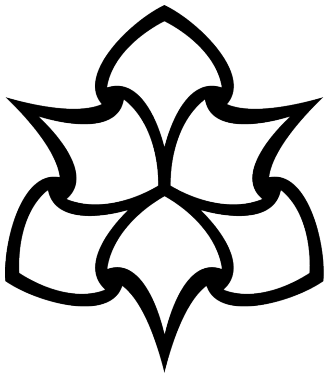

Genetic predisposition score predicts the increase of muscle strength after one-year exercise in healthy elderly

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Abstract

Background. There is very limited evidence of the effect muscle-related genes play on muscular phenotypes and the responses after exercise intervention in healthy elderly.

Methods. 200 participants between 60 and 80 years old were randomly assigned into three groups: fitness (FIT) group, whole-body vibration (WBV) group and control (CON) group. Participants in FIT and WBV groups performed a one-year exercise program. Whole-body skeletal muscle mass (SMM) and peak isometric knee extension torque at a knee flexion angle of 60° (PT_{IM60}) were tested before and after the intervention. Relative change of each parameter was calculated for further analysis. Genotype of each participant was obtained from blood sample. Data-driven genetic predisposition score (GPS) was calculated by adding up predisposing alleles of single nucleotide polymorphisms (SNPs) which were closely related to respective parameter from a 224 muscle-related SNP pool.

Results. PT_{IM60} increased ($p < .05$) in exercise groups after one-year intervention. Its relative changes were also greater than that in CON group ($p < .05$). Similar relative changes of SMM ($p = .299$) were found among the three groups over one year with an average value from 2.21% to 3.96%. GPS was closely related to baseline PTI_{M60}, relative changes of SMM and PTI_{M60} in exercise groups. GPS explained the variance of corresponding parameter by 3.2%, 14% and 27%, respectively.

Conclusion. GPS is positively related to baseline knee strength and muscular adaptations towards exercise in healthy elderly. It can partly explain the inter-individual variance of muscle responses after training and suggests a new training approach of involving genetic information in exercise regimen design.

Key Words: Exercise—Aging—GPS—Prediction—Muscular responses

Introduction

Increasing longevity throughout the world in recent decades has brought healthy aging to the attention of both gerontology and kinesiology researchers. Past studies have found a loss of muscle mass and decrease in muscle performance as two of the most prominent features during the aging process. Such age-associated muscular decline is known as sarcopenia[1]. Using magnetic resonance imaging, Janssen et al. discovered an onset of muscle mass degeneration among the subjects in their thirties, with the decay reaching a significant level in the fifth decade[2]. This decrease was mainly caused by the loss of muscle mass in the lower body[2,3]. Similar to muscle mass loss, muscle strength also decreases with aging, but at a faster rate[4]. This functional weakness is thought to be associated with many factors such as denervation in aged muscle[5], declined function in mitochondria[6,7], elevated type I/type II fiber ratio[1,8] and alteration in contractile properties[9]. Moreover, these muscular declines were found closely related to elderly mortality rate[10].

It is now well reported that regular participation in exercise programs can help reduce aging-associated functional declines. Multiple exercise methods have been reported as effective in slowing the muscular aging process. Resistance training and combined aerobic and resistance training have been proven to maintain muscle performance[11–13]. A 26-week exercise intervention on obese elderly has found a 18% improvement in strength after combined training and a 19% strength increment after resistance

training[14]. Meanwhile, whole-body vibration (WBV) training has also been introduced as an exercise intervention for the elderly. It has proven to counteract the decay of muscle mass and strength during aging[15,16]. Despite the benefits of exercise, muscle strength and mass responses after resistance training showed individual response variances among subjects, while those responses were not affected by age and sex[17]. Sibling and twin studies estimated the heritability of muscle strength and muscle mass, indicating that the individual genetic makeup can exert an influence on the development of muscle mass and strength[18]. This indicates that the response variability resulting from exercise might be related to inherited characteristics[19,20].

Since early reports on exercise capacity-related genes at the end of twentieth century[21,22], many studies have shown the relation between hereditary characteristics and physical performance[23]. However, a considerable number of these studies focused merely on one or a limited number of genes. Since muscular performance can be affected by the combined influences of multiple genes, a new method needs to be applied in order to study the overall effect of multiple genes. With the development of genome-wide association studies, the method of data-driven genetic predisposition score (GPS) has gradually been introduced into exercise genomics. Through the usage of GPS, heritability studies have been able to show the role genetic factors plays in the changes of muscular phenotypes after exercise intervention. Recent studies have been made on elite athletes[24,25] and patients with coronary artery disease[19,20]. To the best of our knowledge, no studies have been performed combining muscle-related genes with GPS to explain baseline muscular phenotypes and exercise-induced muscular changes in a healthy elderly population. Yet, such studies might help us better understand individual adaptive variations towards exercise and can be useful for the design of exercise prescription in the future.

Therefore, the aim of the research was to study the predictive power of GPS on baseline muscular phenotypes and muscular changes after exercise in a healthy elderly population. We hypothesized that elderly people with higher GPS might have a better baseline value and greater muscular improvement than those with lower GPS.

Materials and Methods

Subjects

Elderly people between 60 to 80 years old were recruited from the local communities of the city of Leuven and its surrounding areas. They were the same group as that recruited in the study of Bogaerts et al[26]. All the subjects went through a series of medical examinations. Exclusion criteria were skeletomuscular, neuromuscular and cardiovascular disorders that may prohibit training process and strength-related tests. People with recent training experience were also excluded. This study was approved by the University's Human Ethics Committee in accordance with the Declaration of Helsinki. Informed consent was given by each subject. 200 participants (104 men, 96 women) agreed to provide blood sample for DNA analyses and their data were included in this study.

Training protocols

Of the 200 participants providing a blood sample, 54 of them were from the fitness (FIT) group, 85 of them were in the WBV group and the rest came from the control (CON) group. Subjects in the FIT and the WBV groups received training three times a week on nonconsecutive days over a period of one year. All training programs were performed at Leuven University's Training Center under the guidance and supervision of qualified health and fitness instructors.

The training programs in the FIT group consisted of aerobic, resistance, balance and flexibility training. It was designed based on the exercise prescriptions for elderly recommended by American College of

Sports Medicine (ACSM) guidelines[27]. Subjects firstly performed the aerobic session through one of the four exercises: walking, running, cycling or stepping. The training intensity varied from 70% to 85% of the individual heart rate reserve. The duration of this session was 20 minutes in the starting week and was gradually increased to 45 minutes in the end. In the resistance training session, subjects performed leg press, leg extension, leg curl (lower body), chest press, vertical row, shoulder press, vertical traction, arm curl (upper body), abdominal crunch and back extension (abdominal region) on strength trainers (Technogym Systems, Gambotella, Italy). Before the resistance training, the 1 repetition maximum (RM) of participant was assessed by qualified instructors in each exercise. The load of the training started at 50% of 1-RM with 15 repetitions and was gradually increased to 80% of 1-RM with 8 repetitions. 15 minutes of balance exercise and 10 minutes of stretching were performed after each training session. The training programs were described in detail in the study of Bogaerts et al.[26] (Supplementary Table 1).

Participants in the WBV group performed exercises on a vibration platform (Power Plate®, Amsterdam, Netherlands) with a maximum duration of 40 minutes. The exercises included body weight squat, deep squat, wide stance squat, toes-stand, toes-stand deep, one-legged squat and lunge. The duration of each exercise started from 30 seconds and was finally increased to 60 seconds. A detailed training protocol can also be found in the study of Bogaerts' et al.[28] (Supplementary Table 1).

Subjects in the CON group did not undertake any training program. They were advised to maintain their original lifestyle during the study and to not engage in any new physical activity.

Genotyping

Blood samples were taken from each participant. Genotyping was done with the Illumina GoldenGate platform (Illumina, Inc., San Diego, CA, USA) at the Genomics Core Facility (UZ/KU Leuven). The selection of genes was based on published articles (up to August 2014) and expression quantitative trait loci (eQTL) analysis. Detailed selecting process can be found in Ruben's study[29]. These potential candidate genes were identified for muscular strength or muscular endurance development or regulation. 224 single nucleotide polymorphisms (SNPs) (Supplementary Table 2) came out as muscle-related SNPs. Through blood testing, 12 SNPs failed to be tested out and 3 SNPs presented the same genotypes among all subjects. Those 15 SNPs were ruled out from the 224-SNP pool. Results of linkage disequilibrium test showed that 58 SNPs were highly linked as 19 subgroups and one representative was selected from each of these subgroups. Combined with those that were lowly linked, a total number of 170 SNPs were withheld for further analyses.

Muscular phenotype measurements

Whole-body skeletal muscle mass (SMM) was calculated through bioelectrical impedance analysis (BIA). Resistance of BIA was measured by Bodystat 1500MDD (Bodystat Ltd, Douglas, UK) before and after the one-year intervention. Before the test, participants were asked to lie down in a supine position for one minute. During the measurement, two electrodes were put on the right hand and right foot as instructed in the manual. SMM was calculated for further analyses, using the following regression equation that has been assessed for validity in elderly particpants[30]:

$$\text{SM mass (kg)} = (Ht^2/R \times 0.401) + (\text{sex} \times 3.825) + [\text{age} \times (-0.071)] + 5.102$$

where Ht stands for height in centimeters; R stands for BIA resistance in ohms; in sex, men = 1 and women = 0; age is in years.

Biodex Medical System 3 dynamometer (Biodex Company, New York, USA) was used for the measurements of isometric, isotonic and isokinetic strength of knee extensors. These measurements were done by the same operator before and after the intervention. Before testing, participants were

asked to complete a 5-minute warm up on a free-loaded ergometer. Two trials were performed before formal test to allow participants better understand the measuring process. Maximal isometric knee extension were evaluated at knee flexion angles of 60° (PT_{IM60}) with 0° representing full extension. Peak torque (PT_{IM60}) was withheld for further analyses.

Statistical analyses

All the data were reported as mean ± standard deviation (SD) and were analyzed using SAS statistical software version 9.4 for Windows (SAS Institute Inc, Cary, NC). Stepwise regression analysis was first used in the detection of SNPs that were significantly related to muscular phenotypes. The significance level for entry was 0.1 and that for stay was 0.05. Alleles that were found positively related to muscular phenotypes from the analysis were regarded as phenotype-related predisposing alleles. Based on the selected significant SNPs from stepwise regression analysis, muscular phenotype-related GPS was calculated with the method used in the calculation of data-driven GPS in the study of Charlier et al[29]. Since the weights of alleles in muscle-related SNPs were not well defined, an accumulative effect was hypothesized and equal weight was given to each predisposing allele. Thus, data-driven GPS of each individual was calculated by adding up all the corresponding predisposing alleles.

Two-way analysis of variance (ANOVA) was applied to evaluate between-group comparisons at baseline and one-year relative changes with gender and group as factors. Bonferroni method was used as post-hoc test. Repeated measures ANOVA was used for within-group comparisons of muscular phenotypes between baseline and post-intervention level with gender as a factor. To analyze the influence GPS played on baseline muscular parameters, linear test between GPS and corresponding muscular phenotype was performed by analysis of covariance (ANCOVA) with age, height, gender and baseline SMM as covariates. In exercise groups, the relations between GPS and relative changes of phenotypes after exercise were also analyzed through ANCOVA with age, height, sex and corresponding baseline muscular value as covariates. P value of 0.05 was set as the level of significance.

Results

Descriptive data

Descriptive data of subjects in each group are presented in table 1. Participants in the three groups had similar age, height and body mass before the intervention. No significant difference in body mass change was found among the three groups after one year.

Table 1. Descriptive data of subjects (mean ± SD)

Group	Number	Age (year)	Height (cm)	Body Mass (kg)		
				Pre-intervention	Post-intervention	$\Delta_{post-pre}$ (%)
CON	61	68.23 ± 5.38	167.45 ± 8.54	75.43 ± 10.86	74.49 ± 10.78	-0.98 ± 3.38
FIT	54	67.00 ± 3.88	167.7 ± 9.98	76.13 ± 11.98	74.63 ± 12.19	-1.78 ± 2.99
WBV	85	67.44 ± 4.83	167.22 ± 8.51	75.21 ± 12.62	73.8 ± 11.67	-1.2 ± 3.15

Baseline muscular phenotypes and training effects

The baseline values and training effects of muscular phenotypes are presented in table 2. At baseline level, SMM and PT_{IM60} showed no significant difference among groups ($p = .486$ and $p = .805$, respectively). Significant increases of SMM (CON: $p < .001$, FIT: $p = .006$, WBV: $p = .029$) was found in all groups after one year, but these changes among the three groups did not show any significant differences ($p = .299$). After one-year training, PT_{IM60} increased significantly in the two exercise groups (FIT: $p < .001$, WBV: $p < .001$) while in CON group there was no pre-post difference ($p= .744$). Moreover, two-way ANOVA results showed significant differences in relative changes of PT_{IM60}

among the three groups ($p < .001$). Post-hoc test further found that exercise groups had significant increments than that in CON group ($p < .05$).

Table 2. Muscular phenotypes before and after one-year intervention (mean ± SD)

Parameter	Baseline	Post-intervention	$\Delta_{post\text{-}baseline}$ (%)
SMM (kg)			
CON	23.68 ± 6.82	24.01 ± 6.09^{+++}	3.96 ± 5.92
FIT	23.65 ± 6.27	24.59 ± 6.65^{++}	3.38 ± 8.06
WBV	23.94 ± 6.50	24.32 ± 6.57^{+}	2.21 ± 6.79
PT_{IM60} (Nm)			
CON	136.29 ± 44.25	138.17 ± 43.51	0.19 ± 16.06
FIT	141.70 ± 39.65	162.43 ± 37.89*$^{+++}$	14.97 ± 15.57*
WBV	136.92 ± 41.77	151.32 ± 43.47^{+++}	12.09 ± 15.51*

* significant difference when compared with CON group ($p < .05$)
$^{+}$ significant difference when compared with baseline value ($p < .05$)
$^{++}$ significant difference when compared with baseline value ($p < .01$)
$^{+++}$ significant difference when compared with baseline value ($p < .001$)

Genetic predisposition score

SNPs closely related to muscular phenotypes were selected through stepwise regression analysis (Supplementary Table 3). Linear relations between GPS and corresponding muscular phenotypes at baseline level are shown in table 3. Since stepwise regression was made separately on each muscular parameter, the number of data-driven SNPs varied with each parameter. As presented in table 3, Four SNPs (*ACVR1B*: rs2854464; *FST*: rs3797297; *IGFBP3*: rs3110697; *TTN*: rs10497520) were found significantly related to baseline PT_{IM60}. Data-driven GPS could explain 3.2% of the variance in isometric knee extensor. With a single increase of GPS, baseline PT_{IM60} would be increased by 4.73 Nm. From ANCOVA analysis, sex, age and baseline SMM were also significantly related to baseline PT_{IM60}. Although five SNPs (*ACVR1B*: rs2854464; *IGFBP3*: rs3110697, rs6670; *MTRR*: rs327588; *VDR*: rs731236) were found closely related to baseline SMM, ANCOVA result did not show a significant relation between baseline SMM and GPS ($p = .250$).

Table 3. ANCOVA results of baseline muscular phenotypes

	SMM (kg)				PT_{IM60} (Nm)			
	Estimate	β value	r^2	p	Estimate	β value	r^2	p
GPS	0.17	0.04	.007	.250	4.73*	0.12	.032	.016
SEX (M=1,F=0)	8.54***	0.66	.560	<.0001	18.95*	0.23	.025	.034
AGE	-0.06	-0.04	.011	.141	-2.01***	-0.23	.106	<.0001
HEIGHT	0.23***	0.31	.235	<.0001	0.64	0.13	.017	.085
$SMM_{baseline}$	-	-	-	-	2.38**	0.37	.052	.002
Intercept	-16.01	-	-	-	76.22	-	-	-
Adj. r^2		.839				.577		
No. of SNPs		5				4		

* $p < .05$, ** $p < .01$, *** $p < .0001$

Results of ANCOVA on GPS and training responses of FIT and WBV groups are presented in table 4. SNPs closed related to muscular adaptations were selected through stepwise regression analysis (Supplementary Table 3). Stepwise result had found 6 SNPs (*CCL2*: rs4586; *CCR2*: rs768539; *GR/NR3C1*: rs6190; *METTL21C*: rs2390760; *MSTN*: rs2390760; *SPP1*: rs10516796) significantly related to SMM changes in exercise groups. As table 4 shows, GPS, sex, height and baseline SMM were closely related to SMM changes in exercise groups. Age and training methods (FIT or WBV) did not

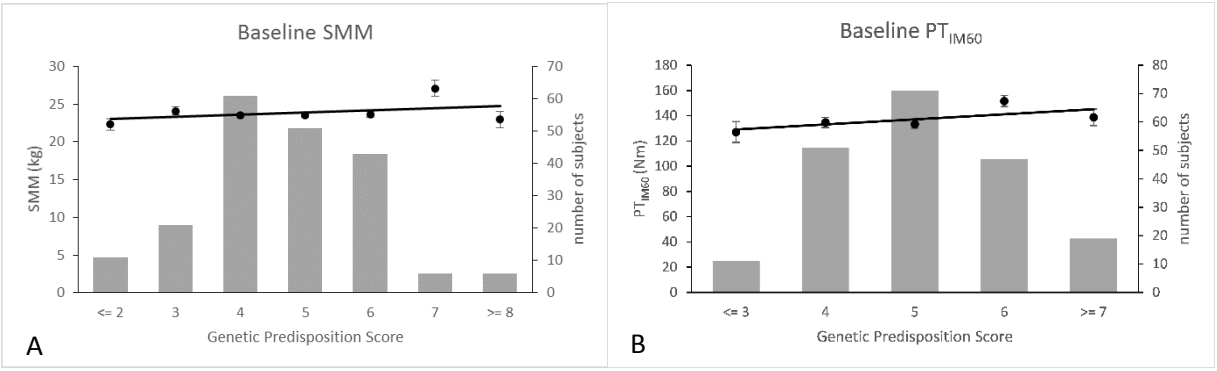
significantly affect the changes over the one year period. From ANCOVA result, GPS alone could explain 14% of the adaptive change in SMM and an additional increase of GPS could bring a 1.78 % increase in SMM change. In the training response of PT_{IM60}, 8 SNPs (*AKT1*: rs1130214; *DNMT3L*: rs7354779; *IGFBP3*: rs3110697; *IL15RA*: rs2228059; *MSTN*: rs1805086; *MTRR*: rs162040, rs7703033; *SPP1*: rs10516796) were found significantly related to it. The analysis result showed that GPS, sex and baseline PT_{IM60} were closely related to PT_{IM60} change in exercise groups. GPS alone could explain 27% of the adaptive change. Moreover, with an additional increase of GPS, PT_{IM60} change in exercise groups could be increased by 3.86%.

Table 4. ANCOVA results of relative changes in muscular phenotypes of exercise groups

	ΔSMM (%)				ΔPT$_{IM60}$ (%)			
	Estimate	β value	r^2	p	Estimate	β value	r^2	p
GPS	1.78***	0.34	.140	<.0001	3.86***	0.45	.270	<.0001
SEX (M=1,F=0)	10.83***	0.74	.146	<.0001	11.53**	0.37	0.110	.001
EXE (FIT=1,WBV=0)	-0.32	-0.02	.001	.770	3.25	0.10	.022	.139
AGE	-0.10	-0.06	.005	.423	-0.41	-0.12	.024	.128
HEIGHT	0.25**	0.32	.054	.009	0.22	0.12	.015	.232
$SMM_{baseline}$	-1.20***	-1.07	.217	<.0001	-	-	-	-
$PT_{IM60\_baseline}$	-	-	-	-	-0.24***	-0.65	.273	<.0001
Intercept	-19.54	-	-	-	-3.00	-	-	-
Adj. r^2	.350				.511			
No. of SNPs	6				8			

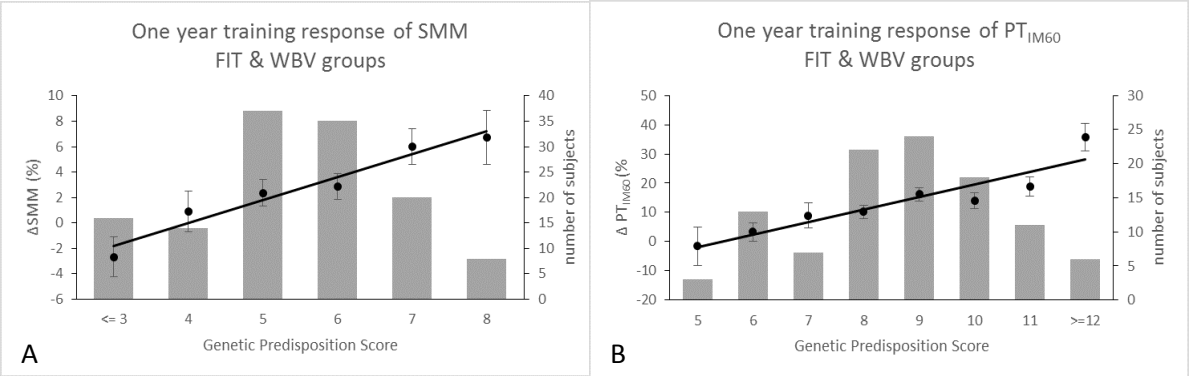
** p < .01, *** p < .0001

Figure 1 Distribution of GPS and its linear regression model with baseline muscular phenotypes



A: linear regression between genetic predisposition score (GPS) and whole-body skeletal muscle mass (SMM) at baseline. Baseline SMM values are presented on the left y-axis. The trend line shows the relation between GPS and baseline SMM. Mean value of SMM in each GPS is presented as dot and least squares mean is presented as error bar. Distribution of participants in each GPS is presented in the histogram with number of participants on the right y-axis.
B: linear regression between GPS and peak isometric knee extension torque at a knee flexion angle of 60° (PT_{IM60}) at baseline.

Figure 2 Distribution of GPS and its linear regression model with muscular phenotype changes in exercise groups after one-year training



A: linear regression between genetic predisposition score (GPS) and relative changes of skeletal muscle mass (ΔSMM) after one year. Since no significant difference was found in ΔSMM amount the three groups, linear regression was completed by analyzing the data together. ΔSMM is presented on the left y-axis. The trend line shows the relation between GPS and ΔSMM. Mean value of ΔSMM in each GPS is presented as dot and least squares mean is presented as error bar. Distribution of participants in each GPS is presented in the histogram with number of participants on the right y-axis.
B: linear regression between GPS and relative changes of peak isometric knee extension torque at a knee flexion angle of 60° (ΔPTIM60) after one year.

The distribution of GPS and its linear relation with muscular parameters are shown in figure 1 and figure 2. GPS with less than three subjects were pooled together at the lower and upper end of the distribution. As shown in the graphs, Subjects with higher GPS tended to have higher baseline values and they also had the tendency of more increment after one year exercise training.

Discussion

To our knowledge, this is the first study using GPS to explain the effects of genetic factors on baseline muscular phenotypes and exercise-induced muscular changes in healthy elderly population. Unlike previous researches that studied muscular phenotypes with single or small number of genes, this study performed analyses with a set of 224 muscle-related SNPs. From the present ANCOVA results, data-driven GPS was positively related to baseline PT_{IM60} and changes of SMM and PT_{IM60} in the exercise group after one year training. Specifically, in models of training adaptations, a sex-related variance in training responses was found. This could be partly explained by different hormonal adaptations in men and women towards exercise[31]. Meanwhile, participants with lower strength and muscle mass would improve more than their stronger peers after the same training. The insignificance of age might be due to close ages of participants and limited training period. Although the findings of Thomaes' study[20] which did not find a significant relation between GPS and isometric knee extension strength, ANCOVA analysis in our study showed that GPS was positively related to baseline PT_{IM60}. This might be attributed to a larger SNP pool in the present study. Considering the fact that muscular phenotypes are the result of multifactorial and polygenic effects, a larger SNP pool might increase the accuracy of results on genetic influences. Similar to the present study, a significant relationship was also found between GPS and baseline PT_{IM60} in Charlier's study[29] with a beta-coefficient of 0.15, despite a large lifespan of participants that was used.

Through stepwise regression analyses, six genes were found closely related to baseline SMM and PT_{IM60}. Among these genes, two of them (*ACVR1B*: rs2854464, *IGFBP3*: rs3110697) were associated with both parameters. SNP rs2854464 in *ACVR1B* gene was found strongly associated with isometric knee extensor strength at a knee flexion degree of 60[32]. Specifically, AA individuals had significant stronger isokinetic knee extensor strength than G-allele carriers, but isometric strength remained similar between the two groups. However, such association of A-allele and sprint/power performance were not found in Brazilian and Japanese populations[33,34]. Based on our data-driven regression result, G-allele was found predisposed to a higher isometric knee strength. *IGFBP3* gene was selected into this

study because it facilitates myoblast differentiation; specifically the production and secretion of IGFBP3 was in accordance with the differentiation level of myoblast[35]. Rs3110697 was also reported as one of the polymorphisms closely related to IGFBP3 blood level[36]. Another SNP that was closely related to baseline PT_{IM60} is rs3797297 from *FST* gene, which codes for follistatin. Acting as an inhibitor of myostatin receptor[37], the overexpression of follistatin could cause dramatic increases in muscle growth[38]. Previously, sex-specific fat free mass was found to be associated with the *FST* gene[39]. In our study, rs10497520 was found related to baseline PT_{IM60}. The finding of this strength-related SNP was in line with that of Stebbings' study, which showed that T-allele at rs10497520 in the *TTN* gene was associated with shorter skeletal muscle fascicle length and conveys an advantage for marathon running performance in habitually trained men[40]. Finally, *VDR* gene codes for vitamin D receptor, which plays an important role in calcium homeostasis and muscle function[41]. Rs731236 in *VDR* gene is associated with hand grip strength[42]. Inconsistent with the finding of Windelinckx[43], which showed a sex-specific relation between *VDR* polymorphisms and knee strength, our result did not find a significance between *VDR* gene and isometric knee strength.

Training responses of SMM and knee strength were found closely related to 11 genes. *MSTN* and *MTRR* gene contributed two SNPs while others only contributed one. *MSTN* gene encodes myostatin, a protein which negatively regulates the growth of muscle cell. Myostatin deficient mice were found with larger muscle mass, more IIB type fibers and lower force generation ability than wild types[44]. AKT is a critical regulator of muscle growth through IGF1-Akt/PKB pathway[45]. Animal experiment revealed that disruption of *AKT1* gene could lead to growth retardation and increased apoptosis[46]. Our result showed that *AKT1* gene was related to training response of PT_{IM60}. The presence of *CCL2* and *CCR2* gene in adaptive changes rather than baseline values supported the idea that these two genes were more related to muscular adaptations. CCL2 is expressed by macrophages and muscle satellite cells, its expression is dramatically increased following muscle damage. CCR2 is the receptor of CCL2. Previous studies have found that the expressions of both genes were associated with muscle exercise-induced damage and the speed of recovery, which varied with individuals[47,48]. *NR3C1* polymorphisms have been reported related to many sex-specific body composition and muscular phenotypes[49]. Recent study also found *NR3C1* polymorphisms (*NR3C1*-2722, -1887, 1017) associated with muscle strength and size response towards a 3-month resistance training[50]. Our results showed another SNP (rs6190) in *NR3C1* gene that was related to knee strength changes after training. METTL21C not only had protein-lysine methyltransferase activity but was found to affect bone and muscle metabolism as well[51]. Hangelbroek et al. found that higher expression of *METTL21C* gene was associated with frail status in both young and elderly subjects while we found this gene was related to exercise-induced SMM change[52]. *IL15RA* gene was found related to skeletal muscle size and performance[53,54]. A-allele in rs2228059 was reported associated with larger muscle volume but lower muscle quality in men[55]. However, in our study, rs2228059 was only found related to knee strength adaptation after training. Study on Duchenne muscular dystrophy patients showed *SPP1* gene as a determinant of this disease with G-allele carriers in SNP rs28357094 suffered from a more rapid degenerating progress[56]. Although that SNP was also included in our initial SNP pool, rs10516796 came out as the only SNP in *SPP1* gene that showed close relation with muscular changes after exercise. Yet, the two opposite directions of the effect of rs10516796 on muscle mass and knee strength changes might be related to the result of its interaction with other SNPs in the regression model.

Noticeably, through stepwise regression, *MTRR* gene were identified closely related to both baseline SMM and one-year PT_{IM60} response. Gene *MTRR* expresses methionine synthase reductase which participates in the metabolic cycle that provides methyl groups to DNA[57]. Heterozygotes in *MTRR* gene was thought to impair the catalytic functions of corresponding enzyme and was found more frequently in athletes when compared with non-athletes[58]. Since this gene was reported to affect muscular

metabolism through DNA methylation[58,59], our results indicated that DNA methylation may contribute to the adaptations of muscle after exercise. In our study, one year exercise training may induce DNA hypomethylation in *MTRR* gene region which lead to an increase in myogenic proteins[58], resulting in an improvement of knee isometric strength. Furthermore, the discovery of *DNMT3L* gene also supported the idea of the occurrence of DNA methylation during training. Study has found that DNMT3L plays a crucial role in the activation of DNMT3a2 while the latter is the major DNA methyltransferase in male germ cells[60].

From results of ANCOVA in exercise groups after training, GPS could only explain 14% of the variance of SMM change and 27% of that in PT_{IM60}. Figures of GPS distribution and its linear regression with muscular parameters also showed individual variances among subjects within the same GPS group. Such findings indicated that there might be other unknown exercise-related genes and genetic composition is not the only factor that affect muscular phenotypes. In fact, the expression of gene can also be affected without the alteration of genetic sequence, this process is known as epigenetics[61]. Many external factors, such as food habit, activity level and living environment can contribute to the modification of DNA (de-)methylation[62]. The involvement of *MTRR*, *DNMT3L* and *METTL21C* gene discussed above also suggested the existence of epigenetics in training adaptation process. Thus, further research on the relation between epigenetic factors and aging muscle is also needed.

The limited sample size can be a weakness in this study because the small number of subjects in each group might affect the effect size and reproducibility of the results. As only Caucasian subjects were recruited, ethnic difference in relation between GPS and muscular phenotypes was not tested. Moreover, since only a limited number of these participants received upper leg computed tomography scan, data of thigh muscle mass were not sufficient enough for statistics. As a substitute, SMM was then used. This might undermine the accuracy in data analyses and further explanations. In the calculation of GPS, each predisposing allele was given equal weight. This could ignore the fact that these alleles might contribute differently towards certain muscular phenotypes. Thus, other GPS calculation methods, such as total weighting genotype score[63], LASSO and Elastic Nets[64] can provide new ways to study the relation between gene and aging muscle.

In conclusion, we found that data-drive GPS was positively related to baseline isometric knee strength and adaptive changes of muscle mass and knee strength after one-year exercise in healthy elderly population. Specifically, GPS could partly explain the inter-individual variance of training response while DNA methylation was also involved in the adaptive process. Moreover, a pilot study has already indicated an enhanced efficiency of resistance training when individual's genotype was included in the design of exercise prescription[65]. Thus, our results can provide supportive genetic information for the design of personalized exercise regimen.

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