while consistent with the findings by Liu et al. (2009), Chapters 5 and 6 showed the possibility of a dominant positive influence of *TRHR* rs7832552 genotype on IMTP variables and in-game performance. A plausible mechanism for these observations could be via the variant's influence on lean body mass – by enhancing maximal force generation that in turn, provides an advantage for in-game performance. To the author's knowledge, one study has attempted to investigate the functionality of the rs7832552 polymorphism, and this was done *in vitro* using mice skeletal muscle cell lines – with the method used considered a main limitation of that study by the same authors (Fuku et al., 2015). Whilst the *TRHR* rs7832552 variant appears promising for its advantageous role on lean mass, muscle strength and possibly in-game performance, previous literature combined with the collective results in this thesis suggest that further research that could elucidate the molecular mechanisms by which rs7832552 exerts its effect on muscle development and increased lean mass is warranted.

7.3.3 *FTO* rs9939609

As hypothesised, *FTO* rs9939609 was associated with athlete status and playing position in Chapter 4. While consistent with Heffernan et al. (2017) but with an increased cohort size, Chapter 4 showed that athletes had more A allele carriers and less TT homozygotes than non-athletes. Similarly, forwards contained more A allele carriers and less TT homozygotes than non-athletes, notably in the opposite direction to the comparison between backs and non-athletes, in that backs had more T allele carriers. Collectively, the results of Chapter 4 showed that the T allele and TT genotype at rs9939609 are advantageous for backs who are more reliant on a lean phenotype (Smart et al., 2013; Brazier et al., 2020) and on their ability to produce high levels of lower body mechanical power relative to body mass (Crewther et al., 2012) (Chapter 3), whilst the A allele and AA genotype appear advantageous for forwards who depend on greater body mass (Sedeaud et al., 2012; Sedeaud et al., 2013) and ability to generate the highest absolute force and power output (Brazier et al., 2020) – evidenced in Chapter 3.

Chapter 5 showed that A allele carriers for *FTO* rs9939609 achieved 7.1% greater CMJ maximum concentric power (Cohen's d = 0.52) than TT homozygotes. This could have reflected the overrepresentation of the A allele and AA genotype in forwards found in Chapter 4 (and therefore more forwards constituted the A allele carriers groups in Chapter 5), combined with their greater CMJ maximum concentric power – demonstrated in Chapter 3. However, in contrast to the hypothesis, no associations were found between *FTO* rs9939609 and in-game performance variables within either the forwards or backs in Chapter 6, although there was a tendency of an association (not significant after Benjamini-Hochberg correction) between TT genotype and the number of tries within the backs. Heffernan et al. (2017) found the T allele at rs9939609 was more common in the back three and centre athletes who are

most reliant on lean mass rather than total body mass for success, and accordingly these athletes showed greater CMJ relative maximum concentric power, compared to other athletes in that study (in agreement with Chapter 3 and Chapter 4). These observations combined with the association of *FTO* with CMJ found in Chapter 5 (where athletes were analysed as one group) and the absence of associations in Chapter 6 (where the strength/power influence on in-game performance phenotypes could have been less diluted due to forwards and backs being analysed separately) suggests that the association of rs9939609 with increased strength could occur via the additional mass of the heavier forwards. However, this latter interpretation should be taken only as speculative and thus, further genotype-phenotype association studies that investigate measures of strength/power and in-game performance in relation to *FTO,* possibly in larger samples of elite rugby athletes are warranted to further elucidate the role of *FTO* for different playing positions in rugby.

7.3.4 *NOS3* rs2070744

NOS3 rs2070744 was not associated with athlete status or playing position in Chapter 4 however, Chapter 5 showed that the C allele at rs2070744 could be advantageous for generating maximal isometric strength and relative isometric strength. Specifically, CC homozygotes and CT heterozygotes (individually) achieved greater peak force and relative peak force in the IMTP than their TT counterparts. Interestingly, these results showed a positive additive effect of the C allele on both IMTP variables, in that each C allele contributed to ~7.5% greater peak force and ~10% greater relative peak force, compared to TT homozygotes. No associations were observed between *NOS3* and in-game performance variables in Chapter 6, although there was a tendency for association between rs2070744 and tackling frequency within the backs, with C allele carriers showing greater tackling involvement than their TT genotype counterparts.

Given that the C allele at rs2070744 reduces gene promoter activity and reduced endothelial nitric oxide synthesis (Nakayama et al., 1999) that can reduce oxygenated blood flow to working muscles, *NOS3* was proposed as a potential candidate for endurance performance. Based on this, Gómez-Gallego et al. (2009) hypothesised that the T allele would be overrepresented in endurance oriented compared with power oriented athletes. However, in contrast to their hypothesis the T allele and the TT genotype were overrepresented in power athletes compared to either endurance athletes or non-athletes. These findings were supported by Sessa et al. (2011) and Drozdovska et al. (2013) in athletes and thus, in the current thesis, it was hypothesised that associations would be detected in relation to *NOS3,* with the T allele at rs2070744 being advantageous. Nevertheless, the results in Chapter 5 showed that the C allele at rs2070744 could be advantageous for the generation of maximal isometric force and thus, further research in relation to *NOS3* in larger rugby athlete cohorts that allow for more powerful study designs in terms of both athlete categorisation (e.g. forwards and backs) and sample sizes seems appropriate.

7.3.5 *PTK2* rs7460 and rs7843014

Chapter 5 showed that AT genotype at rs7843014 and rs7460 could be advantageous for producing lower body maximal power and impulse (force × time); the latter determines take-off velocity and therefore, jump height (Enoka, 2002). It was hypothesised that the A allele at both rs7460 and rs7843014 would be advantageous for CMJ and IMTP performance, and that AA homozygotes would probably demonstrate the best performance. Nevertheless, Chapter 5 indicated that the potential advantage of a favourable *PTK2* genotype could be met by one A allele at both polymorphisms; also demonstrated by the highest IMTP peak force achieved by AT heterozygotes at rs7843014. *PTK2* was not associated with athlete status or playing position in Chapter 4, and no associations were observed between *PTK2* and in-game

performance in Chapter 6. *PTK2* encodes focal adhesion kinase, a protein integral for the formation and turnover of muscle costameres (Quach and Rando, 2006) that have a major role in lateral force transmission. Erskine et al. (2012) suggested that an increase in costamere density could occur in response to resistance training, and this costamere increase may enhance muscle specific force (maximum force per unit physiological cross-sectional area) – shedding light on the potential advantages of favourable genotypes at *PTK2* polymorphisms for increased strength via enhanced adaptability to resistance training.

The mechanisms underlying the association between *PTK2* polymorphisms and muscle specific force are unclear. Previous literature has shown that focal adhesion null cells form stronger adhesions, demonstrate enhanced contractile properties and migrate at a slower rate than their wild type counterparts (Furuta et al., 1995; Chen et al., 2002; Ren et al., 2000). Favourable genotypes at rs7460 and rs7843014 might alter gene expression and subsequent differences in focal adhesion kinase expression. This could explain the association between *PTK2* and muscle specific force and thus, some of the variability in strength and power of the athletes in the IMTP and CMJ observed in Chapter 5. Nonetheless, this thesis provides the first evidence of a role for *PTK2* in athletic performance, although there is a requirement for future research to extend these observations to other important strength/power phenotypes in elite rugby. For example, it could be very useful to investigate muscle specific force in the elite athletes to explore the variability in this phenotype which could be attributable to genotypes of *PTK2.*

7.3.6 *HIF1A* rs11549465

For *HIF1A* rs11549465, Chapter 4 showed that forwards had a greater T allele frequency than non-athletes, and showed 1.5 times greater odds of possessing T alleles than non-athletes. In

addition, forwards had more T allele carriers and T alleles than backs, and had almost twice the odds of being T allele carriers than CC homozygotes. *HIF1A* was not associated with IMTP or CMJ in Chapter 5, or with in-game performance in Chapter 6. Given the importance of HIF-1α in regulating the expression of a number of genes that are implicated in various cellular functions (Ke and Costa, 2006), it was reasonable to assume that the frequency of the functional missense polymorphism (C→T transition; Pro582Ser) at rs11549465, that is known to effect HIF-1α protein stability and transcriptional activity (Tanimoto et al., 2003), would differ between athletes and non-athletes and/or between forwards and backs. Data in Chapter 4 suggests that an advantageous role of the T allele at rs11549465 could explain some of the variation in strength and power between athletes and non-athletes (observed in Chapter 3), and support previous investigations reporting an overrepresentation of the T allele in athletes from strength- and power-oriented sports compared to non-athletes (Ahmetov et al., 2008; Cięszczyk et al., 2011; Gabbasov et al., 2013). An additional novel finding in Chapter 4 was that the frequency of CT genotype and T allele at rs11549465 differed between rugby forwards and backs, suggesting that *HIF1A* might explain a proportion of the variability in force generation and power production capabilities within elite rugby athletes of different playing positions.

Previous literature reported that increasing frequency of the T allele in weightlifters paralleled their level of achievement (Ahmetov et al., 2008), whilst Gabbasov et al. (2013) demonstrated that the T allele could distinguish wrestlers from weightlifters (T allele higher in wrestlers) of regional or national competitive levels. There is also scientific evidence suggesting the association between the rs11549465 polymorphism and athletic performance could be mediated via differences in muscle fibre type proportion (Ahmetov et al., 2008), shedding light on the potential advantage of the C allele at rs11549465 for endurance

performance. Collectively, previous evidence and data from Chapter 4 indicate that *HIF1A* rs11549465 might belong to a growing panel of variants that influence elite performance and playing position in rugby.

7.4 Polygenic profiling

To date, the concept that strength performance is likely to be determined by the simultaneous presence of multiple genetic variants deemed advantageous for strength/power phenotypes has been addressed in principle (Hughes et al., 2011) or in a mixed cohort of strength/power athletes (Ruiz et al., 2010; Guilherme and Lancha, 2017; Guilherme et al., 2018). Some investigations have sought to define or quantify the impact of multiple genotype combinations that influence strength/power athlete status or performances for two or three variants (Ahmetov et al., 2013; Gineviciene et al., 2016; Ben-Zaken et al., 2019; Grishina et al., 2019), with one study including 28 variants in a cohort of 53 elite Russian weightlifters and 30 powerlifters (Moreland et al., 2022) – supported by Kikuchi et al. (2021) in a cohort that included 53 Russian elite weightlifters and 100 sub-elite Japanese weightlifters. This thesis has extended this concept by using a genotype-phenotype design within an elite rugby union context – focusing on the combined effect of seven polymorphisms on laboratory-based strength/power measures and in-game performance.

In Chapter 5, no associations were found between either TGS or FAC and variables in CMJ and IMTP, but Chapter 6 captured the polygenic influence of seven SNPs on in-game performance. Specifically, TGS was associated with the number of tackles in forwards (r = 0.23), with the polygenic influence on the same variable also being captured by the FAC approach using three categories. For FAC, forwards possessing 10-14 favourable alleles were involved in 16.5% more successful tackles (effect size (r) = 0.30) than those possessing 1-7

favourable alleles. In addition, forwards possessing 8-9 favourable alleles were involved in 9.5% more successful tackles (effect size (*r*) = 0.23) than those possessing 1-7 favourable alleles. Furthermore, the FAC approach using two categories showed that backs possessing 9-14 favourable alleles gained 26.3% more territory (effect size (r) = 0.24) than those possessing 1-8 favourable alleles. Therefore, despite the fact that no associations were detected within the forwards for individual polymorphisms, or within either the forwards or backs in relation to the ability to gain territory, these findings demonstrate that both TGS and FAC approaches could be powerful in detecting the cumulative effect of individual SNPs that show tendencies for associations with the phenotype of interest.

It is most probable that as additional SNPs influencing muscular strength/power are included for polygenic profiling, the cumulative effect will approach statistical significance. Notably, and in relation to this, the polygenic analyses adopted in Chapters 5 and 6 followed the original intention of Williams and Folland (2008) for the TGS, in that the allocation of the allele deemed advantageous was based purely on previous literature. This is in contrast to the data-driven approach (Bouchard et al., 2011; Thomaes et al., 2011; Thomaes et al., 2013) adopted in some investigations using polygenic analyses, where those alleles that have demonstrated an advantage for the phenotype investigated in the same work were considered advantageous for the polygenic profiling, and the variants that have not shown any association were simply excluded from the analyses. Of note is that more recently, Massidda et al. (2014) constructed a weighted genetic predisposition score, in which a weight was given to each genotype, based on the explained variance of the phenotypic traits by this gene variant. While the usefulness of such adapted approaches is acknowledged, the polygenic analyses in this thesis relied on data from previous literature via which the allele considered beneficial was the basis of all analyses, including whether the models analysed were

considered dominant or recessive for individual SNPs. Nevertheless, despite the exclusion of three variants and ~38% of the athletes, and the non-parametric nature of the data sets for the in-game variables (and associated non-parametric analyses that are, arguably, less powerful than their parametric counterparts), combined with the unexpected direction of association for some of the individual SNPs, these results suggest in an unbiased way that favourable alleles in several strength/power related SNPs might exert a combined influence on in-game performance variables in elite rugby union. Further research that uses TGS or other genetic predisposition scores (Charlier et al., 2017) in larger samples of elite rugby athletes is warranted, as this appears a potentially powerful approach serving to further our understanding of the highly polygenic nature of strength/power phenotypes.

7.5 Consideration of gene-environment interactions

Undoubtedly, the most obvious environmental factors influencing athletic performance are training and nutrition. The long-established fact that muscle hypertrophy occurs in response to resistance training and in the presence of amino acids (Tiidus et al., 2012) exemplifies the importance of both environmental factors in promoting muscle size and strength. Rugby athletes are exposed to high training loads, resulting in substantial energy outputs (Bradley et al., 2015; Morehen et al., 2016), glycogen utilisation (Bradley et al., 2016; Bradley et al., 2017), exercise-induced muscle damage from training and game-play (Pollard et al., 2018), and extensive impact-induced muscle damage, particularly from collisions during competition (Costello et al., 2018). Therefore, to optimally prepare, fuel and recover from the demands of training and competition, athletes must consume adequate amounts of food to meet energy and macronutrient requirements that augment glycogen restoration, muscle regeneration, immune support, as well as reduce fatigue (Heaton et al., 2017). Accordingly, recent research provided evidence that the amount, composition, and timing of food intake

can profoundly affect athletes' performance (Collins et al., 2021; Jonvik et al., 2022); and this strengthens the well-established fact that both training and nutrition are of utmost importance for elite athletes. Thus, it is unsurprising that a gene-training or gene-diet interaction effect of a specific genetic variant on a quantitative trait (such as muscle strength) could lead to differences in variance of the trait among groups of individuals with different variant genotypes (Roth, 2007; Wang et al., 2019).

Complex human phenotypes (e.g. skeletal structure, muscle strength, and heart and lung size) are made more complex by the fact that environmental factors can interact with genetic factors such that any one gene and environmental factor by itself may have minimal influence, but acting together the various factors can have a large influence on a phenotype. Each of the vast array of complex phenotypes that influence athletic performance result from a complex interaction between a myriad of anatomical, biochemical, and physiological systems (Roth, 2007). For example, muscle strength/power are influenced by the number of muscle fibres, fibre type proportions, angle of pennation, number of motor neurons, and blood flow (Folland and Williams, 2007), to name but a few (Section 1.3). These phenotypes themselves will be influenced by a variety of other processes (e.g. dietary volume and characteristics, muscle protein synthesis, and appetite) and cellular types governing these processes, such as muscle proteolysis and synthesis pathways, gut epithelium, and hepatic transport (Puthucheary et al., 2011) – all considered intermediate phenotypes of the broader strength phenotype. In turn, each of these intermediate phenotypes is influenced by many individual genes; such that the broader the phenotype, the larger the number of contributing genes. Thus, our final form and function will be determined by the interaction between these numerous genetic factors and the diverse environmental stimuli to which we are exposed (Puthucheary et al., 2011). In other words, gene-environment interactions occur when a

specific environmental factor influences a phenotype differently depending on the different alleles present at a specific locus within a gene that is important for that phenotype.

There is evidence that at least 62 genetic markers are linked to elite strength/power athlete status (Ahmetov et al., 2016), with some of these markers also associated with strength/power performance in athletes (e.g. Fedotovskaia et al. (2012); Gabbasov et al. (2013); Ben-Zaken et al. (2019)). These genes are implicated in growth and development, skeletal muscle contraction, glycolysis, metabolism, energy homeostasis, and neurogenesis (Ahmetov et al., 2016). Whilst we acknowledge the limitations of each investigation in this thesis (detailed in each respective Chapter), our results are the first to show that some genetic variants (previously associated with strength/power) may be indicative of superior abilities in established measures of strength/power and in-game performance within elite rugby union athletes. Because elite athletes are, arguably, exposed to somewhat similar and controlled regimes of training and nutrition (where strength and conditioning processes as well as physiological monitoring are of the highest standard), the associations found in this work may indicate that alleles at specific loci are interacting with the athletes' environment to influence (to some extent) their strength/power and in-game performance. While there is some variability between individual athletes and playing positions in training load and aspects of nutrition, compared to the wider population of athletes from a range of different sports and the vast majority of people who are non-athletes, elite rugby players are *relatively* homogenous in terms of training load (Jones et al., 2016; Weaving et al., 2020) and nutrition (Kelly et al., 2020; Posthumus et al., 2021). i.e. none are sedentary or even close to that, and all will do strength, speed, repeated sprint, agility, and rugby skills training. Similarly, no elite rugby athletes are likely to have low protein, low carbohydrate, or high fat diets, and none will be severely restricting energy intake, nor doing intermittent fasting of prolonged

durations. Thus, while gene-environment interaction means that some players might respond differently to others to the training and nutrition they're exposed to, at least that exposure to the environmental stimuli of training and diet is likely to be relatively common in all rugby athletes. In a hypothetical example where it is assumed that athletes are exposed to precisely the same training and nutrition (which is neither desired nor possible in the real world), the observed phenotypic differences could be attributed to genotype differences between individual athletes (although this hypothetical example assumes there is no variability due to the influence of epigenetic regulation). It is acknowledged, however, that the way by which genetic factors, environmental factors, and gene-environment interactions work in combination to determine the athletes' strength/power abilities and competitive performance is far more complex than once anticipated. In fact, most human traits are complex because they are affected by many genetic and environmental factors as well as potential interactions between them (Wang et al., 2019). Consequently, a main challenge in sports genetic research is to understand the molecular mechanisms by which alleles at relevant polymorphisms interact with the environment to exert their effect on phenotypes that underpin performance.

7.6 Strength- and power-related variants – potential use in genetic testing

There is considerable interest in the utilization of genetic information as a tool to predict future elite athletes and aid selection processes. Genetic testing that is marketed directly to the public, coaches or parents is known as direct-to-consumer genetic testing and is used for the purpose of talent identification and to assess potential for sports performance. In their consensus statement, Webborn et al. (2015) (comprising experts in the field of genomics, exercise, sport performance, disease, injury and antidoping) reported that there is a concern among the scientific community that knowledge of sports genetics is being misrepresented for commercial purposes in relation to direct-to-consumer testing. The authors added that

there is concern over the lack of clarity of information over which specific genes or variants are being tested, and the almost universal lack of appropriate genetic counselling for the interpretation of the genetic data to consumers (Webborn et al., 2015). Varley et al. (2018) aimed to investigate the use of genetic testing in UK elite sport and assess how genetic testing might be received by athletes and those employed. The authors estimated (via questionnaires) that genetic testing for sports performance and injury susceptibility occurs in UK elite sport, albeit it is not commonly conducted. Interestingly, the authors found that the majority of athletes and supporting staff believe that genetics are important in determining an athlete and appear willing to engage in genetic testing for individualising training to improve performance and reduce injury risk (Varley et al., 2018).

Overall, the scientific literature shows that despite continued progress in sports genetics, with some genetic variations appearing reasonably promising candidates for influencing athlete status (Ma et al., 2013; Ahmetov et al., 2016; Moreland et al., 2022), for most genes and variants of interest the impact on sport performance remains inconclusive (Bouchard, 2015; Pitsiladis et al., 2016; Varley et al., 2018). In addition, the published literature has not demonstrated effective practical applications of genetic testing for sport performance or injury susceptibility (Webborn et al., 2015; Varley et al., 2018). The inherent challenges in assessing gene-environment interactions, and the polygenic nature of complex sport- and exercise-related traits that contribute to athletic prowess, suggest very limited applied use in assessing or predicting athletic excellence. Moreover, the need to understand gene-gene interactions (Bouchard, 2015) and gene hierarchy (Williams and Folland, 2008) provide further challenges for the predictive potential of genetic testing in sports. However, despite this limited knowledge at present, the value of genetic testing in athletes for research

purposes should not be underestimated. The need and potential for more substantial and evidence based future applications nevertheless remains.

7.7 Role of polymorphisms in disease

While genetic variation may have a positive influence on a sport-related trait, some genetic variants associated with a phenotype that underpins some aspect of athletic performance are found to have a role in disease or its predisposition (Roth, 2007). For example, it was previously thought that the *ACTN3* R577X polymorphism could have a role in certain types of muscular dystrophy. North and Beggs (1996) studied the expression of skeletal muscle isoforms α-actinins in muscle biopsies from 17 patients with congenital muscular dystrophy (CMD) and other neuromuscular disorders, and found that four specimens of patients with CMD had no detectable expression of α-actinin-3; suggesting that α-actinin-3 protein deficiency may be a marker for CMD. Subsequently, Vainzof et al. (1997) reported that nine of the 54 patients studied (all with CMD) were deficient in α-actinin-3, however, the authors suggested that this could be a secondary effect in muscular dystrophy patients. The R577X was identified by North et al. (1999), with the authors reporting a complete absence of α-actinin-3 protein in XX genotype, and an X allele frequency of 25–50 % in the general population – hinting that R577X might be associated with certain types of muscular disease. Since then, different genotypes of the R577X polymorphism have been associated with both elite power- and endurance-oriented athlete status, with the associations replicated in many independent cohorts (Section 1.9.2). However, recent evidence shows that the α-actinin-3 deficiency results in significantly reduced muscle strength and poorer walking ability in young patients with Duchenne muscular dystrophy (DMD) (Hogarth et al., 2017), and lower left ventricular dilation-free survival rate in patients with DMD (Nagai et al., 2020),

confirming the role of R557X (arguably the most studied polymorphism in sport) in certain types of muscular dystrophy disease.

Genetic variations can also have dramatic consequences for a phenotype, resulting in overt disease or a strong disease predisposition for an individual (Roth, 2007). This could be the case in athletes where such variants do not manifest in previously identified obvious and/or visible phenotypic features. For example, physical exercise, although associated with several health benefits, can trigger life-threatening arrhythmias and sudden cardiac death in athletes affected by an underlying cardiac disease (Pelliccia et al., 2021). Inherited cardiovascular diseases that predispose to lethal arrhythmias are the most common causes of sudden cardiac death in athletes (Maron et al., 1980; Maron, 2015). The diagnosis of inherited cardiovascular diseases in athletes may be challenging, mainly due to changes in the electrical, structural and functional properties occurring in the heart as a physiological adaptation in response to the increased demands imposed by the sport (Castelletti et al., 2022). Thus, these adaptive changes (the so-called 'athlete's heart') may overlap with inherited cardiovascular diseases, such as cardiomyopathies and cardiac ion channel diseases (Pelliccia et al., 2021; Castelletti and Gati, 2021). Clinical molecular laboratories are increasingly detecting novel DNA sequence variants in the course of testing patient specimens – resulting in a rampant increase in number of genes linked to genetic disorders (Richards et al., 2015). Accordingly, in an attempt to facilitate the process of variant interpretation, research efforts have been made to develop a universally applicable workflow to classify variants related to Mendelian diseases – based on criteria using typical types of variant evidence; such as population data, computational data, functional data and segregation data (Richards et al., 2008; Richards et al., 2015).

An individual's family history plays an important role in a clinical assessment for a disease and may mediate the decision to conduct a genetic test (Merghani and Sharma, 2012; Landry et al., 2017; Stormholt et al., 2021). Subsequently, genetic testing is conducted to support the clinical diagnosis of inherited diseases through the identification of pathogenic genetic variants, and to make a pre-clinical genetic diagnosis among proband's family members, more commonly known as 'cascade family screening'. Castelletti et al. (2022) suggested that for children and adolescents without signs of the phenotype but with a familial diagnosis of a genetic disease, the benefits of a predictive genetic testing on diagnosis, management and risk stratification should outweigh any negative psychological and emotional consequences associated with non-eligibility to compete. Therefore, it is important to know the yield of molecular genetic testing for different genetic diseases as well as the potential implications of the test results for clinical management as part of talent identification processes.

7.8 Conclusions

The current thesis investigated 10 strength/power associated genetic variants in elite rugby union athletes and explored how the variants might influence the athletes' capabilities of achieving athlete status, and their strength/power and performance during match play. Consequently, this work has extended the growing body of research that evidences the role of genetics for athletic performance by using an athlete cohort from one sport and non-athletes. There appears to be a genetic association with established measures of strength/power, and that influence appears to be partly reflected in athlete in-game performance. The work presented here has implications for the physical monitoring of elite rugby athletes and for talent identification in the future.

7.9 Direction for Future Research

Muscular strength and power are highly polygenic, and the collective findings in this thesis provide further evidence of the possible individual and combined influence of some polymorphisms on rugby athletes' strength/power and performance during match play. Although the analyses employed revealed a number of novel associations with the phenotypes investigated, it is pertinent to state that most probably many existing associations were not detected; and assuming that this statement is correct, the only plausible attribution of such lack of detection could be sub-optimal statistical power, with such limitation becoming more evident when combined with the small inter-individual variability in the phenotype (often less than 1%) that each variant can most probably explain (Bouchard, 2015). The aforementioned factors indicate that a main challenge in sports genetics is to recruit large numbers of athletes (Heffernan et al., 2015; Pitsiladis et al., 2016). Thus, collaborative research efforts should aim to recruit larger sample sizes of rugby athletes across a range of competitive levels, from different geographic ancestries, and athletes of both sexes – to explore the broader range of strength and power phenotypes in rugby.

Of utmost importance for future research is to maximise the genetic information gathered from athletes' genomes, and this can only bey accomplished by using the most advanced genotyping techniques. Undoubtedly, GWAS would probably be a valuable hypothesis-free approach to identify novel candidate genes that influence phenotypes of muscle mass, strength, and power. GWAS involves the process of investigating large numbers of known polymorphisms simultaneously (e.g. ~2 million) for a given complex trait, although it requires that sample size increases in concert and although its current application is somewhat (and arguably) premature, it is an inevitable approach in sports genomics (Pitsiladis et al., 2016). The ultimate advancement in genotyping, providing that the required

resources such as cohort sizes and financial resources are available, would be to sequence the whole genome of a large number of athletes. Undoubtedly this would be the most comprehensive method to identify many variants of functional importance to complex traits such as muscular strength/power and associated sub-phenotypes. At that point, genetics will move from being primarily a methodological problem to being a bioinformatics and ethical problem (due to the potentially highly informative nature of data).

A potential application of genetic information is in identifying talent. However, as accentuated throughout this thesis, the current state of knowledge that supports such application is inadequate (Webborn et al., 2015; Vlahovich et al., 2017); the currently available genetic tests and analysis methods are not powerful enough to inform important decisions in identifying talent in sport to a substantial degree. Furthermore, there are broad ethical concerns regarding issues such as the confidentiality of genetic data and its use in minors, with such concerns increasing further in concert as genetic data become more informative in future. For example, such tests could be used to make decisions for or against a sporting career, whilst a more extreme scenario could be the use of genetic tests to select embryos on the basis of athletic and/or intellectual abilities. With regards to the eventuality of substantial progress in sports genetics, it was suggested that a thorough multidisciplinary approach would need to be conducted to analyse the usefulness and efficacy of genetic testing and its use in talent identification (Williams et al., 2016). This will serve to determine whether genetic data can provide information not already captured within other traditional non-genetic tests (such as physiological and anthropometric tests) that are already routinely used in talent identification and development programmes in rugby (Section 1.6).

An important consideration relates to the determination as to whether talent should be identified by genetic tests, non-genetic tests or a combination of both. From a legal and ethical point of view, the term ‘genetic exceptionalism’ (Green and Botkin (2003); meaning that genetic tests are somewhat special and therefore require specific and different legislation from that used for other biological tests) was previously used in the medical literature to debate the appropriateness of genetic tests to predict disease. However, from an applied physiological point of view, it appears that recent research literature (Pickering et al., 2019; Kikuchi et al., 2021; Moreland et al., 2022) suggests the combined use of the currently employed physiological tests, plus information derived from genetic tests with substantial (and validated) predictive power. An important aspect that differentiates these two broad categorisations of tests is that the provision of information from genetic tests does not change with age, whereas the information gained from a traditional performance test, and consequently the predictive quality of that test, does change throughout an individual’s life (Williams et al., 2016). However, whilst commercial pressures undoubtedly exist, it would be more responsible to wait for the necessary progress in sports genetics to occur before using genetic information to aid talent identification and development in rugby.

APPENDIX A

- Information Sheet for Participants (ISP)
- Informed Consent Form (ICF)
- Questionnaire for control participants
- Questionnaire for Athletes
- *ACTN3* feedback sheet for participants

APPENDIX A

Manchester Metropolitan University — Cheshire —

MANCHESTER METROPOLITAN UNIVERSITY

Department of Exercise and Sport Science

Information Sheet for Participants

Title of Study:

The Genetic Profile of Elite Athletes

Ethics Committee Reference Number: 12.07.11 (i)

1) This is an invitation to take part in a piece of research.
You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

2) What is the purpose of the research?
The main purpose of the project is to investigate the influence of genetic differences on elite strength/power, mixed-demand and endurance athlete status compared to non-athletes.

3) Why is the study being performed?
Previous scientific studies have demonstrated that differences in genetic make-up are linked to elite athlete status, although relatively few gene variants have been identified and sometimes not enough participants have been recruited to find meaningful results. This study is being performed in very large groups of elite strength/power, mixed-demand and endurance athletes, as well as non-athletes, in order to learn more about which genes influence elite athlete status.

4) Why am I being asked to take part?
We are trying to recruit people from different populations, i.e. top-level strength/power, mixed-demand and endurance athletes, as well as non-athletes. This way we can compare the genetics of elite athletes with the general population.

5) Do I have to take part?
You are under no obligation to take part in this study. If, after reading this information sheet and asking any additional questions, you do not feel comfortable participating in the study you do not have to. If you do decide to take part, you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you, on written request to the principal investigator, and this will not be included when the research is reported. If you decide not to take part or withdraw from the study it will not affect your relationship with any of the staff at the Manchester Metropolitan University. If you do decide to take part you will be asked to sign an informed consent form stating your agreement to take part and you will be given a copy together with this information sheet to keep.

6) What will happen to me if I agree to take part?
In order to obtain the following information, you will either i) be visited once by one of the investigators, ii) asked to come to the Crewe campus of the University on one occasion, or iii) sent a pack, then asked to complete information sheets and simple DNA-collection procedures (saliva sample), and send them to the investigators. In the case of iii), you will be guided through the correct procedures during a telephone call made by one of the named members of the research team (please see below).

Questionnaires. We will ask you to complete a short survey that will give us an indication of your physical activity level, general health, ethnicity, sporting discipline and performance achievement, recent exercise and diet, injury history and one psychological trait questionnaire known as ‘mental toughness’. If you are female, you will also be asked about your menstruation status. The questionnaires will take about 30 minutes to complete.

Body height, weight and somatotype. We will measure your height and weight using standard equipment, from which we will be able to calculate your body mass index. This will take 5 minutes to complete. If you are comfortable doing so and if time permits, we will also determine your somatotype, which will involve taking skinfold measurements from multiple sites on your body (you will need to wear shorts and t-shirt/sports-bra). This will take a further 20 minutes to complete.

Blood sample/saliva sample. If you are visited by one of the investigators or you are asked to visit the University laboratory, you will be asked to provide a small blood sample, from which we will be able to analyse your DNA, RNA and protein levels. While you are lying down, a qualified phlebotomist will take 10 mL blood from a vein in your arm. This is a relatively painless procedure and will take less than a minute to complete. Alternatively, if blood sampling is not possible, you will be asked to dribble 2 mL of saliva into a tube for a few minutes and send the sample to the investigators. These procedures are completely harmless and painless and you will be guided through the correct procedures by one of the research team members.

Bone mineral density. You will be asked to undertake a bone ultrasound or peripheral quantitative computed tomography (pQCT) scan to give us information about your bone mineral density. The ultrasound scan involves a member of the research team scanning your shinbone with a small probe on your skin with the help of a lubricating gel for approximately 5 minutes. This procedure is completely harmless and painless. The pQCT scan (a bit like a medical X-ray) lasts approximately 10 minutes and involves your lower leg being placed in a supported, still position in the centre of the scanner. If you visit the university laboratory you will be asked to complete a dual-energy X-ray absorptiometry (DEXA) scan which gives us information about your bone mineral density as well as muscle mass and fat mass. This procedure is also somewhat similar to that of a medical X-ray. You will be asked to lie on a plinth and remain as still as possible throughout the scan which will last approximately 8 minutes. Each DEXA and pQCT scan exposes you to an extremely minimal dose of radiation, which is well below the maximum recommended dose regarded as safe (see question 7 for more details).

Muscular strength and power. Muscular strength and power will measured using a cycle ergometer and a force platform. You will be asked to cycle as hard as you can for a short time (6 seconds) on the cycle ergometer. Using the force platform, you will be asked to do (i) a maximal jump and (ii) a maximal pull on a static bar while you are standing almost upright (knees slightly bent). You will be assisted by a member of the research team through all tests which will include warm-up and cool-down periods. To complete all strength and power tests will require up to 30 minutes.

7) Are there any disadvantages or risks in taking part?

Blood sample. This is not a painful procedure but some people are a little squeamish about blood and tend to faint. Therefore, you will be seated/lying down while we take the blood and you do not have to see anything. Sometimes there is a little bruising but this should disappear in a matter of a few days, even in the most extreme cases. To prevent further bleeding, you might be asked to place a cotton wool ball over the punctured site and to hold it in place for a few minutes or until bleeding has stopped. Blood samples will be stored in a locked freezer until analysis at a later date. Only appropriately qualified personnel will be used for taking the blood sample. *Any personal information provided by you in connection with the blood donation will be held in strict confidence. Furthermore, all data will be anonymised and stored in secure locations to prevent identification of an individual.*

DEXA/pQCT Scan. Should you decide to take part in this research, you will be exposed to a very small amount of radiation, specifically 8 µSv (DEXA) or 10 µSv (pQCT), depending on which scan is completed. This dose is extremely minimal and is equivalent to the amount of radiation you are subjected to over an average 2-3 days of your life or simply travelling 30-40 miles in a car.

Muscular strength and power. The three tests of muscle strength and power require short, maximal efforts, so you should not find them tiring. Like any physical effort there is a risk of muscle strain, but by ensuring you are fully warmed up this risk will be kept to a minimum.

8) What are the possible benefits of taking part?
The broad benefits of the research are linked to the potential for the study to highlight new links between gene variants and elite athlete status. This information will improve our understanding of what contributes to making an elite athlete 'elite' and which genes are responsible for determining the strength, size, power and endurance capacity of human muscle. By providing the necessary information, you will be contributing to our further understanding of how the body works and what makes us different from one another. Furthermore, we can provide you with immediate feedback concerning your bone mineral density and muscle strength and power, plus feedback concerning *ACTN3* genotype once the genetic analysis has taken place at a later date. This gene variant has been associated with elite strength/power and endurance athlete status.

9) Who are the members of the research team?
- Dr Alun Williams (MMU): Principal Investigator; responsible for overall project design and management, collecting blood and saliva samples, conducting other measurements and administering questionnaires, data analysis and interpretation.
- Dr Stephen Day (MMU), Dr Georgina Stebbings (MMU), Dr Robert Erskine (Liverpool John Moores University), Prof Craig Sale (Nottingham Trent University) Dr Philip Hennis and Prof Hugh Montgomery (both University College London): responsible for project design, collecting blood and saliva samples, conducting other measurements and administering questionnaires, data analysis and interpretation.
- Sarah Lockey, Shane Heffernan, Adam Herbert, Jon Brazier, Mark Antrobus, Peter Callus (MMU): PhD Students; responsible for collecting blood and saliva samples, conducting other measurements and administering questionnaires, data analysis and interpretation.

10) Who is funding the research?
This project is being funded by the Health, Exercise and Active Living Research Centre at Manchester Metropolitan University.

11) Who will have access to the data?
All information collected during the course of this research project will be kept confidential and will only be used for the purposes of the study. The data will be stored anonymously; only the members of the research team named above will have access to it.

The results from the study will be communicated at scientific conferences and published in peer-reviewed scientific journals some time in the future but in a manner that does not allow an individual's identity to be determined. You may obtain a copy of any publication that result from the research by contacting the Principal Investigator (see below - Section 13).

12) Who do I contact if I feel my rights have been violated?
If you wish to make a complaint regarding your involvement in the study, please contact:

MMU Ethics Committee

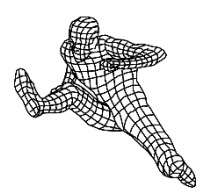
Registrar & Clerk to the Board of Governors
Head of Governance and Secretariat Team
Manchester Metropolitan University
All Saints Building, All Saints
Manchester M15 6BH
Tel: 0161 247 1390

13) Finally, thank you for considering to participate in our research study. If you require any more information, please contact:

Dr Alun Williams
Email: a.g.williams@mmu.ac.uk
Tel: 0161 247 5523

Manchester Metropolitan University
— Cheshire —

Department of Exercise and Sport Science



Informed Consent Form

(Both the investigator and participant should retain a copy of this form)

Name of Participant:

Principal Investigator: Dr Alun Williams

Project Title: The Genetic Profile of Elite Athletes

Ethics Committee Approval Number: 12.07.11 (i)

Participant Statement

I have read the participant information sheet for this study and understand what is involved in taking part. Any questions I have about the study, or my participation in it, have been answered to my satisfaction. I understand that I do not have to take part and that I may decide to withdraw from the study at any point without giving a reason. Any concerns I have raised regarding this study have been answered and I understand that any further concerns that arise during the time of the study will be addressed by the investigator. I therefore agree to participate in the study.

It has been made clear to me that, should I feel that my rights are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform the The University Secretary and Clerk to the Board of Governors, Manchester Metropolitan University, Ormond Building, Manchester, M15 6BX. Tel: 0161 247 3400 who will undertake to investigate my complaint.

Signed (Participant)		Date	
Signed (Investigator)		Date	

The Genetic Profile of Elite Athletes
Questionnaire: Physical Activity & General Health

Manchester Metropolitan University

Thank you for participating in this research study. We would like you to answer a few questions concerning your general health and physical activity level. Please answer the following questions as honestly as you can.

Participant ID code: _____ Date of birth: _____

Gender (please tick): Male ☐ / Female ☐ Height: _____

Nationality (as on passport, e.g. British): _____ Body weight: _____

What is your ethnic group? Please tick the appropriate box.

A) White: English ☐ Scottish ☐ Welsh ☐ N. Irish ☐ Irish ☐
French ☐ South African ☐ New Zealander ☐ Australian ☐ Other ☐
If other, please state here: _____

B) Mixed: White & Black British ☐ White & Black Caribbean ☐ White & Black African ☐ White & Asian ☐ White & Latin American ☐ Other ☐
If other, please state here: _____

C) Asian: British ☐ Indian ☐ Pakistani ☐ Chinese ☐ Japanese ☐ Other ☐
If other, please state here: _____

D) Black: British ☐ Caribbean ☐ African ☐ Other ☐
If other, please state here: _____

E) Latin American: Brazilian ☐ Argentinian ☐ Mexican ☐ Colombian ☐ Other ☐
If other, please state here: _____

F) Pacific Islands: Samoa ☐ Fiji ☐ Tonga ☐ PNG ☐ Other ☐
If other, please state here: _____

G) Other ethnic background: ☐ Please state here: _____

I do not wish to state my ethnic origin ☐
Using the ethnic groups above as a guide, please tell us the ethnic origin of your:
Mother: _____ Do not know: ☐
Father: _____ Do not know: ☐
Mother's mother: _____ Do not know: ☐
Mother's father: _____ Do not know: ☐
Father's mother: _____ Do not know: ☐
Father's father: _____ Do not know: ☐

Blood donation
We would like to take a small (10 mL) blood sample from a vein in your arm. Before doing so, please answer the following safety questions.
1. Have you ever been infected with a blood-borne disease? _____ Yes ☐ No ☐
2. Are you anaemic or receiving treatment for anaemia or iron deficiency? __ Yes ☐ No ☐

If you have answered YES to any of these questions and/or you would prefer not to provide a blood sample, a saliva sample may be provided instead.

Your general health

1. **At present,** do you have any health problem for which you are:

a) on medication, prescribed (by a doctor) or otherwise _____ Yes ☐ No ☐
b) attending (visiting) your doctor _____ Yes ☐ No ☐
c) on a hospital waiting list _____ Yes ☐ No ☐

2. **Have you ever** had any of the following?

a) Your doctor advised you not to take vigorous exercise _____ Yes ☐ No ☐
b) Pain in your chest when you undertake physical activity? _____ Yes ☐ No ☐
c) Central Nervous System disease, such as Parkinson, Alzheimer, Convulsions/epilepsy _____ Yes ☐ No ☐
d) Have you any history of chest problems, such as bronchitis, asthma or wheezy chest _____ Yes ☐ No ☐
e) Major illness, such as viral hepatitis, cancer _____ Yes ☐ No ☐
f) Eczema _____ Yes ☐ No ☐
g) Diabetes _____ Yes ☐ No ☐
h) High blood pressure _____ Yes ☐ No ☐
i) A limb fracture _____ Yes ☐ No ☐
j) Blood disorder, such as clotting problems, thrombosis, aneurysm, embolus) _____ Yes ☐ No ☐
k) Head injury _____ Yes ☐ No ☐
l) Digestive problems _____ Yes ☐ No ☐
m) Heart problems, such as heart attack, valve disease, palpitations, angina _____ Yes ☐ No ☐
n) Problems with bones, such as osteoporosis or osteoarthritis _____ Yes ☐ No ☐
o) Problems with joints, such as rheumatoid arthritis, any persistent pain, or any surgery on your joints _____ Yes ☐ No ☐
p) Back problems _____ Yes ☐ No ☐
q) Disturbance of balance/co-ordination, such as dizziness or balance-system dysfunction _____ Yes ☐ No ☐
r) Numbness in hands or feet _____ Yes ☐ No ☐
s) Disturbance of vision _____ Yes ☐ No ☐
t) Physical limitations, such as visual, hearing, walking problems ____ Yes ☐ No ☐
u) Thyroid problems, e.g. rapid loss or gain of weight _____ Yes ☐ No ☐
v) Kidney or liver problems _____ Yes ☐ No ☐
w) A severe allergic reaction, e.g. swelling, breathing difficulties in response to an external stimulus _____ Yes ☐ No ☐
x) Emotional or psychiatric problems _____ Yes ☐ No ☐
y) Any other illness or condition that affects your general health or interferes with your daily activities _____ Yes ☐ No ☐

4. If you answered **YES** to any of the questions above, please describe the details briefly below or to the investigator if you wish.

_____

_____

5. Are you currently involved in any other research studies at the University or elsewhere? Yes ☐ No ☐
If **YES** please provide details of the study:

_____

The Genetic Profile of Elite Athletes: Questionnaire

Thank you for your interest in our research study. Please answer the following questions about your ethnic origin, athlete status, and your training, diet and injury history.

SECTION A: Questions concerning your ethnic background.

Participant ID code: _____ Date of birth: _____

Gender (please tick): Male ☐ / Female ☐ Height (in metres): _____

Nationality (as on passport, e.g. British): _____ Body weight (in kg): _____

What is your ethnic group? Please tick the appropriate box.

A) White: English ☐ Scottish ☐ Welsh ☐ N. Irish ☐ Irish ☐
French ☐ South African ☐ New Zealander ☐ Australian ☐ Other ☐
If other, please state here: _____

B) Mixed: White & Black British ☐ White & Black Caribbean ☐ White & Black African ☐ White & Asian ☐ White & Latin American ☐ Other ☐
If other, please state here: _____

C) Asian: British ☐ Indian ☐ Pakistani ☐ Chinese ☐ Japanese ☐ Other ☐
If other, please state here: _____

D) Black: British ☐ Caribbean ☐ African ☐ Other ☐
If other, please state here: _____

E) Latin American: Brazilian ☐ Argentinian ☐ Mexican ☐ Colombian ☐ Other ☐
If other, please state here: _____

F) Pacific Islands: Samoa ☐ Fiji ☐ Tonga ☐ PNG ☐ Other ☐
If other, please state here: _____

G) Other ethnic background: ☐ Please state here: _____

I do not wish to state my ethnic origin ☐
Using the ethnic groups above as a guide, please tell us the ethnic origin of your:
Mother: _____ Don't know: ☐
Father: _____ Don't know: ☐
Mother's mother: _____ Don't know: ☐
Mother's father: _____ Don't know: ☐
Father's mother: _____ Don't know: ☐
Father's father: _____ Don't know: ☐

Blood donation
We would like to take a small (10 mL) blood sample from a vein in your arm. Before doing so, please answer the following safety questions.
1. Have you ever been infected with a blood-borne disease? _____ Yes ☐ No ☐
2. Are you anaemic or receiving treatment for anaemia or iron deficiency? __ Yes ☐ No ☐

If you have answered YES to any of these questions and/or you would prefer not to provide a blood sample, a saliva sample may be provided instead.

PLEASE TURN OVER

SECTION B: Questions concerning your athlete status.

1. What is/was your main playing position (if team sport) or your main event (if individual sport). If multiple, please state preferred position/event.
   _____
   _____

2. Please state the number of seasons you have competed as a professional: _____

3. Please state all the professional clubs you have competed for so far in your career:
   _____
   _____

4. Please state the highest level that you have competed, including number of caps earned e.g. England under 16s (4 caps), 18s (10 caps), senior (21 caps):
   _____
   _____

5. Have you any other athletic achievements? If so please state highest achievements and include relevant details:
   _____
   _____

SECTION C: Questions concerning your training.

1. Typically, how many hours do you train a week?

   _____

2. Typically, what is your average running distance per week?

   _____

PLEASE TURN OVER

SECTION D: Questions concerning your injury history.

1. We would like to know about your injury history, so that we can explore any genetic link with the type and/or number of injuries. Your club may have information about this and it would be really useful if we could use this for our research. By ticking the box below, you give us your written informed consent to contact your club and ask if we may have access to this information. ☐

2. Have you ever fractured a bone? Yes ☐ No ☐

3. If Yes, please give details of the bone(s) you broke and at what age you broke them.

Bone	**Age**
e.g. upper leg/femur	*e.g. 20*

4. Have you ever been told that you have had a STRESS FRACTURE *(or micro-fracture)* injury? Yes ☐ No ☐

5. If Yes, please give details of the bone/bones where the stress fracture occurred and at what age the fracture occurred.

Bone	**Age**
e.g. shin/tibia	*e.g. 20*

6. If Yes, was it confirmed by a bone scan, e.g. MRI, X-ray, CT scan? Yes ☐ No ☐

7. Have you ever suffered from prolonged shin pain during exercise that does not go away for weeks? Yes ☐ No ☐

8. Does anyone in your close family suffer from OSTEOPOROSIS or FRAGILE bones? Yes ☐ No ☐ Don't know ☐

9. Has anyone in your close family ever had a STRESS FRACTURE? Yes ☐ No ☐ Don't know ☐

PLEASE TURN OVER

10. Have you ever ruptured your tendon? Yes ☐ If yes, which tendon? *e.g. Achilles* No ☐

11. If Yes, please give details of how this occurred and at what age. **Activity** *e.g. sprinting* **Age** *e.g. 20*

12. Have you ever suffered from prolonged tendon pain during exercise that does not go away for weeks? Yes ☐ If yes, which tendon? *e.g. Achilles* No ☐

13. Have you ever been told that you have had tendinopathy? Yes ☐ If yes, which tendon? *e.g. Achilles* No ☐

14. If Yes, was it confirmed by a scan, e.g. MRI or ultrasound? Yes ☐ No ☐

15. Does anyone in your close family suffer from tendinopathy? Yes ☐ No ☐ Don't know ☐

If yes, which tendon? *e.g. Achilles*

16. Has anyone in your close family ever ruptured a tendon? Yes ☐ No ☐ Don't know ☐

If yes, which tendon? *e.g. Achilles*

PLEASE TURN OVER

17. Have you ever fully ruptured a ligament? Yes ☐ If yes, which ligament? *e.g. ACL* No ☐

18. If Yes, please give details of how this occurred and at what age.

	Contact	**Non-contact**	**Age**
	e.g. tackled from the side	*e.g. landing from a jump*	*e.g. 20*

19. Have you ever been told that you have had a ligament sprain/tear? Yes ☐ If yes, which ligament? *e.g. ACL* No ☐

20. If Yes, was it confirmed by a scan, e.g. MRI or ultrasound? Yes ☐ No ☐

21. Has anyone in your close family ever ruptured a ligament? Yes ☐ No ☐ Don't know ☐

If yes, which ligament? *e.g. ACL*

PLEASE TURN OVER

22. Have you ever been concussed or knocked out? Yes ☐ No ☐

23. If Yes, how many times have you been concussed or knocked out? _____ times

24. What were you doing at the time of the injury(ies)? E.g. rugby tackle, boxing, road accident. _____
_____

25. If Yes, how long was your recovery period, until the day when you had no signs and symptoms and were free to train and play fully? (tick, multiple times if necessary, any recovery periods that apply for the different occasions)
<7 days ☐ 7-10 days ☐ 10-20 days ☐
20-40 days ☐ 40-60 days ☐ >60 days ☐

26. If Yes, was/were your concussion(s) or knock-out(s) diagnosed by a medical professional? (tick, multiple times if necessary, any that apply) Yes ☐ No ☐

27. Does anyone in your close family (parents, siblings or grandparents) suffer from a neurological condition, such as:
Dementia, Alzheimer's disease, chronic traumatic encephalopathy (CTE), cognitive impairment, movement disorders, psychiatric disorders, motor neuron disease
Yes ☐ No ☐ Don't know ☐

Who and which condition(s)?
e.g. grandfather, dementia

_____

_____

Contact Details

Manchester Metropolitan University

Thank you for your interest in this research study. Please provide us with your contact details, so that we may contact you with information at a later date.

Name (PLEASE PRINT CLEARLY): _____

Email (PLEASE PRINT CLEARLY): _____

Telephone (ONLY REQUIRED IF NOT USING EMAIL): _____

Postal address (ONLY REQUIRED IF NOT USING EMAIL): _____

_____

_____

The ACTN3 R577X gene variant

We all have two copies of the *ACTN3* gene, one inherited from each parent. At a certain point along the length of the gene the structure can vary slightly, which means that a particular protein (alpha actinin-3) can/cannot be produced in the muscle. Each person is either RR, RX or XX genotype for the *ACTN3* R577X gene variant. If you are XX genotype, you cannot produce alpha actinin-3, which is only found in fast-twitch muscle fibres. As these muscle fibres are important for producing force and power during high-speed muscle contractions, not having the protein might be detrimental for power generation. RR genotype is generally associated with strength, power and greater muscle size, while XX is linked to elite endurance athlete status.

Are you interested in receiving feedback regarding your ACTN3 R577X genotype?

Yes ☐ No ☐

If yes, would you prefer to receive that feedback via email?

Yes ☐ No ☐

Thank you for taking part in this project. All information will be kept strictly confidential.

APPENDIX B

- Operation of portable Kistler Force Plate and Isometric Mid-Thigh Pull (IMTP) rig.

APPENDIX B

Operation of portable Kistler Force Plate type 9286AA and Isometric Mid-Thigh Pull (IMTP) rig loaned from Swansea University.

The Kistler force plate type 9286AA, and associated equipment (see below) was borrowed from Swansea University on April 2017 with the intention to be used by research members of the RugbyGene project. The force plate together with its bespoke IMTP rig, analogue to digital convertor, laptop, and necessary cabling was used previously by Professor Liam Kilduff and his research team for the measurement of various muscular strength and power parameters of professional rugby players and other athletes.

Equipment – Force plate, Data Acquisition system (DAQ), laptop (with BioWare), and IMTP rig.

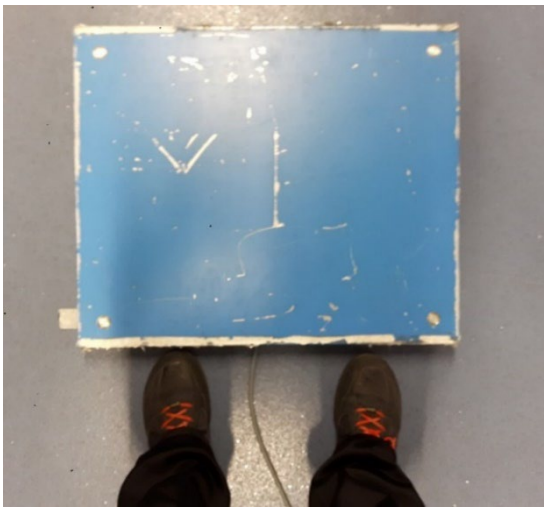


Figure 1a. Portable Kistler Force Plate with built-in charge amplifier (force plate type 2986AA; serial number 1793248).

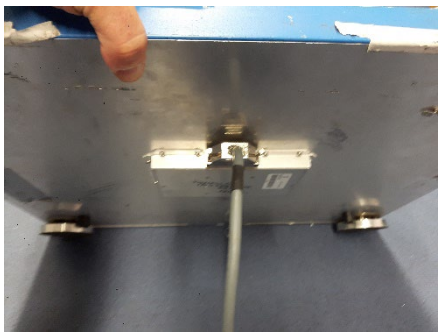


Figure 1b. Underside of Kistler portable force plate showing connecting cable entry to force plate by means of plug. Connecting cable connects force plate with DAQ system.

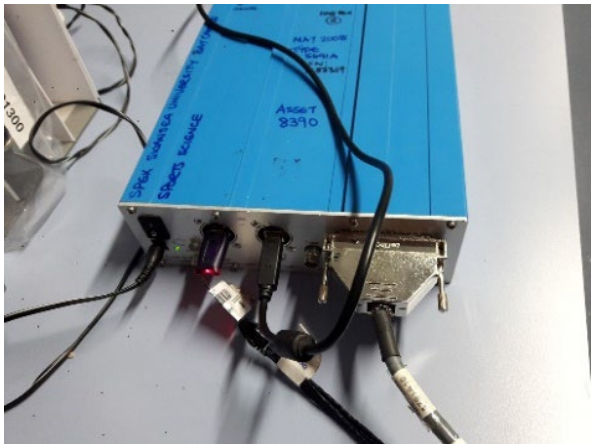


Figure 2. Data Acquisition (DAQ) system enclosure (type 5691) and connecting cabling. *Left to right* – Power supply; dongle (licence); computer interface (USB computer end); connecting cable to force plate (upper socket – force plate 1. port).

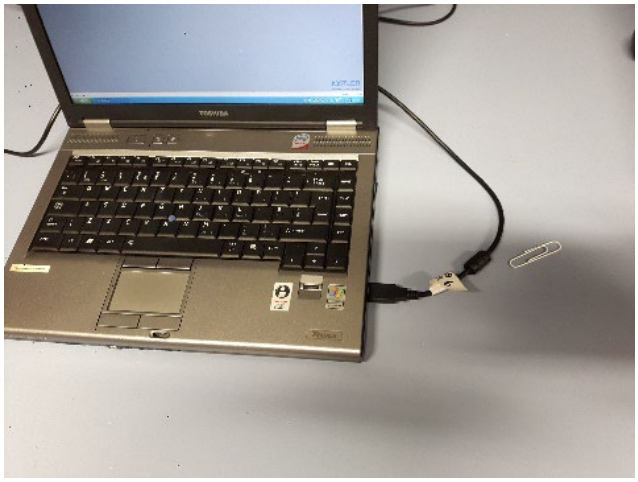
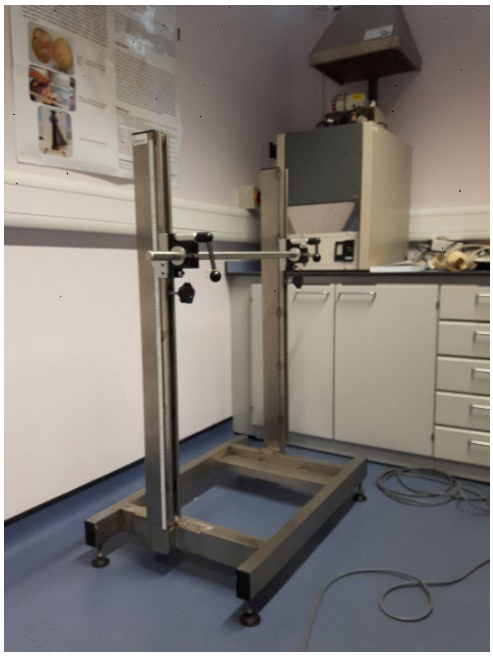
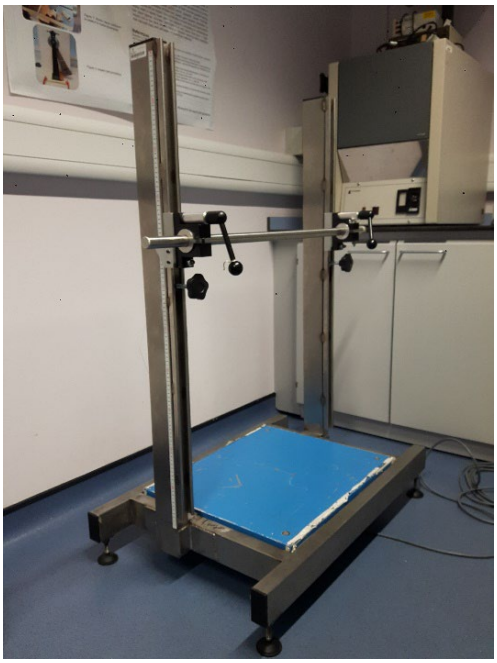


Figure 3. Computer (Toshiba 14 inch laptop) with BioWare data acquisition and signal conditioning software (type 2812A; version 4.0.0.0) installed. USB used for interface with DAQ system (see Figure 2.).





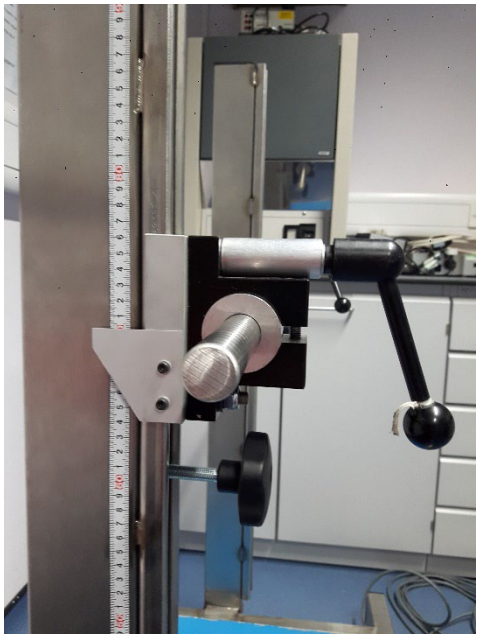


Figure 4.

Top left - IMTP Rig designed at the Sport and Exercise Science Research Centre at Swansea University (without force plate);
Top right - IMTP rig with Kistler portable force plate in its place on the IMTP rig.
Bottom left – Right side trail mechanism for fixing bar at the desired height according to each participant's anatomy and positioning as during the second pull phase of the clean movement. The knob (below bar) and lever (above bar) are used to tighten (and fix) the trail mechanism to the upright metal channel;
Bottom right – IMPT rig and force plate (placed at the front side of rig) showing connecting cable entry from the rig's underside. Note the shims placed on the force plate enclosure bottom (rear right side of the rig).

Equipment preparation and cabling

- Place the portable force plate on a solid level floor (for CMJ and DJ) or in the pull rig (for IMTP).
- With plate in position (on floor or in rig's plate enclosure), test manually for stability by rocking opposite corners and adjust using metal shims to eliminate rocking if necessary.
- Lay cabling and connect DAQ system with force plate and laptop computer (refer to figures 1 – 4).

NB. Computer and DAQ system need supply from a 220V power source by means of their respective power lead.

Equipment set-up before acquiring data

- Turn on laptop first until desktop shown, then turn on DAQ (force plate doesn't have a direct power supply).
- Create folders as required – e.g. named for testing group and type of test, e.g. "Non-athletes CMJ" or "Leinster IMTP".
- Run BioWare software. If Kistler plate not recognised or set up (it should be), choose <No>, shut down BioWare, run Instacal software that should detect a USB device (the DAQ), accept and shut down Instacal, then restart BioWare.

Acquiring data during a CMJ

Instruction: "minimum time at lowest position and jump as high as possible"

- Data collection duration (time) is 10 s and frequency 1000 Hz.
- <Start> = zeroing of plate (no participant).
- Participant onto plate, hands fixed on hips, stationary.
- Click "Enter to begin…" for 10 s collection.
- Count 3…2…1 and prompt participant to initiate jump after 5s from pressing the 'Enter' key.

Visual checks of 'good' trial:

- ✓ Stable and realistic body weight from 4-5 s.
- ✓ Jump begins > 5 s and completed Ok.
- ✓ During flight phase, trace at 0 N.

NB. Body weight assessed during 4-5 s, so participant must be still, with jump between 5-10 s prompted by tester. As long as take-off begins <10 s (i.e. participant has left plate during jump), main data are recorded, but should get take-off and landing and that provides a little extra data.

After completion, click X at top right of open window, get prompted save? Choose Yes (if good trial), give filename and save into correct folder.

Historically 2-3 good trials have been conducted with athletes, usually 2 because they are familiar with the test and a third usually provides no increase in performance and takes more time. We also suggest 2-3 good trials on a single test occasion in athletes familiar with CMJ. In non-athletes, at least 3 good trials on 2 separate test days are recommended.

Acquiring data during a DJ (40 cm typically used, or 20 cm for single leg)

Instruction: "short contact time and jump as high as possible"

- Data collection duration (time) is 5s (just to help speed of testing) and frequency 1000 Hz.
- <Start> = zeroing of plate (participant on step behind force plate, hands fixed on hips, stationary).
- Count 3…2…1, and press 'Enter' while prompting participant to initiate jump by dropping on force plate.

Visual checks of 'good' trial:

- ✓ Hands on hips throughout.
- ✓ Don't "lower down gently" or "jump up".
- ✓ Two contacts on plate required for good trial – 1st and 2nd landing, on approximately the same spot (i.e. jump after 1st contact is vertical).
- ✓ Good trace includes zero baseline, 1st contact, 2nd contact (doesn't need to reach stable trace).
- ✓ During flight phase, trace at 0 N.

NB. After completion, click X at top right of open window, get prompted save? Choose Yes (if good trial), give filename and save into correct folder.

We suggest 2-3 good trials on a single test occasion in athletes familiar with DJ.

Acquiring data during an IMTP on the bespoke rig.

Instruction: "Pull as fast and as hard as possible, and keep pulling until told to stop".

- Can get participant strapped to bar before zeroing plate as long as participant not on plate (just to help speed of testing if required).
- Height of bar adjusted to be "mid-thigh" of participant, judged visually - this has been approach used by Liam Kilduff et al. for several years, including in publications and in the data we have received from them, so for consistency we should use the same approach.
- Use weightlifting straps.
- Data collection duration (time) is 16s and frequency 1000 Hz.

- Ensure participant is in good posture on plate, ready to pull **vertically**.
- Duration of pull is 5 s.
- After RFD variables, only peak force is required, but because trace not on screen in real time 5 s used in case peak occurs in latter part of 5 s (unlikely – typically peak is at 1-3 s)
- BioWare needs at least 3 s before pull begins, so recommended sequence:
    - Click Enter to begin
    - After 1-2 s begin instruction "3-2-1-PULL" and maintain verbal encouragement for 5 s

Visual checks of 'good' trial:

- ✓ Good posture maintained throughout
- ✓ No obvious countermovement observed when looking at participant
- ✓ On trace:
    a. Reasonably stable trace before pull begins, although less important than in CMJ because not used for calculations because body weight is taken from a CMJ trial.
    b. Little/no sign of countermovement – i.e. no/only very small dip in trace before pull begins.
    c. If participant happens to step off plate before 16 s ends, trace should return to 0 N.

NB. After completion, click X at top right of open window, get prompted save? Choose Yes (if good trial), give filename and save into correct folder.

Use 2-3 good trials in athletes familiar with the IMTP. In non-athletes, at least 3 good trials on 2 separate test days are recommended.

To export data of CMJ, DJ and IMTP to '.txt files' to send to Dan Cunningham for analysis using bespoke software:

- Close all files in BioWare.
- Choose <File>, <Export data>.
- Select all relevant '.dat' files – e.g. all in a given folder.
- Click <Open>.
- Ensure only Fz is checked.
- Click <Export data>.
- A new '.txt' file will be created for each '.dat' file using the same main filename.

NB. Please make sure everything is backed-up regularly!

APPENDIX C

- Table. AppC4.1 Nationality and physical characteristics of participants

- Tables. AppC4.2a and AppC4.2b Hardy-Weinberg equilibrium compliance test results.

- Table. AppC4.3 Genotype and allele frequency distributions

- Tables. AppC4.4 Comparisons between athlete groups and non-athletes for 10 SNPs and four models, including test results of chi-square, odds ratio, confidence interval and *P* value, and Benjamini-Hochberg adjustment.

- Table. AppC6.2a *P*-values for all comparisons within forwards between genotypes for all performance variables and SNPs.

- Table. AppC6.2b *P*-values for all comparisons within backs between genotypes for all performance variables and SNPs.

APPENDIX C

Table AppC4.1 Nationality of participants and physical characteristics of athletes.

	Athletes n = 567						Non-athletes n = 1171	
	Athletes (All)	Front five n = 223	Back row n = 103	Half backs n = 89	Centres n = 63	Back three n = 89	Male n=508	Female n=663
Nationality	(% of group)						(% of group)	
British	57	54	57	54	64	62	84	99
Irish	13	13	11	11	11	18	1	--
South African	16	18	20	19	8	11	14	--
Italian	11	12	7	10	16	8	--	--
New Zealand	2	2	2	3	2	0	--	--
Other	1	1	3	3	0	1	1	1
Physical characteristics	Mean (SD)							
Height (m)	1.859 (7.3)	188 (8.3)	190 (4.5)	179.3 (4.9)	185.7 (4.5)	182.9 (5.1)		
Body mass (kg)	102.8 (12.5)	113.9 (7.66)	106 (5.9)	87.3 (5.8)	96.5 (5.1)	91.5 (7.0)		
BMI ($kg \cdot m^{-2}$)	29.72 (3.14)	32.33 (3.0)	29.4 (1.46)	27.16 (1.65)	28.01 (1.27)	27.37 (1.59)		

Table. AppC4.2a Hardy-Weinberg Equilibrium compliance test results for athlete groups and non-athletes. Data are presented in chi-square (χ^2).

	Non-athletes	Athletes	Forwards	Front five	Back row	Backs	Half backs	Centres	Back three
ACE rs4341	0.013	0.215	0.325	0.230	0.157	0.003	0.220	1.270	0.374
ACTN3 rs1815739	3.033	**5.204***	1.403	1.970	0.001	**3.988***	**4.425***	0.573	0.140
AMPD1 rs17602729	0.328	0.098	1.027	1.340	0.024	2.244	1.232	1.738	0.087
FTO rs9939609	0.004	1.167	0.048	1.020	1.145	2.818	0.061	**3.857***	2.626
HIF1A rs11549465	**4.062***	1.974	1.590	1.432	0.191	0.056	1.728	0.077	0.605
KDR rs1870377	0.439	0.006	1.300	0.251	1.536	2.095	1.272	0.000	1.654
NOS3 rs2070744	3.738	0.008	0.007	0.057	0.045	0.000	0.216	0.007	0.073
PTK2 rs7460	0.412	1.846	1.051	2.060	0.051	0.797	0.001	0.105	1.291
PTK2 rs7843014	0.769	1.967	3.279	**4.147***	0.046	0.002	1.656	1.535	0.109
TRHR rs7832552	0.059	0.062	0.108	0.480	0.170	0.000	0.162	0.061	0.227

* Indicates critical values for $\chi 2$ that are above 3.84 (degrees of freedom = 1), with a corresponding *p* value of < 0.05.

Table AppC4.2b Hardy-Weinberg Equilibrium compliance test results for athletes (combined), athlete subgroups (forwards and backs) and non- athletes, for each SNP. Data are presented as chi-square with number of participants for each group in parenthesis.

		Non-athletes	Athletes	Forwards	Backs
ACE	rs4341	0.013 (n=925)	0.215 (n=566)	0.325 (n=325)	0.003 (n=241)
ACTN3	rs1815739	3.033 (n=1140)	**5.204*** (n=544)	1.403 (n=312)	**3.988* (n=232)**
AMPD1	rs17602729	0.328 (n=459)	0.098 (n=411)	1.027 (n=233)	2.244 (n=178)
FTO	rs9939609	0.004 (n=898)	1.167 (n=486)	0.048 (n=279)	2.818 (n=207)
HIF1A	rs11549465	**4.062*** (n=606)	1.974 (n=247)	1.590 (n=135)	0.056 (n=112)
NOS3	rs1870377	0.439 (n=726)	0.006 (n=536)	1.300 (n=311)	2.095 (n=225)
KDR	rs2070744	3.738 (n=728)	0.008 (n=539)	0.007 (n=311)	0.000 (n=228)
PTK2	rs7460	0.412 (n=606)	1.846 (n=384)	1.051 (n=223)	0.797 (n=161)
PTK2	rs7843014	0.769 (n=665)	1.967 (n=377)	3.279 (n=217)	0.002 (n=160)
TRHR	rs7832552	0.059 (n=610)	0.062 (n=426)	0.108 (n=240)	0.000 (n=186)

NB. * indicates a significant chi-square value at the 0.05 alpha level. The chi-square critical value for 1 degrees of freedom at $p < 0.05$ is 3.84.

Table AppC4.3 Genotype and allele distributions in athlete groups and non-athletes. Data are presented as counts and percentages in parenthesis.

		Non-athletes	Athletes	Forwards	Backs
ACE	**DD**	282 (30.5)	160 (28.3)	89 (27.4)	71 (29.5)
	ID	459 (49.6)	287 (50.7)	167 (51.4)	120 (49.8)
rs4341	**II**	184 (19.9)	119 (21.0)	69 (21.2)	50 (20.7)
	Total	**925**	**566**	**325**	**241**
	D Carriers	741 (80.1)	447 (79.0)	256 (78.8)	191 (79.3)
	I Carriers	643 (69.5)	406 (71.7)	236 (72.6)	170 (70.5)
	D allele	1023 (55.3)	607 (53.6)	345 (53.1)	262 (54.4)
	I allele	827 (44.7)	525 (46.4)	305 (46.9)	220 (45.6)
ACTN3	**CC (RR)**	389 (34.1)	183 (33.6)	93 (29.8)	90 (38.8)
	CT (RX)	529 (46.4)	242 (44.5)	145 (46.5)	97 (41.8)
rs1815739	**TT (XX)**	222 (19.5)	119 (21.9)	74 (23.7)	45 (19.4)
	Total	**1140**	**544**	**312**	**232**
	C Carriers	918 (80.5)	425 (78.1)	238 (76.3)	187 (80.6)
	T Carriers	751 (65.9)	361 (66.4)	219 (70.2)	142 (61.2)
	C allele	1307 (57.3)	608 (55.9)	331 (53.0)	277 (59.7)
	T allele	973 (42.7)	480 (44.1)	293 (47.0)	187 (40.3)
AMPD1	**GG**	363 (79.1)	312 (75.9)	182 (78.1)	130 (73.0)
	GA	89 (19.4)	93 (22.6)	46 (19.7)	47 (26.4)
rs17602729	**AA**	7 (1.5)	6 (1.5)	5 (2.1)	1 (0.6)
	Total	**459**	**411**	**233**	**178**
	G Carriers	452 (98.5)	405 (98.5)	228 (97.9)	177 (99.4)
	A Carriers	96 (20.9)	99 (24.1)	51 (21.9)	48 (27.0)
	G allele	815 (88.8)	717 (87.2)	410 (88.0)	307 (86.2)
	A allele	103 (11.2)	105 (12.8)	56 (12.0)	49 (13.8)
FTO	**TT**	330 (36.7)	159 (32.7)	83 (29.7)	76 (36.7)
	TA	428 (47.7)	248 (51.0)	140 (50.2)	108 (52.2
rs9939609	**AA**	140 (15.6)	79 (16.3)	56 (20.1)	23 (11.1)
	Total	**898**	**486**	**279**	**207**
	T Carriers	758 (84.4)	407 (83.7)	223 (79.9)	184 (88.9)
	A Carriers	568 (63.3)	327 (67.3)	196 (70.3)	131 (63.3)
	T allele	1088 (60.6)	566 (58.2)	306 (54.8)	260 (62.8)
	A allele	708 (39.4)	406 (41.8)	252 (45.2)	154 (37.2)
HIF1A	**CC**	481 (79.4)	193 (78.1)	99 (73.3)	94 (83.9)
	CT	123 (20.3)	48 (19.4)	31 (23.0)	17 (15.2)
rs11549465	**TT**	2 (0.3)	6 (2.4)	5 (3.7)	1 (0.9)
	Total	**606**	**247**	**135**	**112**
	C Carriers	604 (99.7)	241 (97.6)	130 (96.3)	111 (99.1)
	T Carriers	125 (20.6)	54 (21.9)	36 (26.7)	18 (16.1)
	C allele	1085 (89.5)	434 (87.9)	229 (84.8)	205 (91.5)
	T allele	127 (10.5)	60 (12.1)	41 (15.2)	19 (8.5)
KDR	**TT**	399 (55.0)	311 (58.0)	185 (59.5)	126 (56.0)
	TA	283 (39.0)	195 (36.4)	105 (33.8)	90 (40.0)
rs1870377	**AA**	44 (6.1)	30 (5.6)	21 (6.8)	9 (4.0)
	Total	**726**	**536**	**311**	**225**
	T Carriers	682 (93.9)	506 (94.4)	290 (93.2)	216 (96.0)
	A Carriers	327 (45.0)	225 (42.0)	126 (40.5)	99 (44.0)
	T allele	1081 (74.4)	817 (76.2)	475 (76.4)	342 (76.0)
	A allele	371 (25.6)	255 (23.8)	147 (23.6)	108 (24.0)
NOS3	**TT**	282 (38.7)	205 (38.0)	114 (36.7)	91 (39.9)
	CT	322 (44.2)	254 (47.1)	148 (47.6)	106 (46.5)
rs2070744	**CC**	124 (17.0)	80 (14.8)	49 (15.8)	31 (13.6)
	Total	**728**	**539**	**311**	**228**
	T Carriers	604 (83.0)	459 (85.2)	262 (84.2)	197 (86.4)
	C Carriers	446 (61.3)	334 (62.0)	197 (63.3)	137 (60.1)
	T allele	886 (60.9)	664 (61.6)	376 (60.5)	288 (63.2)
	C allele	570 (39.1)	414 (38.4)	246 (39.5)	168 (36.8)
PTK2	**AA**	161 (26.6)	97 (25.3)	56 (25.1)	41 (25.5)
	AT	295 (48.7)	205 (53.4)	119 (53.4)	86 (53.4)
rs7460	**TT**	150 (24.8)	82 (21.4)	48 (21.5)	34 (21.1)
	Total	**606**	**384**	**223**	**161**
	A Carriers	456 (75.2)	302 (78.6)	175 (78.5)	127 (78.9)
	T Carriers	445 (73.4)	287 (74.7)	167 (74.9)	120 (74.5)
	A allele	617 (50.9)	399 (52.0)	231 (51.8)	168 (52.2)
	T allele	595 (49.1)	369 (48.0)	215 (48.2)	154 (47.8)
PTK2	**AA**	212 (31.9)	102 (27.1)	57 (26.3)	45 (28.1)
	AC	317 (47.7)	201 (53.3)	121 (55.8)	80 (50.0)
rs7843014	**CC**	136 (20.5)	74 (19.6)	39 (18.0)	35 (21.9)
	Total	**665**	**377**	**217**	**160**
	A Carriers	529 (79.5)	303 (80.4)	178 (82.0)	125 (78.1)
	C Carriers	453 (68.1)	275 (72.9)	160 (73.7)	115 (71.9)
	A allele	741 (55.7)	405 (53.7)	235 (54.1)	170 (53.1)
	C allele	589 (44.3)	349 (46.3)	199 (45.9)	150 (46.9)
TRHR	**CC**	285 (46.7)	177 (41.5)	97 (40.4)	80 (43.0)
	CT	262 (43.0)	197 (46.2)	113 (47.1)	84 (45.2)
rs7832552	**TT**	63 (10.3)	52 (12.2)	30 (12.5)	22 (11.8)
	Total	**610**	**426**	**240**	**186**
	C Carriers	547 (89.7)	374 (87.8)	210 (87.5)	164 (88.2)
	T Carriers	325 (53.3)	249 (58.5)	143 (59.6)	106 (57.0)
	C allele	832 (68.2)	551 (64.7)	307 (64.0)	244 (65.6)
	T allele	388 (31.8)	301 (35.3)	173 (36.0)	128 (34.4)

Table. AppC4.4 Chi-square, Odds Ratio (OR) with 95% Confidence Intervals (CI) for all comparisons, and for 10 SNPs.

SNP	Model	Athletes *vs* Non-athletes					Forwards *vs* Non-athletes				
		χ^2	*P* value	OR	95% CI	*P* value (OR)	χ^2	*P* value	OR	95% CI	*P* value (OR)
ACE	Additive	1.413,	0.493	0.88	0.649 - 1.186	0.394	1.522	0.467	0.84	0.584 - 1.213	0.355
rs4341	DD/I carriers	1.314,	0.252	0.90	0.714 - 1.131	0.363	1.476	0.224	0.86	0.649 - 1.139	0.293
	D carriers/II	0.456,	0.500	0.93	0.720 - 1.208	0.598	0.366	0.545	0.92	0.675 - 1.257	0.605
	D allele/I allele	1.285,	0.257	0.93	0.806 - 1.084	0.373	1.296	0.255	0.91	0.764 - 1.094	0.328
ACTN3	Additive	2.079,	0.354	0.88	0.661 - 1.165	0.367	4.589	0.101	0.72	0.507 - 1.015	0.061
rs1815739	RR/X carriers	0.056,	0.812	0.98	0.789 - 1.215	0.845	2.584	0.108	0.82	0.625 - 1.076	0.152
	R carriers/XX	2.000,	0.157	0.86	0.672 - 1.110	0.252	3.584	0.058	0.78	0.577 - 1.049	0.100
	R allele/X allele	0.925,	0.336	0.94	0.815 - 1.091	0.429	4.672	0.031	0.84	0.704 - 1.005	0.056
AMPD1	Additive	2.757	0.252	1.00	0.333 3.015	0.996	0.632	0.729	0.702	0.220 2.242	0.5503
rs17602729	GG/A carriers	2.501,	0.114	0.83	0.606 - 1.147	0.263	0.133	0.715	0.94	0.643 - 1.385	0.767
	G carriers/AA	0.012	0.914	1.05	0.348 3.136	0.936	-	-	-	- -	-
	G allele/I allele	1.992,	0.158	0.86	0.646 - 1.153	0.319	0.297	0.586	0.93	0.654 - 1.308	0.660
FTO	Additive	3.444,	0.179	0.85	0.611 - 1.193	0.355	7.685	**0.021***	0.63	0.425 - 0.931	0.021
rs9939609	TT/A carriers	3.400,	**0.065***	0.84	0.663 - 1.057	0.134	5.880	**0.015***	0.73	0.545 - 0.974	0.033
	T carriers/AA	0.163,	0.686	0.95	0.704 - 1.286	0.746	4.258	**0.039***	0.74	0.521 - 1.037	0.080
	T allele/A allele	2.245,	0.134	0.91	0.774 - 1.063	0.229	7.699	**0.006***	0.79	0.652 - 0.957	0.016
HIF1A	Additive						-	-	-	- -	-
rs11549465	CC/T carriers	0.230,	0.631	0.93	0.648 - 1.332	0.688	3.008	0.083	0.71	0.465 - 1.098	0.125
	C carriers/TT						-	-	-	- -	-
	C allele/T allele	1.464,	0.226	0.85	0.611 - 1.174	0.318	6.376	**0.012***	0.65	0.447 - 0.956	0.028
KDR	Additive	2.035,	0.361	1.14	0.702 - 1.861	0.590	3.578	0.167	0.97	0.561 - 1.681	0.918
rs1870377	TT/A carriers	2.032,	0.154	1.13	0.904 - 1.419	0.278	2.575	0.109	1.20	0.919 - 1.576	0.178
	T carriers/AA	0.202,	0.653	1.09	0.675 - 1.755	0.729	0.261	0.609	0.89	0.520 - 1.525	0.674
	T allele/A	1.753,	0.186	1.10	0.915 - 1.321	0.311	1.202	0.273	1.11	0.891 - 1.381	0.355
NOS3	Additive	2.608,	0.271	1.13	0.807 - 1.573	0.483	1.438	0.487	1.02	0.689 - 1.520	0.910
rs2070744	TT/C carriers	0.112,	0.738	0.97	0.772 - 1.221	0.799	0.567	0.451	0.92	0.695 - 1.204	0.527
	T carriers/CC	1.830,	0.176	1.18	0.867 - 1.600	0.295	0.359	0.549	1.10	0.765 - 1.575	0.613
	T allele/C allele	0.250,	0.617	1.03	0.878 - 1.213	0.704	0.042	0.837	0.98	0.811 - 1.192	0.864
PTK2	Additive	3.785,	0.151	1.10	0.762 - 1.593	0.605	2.121	0.346	1.09	0.696 - 1.696	0.714
rs7460	AA/T carriers	0.336,	0.562	0.93	0.697 - 1.251	0.648	0.242	0.623	0.93	0.652 - 1.318	0.673
	A carriers/TT	2.381,	0.123	1.21	0.892 - 1.645	0.219	1.247	0.264	1.20	0.829 - 1.734	0.334
	A allele/T allele	0.336,	0.562	1.04	0.870 - 1.250	0.650	0.140	0.708	1.04	0.834 - 1.287	0.749
PTK2	Additive	5.398,	0.067	0.88	0.612 - 1.278	0.513	5.776	0.056	0.94	0.591 - 1.486	0.784
rs7843014	AA/C carriers	4.040,	0.044	0.79	0.599 - 1.048	0.103	3.148	0.076	0.76	0.540 - 1.073	0.120
	A carriers/TT	0.157,	0.692	1.05	0.767 - 1.444	0.751	0.820	0.365	1.17	0.791 - 1.741	0.427
	A allele/C allele	1.223,	0.269	0.92	0.771 - 1.104	0.378	0.432	0.511	0.94	0.755 - 1.167	0.569
TRHR	Additive	4.971,	**0.083***	1.33	0.880 - 2.007	0.176	4.092	0.129	1.40	0.855 - 2.288	0.181
rs7832552	TT/C carriers	1.624,	0.203	1.21	0.817 - 1.783	0.344	1.223	0.269	1.24	0.781 - 1.971	0.362
	T carriers/CC	4.578,	**0.032***	1.23	0.961 - 1.584	0.100	3.832	**0.050***	1.29	0.955 - 1.750	0.097
	T allele/C allele	4.882,	**0.027***	1.17	0.973 - 1.410	0.094	3.976	**0.046***	1.21	0.968 - 1.509	0.095

SNP	Model	Backs *vs* Non-athletes					Forwards *vs* Backs				
		χ^2	*P* value	OR	95% CI	*P* value (OR)	χ^2	*P* value	OR	95% CI	*P* value (OR)
ACE	Additive	0.173	0.917	0.93	0.617 - 1.392	0.713	0.296	0.863	0.91	0.562 - 1.467	0.694
rs4341	DD/I carriers	0.120	0.729	0.95	0.698 - 1.299	0.758	0.294	0.588	0.90	0.624 - 1.306	0.588
	D carriers/II	0.111	0.740	0.95	0.668 - 1.347	0.768	0.020	0.889	0.97	0.645 - 1.463	0.889
	D allele/I allele	0.172	0.678	0.96	0.787 - 1.177	0.712	0.182	0.669	0.95	0.750 - 1.203	0.669
ACTN3	Additive	2.538	0.281	1.14	0.770 - 1.693	0.511	4.980	0.083	0.63	0.393 - 1.006	0.053
rs1815739	RR/X carriers	2.251	0.134	1.22	0.915 - 1.637	0.174	4.812	**0.028***	0.67	0.468 - 0.959	0.029
	R carriers/XX	0.001	0.976	1.00	0.703 - 1.436	0.978	1.454	0.228	0.77	0.510 - 1.175	0.229
	R allele/X allele	1.069	0.301	1.10	0.900 - 1.351	0.346	4.778	**0.029***	0.76	0.598 - 0.973	0.029
AMPD1	Additive										
rs17602729	GG/A carriers	3.941	0.047	0.72	0.480 - 1.069	0.102	1.423	0.233	1.32	0.837 - 2.075	0.234
	G carriers/AA										
	G allele/I allele	2.313	0.128	0.79	0.550 - 1.140	0.210	0.553	0.457	1.17	0.775 - 1.762	0.458
FTO	Additive	3.548	0.170	1.40	0.845 - 2.327	0.191	7.725	**0.021***	0.45	0.252 - 0.798	0.006
rs9939609	TT/A carriers	0.0001	0.992	1.00	0.730 - 1.366	0.993	2.619	**0.106***	0.73	0.498 - 1.069	0.106
	T carriers/AA	3.156	**0.076***	1.48	0.924 - 2.363	0.103	7.009	**0.008***	0.50	0.295 - 0.840	0.009
	T allele/A allele	0.857	0.355	1.10	0.881 - 1.370	0.403	6.196	**0.013***	0.72	0.555 - 0.933	0.013
HIF1A	Additive										
rs11549465	CC/T carriers	1.420	0.233	1.36	0.790 - 2.332	0.269	4.023	**0.045***	0.53	0.280 - 0.991	0.047
	C carriers/TT										
	C allele/T allele	0.952	0.329	1.26	0.762 - 2.092	0.365	5.155	**0.023***	0.52	0.291 - 0.921	0.025
KDR	Additive	1.681	0.432	1.54	0.733 - 3.250	0.253	3.437	0.179	0.63	0.279 - 1.419	0.264
rs1870377	TT/A carriers	0.099	0.754	1.04	0.772 - 1.410	0.784	0.651	0.420	1.15	0.815 - 1.632	0.420
	T carriers/AA	1.678	0.195	1.55	0.744 - 3.223	0.243	1.872	0.171	0.58	0.258 - 1.281	0.176
	T allele/A	0.569	0.451	1.09	0.849 - 1.390	0.508	0.019	0.889	1.02	0.768 - 1.356	0.889
NOS3	Additive	1.926	0.382	1.29	0.816 - 2.043	0.276	0.814	0.666	0.79	0.468 - 1.343	0.388
rs2070744	TT/C carriers	0.133	0.715	1.05	0.775 - 1.424	0.751	0.592	0.442	0.87	0.613 - 1.238	0.442
	T carriers/CC	1.905	0.167	1.30	0.853 - 1.996	0.220	0.485	0.486	0.84	0.517 - 1.368	0.486
	T allele/C allele	1.018	0.313	1.10	0.887 - 1.371	0.378	0.815	0.367	0.89	0.695 - 1.144	0.367
PTK2	Additive	1.675	0.433	1.12	0.677 - 1.864	0.652	0.012	0.994	0.97	0.533 - 1.756	0.913
rs7460	AA/T carriers	0.100	0.752	0.94	0.634 - 1.406	0.778	0.006	0.937	0.98	0.616 - 1.564	0.937
	A carriers/TT	1.142	0.285	1.23	0.806 - 1.872	0.338	0.009	0.924	0.98	0.595 - 1.602	0.924
	A allele/T allele	0.207	0.649	1.05	0.823 - 1.345	0.696	0.011	0.917	0.98	0.739 - 1.312	0.917
PTK2	Additive	1.049	0.592	0.82	0.505 - 1.348	0.442	1.405	0.495	1.14	0.623 - 2.073	0.676
rs7843014	AA/C carriers	1.039	0.308	0.84	0.571 - 1.224	0.358	0.161	0.688	0.91	0.576 - 1.440	0.688
	A carriers/CC	0.199	0.655	0.92	0.604 - 1.397	0.690	0.889	0.346	1.28	0.767 - 2.129	0.346
	A allele/C allele	0.870	0.351	0.90	0.705 - 1.151	0.403	0.077	0.781	1.04	0.780 - 1.392	0.781
TRHR	Additive	1.165	0.559	1.24	0.721 - 2.146	0.432	0.292	0.864	1.12	0.602 - 2.101	0.713
rs7832552	TT/C carriers	0.452	0.501	1.16	0.695 - 1.951	0.562	0.044	0.834	1.06	0.592 - 1.915	0.834
	T carriers/CC	1.029	0.310	1.16	0.834 - 1.618	0.374	0.290	0.590	1.11	0.755 - 1.640	0.590
	T allele/C allele	1.164	0.281	1.12	0.880 - 1.438	0.347	0.245	0.621	1.07	0.809 - 1.427	0.621

SNP	Model	Back three *vs* Non-athletes						Forwards *vs* Back three					
		χ^2	*P* value	OR	95% CI		*P* value (OR)	χ^2	*P* value	OR	95% CI		*P* value (OR)
ACTN3 rs1815739	Additive	1.370	0.504	1.45	0.747	2.810	0.273	3.937	0.140	0.50	0.24	1.01	0.053
	RR/X carriers	0.996	0.318	1.25	0.793	1.968	0.338	2.741	0.098	0.66	0.40	1.08	0.099
	R carriers/XX	0.856	0.355	1.32	0.718	2.429	0.371	2.622	0.105	0.59	0.31	1.12	0.108
	R allele/X allele	1.441	0.230	1.21	0.876	1.670	0.247	4.197	**0.041***	0.70	0.49	0.99	0.041

SNP	Model	Half backs and Centres vs Back three					
		χ^2	*P* value	OR	95% CI		*P* value (OR)
ACTN3 rs1815739	Additive	1.422	0.491	0.70	0.32	1.52	0.370
	RR/X carriers	0.013	0.908	0.97	0.56	1.68	0.908
	R carriers/XX	1.294	0.255	0.66	0.33	1.35	0.257
	R allele/X allele	0.533	0.465	0.87	0.59	1.28	0.466

SNP	Model	Back three & Centres *vs* Non-athletes						Back three & Centres *vs* Forwards					
		χ^2	*P* value	OR	95% CI		*P* value (OR)	χ^2	*P* value	OR	95% CI		*P* value (OR)
FTO rs9939609	Additive	6.829	**0.033***	2.04	1.00	4.14	0.049	10.310	**0.006***	3.24	1.51	6.93	0.003
	TT/A carriers	0.002	0.967	1.01	0.69	1.48	0.969	2.096	**0.148***	1.38	0.89	2.14	0.148
	T carriers/AA	6.162	**0.013***	2.22	1.13	4.33	0.020	10.042	**0.002***	3.01	1.48	6.12	0.002
	T allele/A allele	1.774	0.183	1.19	0.91	1.56	0.213	6.957	**0.008***	1.50	1.11	2.04	0.009

SNP	Model	Back three & Centres vs Forwards & Half backs					
		χ^2	*P* value	OR	95% CI		*P* value (OR)
FTO rs9939609	Additive	9.625	**0.008***	2.98	1.42	6.28	0.004
	TT/A carriers	1.427	0.232	1.29	0.85	1.97	0.233
	T carriers/AA	9.559	**0.002***	2.89	1.44	5.79	0.003
	T allele/A allele	5.949	**0.015***	1.44	1.07	1.93	0.015

*indicates significance for *p* values after Benjamini-Hochberg correction.

Table AppC6.1a Comparisons within forwards between genotypes, for six performance variables and all SNPs.

Forwards	SCORE	ATTACK				TACKLES
	Tries	Carries	Metres	Clean breaks	Defenders beaten	Tackles
	p-value for genotype comparisons					
ACE **(n=171)** rs4341						
DD vs ID vs II	0.291	0.288	0.400	0.446	0.257	0.470
D carriers vs II	0.189	0.542	0.769	0.494	0.619	0.263
I carriers vs DD	0.685	0.234	0.245	0.423	0.099	0.896
ACTN3 **(n=159)** rs1815739						
CC (RR) vs CT vs TT	0.220	0.757	0.474	0.938	0.829	0.778
C carriers vs TT	0.135	0.670	0.509	0.723	0.669	0.481
T carriers vs CC	0.752	0.470	0.233	0.865	0.577	0.760
AMPD1 **(n=105)** rs17602729						
GG vs GA vs AA	0.663	0.708	0.990	0.559	0.730	0.342
G carriers vs AA	0.515	0.515	0.897	0.543	0.990	0.411
A carriers vs GG	0.755	0.524	0.946	0.540	0.454	0.405
FTO **(n=133)** rs9939609						
TT vs TA vs AA	0.129	0.195	0.196	0.458	0.163	0.283
T carriers vs AA	0.044	0.088	0.080	0.266	0.083	0.133
A carriers vs TT	0.434	0.967	0.905	0.845	0.825	0.338
HIF1A **(n=71)** rs11549465						
CC vs CT vs TT	0.553	0.750	0.946	0.923	0.974	0.606
C carriers vs TT	0.577	0.756	0.882	0.715	0.840	0.715
T carriers vs CC	0.290	0.451	0.835	0.902	0.912	0.317

NB. no significant associations after B-H correction.

Table AppC6.1a continued.

Forwards	SCORE	ATTACK				TACKLES
	Tries	Carries	Metres	Clean breaks	Defenders beaten	Tackles
	p-value for genotype comparisons					
KDR **(n=165)** rs1870377						
TT vs TA vs AA	0.579	0.739	0.216	0.131	0.579	0.788
T carriers vs AA	0.366	0.455	0.638	0.488	0.574	0.754
A carriers vs TT	0.867	0.949	0.159	0.129	0.533	0.643
NOS3 **(n=167)** rs2070744						
TT vs CT vs CC	0.484	0.089	0.166	0.283	0.168	0.849
T carriers vs CC	0.381	0.200	0.558	0.776	0.452	0.693
C carriers vs TT	0.631	0.034	0.058	0.170	0.059	0.798
PTK2 **(n=111)** rs7460						
AA vs AT vs TT	0.749	0.702	0.894	0.517	0.516	0.473
A carriers vs TT	0.644	0.778	0.644	0.331	0.351	0.280
T carriers vs AA	0.485	0.483	0.835	0.408	0.380	0.746
PTK2 **(n=105)** rs7843014						
AA vs AC vs CC	0.445	0.646	0.961	0.962	0.845	0.121
A carriers vs CC	0.211	0.361	0.821	0.914	0.996	0.509
C carriers vs AA	0.631	0.994	0.832	0.819	0.571	0.076
TRHR **(n=109)** rs7832552						
CC vs CT vs TT	0.401	0.510	0.640	0.370	0.817	0.272
C carriers vs TT	0.961	0.427	0.608	0.670	0.574	0.117
T carriers vs CC	0.201	0.562	0.541	0.158	0.902	0.411

NB. no significant associations after B-H correction.

Table AppC6.1b Comparisons within backs between genotypes, for six performance variables and all SNPs.

Backs	SCORE	ATTACK				TACKLES
	Tries	Carries	Metres	Clean breaks	Defenders beaten	Tackles
	p-value for genotype comparisons					
ACE **(n=120)** rs4341						
DD vs ID vs II	0.113	0.587	0.926	0.871	0.899	0.847
D carriers vs II	0.047	0.864	0.696	0.816	0.644	0.581
I carriers vs DD	0.965	0.364	0.884	0.602	0.898	0.981
ACTN3 **(n=112)** rs1815739						
CC (RR) vs CT vs TT	0.321	0.892	0.825	0.923	0.945	**0.010
C carriers vs TT	0.893	0.762	0.697	0.907	0.296	0.048
T carriers vs CC	0.158	0.643	0.813	0.693	0.337	0.229
AMPD1 **(n=80)** rs17602729						
GG vs GA vs AA	0.233	0.557	0.586	0.249	0.349	0.669
G carriers vs AA	0.050	0.950	0.450	0.250	0.225	0.500
A carriers vs GG	0.970	0.281	0.872	0.447	0.813	0.966
FTO **(n=95)** rs9939609						
TT vs TA vs AA	0.117	0.988	0.926	0.769	0.613	0.620
T carriers vs AA	0.405	0.963	0.926	0.889	0.621	0.727
A carriers vs TT	0.040	0.876	0.696	0.470	0.487	0.330
HIF1A **(n=50)** rs11549465						
CC vs CT vs TT	0.415	0.149	0.327	0.519	0.171	0.537
C carriers vs TT	0.400	0.080	0.200	0.360	0.040	0.400
T carriers vs CC	0.282	0.119	0.364	0.661	0.184	0.935

**significant after B-H correction.

Table AppC6.1b continued.

Backs	SCORE	ATTACK				TACKLES
	Tries	Carries	Metres	Clean breaks	Defenders beaten	Tackles
	p-value for genotype comparisons					
KDR **(n=116)** rs1870377						
TT vs TA vs AA	0.639	0.750	0.927	0.978	0.708	0.921
T carriers vs AA	0.466	0.467	0.698	0.865	0.639	0.930
A carriers vs TT	0.443	0.698	0.949	0.940	0.584	0.718
NOS3 **(n=117)** rs2070744						
TT vs CT vs CC	0.238	0.658	0.182	0.594	0.267	0.143
T carriers vs CC	0.167	0.896	0.221	0.761	0.422	0.182
C carriers vs TT	0.183	0.406	0.094	0.307	0.114	0.076
PTK2 **(n=72)** rs7460						
AA vs AT vs TT	0.183	0.406	0.094	0.307	0.114	0.076
A carriers vs TT	0.906	0.603	0.458	0.961	0.672	0.623
T carriers vs AA	0.647	0.075	0.107	0.725	0.091	0.115
PTK2 **(n=71)** rs7843014						
AA vs AC vs CC	0.848	0.303	0.288	0.721	0.207	0.255
A carriers vs CC	0.916	0.125	0.121	0.438	0.107	0.126
C carriers vs AA	0.623	0.718	0.412	0.628	0.209	0.950
TRHR **(n=83)** rs7832552						
CC vs CT vs TT	0.705	0.037	0.176	0.614	0.055	0.721
C carriers vs TT	0.803	0.080	0.132	0.346	0.135	0.971
T carriers vs CC	0.404	*0.017	0.118	0.540	**0.021	0.443

**significant after B-H correction.

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