THE ELUCIDATION OF MOLECULAR MECHANISMS IN SYSTEMIC MASTOCYTOSIS

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THE ELUCIDATION OF MOLECULAR MECHANISMS IN SYSTEMIC MASTOCYTOSIS

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Declaration:

With the exception of any statements to the contrary, all the data presented in this report are the result of my own efforts. In addition, no parts of this report have been copied from other sources. I understand that any evidence of plagiarism and/or the use of unacknowledged third party data will be dealt with as a very serious matter.

Signed .....
Date .....

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Acknowledgments

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Finally, but by no means least, thanks go to my Mum, Dad and Ste whose love and guidance are with me in whatever I pursue. They are the most important people in my world and I dedicate this to them.

Tables

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Abbreviations

AML	Acute Myeloproliferative Leukaemia
ASM	Aggressive Systemic Mastocytosis
β2M	Beta 2 Microglobulin
BM	Bone Marrow
BSA	Bovine Serum Albumin
CM	Cutaneous Mastocytosis
CRP	C-Reactive Protein
CXCL7	Platelet basic protein
DAVID	Database Annotation Visualisation and Integrated Discovery
DCM	Diffused Cutaneous Mastocytosis
DDA	Data Dependant Acquisition
DIA	Data Independent Acquisition
DOC	Sodium Deoxycholate
DTT	Dithiothreitol
FDA	Food and Drink Administration
FDR	False Discovery Rate
GO	Gene Ontology
IAA	Iodoacetamide
IL	Interleukin
IM	Imatinib Mesylate
INFα	Interferon alpha
IRTs	Indexed Retention Times
ISM	Indolent Systemic Mastocytosis
LBP	Liposaccharide Binding Protein
MCL	Mast Cell Leukaemia
MCS	Mast Cell Sarcoma
MDS	Myelodysplastic Syndromes
MPCM	Maculopapular Cutaneous Mastocytosis
MS	Mass Spectrometry
PANTHER	Protein Annotation Through Evolutionary Relationships
PCA	Principle Component Analysis
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death protein 1 Ligand
PDGFrβ	Platelet derived growth factor receptor beta
PPI	Protein-protein Interactions
SCF	Stem Cell Factor
SM	Systemic Mastocytosis
SM-AHN	Systemic Mastocytosis with associated Haematological neoplasm
SSM	Smouldering Systemic Mastocytosis
SWATH-MS	Sequential Window Acquisition of all Theoretical Mass Spectrometry
TGFβ1	Transforming Growth Factor Beta 1
WHO	World Health Organisation
2-CdA	2-Chlorodeoxyadenoine

Abstract

Introduction

Mastocytosis, one of the subcategories of myeloproliferative neoplasms, results from a colonel, neoplastic proliferation of morphologically and immunophenotypically abnormal mast cells which accumulate primarily in the skin and bone marrow. Prevalence is reported to be 13 in 100,000 of the population, with an equal male to female preponderance. The clinical spectrum is heterogeneous and ranges from relatively benign cutaneous mastocytosis (CM), with isolated skin lesions, to a more aggressive variant, systemic mastocytosis (SM).

Aim

To carry out both a global discovery and a targeted analysis of the proteome of plasma from peripheral blood of systemic mastocytosis patients and compare to healthy controls, to understand the molecular mechanism of systemic mastocytosis and to identify novel disease biomarkers.

Methods

Peripheral blood was collected by venepuncture at the antecubital fossa from systemic mastocytosis patients (n=13) and healthy controls (n=7). Following immune-depletion of high abundance proteins and tryptic digestion, plasma samples were loaded onto a SciEx 6600 Triple TOF mass-spectrometer.

Results

SWATH-MS identified 1437 proteins, off which 360 were upregulated. Further analysis identified these as being involved in immune system regulation and leukocyte activation. ELISA was used to measure the levels of proteins common to all pathways involved in immune regulation and inflammation. The results of ELISA demonstrated significantly increased circulating plasma levels of CRP, CXCL7, LBP, TGFβ1 and PDGFrβ in systemic mastocytosis compared to controls. Circulating plasma levels of B2M were also increased in patients, although this did not reach the level of statistical significance.

Conclusion

The results of the current investigation demonstrate, the up-regulated proteins identified are involved in immune system regulation and inflammation, suggesting a central role of the inflammatory response in the patho-physiology of systemic mastocytosis.

Chapter 1: Introduction and Literature Review

1.1 Introduction

According to the World Health Organisation (WHO), mastocytosis is considered one of the eight subcategories of myeloproliferative neoplasms. Mastocytosis results from a clonal, neoplastic proliferation of, morphologically and immunophenotypically, abnormal mast cells which accumulate primarily in the skin and bone marrow (Pardanani, 2016).

Mastocytosis has a reported prevalence of 13 in 100,000 of the population, with an equal male to female preponderance, however underdiagnoses is assumed due to clinical misclassification and non-specific symptomology (Brockow, 2014).

The clinical spectrum of mastocytosis is heterogeneous and ranges from relatively benign cutaneous mastocytosis (CM), with isolated skin lesions, particularly in paediatric patients who generally experience spontaneous regression at puberty and is associated with an excellent prognosis (Magliacane et al., 2014), to a more aggressive variant, systemic mastocytosis (SM). Systemic mastocytosis generally occurs in adults and is associated with widespread systemic involvement associated with multiorgan dysfunction and reduced survival (Pardanani, 2016).

Mastocytosis patients present with a broad spectrum of widely varying clinical signs and symptoms which, apart from the characteristic skin lesions of urticaria pigmoentosa, lack specificity to point clearly to a definitive diagnosis (Horney et al., 2008). Nevertheless clinical diagnosis of mastocytosis is determined according to the 2016 WHO criteria, which includes the identification of neoplastic mast cells using morphological, immunophenotypic and/or molecular criteria (Pardanani, 2016).

The clinical signs and symptoms of mastocytosis include fatigue, pruritus, flushing, tachycardia, abdominal pain, bone pain and rarely neuro-psychiatric symptoms. The clinical signs and symptoms are mediated by mast cell activation and release of inflammatory and immunological mediators, consequently clinical severity can, in part, be related to the degree of mast cell infiltration and activation (Metcalfe et al., 2017). Clinical management of mastocytosis, regardless of subtype, involves the control of immediate, possibly severe symptoms, by the use of, for example, H1-antihistamines for the reduction of pruritus and flushing, and corticosteroids for bone pain (Komi et al., 2017).

1.2 The Mast Cell

In 1878, Paul Ehrlich was the first to describe mast cells or “mastzellen” in connective tissue that stained reddish purple with aniline dyes (Metcalfe, 2008). Ehrlich also went onto describe the pivotal role of mast cells in inflammation. Several developments, such as the discovery of histamine, mast cell growth factors and the role of mast cells in inflammatory diseases, has since occurred (Krishnaswamy et al., 2001). (Figure 1)

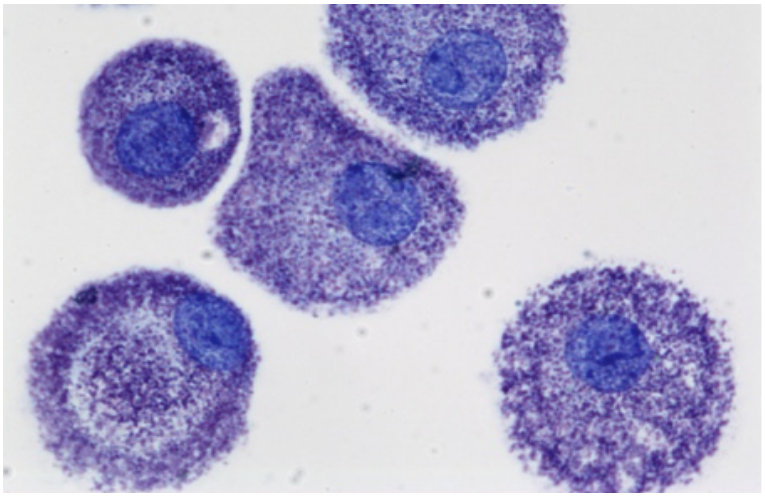


Figure 1: Photomicrograph of Human mast cells (x400 magnification). Human mast cells cultured from peripheral blood in stem cell factor, stained with toluidine blue (Metcalfe, 2008).

Mast cells are found in peripheral tissue, such as connective tissue and the dermal layer of the skin, where they play a central role in mediating inflammation and immediate allergic reactions (Payne and Kam, 2004). Mast cells are heavily granulated, wandering cells which maybe recruited into peripheral tissues, such as lungs, mucosa, dermis of skin and submucosa of the intestines where they differentiate and mature under the influence of mast cell growth factors (Krishnaswamy et al., 2001).

Mast cells are derived from haematopoietic progenitor cells from bone marrow. Circulating pluripotent progenitor ($CD34^{+}/CD117^{+}$) cells, migrate into the peripheral tissue and mature under the influence of stem cell factor (SCF) into ($Fc\varepsilon R1^{+}/CD117^{+}$) mast cells. Stem cell factor, which is produced by a number of cells, notably fibroblasts and endothelial cells, is a ligand for the c-KIT (CD117) receptor expressed by mature mast cells, leading to activation of c-KIT to mediate the intracellular signalling pathways (Figure 2).

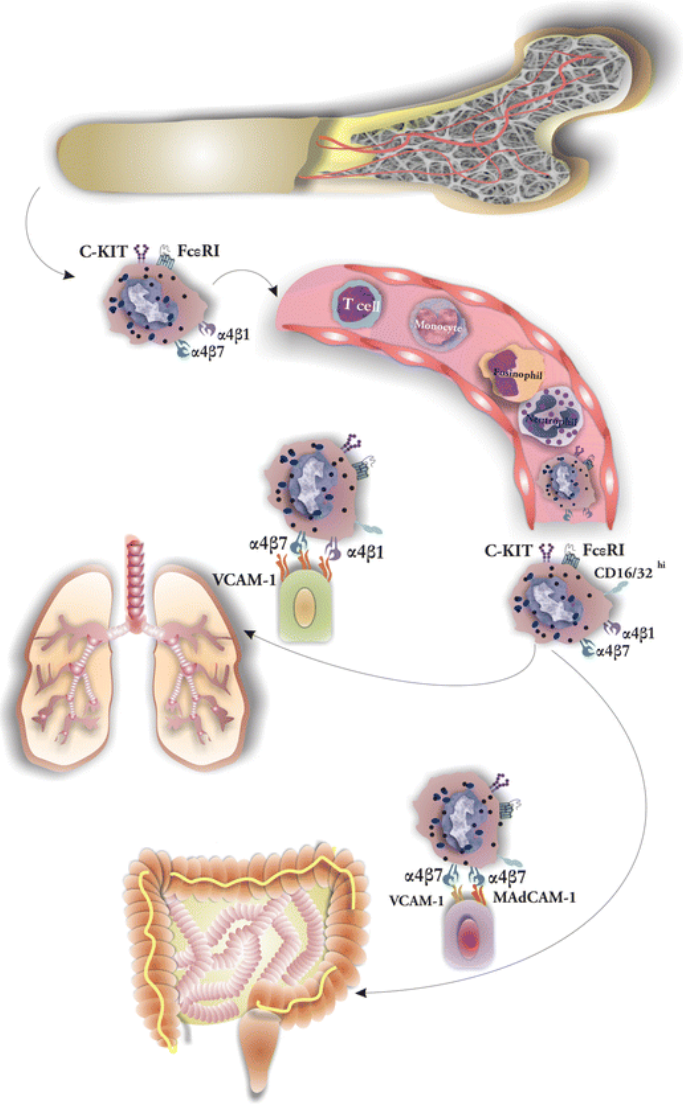


Figure 2: Mast cell development: Mast cells develop from $CD34^{+}$ /$CD117^{+}$ progenitors that originate from the bone marrow. Once the bone marrow progenitors are released from the bone marrow, they enter circulation where they follow a controlled trafficking pattern with the help of interactions between integrin's and their receptors. Upon reaching their target tissues, they mature into mast cells under the influence of growth factors (Komi *et al*,. 2017).

Mast cells are specialised secretory cells of the innate immune system. They play an important role in host defence by producing and releasing proinflammatory mediators, chemotactic factors and immunoregulatory cytokines (Komi et al., 2017) (Table 1). Within their cytoplasm, mast cells contain dense metachromatic granules containing heparin, histamine and a variety of proteases. Upon stimulation of the

cell surface Fc receptors with their physiological ligand IgE, the mast cells immediately release the contents of the secretory granules in the process of degranulation. In addition, degranulation may be induced by a number of physical factors, including toxins and venoms (Payne and Kam, 2004).

Table 1: Mast cell proinflammatory mediators, chemotactic factors and immunoregulatory cytokines release. Major human mast cell derived mediators released upon degranulation and their physiological effects upon the body (Adapted from Metcalfe, 2008).

Class	*Mediators*	*Physiological effects*
Preformed mediators	Histamine, Serotonin, Heparin, Neutral Proteases (tryptase and chymase), Carboxypeptidase, Cathepsin G, Major basic protein, Acid Hydrolases, Peroxidase, Phospholipases	Vasodilation, Vasoconstriction, Angiogenesis, Mitogenisis, Pain, Protein processing/degradation, lipid/proteoglycan hydrolysis, Arachidonic acid generation, Tissue damaged and repair, Inflammation
Lipid mediators	LTB4, LTC4, PGE2, PGD2, PAF	Leukocyte chemotaxis, Vasoconstriction, Bronchoconstriction, Platelet activation, Vasodilation
Cytokines	TNF-α, TGF-β, IFN-α, IFN-, β IL-1α, IL-1 β, IL-5, IL-6, IL-13, IL-16, IL-18	Inflammation, leukocyte, migration/proliferation
Chemokines	IL-8, I-309, MCP-1, MIP-1αS, MIP1 β, MCP-3, RANTES, EOTAXIN, MCAF	Chemoattraction and tissue infiltration of leukocytes
Growth Factors	SCF, M-CSF, GM-CSF, BFGF, VEGF, NGF, PDGF	Growth of various cell types, Vasodilation, Neovascularization, Angiogenesis.

The mast cell receptor c-KIT (CD117), is a type III tyrosine kinase (Komi et al., 2017), consisting of five extracellular immunoglobulin like domains and a single transmembrane spanning region (Figure 3). Three of the Ig domains possess complementary shape and charge and are the ligand binding site for SCF, while domains four and five permit receptor dimerization (Metcalfe, 2008). The intracellular juxtamembrane region, located between the plasma membrane and the kinase domain, controls the c-KIT kinase activity. In addition, the kinase domain

is composed of 2 sub-domains designated, tyrosine kinase domain 1 and 2. Stem cell factor mediated c-KIT signalling plays important roles in mediating angiogenesis, migration, cell survival and proliferation of mast cells (Komi et al., 2017). Binding of KIT leads to homodimerization of c-KIT due to the interactions of Ig-like domain 4/5 of two monomeric KIT receptors. These interactions lead to the consecutive transphophylation in the regions juxtamembrane, kinase insert, kinase domain and COOH terminal. The phosphorylated residues act like docking sites for signalling molecules Src and Shc kinase, PI3K and PLCy. Sos, PI3K, PLCy and JAK2 active MAPK cascade, and as a result causes Ca_2 influx and activation of transcription factors required for mast cell activation. This SCF induced activation of the JAK2 results in STAT5 and STAT6 activation which intern promotes mast cell development, survival and proliferation (Figure 4).

Cross linking of IgE bound allergen triggers the Fc_ER1 signalling pathway via the recruitment of the Syk, tyrosine kinase, to the y-chain- ITAMs following translocation to the phosphorylation of specific tyrosine within these motifs by Lyn, resulting in activation of Syk, phosphorylation of the transmembrane adaptor module LAT, which in turn coordinates downstream signalling pathways. This results in the activation of PLCy1/2, which are essential signals for mast cell mediator release.

A parallel pathway is also initiated by tyrosine kinase, Fyn, which leads to the activation of PI3K, which are also required for optimal degranulation and cytokine production (Figure 5).

GTP exchangers Sos and Vav activate the Ras-Raf-Mapk pathway, which contributes to the activation of specific transcription factors required for cytokine production (Figure 5), (Metcalfe, 2008 and Komi, 2017).

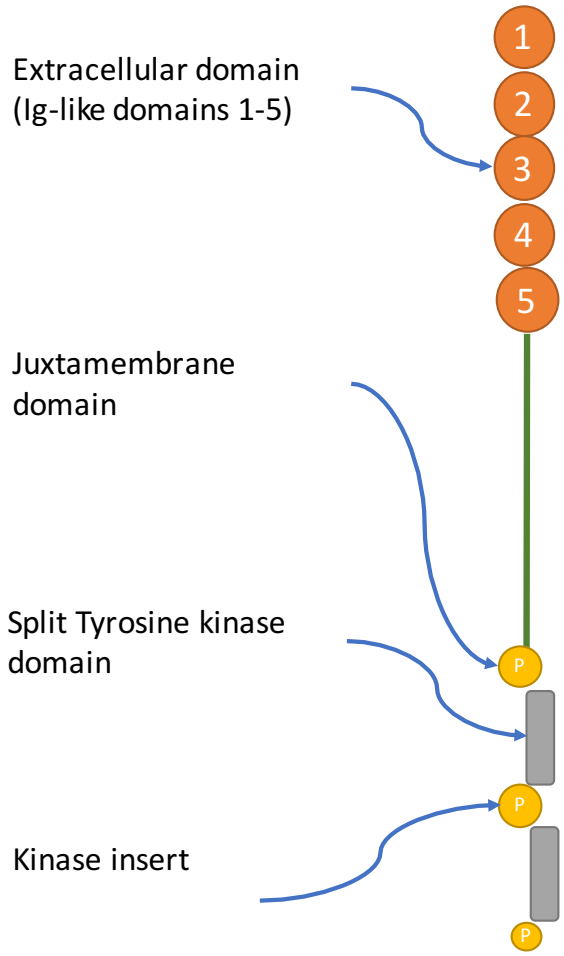


Figure 3: Mast cell receptor c-KIT (CD117). The c-KIT receptor is a type III tyrosine kinase consisting of five extracellular immunoglobulin like domains and a single transmembrane spanning region. Three of the Ig domains possess complementary shape and charge and are the ligand binding site for SCF, while domains four and five permit receptor dimerization (Figure adapted from Metcalfe, 2008 and Komi, 2017).

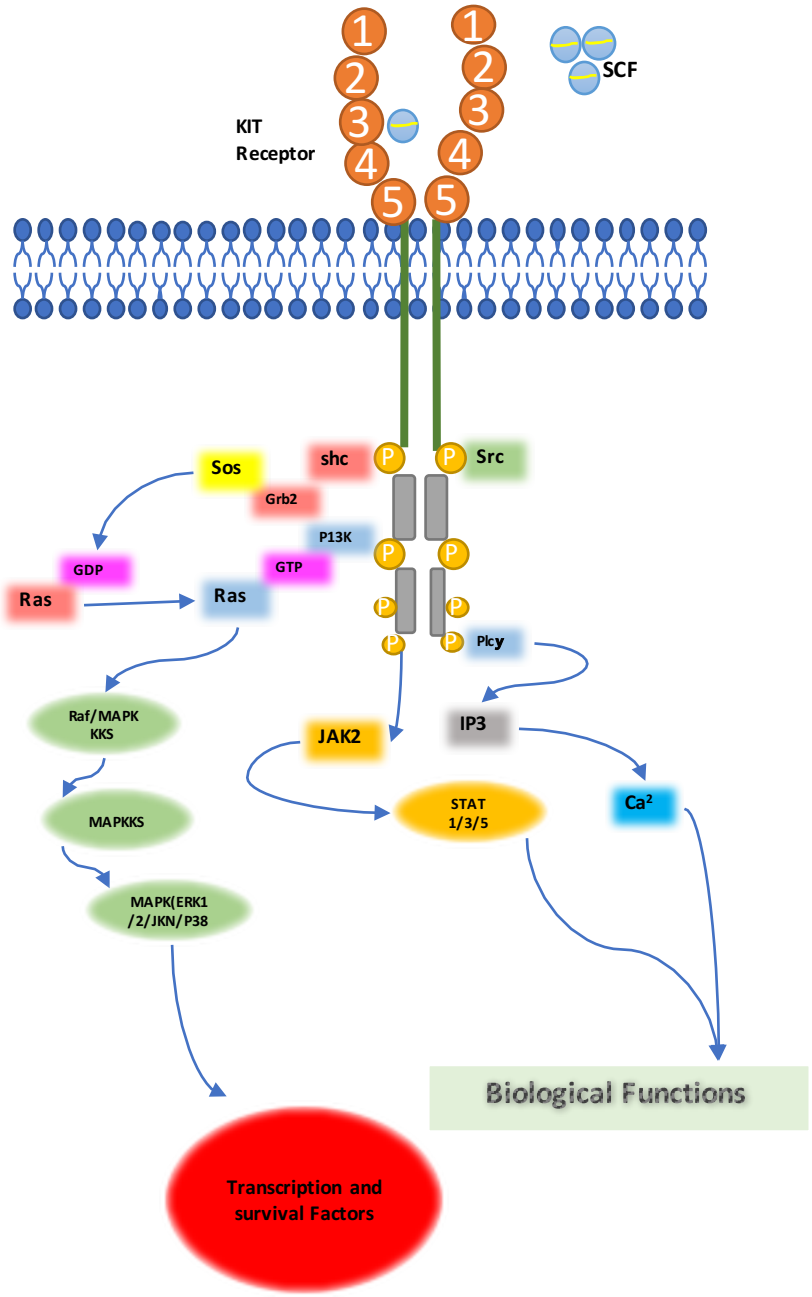


Figure 4: Stem cell factor mediated c-KIT signalling pathway. SCF mediated c-KIT signalling plays important roles in mediating angiogenesis, migration, cell survival and proliferation of mast cells (Figure adapted from Metcalfe, 2008 and Komi, 2017).

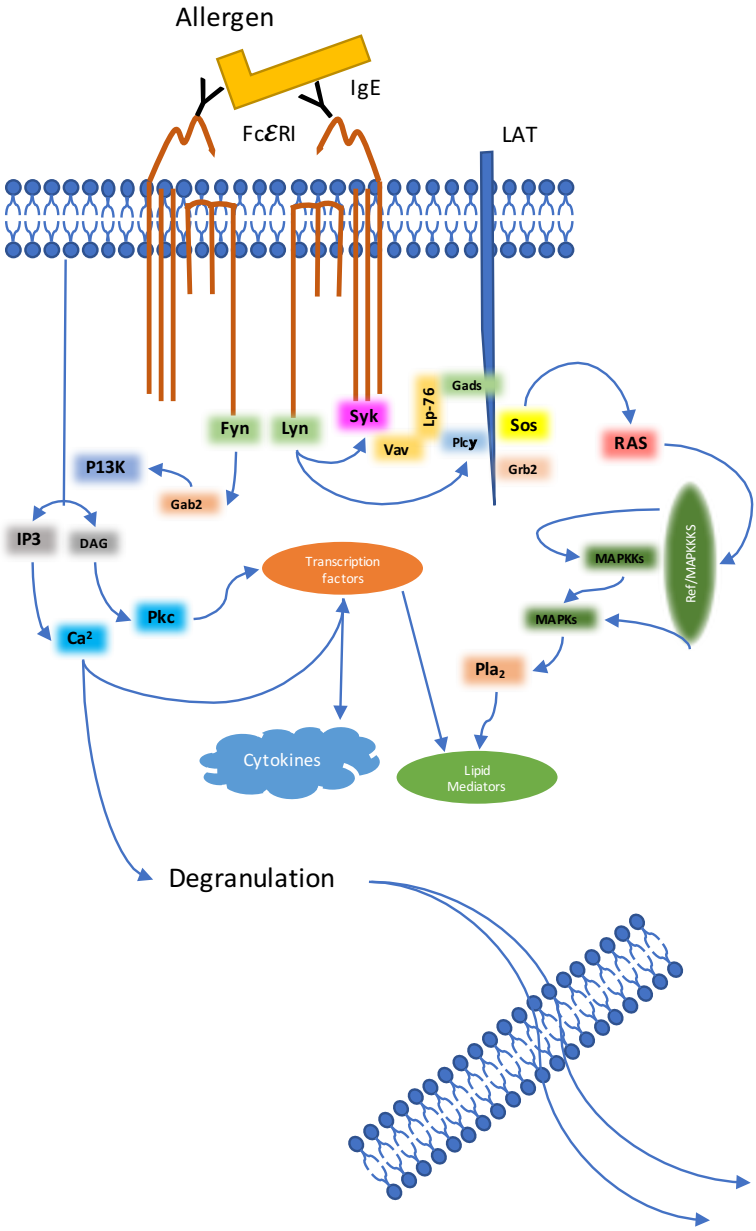


Figure 5: Cross linking of IgE bound allergen and the Fc_ER1 signalling pathway in the mast cell. Fc_ER1 signalling pathway is associated with the production of mediators and degranulation (Figure adapted from Metcalfe, 2008 and Komi, 2017).

1.3 Mastocytosis, Variants and Prevalence

Mastocytosis is considered a rare disease with various studies calculating an estimated incidence of 5 to 10 new cases per 1 million population per year (Hartmann et al., 2001). A prevalence of 1 in 60,000 was reported by the authors

of an epidemiological study in Europe and the United States (Magliacane et al., 2014).

Nettleship and Tay (1869) first described mastocytosis in 1869 as a rare form of urticaria which was later termed urticaria pigmentosa by Sanger (Sanger, 1878). In 1887 Unna, reported an association between the lesions and an increase in dermal mast cell numbers (Unna, 1887). In 1949 the first case of SM was reported (Ellis, 1949) with different sub classifications of cutaneous mastocytosis (CM) and systemic mastocytosis (SM) being described over the following decades (Valent et al., 2017). It has now been established, based on clinical observations, that SM can present with or without skin lesions and can display an indolent or aggressive clinical course, in some instances with co-existence of a clonal non-mast cell lineage disorder such as a myeloproliferative disorder or myelodysplastic syndrome (Metcalfe et al., 2017). In current clinical practice the diagnosis of mastocytosis is based on the World Health Organisation (WHO) Criteria developed in 2001 and updated in 2008 and 2016 (Valent, 2017). The WHO diagnostic criteria includes a classification of mastocytosis based on molecular markers, haematological findings, serum tryptase levels, CD markers and the identification of associated mutations (Metcalfe et al., 2017).

Further, the 2016 updated World Health organisation (WHO) classification discriminates cutaneous mastocytosis (CM), disease confined to the skin, and systemic variants which reflect different clinical presentation, prevalence and outcome (Valent et al., 2017), (Figure 6).

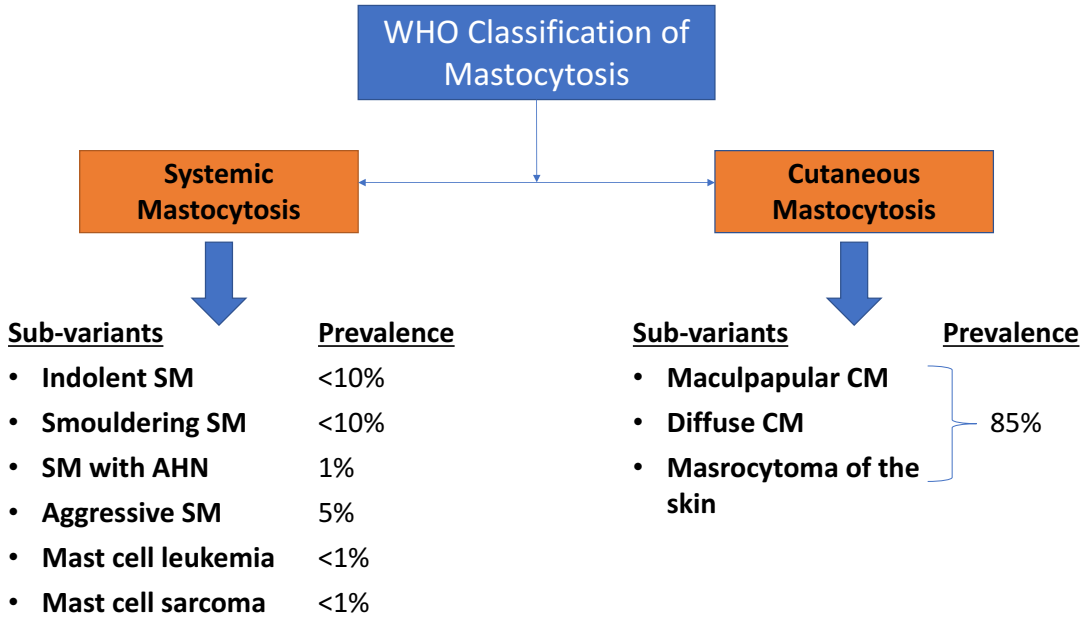


Figure 6: The 2016 updated WHO classification and prevalence of Mastocytosis. Variants and prevalence of mastocytosis, Systemic Mastocytosis and Cutaneous Mastocytosis. Systemic mastocytosis is further subdivided into Indolent SM (ISM), Smouldering SM (SSM), SM with associated hematologic neoplasm (SM with AHN), Aggravated SM and Mast cell leukaemia. Cutaneous Mastocytosis is further subdivided into maculopapular CM (MPCM), Diffused CM (DCM) and localised Mastocytoma of the skin (Figure adapted from Valent *et al*., 2017 and Magliacane *et al.,* 2014).

The most frequent variants of mastocytosis are CM and Indolent SM, with the rarest found to be mast cell leukaemia. The cutaneous form of the disease, which is limited to the skin, comprises of three clinical variants including maculopapular CM (MPCM), diffused CM (DCM) and cutaneous mastcocytoma (Figure 6). All the cutaneous manifestations are more common in children with 80% of cases occurring during the first year of life. Many cases spontaneously regress at, or during, puberty and are associated with a good lifetime prognosis (Fried and Akin, 2013).

In contrast, the systemic form of the disease, which is a clonal and disseminated condition, comprises six clinical variants including, indolent systemic mastocytosis (ISM), smouldering systemic mastocytosis (SSM), systemic mastocytosis with associated hematologic neoplasm (SM-AHN), mast cell leukaemia (MCL) and mast cell sarcoma (MCS). Systemic mastocytosis mainly affects adults, with a peak incidence in adults between 20-40 years of age. Moreover systemic mastocytosis is a chronic condition associated with a poorer prognosis than CM (Lykkegaard Anderson et al., 2012). In systemic mastocytosis, epidemiological evidence suggests that ISM and SM-AHN account for approximately 85% of patients (Lim et al., 2009b) (Figure 6).

1.4 Clinical Features

Mastocytosis is a condition with a broad, none specific symptomology related to mast cell release and, in some cases, multi-organ infiltration (Anderson et al., 2012). Skin symptoms are common in mastocytosis with urticaria pigmentosa being the most frequent. Urticarial pigmentosa is characterised by brownish- red skin lesions and is a universal sign of CM and in addition, occurs in 90% of SM patients including in up to 50% of patients with either SM-AHNMD or aggressive SM (ASM) (Soter, 2000) meaning it is a sensitive but non-specific sign of mastocytosis. In adults, cutaneous lesions are generally <0.5cm in dimeter and primarily occur on thighs and trunk (Horney et al., 2008) (Figure 7a). In children, cutaneous lesions are usually larger, approximately 0.5cm- 3cm in diameter, and affect the body, face and head. (figure 7b).

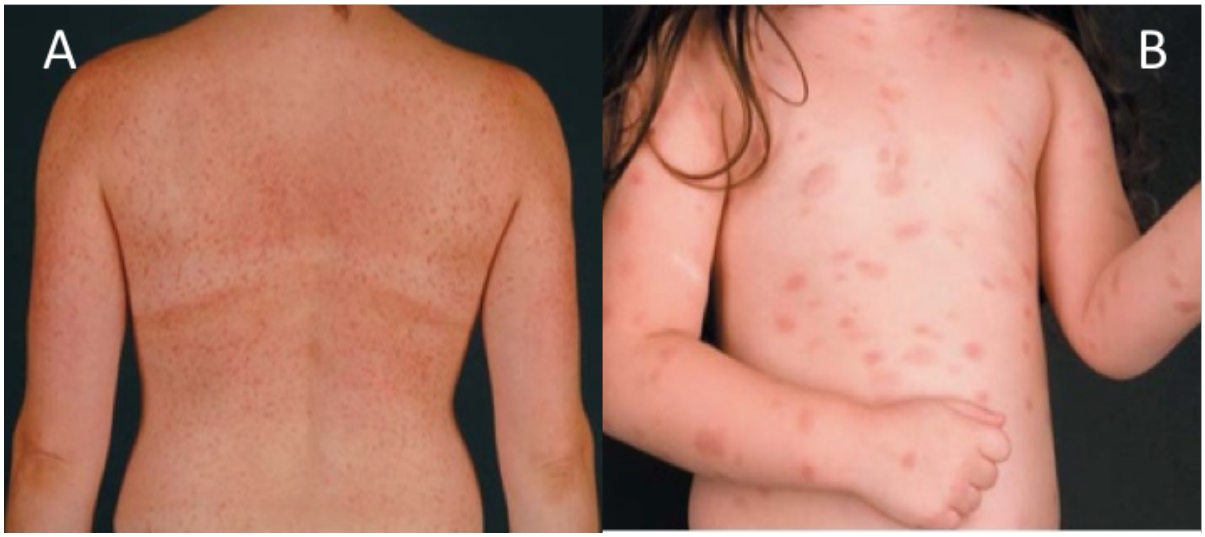


Figure 7: Urticara Pigmentosa (Maculopapular skin lesions) in both adult and paediatric patients. A) Typical Urticara Pigmentosa skin lesions seen in adult with CM or SM. B) Typical Urticara Pigmentosa skin lesions, usually larger in paediatric patients: as a rule, there is no SM involvement (Image from Horney *et a*l., 2008)

One of the most common complaints is pruritus initiated by changes in temperature (for instance hot baths), certain foods, physical activity, alcohol or drugs (Horney et al., 2008, Lykkegaard-Anderson et al., 2012).

Symptoms related to mast cell activation, degranulation and release occur in both CM and SM with the mast cell mediators inducing vasodilation, hypotension, flushing, itching and syncope. Symptoms can vary in intensity, related to disease burden and extent of mast cell degranulation, from mild allergic reactions to severe life threating anaphylaxis and anaphylactic shock (Austen, 1992). In addition, mast cell release can also result in chronic symptoms, such as persistent gastrointestinal complaints (including vomiting and diarrhoea), however these are less frequent. Symptom onset may be initiated by a range of factors, such as drugs or mediated

via cross linking of IgE, infection or emotional stress (Lykkegaard-Anderson et al., 2012).

The symptomology of mastocytosis may also be suggestive of the systemic variety and include anaemia, thrombocytopenia, malabsorption, splenomegaly, hepatomegaly and bone disease in the form of lytic lesions and pathological fractures (Austen, 1992). These more severe chronic symptoms result from multi system organ infiltration causing secondary organ dysfunction (Lykkegaard-Anderson et al., 2012) .

1.5 Pathogenesis

Initial small scale studies aimed to determine if SCF, the principal human mast cell growth factor, was elevated in mastocytosis. When considered in its entirety the evidence is contradictory with some authors reporting elevated levels in mastocytotic skin lesions by immunohistochemistry, however later studies measuring SCF levels in skin and blood, by immunoassay, did not support these initial findings in adult patients. It is important to note however that many of these were small number series or case reports. Therefore research began to focus on the SCF receptor, c-KIT (Metcalfe, 2008).

In 1995, Nagata and colleagues, identified a point mutation consisting of a substitution of aspartate to valine in the catalytic domain of *c-KIT* (*ASP816VAL* or *D816V*) in the peripheral blood of patient with mastocytosis (Komi et al., 2017). In 1996, the same mutation was identified in CM and ASM in both skin and spleen

(Metcalfe et al., 2017). In normal mature mast cells activation of the c-KIT receptor by its ligand, SCF, results in increased mast cell proliferation, prolonged survival and an intense release in mediators. These functions are reinforced in mastocytosis resulting in autocrine activation and degranulation of the mast cell. (Horney et al., 2008).

Recent research, in bone marrow derived mononuclear cells, has reported that >80-90% of patients with SM have a somatic gain of function mutation in the KIT receptor tyrosine kinase domain, primarily consisting of an aspartic acid to valine substitution (*D816V*) in the second catalytic domain, causing enhanced survival and mast cell proliferation (Komi et al., 2017). Further research has demonstrated that in a subset of patients mast cell proliferation increases to such an extent that mast cells may be detected in the peripheral circulation (Metcalfe, 2008). Results from molecular studies and clinical profiling support the contention that mastocytosis can arise due to over activity of the c-KIT receptor and that this is associated with a more severe disease phenotype (Metcalfe et al., 2017). While the *D816V* mutation would appear to be the most common, others have been reported including *V560G*, within the juxtamembranne domain of KIT, the *E839K* dominant inactivating mutation and the rare germ line mutation *F522C* (Metcalfe, 2008) . Further, other non-specific oncogenic mutations have been recently identified in mastocytosis patients including, *TET2*, a putative tumour suppressor gene and N-RAS an oncogene (Magliacane et al., 2014). Although evidence suggests *KIT* mutations contribute to the pathogenesis of mastocytosis, they are also the most common additional genetic abnormalities in Acute Myeloproliferative Leukaemia (AML) with a reported incidence ranging from 26- 47%. The *c-KIT* mutation is

considered a pro prognostic indicator of AML, hence limiting their specificity in clinical practice (Pullarkat et al., 2009).

In addition, the pathogenesis of mastocytosis is not limited to the activation of the KIT receptor. Authors have reported abnormal mast cell apoptosis with a preponderance to anti-apoptosis contributing to increased cell survival (Komi et al., 2017). Lange *et al*., (2012) have reported upregulation of the anti-apoptotic protein Bcl-2, in aggressive mastocytosis, along with the upregulation of Bcl-X, another anti-apoptotic protein, in the bone marrow of patients with ISM (Lange et al., 2012). The role of programmed cell death protein-1 (PD-1) has recently been investigated in mastocytosis. The PD-1 ligand (PD-L1) is expressed on tumour cells while the PD-1 receptor is expressed on T and B lymphocytes. PD-L1, expression on mast cells has been reported (Kuklinski and Kim, 2016), enabling abnormal mast cells to evade immune surveillance (Komi et al., 2017). In their 2017 study, Kuklinski and Kim reported increased expression of PD-L1 in mast cells, by immunohistochemistry, in skin biopsies obtained from patients with mastocytosis, although the exact mastocytosis variants were not reported. These results are supported by other investigators who have shown expression of the PD-1 receptor in clinical samples of human CM and in laboratory studies utilising the human mastocytosis cell line LAD2.

In addition, intracellular signalling molecules, involved in KIT signalling, have been investigated as possible therapeutic targets for mastocytosis. Bibi and colleagues (2014) have reported increased phosphorylation of AKT in HMC-1 cells, a human mast cell line potentially implicating a role for the PI3 kinase pathway in the

pathogenesis of mastocytosis. These initial preclinical results have been supported by Komi and colleagues (2017) who have recently reported increased phosphorylation of AKT in patients with *KIT D816V* SM (Komi et al., 2017). Further molecular studies by Chan and colleagues (2013), described alterations in *KIT* mRNA transcription in SM patients after identifying novel KIT transcripts in aggressive mast cell tumours (Chan et al., 2013).

1.6 Diagnosis and the WHO criteria

The classification and diagnosis of mastocytosis is based on established WHO criteria as developed and refined in 2008 & 2016, by the identification of neoplastic mast cells utilising morphological, immunophenotypic, and/or genetic criteria (Pardanani, 2016) The WHO diagnostic criteria are summarised in Table 2.

Table 2: WHO 2016 diagnostic criteria for Mastocytosis. Table showing major, minor, B findings and C findings criteria for the diagnosis of mastocytosis (Adapted from Metcalfe *et al.,* 2017).

CRITERIA	DEFINITION
MAJOR CRITERION	• Detected in sections in BM and/or other extracutaneous organs – multifocal dense infiltrates of MC (>15 MC in aggregates)
MINOR CRITERION	• Detected in sections in BM or other extracutaneous organs – >25% of MC in the infiltrate are spindle-shaped or have atypical morphology, • or of all MC in BM aspirate smears, >25% are immature or atypical.
	• Detection in blood, BM, or another extracutaneous organ – A activating point mutation at codon 816 of KIT
	• Detection in blood, BM, or another extracutaneous organ – mast cells expressing CD25, with or without CD2, in addition to MC markers.
	• Serum total tryptase > 20 ng/mL
B FINDINGS	• BM biopsy showing - >30% infiltration by MC and/or serum total tryptase level >200 ng/mL
	• Signs of myeloproliferation or dysplasia in non-MC lineages but insufficient criteria for definitive diagnosis of a haematopoietic neoplasm with normal or slightly increased blood counts.
	• Hepatomegaly without impairment of liver function, • and/or palpable splenomegaly without hyperplenism • and/or lymphadenopathy on palpation or imaging
C FINDINGS	• BM dysfunction manifested by 1 or more cytopenias caused by neoplastic mast cell infiltration.
	• Hepatomegaly with impairment of liver function • Ascites • And/or portal hypertension
	• Skeletal involvement with large, osteolytic lesions and/or pathological fractures.
	• Malabsorption with weight loss due to gastrointestinal MC infiltrates
	• Splenomegaly with hypersplenism

Table 3: Diagnosis of Mastocytosis Variants. Table showing the WHO diagnosis criteria for mastocytosis variants (Adapted from Metcalfe et al., 2017).

VARIANT	DIAGNOSIS
• **CUTANEOUS MASTOCYTOSIS**	Typical mast cell infiltrates in a multifocal or diffused pattern on biopsy.
• **SYSTEMIC MASTOCYTOSIS**	1 major or 1 minor criterion, or at least 3 minor are present (see table 2)
• **INDOLENT SM**	SM and no C findings, no sign of associated haematological neoplasm. (see table 2)
• **SMOLDERING SM**	ISM but with 2 or more B findings and no C findings. No sign of associated haematological neoplasm or MCL. (see table 2)
• **SM WITH ASSIOSIATED HAEMATOLOGIC NEOPLASM**	Meets criteria for SM and for an associated clonal haematological non-mast cell lineage disorder, lymphoma or other haematological neoplasm (see table 2)
• **AGGRESSIVE SM**	SM with 1 or more C findings. No evidence of MCL (see table 2)
• **MAST CELL LEUKEMIA**	SM and bone marrow shows diffuse infiltration by atypical, immature mast cells: bone marrow aspirate smears show >20% mast cells.
• **MAST CELL SARCOMA**	Unifocal mast cell tumour with no evidence of SM, high grade cytology.

1.6.1 Bone Marrow Histology

As the bone marrow is universally involved in SM, bone marrow aspiration and examination remains the diagnostic investigation of choice in suspected SM. However histological examination and identification of the key histological abnormalities (dense mast cell aggregates in the perivascular and/or paratrabecular bone marrow) may be hampered by poor uptake of standard stains

such as Giemsa, in particular when mast cell hypergranulation or abnormal nuclear morphology is present (Pardanani, 2016). Immunoreactive tryptase has been reported as the most sensitive immunohistochemical marker of mastocytosis, however immunohistochemical identification of tryptase, and KIT/CD117 lacks the diagnostic specificity to differentiate normal and abnormal mast cells. The most specific immunohistochemical marker has been reported to be CD30 due to its ability to detect all abnormal mast cells in patients with SM (Sotlar et al., 2004) (Figure 8).

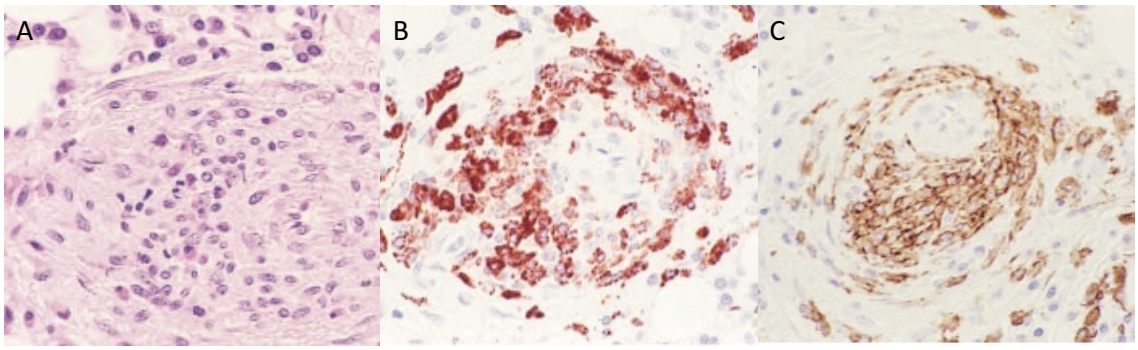


Figure 8: Bone Marrow trephine biopsy, showing a pathognomonic mast cell aggregate comprised of morphologically a typical mast cells revelled by A) Hematoxylin-eosin stain, B) Tryptase immunostaining, C) c-kit immunostaining (Image from Patnaik et al., 2007).

1.6.2 Mast Cell Immunophenotyping

Mast cell immunophenotyping by flow cytometry to identify and quantify cell surface antigen expression contributes towards the minor diagnostic criteria set by WHO. Normal mast cells usually express c-KIT/CD117 and FcεR1 and the typical profile of normal mast cells is $CD117^{+}$/ $Fc\varepsilon R1^{+}$/ $CD34^{-}$/$CD38^{-}$ /$CD33^{+}$/$CD45^{+}$/$CD11c^{+}$/$CD71^{+}$. It is also worthy of note that myeloid markers, such as CD14 and CD15, or lymphoid lineage markers, except CD22, are not expressed on normal mast cells (Patnaik et al., 2007)

In contrast, neoplastic mast cell commonly express the surface antigens CD25 and/or CD2 with at least one of these two counting as a minor criterion towards the diagnosis of SM according to the WHO criteria, although CD25 may be a more sensitive and specific marker (Horny *et al*., 2008; Horny and Valent, 2002; Patnaik *et al.,* 2007).

1.6.3 Serum Tryptase Levels

Mast cell derived tryptase has been shown to be a useful disease related marker in SM. Mast cell tryptase include two molecular forms (alpha and beta) with designated subtypes alpha 1 or 2 and beta 1, 2 or 3. Beta 2 tryptase is the molecular form released on mast cell degranulation. It has been suggested therefore that circulating levels of beta 2 tryptase may reflect mast cell burden and the extent of mast cell activation (Patnaik et al., 2007). Circulating mast cell tryptase activity has been evaluated as a diagnostic test, which yielded a diagnostic sensitivity of 83%, and specificity of >98%. In addition, other groups have demonstrated that serum tryptase activity may be useful in evaluating treatment aimed to reduce mast cell numbers in patients with SM (Payne and Kam, 2004 and Valent et al., 2017). The specificity of serum tryptase activity however is limited due to elevated levels being observed in other conditions such as acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML) and myelodysplastic syndromes (MDS) (Sperr et al., 2009). At present there appears to be no direct clinical correlation between the serum tryptase and occurrence or severity of symptoms associated with increased mast cell degranulation or systemic mast cell burden (Pardanani et al., 2010). Further, in patients with indolent mastocytosis,

Paradanani and colleagues (2010) reported, mast cell release levels were significantly correlated with bone marrow burden, but not to mast cell mediator release symptoms for reasons which remain to be elucidated (Pardanani et al., 2010).

1.6.4 Molecular Biology

The identification of *KIT D816V* mutation fulfils a minor criterion according to the WHO diagnostic classification (Metcalfe et al., 2017). With the use of sensitive techniques, it is possible to demonstrate a somatic mutation in the coding sequence of KIT-gene in 90% of adult cases of SM however, with the less sensitive Sanger Sequencing, false negative results may be reported (Kristensen et al., 2011). In a smaller subgroup of adult patients, where *D816V* mutation cannot be demonstrated, other sensitive molecular techniques are able to detect other auto activating mutations of KIT (Lykkegaard Anderson et al., 2012).

By drawing all the diagnostic criteria together, Sanchez-Munoz and colleagues (2011) conducted a study to validate the WHO diagnostic criteria and reported that approximately 20% of ISM patients lack mast cell clusters in the bone marrow and approximately 30% exhibit a total serum tryptase level <20 ng/ml (Sanchez-Munoz et al., 2011). However, the sensitivity for detecting morphologic atypia, aberrant CD25 and/or CD2 expression, or *KIT D816V* in bone marrow mast cells is reported as >90% although the reporting of the diagnostic efficacy of all the investigations undertaken is inconsistent. The full diagnostic validation study however

demonstrates the importance of the minor criteria that form part of the WHO diagnostic criteria for mastocytosis (Sanchez-Munoz et al., 2011).

1.7 Prognosis

In 95% of adult and paediatric patients with CM and ISM, life expectancy is not reduced and a favourable prognosis is determined (Horney et al., 2008). Cutaneous mastocytosis mainly occurs in children, with >50% of recorded cases occurring in the paediatric population. In children, the majority of cases spontaneously regress by the time the patient reaches adolescence. In adults, however, CM usually follows a chronic course and only in a small number of patients with ISM does it tend to regress (Horney et al., 2008). In contrast, the progressive forms of SM, in particular ASM and MCL, are associated with significant mortality, in some instances within a few months, with or without chemotherapy to reduce tumour burden (Valent et al., 2007).

While in an outcome based follow up study, Lim and colleagues (2009) followed 342 adult patients with ISM and ASM. The authors reported an overall mean survival of 198 months, which was reported not to be significantly different from the non-ISM population when matched for age and sex. In the ASM group however, the prognosis was significantly worse, with an overall mean survival of 41 months and 5% of patients with ASM undergoing leukaemic transformation during the follow-up period (Lim et al., 2009). While the outcomes of the study are instructive as to outcome in ISM and ASM, the study is limited by its small number of subjects and further subgroup analysis.

1.8 Current Treatment and New Investigational Agents

The treatment of all categories of mastocytosis involves the control of symptoms with pharmacological agents, that inhibit the action of mast cell mediators such as histamine and leukotrienes (Table 4), together with agents to treat any associated haematological disorder and reduce mast cell burden (Metcalfe et al., 2017). Therapy should be individualised to each patients' clinical presentation and prognosis, due to the heterogeneous nature of mastocytosis.

Table 4: Table of Mastocytosis symptoms and treatments. Treatments include pharmacological agents, that inhibit the action of mast cell mediators (Adapted from Metcalfe, 2017)

Symptoms	*Treatment*
Flushing, Tachycardia, Syncope	Aspirin,
Diarrhoea	Anticholinergics, oral cromolyn sodium
Pruritus, Flushing	H1 and H2 Antihistamines
Gastric Hypersecretion	H2 Antihistamines and Proton Pump Inhibitors.
Osteoporosis	Calcium, Vitamin D and Bisphosphonates.
Associated Inflammation	Glucocorticoids
Systemic anaphylaxis-like reactions	Epinephrine

Control of cutaneous symptoms such as pruritus and flushing, together with gastric hypersecretion can be controlled by antihistamines and proton pumps inhibitors respectfully. Corticosteroids are also used for the treatment of anaphylaxis as well as for the control of malabsorption and ascites (Worobec, 2000). In addition, some authors report the use of aspirin for the treatment of flushing, tachycardia and syncope however, this must be used with caution as it may trigger vascular collapse, in some patients, and exacerbate co-existing peptic ulcer disease

(Metcalfe et al., 2017). At present, chemotherapy does not seem to have a role in the treatment of the cutaneous or indolent forms of mastocytosis.

Currently, no standardised treatment regimens have been documented for use in patients with aggressive forms of mastocytosis, rather treatment for SM-AHN, ASM and MCL are focused on management of any associated medical conditions and the underlying haematological disorder and associated co-morbidities (Hennessy et al., 2004).

1.8.1 Interferon alfa (INF-α)

In patients with symptomatic aggressive SM, INF-α has shown variable therapeutic efficacy, as a first line therapeutic agent, to improve symptoms related to mast cell degranulation, decrease mast cell infiltration of the bone marrow and improve mastocytosis related ascites, hepatosplenomegaly, cytopenias, cutaneous symptoms and osteoporosis. It should be noted, however that these results are derived from small case series, and not randomised controlled trials and hence patient selection will be highly variable, of subject to selection bias, and perhaps contribute to the variable therapeutic efficacy observed in these small series. (Kluin-Nelemans et al., 1992; Worobec et al., 1996; Butterfield, 1998; Lehmann and Lammle, 1999).

Further, using the WHO diagnostic criteria for mastocytosis as a guide to measure treatment efficacy, INFα has been shown to be consistently ineffective when assessed for frequency of a major response. In this context, a major response is defined as compete resolution of one of more baseline C-findings (Table 2). In small

series clinical investigations, INFα has been shown to result in major therapeutic response in only 20-30% of cases (Hennessy et al., 2004), although this can be improved by concomitant administration of glucocorticoid to around 40% (Delaporte et al., 1995). The poor therapeutic efficacy could be related to the fact that the optimal duration and dose of INF-α remains to be elucidated (Delaporte et al., 1995).

INF-α treatment is not without side effects and complications such as fever, bone pain, flu like symptoms, cytopenias, depression and hypothyroidism have been reported in up to 50% of patients, however it should be noted that numbers are small and patient selection criteria highly variable (Pardanani, 2016). Time to therapeutic response has also been variable, with authors reporting up to a year, and in some cases longer, to observe clinical benefit (Pardanani, 2016).

1.8.2 2-Chlorodeoxyadenosine (Cladribine or 2-CdA)

2-Chlorodeoxyadenosine (cladribine or 2-CdA) is a purine nucleoside analog which has been shown to reduce cell proliferation by interfering with DNA replication, including in neoplastic mast cells. Small case series have reported the use of cladribine for the treatment of all types of aggressive mastocytosis including systemic mastocytosis. In a study of 9 patients with systemic mastocytosis, receiving 6 courses of treatment, Kluin-Nelemans *et al.,* (2003) reported an improvement in clinical symptomology and a decrease in circulating serum tryptase levels. The authors concluded that cladribine may be a possible therapeutic regimen for aggressive mastocytosis, although the number of participants is too small to draw any significant conclusions or make a recommendation cladribine use

as a therapeutic agent in the setting of SM. In addition, cladribine is not without significant side effects and may induce pancytopenia and immunosuppression (Metcalfe et al., 2017).

1.8.3 Imatinib Mesylate (IM)

Imatinib Mesylate is a tyrosine kinase inhibitor that has been shown to inhibit the phosphorylation of wild type KIT, including some of the transmembrane and juxtamembrane KIT mutations. Imatinib, however is ineffective at inhibiting the phosphorylation of KIT with the common *D816V* mutation (Pardanani, 2016). Presently, Imatinib is the only therapeutic agent that has been approved by the US Food and Drug Administration (FDA) for patients with SM, without the *KIT D816V* mutation, or when the KIT phenotype is unknown. Evidence for the use of Imatinib however is still limited. In a series of 27 patients with SM, Lim and colleagues (2009) reported an overall response rate, irrespective of KIT phenotype, of 18% during a median duration of treatment of 19.6 months. Response was defined as an improvement in urticarial pigmentosa and a decrease in bone marrow mast cell burden. In this series, however, subgroup analysis of the 86% of patients who carry the D816V mutation reported an overall response rate of 17%, compared to 33% in patients who do not carry the *D816V* mutation. It should be noted however that the number of participants was small and heavily biased towards patients carrying the *D816V* mutation. However an earlier study reported overall response rate of 36% in patients with the *D816V* mutation (Droogendijk et al., 2006). The difference may be due to patient selection or the occurrence of Imatinib selective KIT mutations (Pardanani, 2016). Therefore current evidence has shown that efforts to

inhibit the mutant D816V KIT with tyrosine kinase inhibitors has been largely unsuccessful, although results are variable, and further molecular and proteomic profilling is required to identify potential new targets (Magliacane et al., 2014)

1.8.4 Investigational Agents

Second generation tyrosine kinase inhibitors, such as Midostaurine (PKC412), Dasatinib and Nilotinib, may provide additional therapeutic efficacy that is not evidence with current treatment regimens. (Metcalfe et al., 2017).

Dasatinib, *in vitro*, has shown efficacy against various KIT mutations including *D816V* and appears to have modest activity in *KITD816V* positive SM (Shah et al., 2006). Cumulative published data however, does not clarify which subgroup of SM patients are likely to gain the most benefit. (Pardanani, 2016).

Midostaurine (PKC412) also demonstrates activity against kinase domain KIT mutants (*D816V*) as in a small clinical study, including patients with MCL who harbour the *KITD816V,* resulted in transient clinical benefit (Growney et al., 2005). In addition, midostaurine treatment, in patients with advanced SM, resulted in significant improvement in organ function with concomitant reduction in mast cell burden, in the majority of patients (Pardanani, 2016).

Brentuximab vedotin is an antibody-drug conjugate directed against the cell membrane protein, CD30. CD30 is a member of the tumour necrosis factor receptor superfamily, with reported aberrant expression on neoplastic mast cells, with preferential expression in advanced SM, the role of Brentuximab vedotin in treating advanced SM is currently being investigated (Pardanani, 2016). Recently,

Borate and colleagues (2016) reported the outcome a phase 2 open label trial, where two of four patients with advanced SM demonstrated an improvement in circulating serum tryptase and improvements in circulating and bone marrow mast cell burden (Borate et al., 2016). It should be noted however that these are preliminary results in a small number of patients and that furthermore detailed clinical evaluation of Brentuximab will require further study in a larger patient cohort.

1.8.5 Splenectomy

In patients with ASM or SM-AHN, who present with massive splenomegaly, splenectomy has been shown to decrease mast cell burden aiding the use of myelosuppressive agents (Metcalfe, 2008). Previous work by Friedman and colleagues (1990) has shown an improvement in overall survival (34 vs 26 months in the control group), however the authors note the high surgical risk associated with splenectomy (Friedman et al., 1990 & Metcalfe, 2008).

1.8.6 Stem Cell and Bone Marrow Transplantation

In patients with advanced and potentially fatal SM-AHN, MCL and ASM, stem cell and bone marrow transplantation, following ablative chemotherapy has been proposed. To date, there is limited outcome data to show therapeutic efficacy, and the data that has been published is contradictory (Metcalfe, 2017).

Therefore, drawing all the evidence together for the different therapeutic options for mastocytosis, Vaes and colleagues (2017) proposed a treatment algorithm for Mastocytosis as shown below (Figure 9).

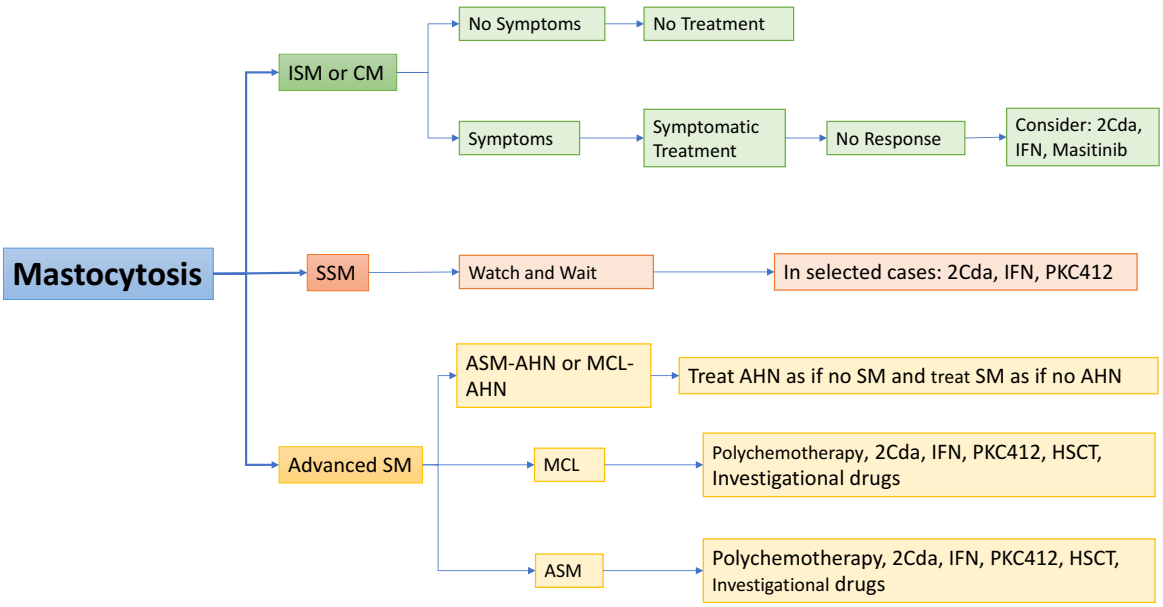


Figure 9: A Proposed treatment algorithm for Mastocytosis sub-variants including: ISM, CM, SSM, ASM, MCL, ASM-AHN (Adapted from Vaes *et al.,* 2017.)

1.9 Biomarkers

Mastocytosis is a difficult disease to diagnose. It is recognised that c-KIT is implicated in its pathogenesis however we do not fully understand what is driving its aetiology. Proteomics is a valuable tool used to elucidate the differences in alterations in proteins and/or protein expression levels thus providing a central contribution to improve the knowledge into physiological and pathological mechanisms (Anjo et al., 2017). By allowing the identification of biomarkers capable of distinguishing healthy from diseased conditions, these approaches are valuable for applied and translational research (Anjo et al., 2017) .

Biomarkers can serve a variety of functions when used in a clinical content such as for diagnosis or risk stratification (Table 5).

Table 5: Biomarker grouping and their applications. Biomarker grouping types and their application when used in a clinical content (Adapted from Biomarkers definition working group, 2001)

Biomarkers	**Application**
Risk stratification biomarkers	Identifying the risk of disease development
Screening biomarkers	Screening for subclinical diseases
Diagnostic biomarkers	Recognising overt diseases
Staging biomarkers	Categorising disease severity
Prognostic biomarker	Predicting future disease course and therapy response

The use of biomarkers in improving personised clinical information relies on reliable pre-analytical and analytical processing of clinical samples (Heaney et al., 2017).

Mass spectrometry (MS) is a powerful qualitative and quantitative analytical technique that is capable of measuring a wide verity of clinical relevant analyte with high levels of reproducibility, precision and accuracy and has a potential to extend current capabilities in biomarker discovery, development and validation (Heaney et al., 2017). Due to its incomparable capacity to analyse the complex protein mixtures found in body fluids and tissues, MS has become the method of choice in proteomic approaches. The biomarker tryptase for mastocytosis does not fully fulfil the characteristics of an idea biomarker therefore, creating the need to

establish and validate sensitive and specific biomarker for mastocytosis (Crutchfield, 2016). One way to measure biomarkers is via Sequential window acquisition of all theoretical Mass spectrometry (SWATH-MS).

1.10 SWATH – MS

Sequential window acquisition of all theoretical Mass spectrometry (SWATH-MS) is a data independent acquisition (DIA) approach that is able to reliably quantify significant numbers of peptides and proteins in an unbiased manner as well as allow peptide identification and quantification (Kang et al., 2017).

Proteomic studies have made remarkable progress recently, facilitated by the widespread application of LC MS/MS, and development of quantification methods (Domon and Aebersold, 2006). The development of TOF MS has enabled many new approaches for data acquisition in proteomic analysis possible. There are generally three types of LC MS/MS strategies, targeted acquisition, precursor dependant acquisition and precursor independent acquisition, in which the application of the different methods are dependent on factors such as goal of analysis, instrumental performance and sample complexity (Kang et al., 2017).

Data dependant acquisition (DDA) is a technique which typically detects only the most abundant peptides from complex samples after extensive fractionation (Hopfgartner et al., 2012). Data-independent acquisition (DIA) is a technique in where fragment ion information for all precursor ions within a determined mass range are acquired, therefore result in an unbiased precursor ion selection (Tate et al., 2013).

One DIA approach is SWATH-MS which utilises the rapid analysis time of TOF and has enhanced ion detection and fragmentation quality resulting in improved reproducibility when compared to DDA approaches (Zhu et al., 2014). Peptide extraction for SWATH-MS data requires a spectral reference list containing information of retention time (IRTs), fragment ions and their relative intensity which are used to extract any given peptide from SWATH-MS/MS maps using the fragmentation chromatograms (Kang et al., 2017).

The SWATH-MS method uses small Q1 windows enabling better selectivity and enhanced high confidence peptide extraction from data files (Kang et al., 2017). All precursors present are associated with their fragment ions resulting in improved identification specificity and therefore providing a more in depth view of protein and peptide species in a complex sample (Figure 10) (Kang et al., 2017).

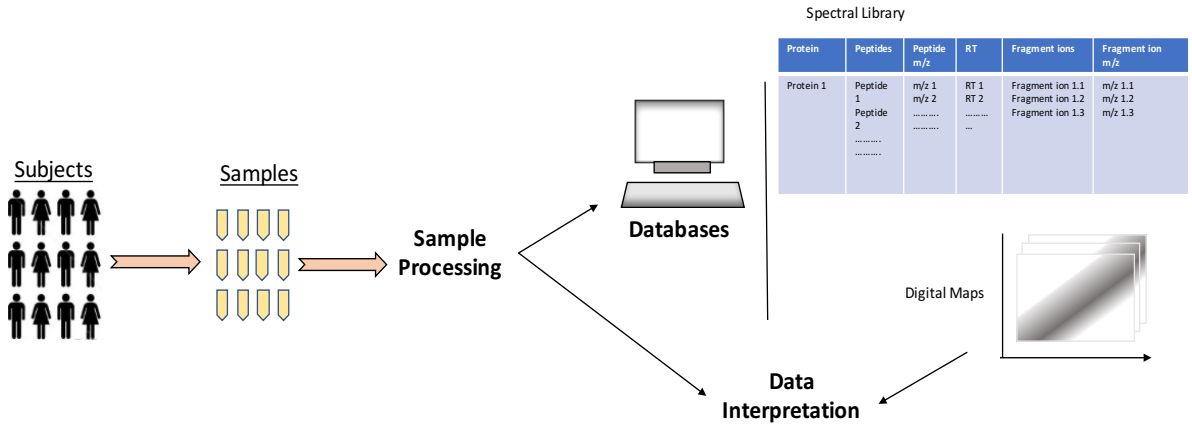


Figure 10: Schematic of the SWATH-MS. Implementation in applied and translational research (Adapted from Anjo et al., 2017)

SWATH-MS has been applied to biological samples such as, urine, serum and plasma together with organ specific tissue (Anjo et al., 2017). The variety of potential analytical sample types suggests that SWATH-MS is a valuable techniques for the identification and validation of biomarkers for diagnostic testing (Anjo et al., 2017).

1.11 Aims and Objectives

AIM: Using SWATH-MS to investigate the underlying mechanisms of disease in systemic mastocytosis.

To carry out global discovery of the proteome of plasma from peripheral blood of systemic mastocytosis patients and compare these to healthy controls.

Sequential Window Acquisition of all Theoretical fragmentation spectra mass spectrometry (SWATH-MS) will be employed to generate, in a single measurement, a complete permanent recording of all the components in a biological sample – a digital map.

The aim is to identify proteins that may discriminate between the two groups (patients diagnosed with SM and healthy controls). A map of those differently expressed proteins in an unbiased manner will be produced, allowing the elucidation of the molecular mechanisms involved in SM.

Chapter 2: Materials and Methods

2.1 Ethics

This study was approved by the NHS Health Research Authority on the 10th May 2016 (London and City East Research Ethics Committee (16/LO/0787). Ethical approval was also granted for this study by the Faculty of Science and Engineering Research Ethics Committee, following the completion of an internal ethics checklist and application. COSHH and risk assessments were also completed before the study was launched. The research is being co-sponsored by the United Lincolnshire Hospitals NHS Trust, University of Lincoln, Manchester Metropolitan University and the University of Manchester. The study was funded by the Mastocytosis Charity.

2.2 Subjects

Fourteen (14) patients with systemic mastocytosis were recruited from the Mastocytosis clinic at the United Lincolnshire Hospitals NHS Trust and Leicester Royal Infirmary. In addition, eleven (11), apparently healthy, controls were recruited from the staff and students of Manchester Metropolitan University. Patients and controls were recruited over a 30-month period between January 2016 - June 2018, by the author and other members of the research team.

Patient inclusion and exclusion criteria are shown below:

Inclusion Criteria:

- Male or female participants ≥ 18 years old.
- Diagnosed with Systemic Mastocytosis according to WHO classification or documented Mastocytosis based on histological criteria.

- Able and willing to provide written informed consent prior to participation in the study.

Exclusion criteria:

- Unable or unwilling to provide written informed consent prior to participation in the study.
- Pregnant or currently breast feeding.
- <18 years old
- other haematological malignancies
- Patients who have received chemotherapy, any investigational drug or undergone majored surgery < 4 weeks prior to the begging of the study.

2.2.1 Patient Recruitment

Patients were selected from either the Mastocytosis database, or from potential patients, according to clinical and/or laboratory suspicion of Mastocytosis and where a bone marrow biopsy was indicated. Potential study participants were invited take part by letter and accompanying patient information sheet, which was forwarded at the same time as their appointment letter for the haematology clinic (Appendix 1). Potential participants were able to discuss the study, if they wished to seek further information or clarification, with a consultant haematologist at a time separate to their clinic appointment. All patients recruited to the study gave written informed consent prior to the collection of a peripheral blood sample at the antecubital fossa (Appendix 2) .

2.2.2 Recruitment of Control Subjects

Heathy controls were recruited from the staff and students at the University of Lincoln and Manchester Metropolitan University. Volunteers were recruited through use of general University of Lincoln and Manchester Metropolitan University email. Volunteers who were interested in taking part were provided with a participant information sheet (Appendix 1) and given the option to discuss the project further with the author or Principal Investigator. Participants were given a minimum of 24 hours to consider study related information before being asked to provide written informed consent (Appendix 3) to collect a peripheral blood sample at the antecubital fossa.

2.3 Experimental Protocol

Each participant was assigned a unique identity number ensuring that all data was anonymised at source. The numbers ranged from LCH01-LCH09 (Lincolnshire Hospitals NHS Trust), LRI01-LRI04 (Leicester Royal Infirmary) and MCC01-MCC09, MMU02 for healthy controls (Manchester Metropolitan University).

2.4 Peripheral Blood Collection

A venous peripheral blood sample was collected at the antecubital fossa by a trained phlebotomist. Two peripheral blood samples, of a total volume of approximately 8-12ml whole blood (2x EDTA-plasma, Vacutainer, BD, Oxford, England, UK), were collected and processed within 24hrs of collection.

2.5 Plasma Sample Preparation

Peripheral EDTA blood (1ml) (EDTA-plasma, Vacutainer, BD, Oxford, England, UK) was centrifuged (SIGMA 3-15KL) at 3000 rpm for 3 minutes at room temperature. Plasma was aliquoted (15 µl) and stored frozen at -80°C.

2.5.1 Immuno-depletion of plasma

Plasma aliquots (15 µl) were thawed on ice and 10 µl added to Top 12 abundant protein depletion spin columns (Pierce, Thermos Scientific, Rockford, USA). These were vortexed and incubated for 60 minutes at room temperature on a rotary mixer at 300 rpm (Rotor Genie 88881002, Thermo Scientific, Paisley, UK), followed by centrifugation at 1000 g for 2 minutes (Mikro 185, Hettich, USA). Following centrifugation, the eluate (500 µl) was transferred to a Amicon ultra 0.5ml centrifugal filter device (Millipour, UK) and centrifuged at 14,000 g for 30 minutes at room temperature. The eluate was discarded, and 400µl of 25mM ammonium bicarbonate (ACROS Organics) added to the Amicon ultra 0.5ml centrifugal filter device followed by centrifugation at 14,000g for 10 minutes at room temperature. Finally, 30µl of 25mM ammonium bicarbonate was added to the Amicon ultra 0.5ml centrifugal filter device which was then inverted into 2ml Eppendorff tubes and centrifuged at 1000g for 2 minutes at room temperate to elute the proteins.

2.5.2 Protein Quantification of Immuno-depleted samples

A Pierce BCA protein Assay Kit, calibrated with bovine serum albumin (BSA), (Thermo Scientific, Rockford, USA) was utilised to measure the protein concentration of the immuno-depleted plasma samples, according to manufactures

instructions. Using BSA and assay diluent, 25mM ammonium bicarbonate, eight standards were prepared across the concertation range 2000µg/µl to 0µg/µl. Standard and immunodepleted samples (10µl) were added to a 96 well flat bottomed microtitre plate in duplicate. Working reagent (200µl) at a ratio of 50:1 was added to each well containing an aliquot of standard or sample. The plate was incubated for 30 mins at 37°C and the absorbance measured at 562nM. A standard curve was prepared to determine the protein concentration in the immunodepleted samples against the BSA standards. In order to standardise the amount of protein subjected to enzymatic digestion, prior to mass spectrometric proteome analysis, protein concentration was standardised by the addition of an appropriate volume of 25 mM ammonium bicarbonate.

2.5.3 Digestion of protein into peptides

Dithiothreitol (DTT) (12µl of 50mM) (Sigma, St Louis, MO, USA) was added to the normalised depleted protein samples to give a final concentration of 5mM DTT. Subsequently, sodium deoxycholate (DOC) (12µl of 10%) was added (at a final concentration of 1% sodium deoxycholate) and the mixture incubated for 30 mins at 60°C with agitation (Thermo Mixer C, Eppendorph). Following incubation Iodoacetamide (IAA) (10µl of 650 mM) was added (final concentration of 50mM Iodoacetamide). The mixture was again incubated for 30 minutes in the dark, at room temperature. Following incubation, enzymic digestion was performed by the addition of trypsin (1:50 at a final concentration of 0.1 µg/µl). The mixture was again incubated at 37°C, overnight. Equal volumes of 1% formic acid were then added (final concentration of 0.5% formic acid) and the samples centrifuged at

12,000 g for 10 mins, the supernatant transferred into new clean 1.5µl microfuge tubes and vacuum concentrated for approximately 90 minutes (Speedvac concentrator, Savant SPD1010, Thermo Sientific). Samples where then freeze dried and stored frozen, prior to mass spectrometry, at -80°C.

2.5.4 Reconstitution of samples

Depleted and digested samples were allowed to thaw on ice and reconstituted to give a final concentration of 1µg of protein per 5µl volume, using 3% Acetonitrile in 0.1% formic acid solution. Internal standard at 0.1% final concentration of IRTs was added to each sample (Index retention time, Biognosys, Switzerland) (Figure 11).

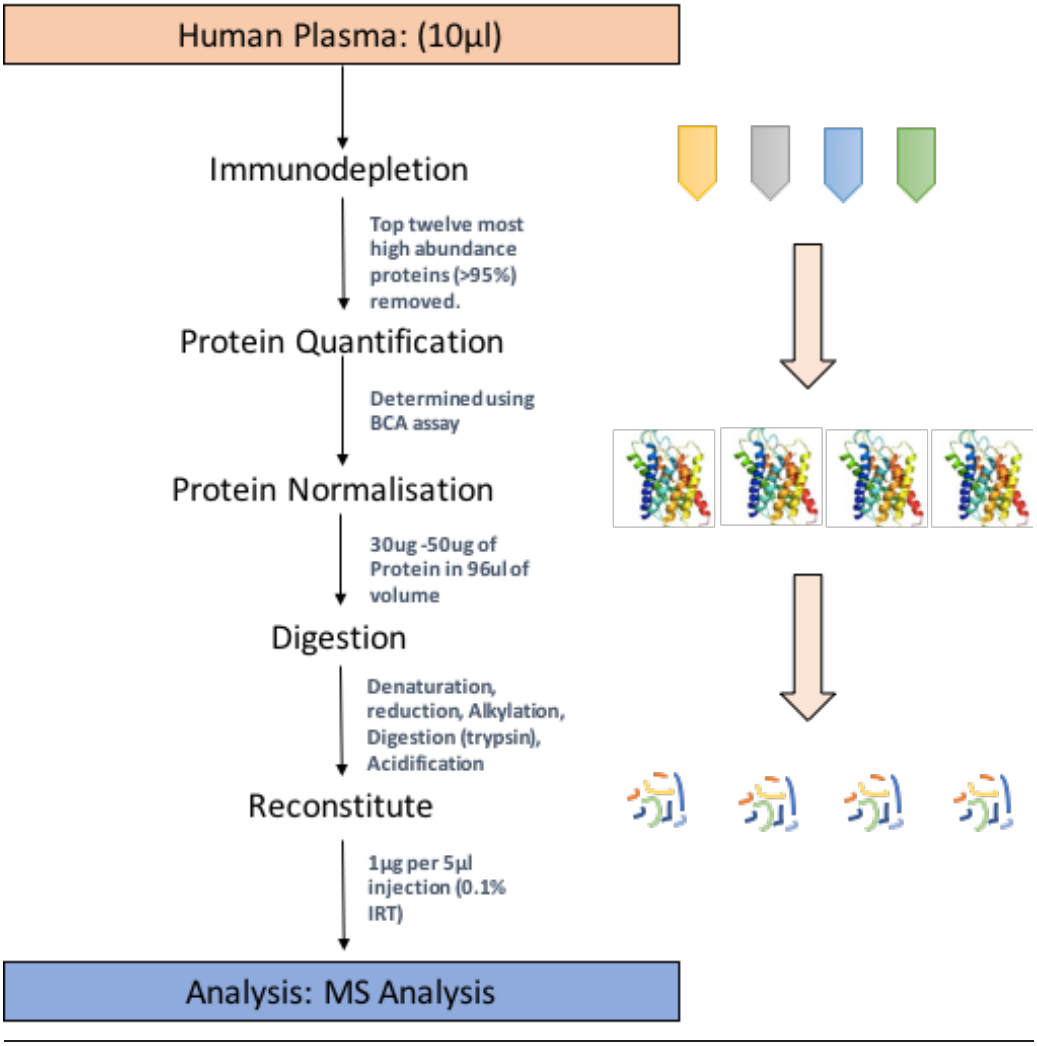


Figure 11: Flow diagram of plasma sample preparation.

2.6 Mass Spectrometry

2.6.1 Triple- TOF MS analysis and SWATH data analysis

Samples from each patient and control subject were loaded onto a 6600 Triple TOF mass spectrometer, in triplicate (AB Sciex, Warrington, UK), with an Eksigent 1D+ Nano LC system (Eksigent, Dublin, CA) for SWATH-MS analysis. The LC gradient consisted of buffer A (2% acetonitrile and 0.1% formic acid in HPLC water) and buffer B (2% water and 0.1% formic acid in acetonitrile). Protein depleted and digested samples were spiked with 1:50 (v:v) iRT peptides. Sample (5µl) was

injected (1µg of protein in 5 µl injection volume) and separated with a linear gradient of 2% buffer A to 35% buffer B over 135 minutes at a flow rate of 0.3µl min^{-1}.

The mass spectrometer was operated and data collected in SWATH acquisition mode using 100 variable windows. SWATH wiff files were converted into mzXML files. The mzXML file was converted to mzML file using OpenMS tool File converter. Spectral alignment and targeted data extraction of DIA samples were performed with SWATH 2.0 processing in Peakveiw (version 2.2, AB Sciex) using an in-house reference spectral library.

2.6.2 Processing of Mass Spec Data

Spectral aliment and targeted extraction of DIA samples were performed in Peakview (version 2.0, Sciex). DIA raw files were loaded in unison using extraction window false discovery rate (FDR) of 1% and peptide confidence of 99%. After data processing, raw data including peak area and retention times were exported from Peakveiw to Microsoft Excel. Data was processed and analysed, using MSStats and Markerviewer (Sciex).

Principle component analysis plots (PCA) were produced using Markerveiwer (Sciex). Volcano plots were generated using GraphPad Prism 7. Data was expressed as mean and the differences were determined using Welch's' t tests to determine differences in regulated proteins. A probability value of < 0.05 with 95% confidence limit was considered statistically significant. A fold chance of >1.5 was

reported as significantly up regulated and 1/1.5 (<0.667) significantly down regulated.

2.6.3 Bioinformatics, Functional and Descriptive analysis

The international standardised gene function classification system of gene ontology (GO) (http://www.geneontology.org/) and the Database Annotation Visualisation and Integrated Discovery (DAVID) database were used to interpret the biological processes, molecular functions and the cellular components of the significantly up regulated identified proteins (P value <0.05), (fold change >1.5).

ClueGo (Version 3.6.1, a Cytoscape plug-in) was used to assess proteins that were significantly enriched (P-value <0.05, fold change >1.5-2). Functional gene ontology (GO) categories in biology processes were reported using right-sided hypergeometric test. Protein-protein interactions were determined using the STRING database (version 10.5) with a high confidence level applied of 0.7.

The PANTHER (v.8.0) (protein annotation through evolutionary relationship) classification system (http://www.pantherdb.org/) was employed to perform a statistical overreprentation test between the 360 significantly enriched proteins identified and a relative reference list in PANTHER (Version 11.0) using a test type of Fishers exact with FDR multiple test correlations. Statistical significant, overrepresented proteins were determined and grouped into Biological process and Reactome pathways (P <0.05), (fold change >2.0).

2.7 ELISA

2.7.1 C-Reactive Protein (CRP)

CRP was measured in neat human plasma using a SimpleStep ELISA kit (Catalogue Number ab181416, Abcam UK) according to manufacturer's instructions. Briefly, standards in the range of 0 - 1000pg/ml were prepared in sample diluent. Samples (1:10000 dilution in sample diluent) or standards (50µl) were added, in duplicate, to each well of a coated mictotitre plate. Antibody cocktail (50µl) was added to each well and the plate incubated for 1 hour at room temperature with agitation. Following incubation, the plate was washed (x3) with plate wash buffer and blotted dry. Substrate (TMB, 100µl) was added to each well and the plate incubated for 10 minutes, with agitation, at room temperature in the dark. Following incubation, stop solution (100µl) was added and the OD at 450nm measured on a plate reader.

2.7.2 Beta-2 Microglobulin (β2M).

β2M was measured in neat human plasma using a SimpleStep ELISA kit (Catalogue Number ab181423, Abcam UK) according to manufacturer's instructions. Briefly, standards in the range of 0 – 2000 pg/ml were prepared in sample diluent. Samples (1:10000 dilution in sample diluent) or standards (50µl) were added, in duplicate, to each well of a coated mictotitre plate. Antibody cocktail (50µl) was added to each well and the plate incubated for 1 hour at room temperature with agitation. Following incubation, the plate was washed (x3) with plate wash buffer and blotted dry. Substrate (TMB, 100µl) was added to each well and the plate

incubated for 10 minutes, with agitation, at room temperature in the dark. Following incubation, stop solution (100µl) was added and the OD at 450nm measured on a plate reader.

2.7.3 Platelet Basic Protein (CXCL7)

CXCL7 was measured in neat human plasma using a SimpleStep ELISA kit (Catalogue Number ab216171, Abcam UK) according to manufacturer's instructions. Briefly, standards in the range of 0 - 300 pg/ml were prepared in sample diluent. Samples (1:10000 dilution in sample diluent) or standards (50µl) were added, in duplicate, to each well of a coated microtitre plate. Antibody cocktail (50µl) was added to each well and the plate incubated for 1 hour at room temperature with agitation. Following incubation, the plate was washed (x3) with plate wash buffer and blotted dry. Substrate (TMB, 100µl) was added to each well and the plate incubated for 10 minutes, with agitation, at room temperature in the dark. Following incubation, stop solution (100µl) was added and the OD at 450nm measured on a plate reader.

2.7.4 Transforming Growth Factor Beta 1 (TGFβ1)

TGFβ1 was measure in neat plasma using a TGF beta 1 Human ELISA KIT (Catalogue Number ab216171, Abcam UK) according to manufactures instructions. Briefly, standards ranged from 0 - 4000 pg/ml were prepared in sample diluent. Samples (1:4 dilution in sample diluent) or standards (100µl) were added, in duplicate, to each well of a coated microtitre plate. The plate was incubated overnight at 4^{o}C with gentle agitation. Following incubation, the plate was washed (x4) with wash

buffer and blotted dry. Biotinylated TGF beta 1 Detection Antibody (100µl) was added to each well and the plate was incubated for 1 hour at room temperature with gentle agitation. Following incubation, the plate was washed (x4) with wash buffer and blotted dry before the addition of HRP- Streptavidin solution (100µl) to each well. The plate was incubated for a further 45 minutes at room temperature with, gentle agitation, before the plate was once again, washed (x4) with wash buffer and blotted dry. Substrate (TMB, 100µl) was added to each well and the plate incubated for 30 minutes, with agitation, at room temperature in the dark. Following incubation, stop solution (100µl) was added and the OD at 450nm measured on a plate reader.

2.7.5 Liposaccharide Binding Protein (LBP)

LBP was measured in neat plasma using Human LBP ELISA KIT (Catalogue Number ab213805, Abcam UK) according to manufactures instructions. Briefly, standards ranged from 0 - 50ng/ml were prepared in sample diluent. Samples (1:100 dilution in sample diluent) and standards (100µl) were added, in duplicates, to each well of a coated microtitre plate. The plate was then incubated at 37 ^{o}C for 90 minutes, before the contents of the plate were discarded and biotinylated anti-human LBP antibody working solution (100µl) added to each well. The plate was then incubated at 37 ^{o}C for 60 minutes before being washed (x3) with 0.01 M PBS. Avidin-Biotin-Peroxidase Complex working solution (100µl) was added to each well and incubated at 37 ^{o}C for 30 minutes. The plate was washed again (x5) before substrate (TMB, 90µl) was added to each well and the plate incubated for 30

minutes, at 37 °C, in the dark. Following incubation, stop solution (100μl) was added and the OD at 450nm measured on a plate reader.

2.7.6 Platelet derived growth factor receptor Beta (PDGFrβ)

PDGF Receptor Beta was measured in neat plasma using a PDGF receptor beta Human ELISA KIT (Catalogue Number ab100626, Abcam UK) according to manufactures instructions. Briefly, standards ranged from 0 - 18,000 pg/ml were prepared in sample diluent. Samples (1:20 dilution in sample diluent) or standards (100μl) were added, in duplicates, to each well of a coated microtitre plate. The plate was incubated overnight at 4°C with gentle agitation. Following incubation, the plate was washed (x4) with wash buffer and blotted dry. Biotinylated PDGF receptor beta Detection Antibody (100μl) was added to each well and the plate incubated for 1 hour at room temperature with gentle agitation. Following incubation, the plate was washed (x4) with wash buffer and blotted dry before the addition of HRP- Streptavidin solution (100μl) to each well. The plate was incubated for a further 45 minutes at room temperature with, gentle agitation, before being, washed (x4) with wash buffer and blotted dry. Substrate (TMB, 100μl) was added to each well and the plate incubated for 30 minutes, with agitation, at room temperature in the dark. Following incubation, stop solution (100μl) was added and the OD at 450nm measured on a plate reader.

All data was uploaded to a master Microsoft Office Excel spreadsheet (Microsoft Excel for Mac, Version 15.30) pending analysis. For statistical analysis, data was imported into Prism 7 for Mac (GraphPad Software, California, USA). Parametric

(Independent T test) tests were used to compare systemic mastocytosis patients and healthy control results. Graphical outputs and box and whisker plots were produced using Prism 7 for Mac (GraphPad Software, California, USA). A $P<0.05$ was accepted as statistically significant.

Chapter 3: Results

3.1 Study participants

A total of thirteen healthy controls, three from University of Lincoln and ten from Manchester Metropolitan University, were invited to participate in the study, which resulted in eleven participants attending the first enrolment visit. From the enrolment visit, four were excluded due to poor venous access, meaning a total of seven healthy controls were included in the proteomic analysis by mass spectrometry (Figure 12).

Fourteen patients with Systemic Mastocytosis, who were attending the Mastocytosis clinics at the United Lincolnshire Hospitals NHS Trust and Leicester Royal Infirmary, were also invited to participate. In total, thirteen patients with systemic mastocytosis were included in the proteomic analysis by mass spectrometry, with one patient sample excluded due to gross haemolysis (Figure 12).

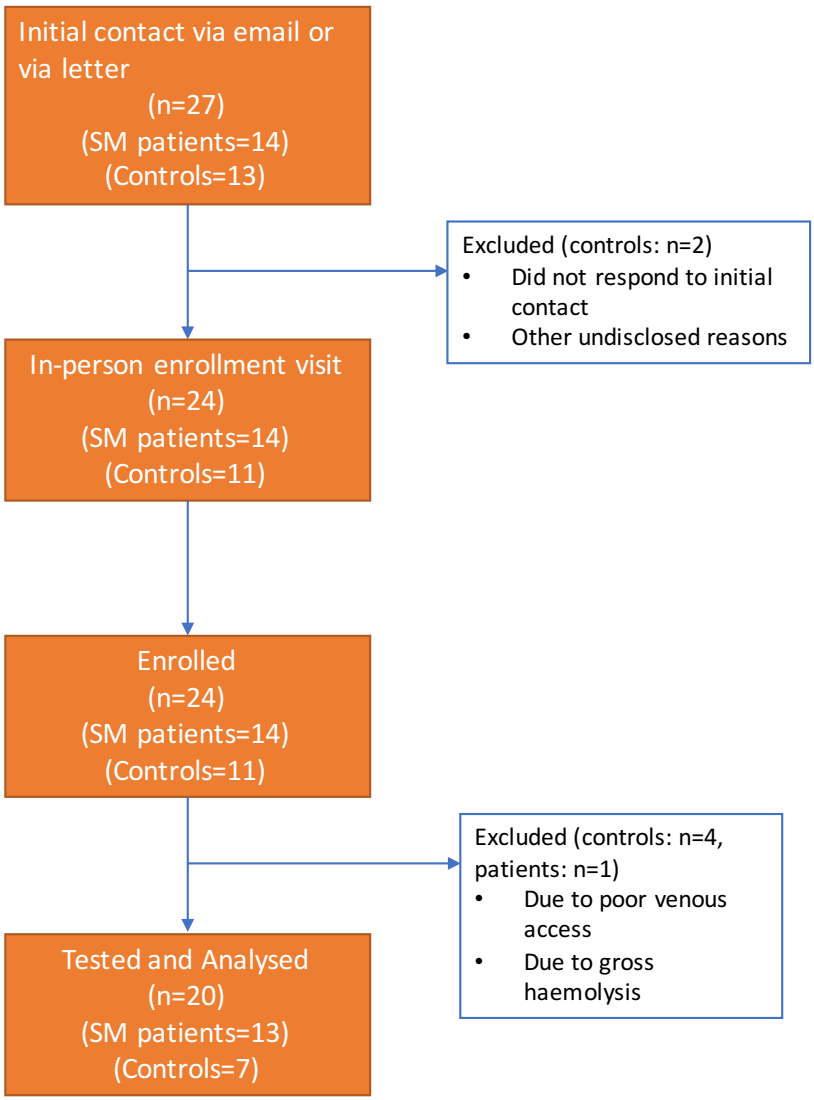


Figure 12: Study Flow Diagram. Flow diagram showing initial recruitment of Systemic Mastocytosis patients and Controls. Diagram also shows the number of participants excluded (patients and controls). The main reasons for exclusion of participants was poor venous access and sample haemolysis.

3.2 Table of participant Characteristics

Participants demographics are shown in table 6 below. There was a greater number of female participants, 9 from the systemic mastocytosis patient group and 1 from the healthy control group, however the gender of 4 participants is unknown. The mean age of the healthy control group is 44 years compared to 58 years in the patients group (P >0.05). A point mutation consisting of a substitution of aspartate

to valine in the catalytic domain of *c-KIT* (*ASP816VAL* or *D816V*) was detected in the peripheral blood of 9, out of the 13, patients diagnosed with systemic mastocytosis. *KIT* status was not determined in the healthy control group. Patients diagnosed with systemic mastocytosis had a mean serum tryptase of 92ng/ml. Healthy control participants had higher mean haemoglobin (138g/l) compared to patients diagnosed with systemic mastocytosis (129g/l), although the difference did not reach the level of statistical significance. The patient group had a higher white cell count and platelet count (6.37 x 10^9/L, 245 x 10^9/L) when compared to the healthy control group (5.53 x 10^9/L, 169 x 10^9/L), which again did not reach the level of statistical significance.

Table 6: Table of participants' characteristics. Demographics include age, gender, kit status, Tryptase (ng/ml), Haemoglobin (g/l), white cell count (WCC, $x10^9$/L), platelets ($x10^9$/L).

Characteristic	***Systemic Mastocytosis Patients***	***Healthy Controls***	***Total Participants***
Subjects *(n)*	*13*	*7*	*20*
Age *(median[range])*	*58 [27-80]*	*44 [26-55]*	*51 [26-80]*
Gender	*Male = 3* *Female = 9* *Unknown = 1*	*Male = 3* *Female= 1* *Unknown = 3*	*Male = 6* *Female = 10* *Unknown = 4*
Kit Status	*Positive = 9* *Negative = 1* *Unknown = 3*	-	*Positive = 9* *Negative = 1* *Unknown = 3*
Tryptase (ng/ml) *(mean [range])*	*92 [20.5->200]*	-	*92 [20.5->200]*
Haemoglobin (g/l) *(mean [range])*	*129 [91-155]*	*138 [124-142]*	*133.5 [91-155]*
WCC **(10x10^9/L)** *(mean [range])*	*6.37 [2.5-9.5]*	*5.53 [2.25-5.99]*	*5.59 [2.25-9.5]*
Platelets (**10x10^9/L)** *(mean [range])*	*245 [112-362]*	*169 [135-205]*	*207 [112-205]*

3.3 Gel Electrophoresis showing the depletion and digestion of Plasma samples

Prior to tryptic digestion, plasma samples were immunodepleted to remove high abundance proteins (Table 7). Previous work has shown that immunodepletion and tryptic digestion are necessary to improve the overall accuracy and precision of proteomic analysis (Pan, *et al* 2009). In order to assess the efficiency of this step, immunodepleted and digested samples were subject to SDS-polyacrylamide gel electrophoresis. Figure 13 shows the results of Immunodepletion and tryptic

digestion. As can be observed, Immunodepletion removed the majority of the high abundance proteins, mainly albumin, while tryptic digestion resulted in the fragmentation of proteins into peptides.

Table 7: List of the top 12 proteins removed by Pierce top 12 abundant protein depletion spin columns (Pierce, Thermos Scientific, Rockford, USA).

Top 12 Proteins Removed by Depletion Spin Columns	
Alpha 1- Acid Glycoprotein	Fibrinogen
Alpha 1- Antitrypsin	Haptoglobin
Alpha 2- Macroglubulin	IgA
Albumin	IgG
Apolipoprotein A-I	IgM
Apolipoprotein A-II	Transferrin

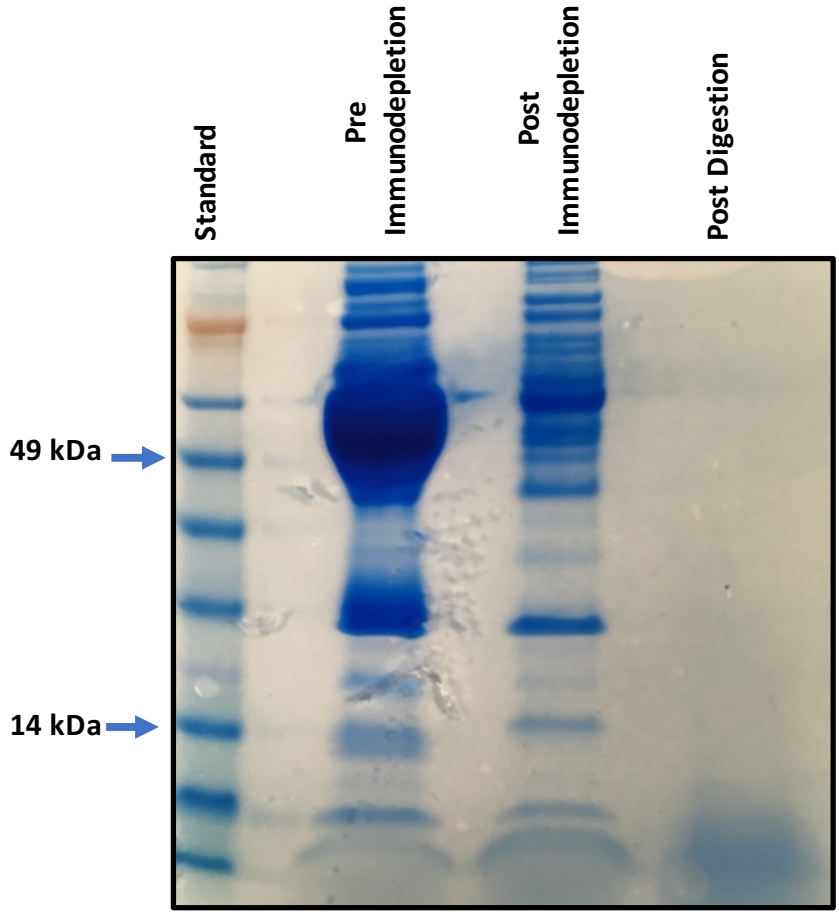


Figure 13: SDS-Polyacrylamide Gel Electrophoresis. This figure shows an image of a gel post electrophoresis using control sample. Pre Immunodepleted plasma (5µl) diluted 1:10/ Post immunodepleted sample, pre-trypsin digest – 20µg /Post Trypsin digested sample. Left hand lane showing SeeBlue Plus2 pre-stained protein molecular weight standards (molecular weight range 3-198 kDa) (Thermos Scientific – UK).

3.4 Proteome difference between patients and controls

Principle component analysis (PCA) was undertaken to show the proteomic differences between the systemic mastocytosis patients and healthy controls. Figure 14 demonstrates that mastocytosis patients can be clearly separated on proteomics from the healthy control population.

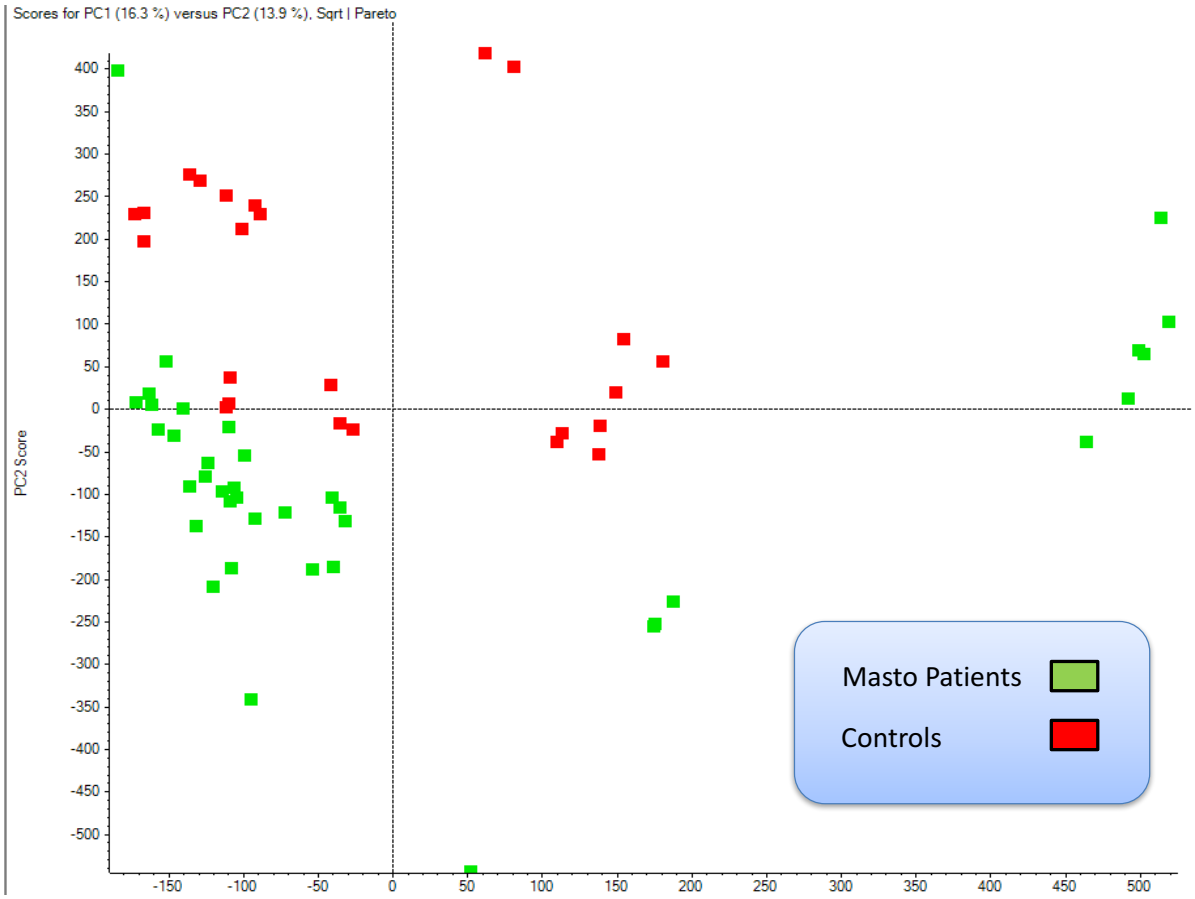


Figure 14: Principle component analysis of Systemic Mastocytosis patents and Control samples.

3.5 Proteins identified and the Profile of the Significantly Enriched Proteins

A high-level coverage of plasma proteome was achieved with a total of 1437 proteins being identified at a 1% FDR and 99% peptide confidence. A table including all 1437 proteins identified, along with their individual P and fold change values, was constructed and is available in Appendix 5.

A volcano plot was constructed to show the results of the differentially expresses proteins, between the patient and control groups, based on fold change versus t-test probability as shown in Figure 15.

When considering patients with Systemic Mastocytosis, 368 (of the 1437) proteins were found to be significantly differentially expressed (>1.5 & < 1/1.5-fold change) when compared to the control population ($P < 0.05$). Of the proteins identified, 360 were found to be significantly up regulated, in patients, with a >1.5-fold change, and 8 found to be significantly down regulated, in patients, with a fold change <1/1.5 (<0.667), when compared to apparently healthy controls. A table of all, 360 significantly upregulated and 8 significantly down regulated proteins identified, along with their individual P value and fold change, was constructed and is available in Appendix 6 & 7. Significantly up regulated proteins included, C-reactive protein (CRP), Transforming Growth Factor Beta 1 (TGFβ1), Beta 2 Microglobulin (β2M), Colony Stimulating Factor 1 Receptor (CSF1R) and Vascular Endothelial Growth Factor Receptor 1 Precursor (FLT1) are represented as red dots seen in Figure 15.

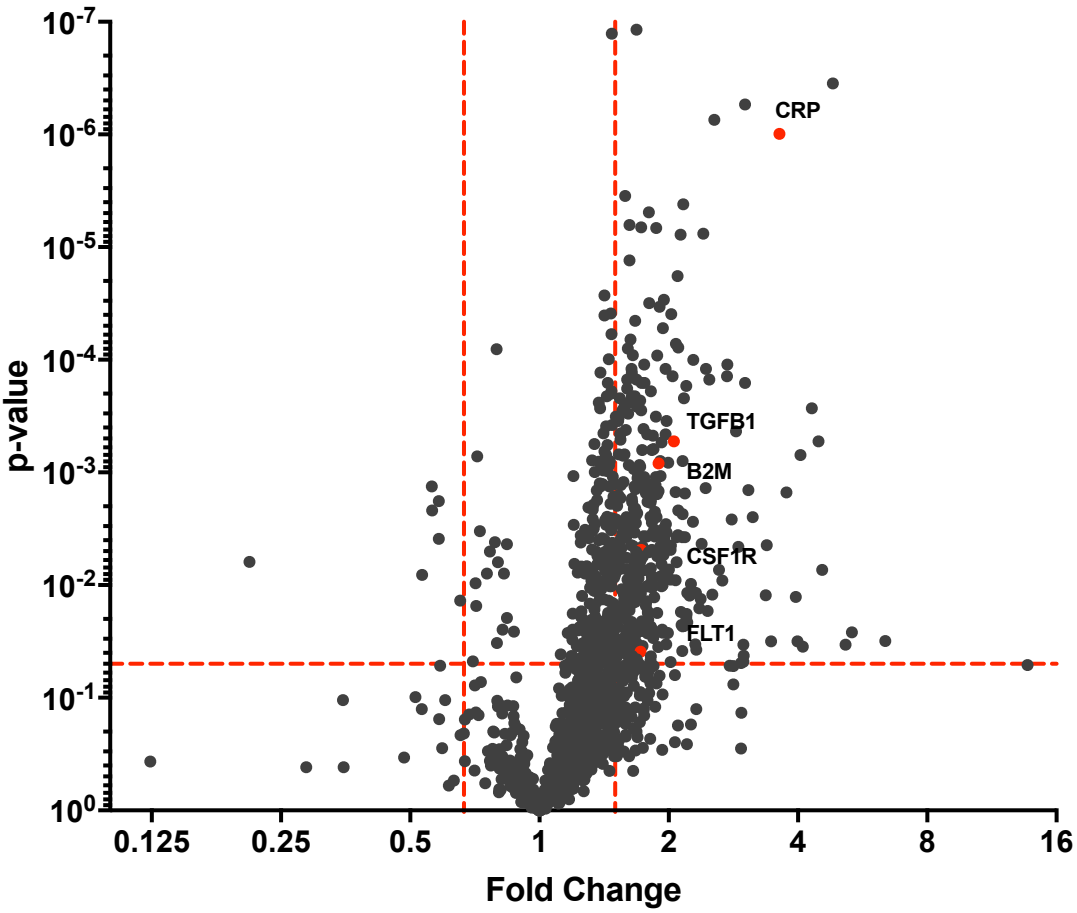


Figure 15: Volcano plot showing differentially expressed proteins. The volcano plot shows the results of differentially expressed proteins between patient and healthy control groups, based on fold change versus t-test probability. Each protein is represented as a black dot and is mapped according to its fold change on the abscissa axis (x) and t-test p-value on the ordinate axis (y). Any proteins with a P <0.05 are found to have significant changes when compared to controls (above horizontal red line). Any proteins found to have a fold change of < 0.667 was considered downregulated when compared to controls (left of red vertical line). Proteins found to have a fold change of > 1.5 were considered up regulated when compared to control group (right of red vertical line). Significantly up regulated proteins included, C-reactive protein (CRP), Transforming Growth Factor Beta 1 (TGF1), Beta 2 Microglobulin (B2M), Colony Stimulating Factor 1 Receptor (CSF1R), Vascular endothelial growth factor receptor 1 precursor (FLT1) (marked as red dots above).

In order to explore protein-protein interactions, STRING (Version 10.5), protein-protein interaction (PPI) network analysis was performed at a high confidence score of 0.7 as illustrated below in figure 16. The results demonstrated a significant association amongst the significantly up regulated proteins.

Further, PPI network analysis (Figure 16) suggests the identified proteins have strong interactions that contribute to numerous biological functions including,

complement, proteasomes, protein synthesis and degradation, inflammation and immune Response, DNA repair and increased cell survival, enhanced protein folding, platelet aggregation and activation and the TGF-β pathway. This suggests a general state of pro-inflammatory and immune response in the pathophysiology of systematic mastocytosis.

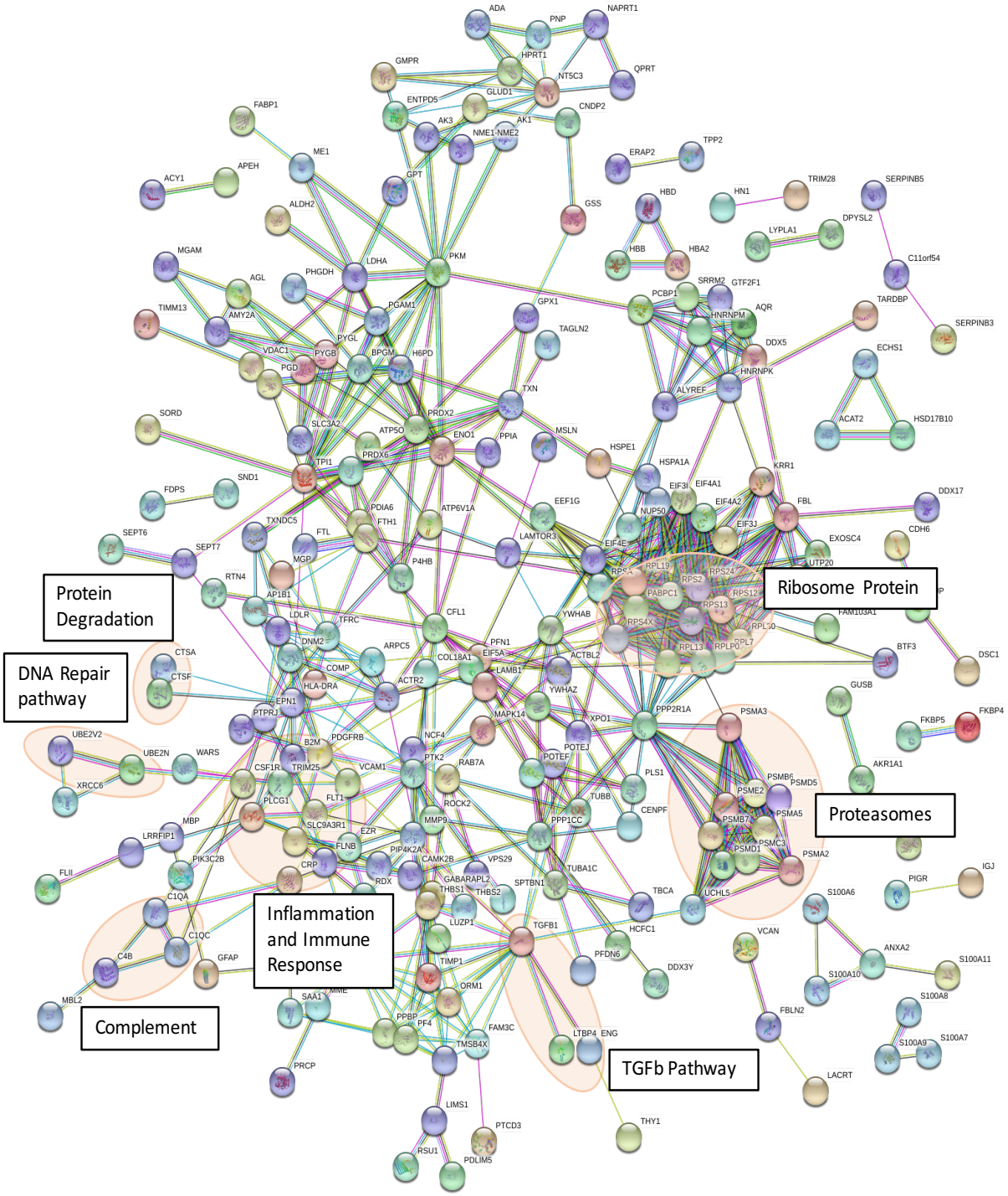


Figure 16: Figure showing the protein-protein interactions for the upregulated proteins identify by SWATH-MS, analysed by STRING (Version 10.5) with a high confidence of 0.7. In the network analysis, the up regulated expressed proteins were represented as nodes.

3.6 Functional Annotation of the Identified Proteins

To assess the biological functions of the significantly upregulated proteins, ClueGo (Version 3.6.1, a Cytoscape plug-in) and DAVID databases were utilised. When analysed for biological processes it was revealed that the identified enriched proteins are largely involved in metabolic processes (35%), immune responses (29%), regulation of development processes (9%), cell adhesion and migration (5%) and cell surface receptor signalling pathways (3%) (Figure 17).

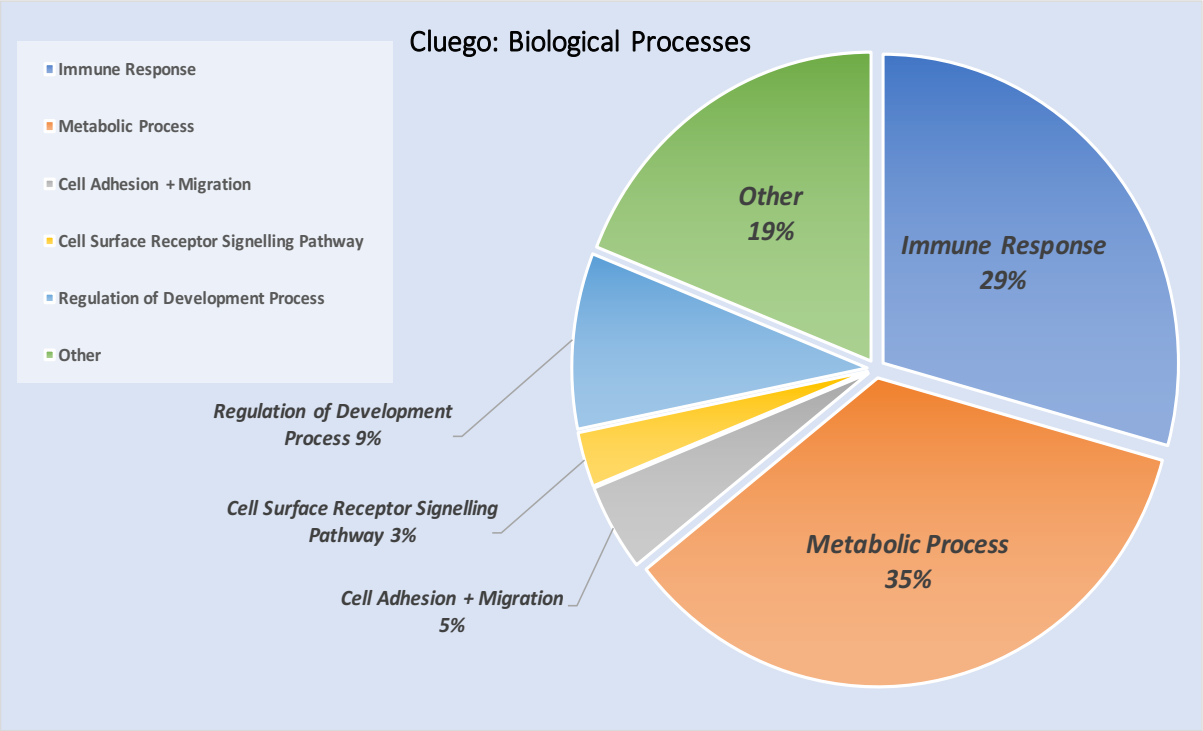


Figure 17: Pie chart representing biological processes of significantly up regulated proteins (ClueGo, Version 3.6.1, a CytoScape plug-in). The biological processes of the significantly up regulated protein were grouped into metabolic response (35%), Immune response (29%), Regulation of development process (9%), Cell adhesion and migration (5%), Cell surface receptor signalling pathway (3%) and other (19%).

Based on functional analysis, the significantly up regulated proteins seem to function largely in the immune response (29%), which could be further sub-divided into immune effector process (13.79%), leukocyte mediated immunity (13.79%)

and defence response (1.72%). According to the KEGG pathway annotation, significantly up regulated proteins, involved with the immune response (29%), were classified into 50 pathways (Figure 18).

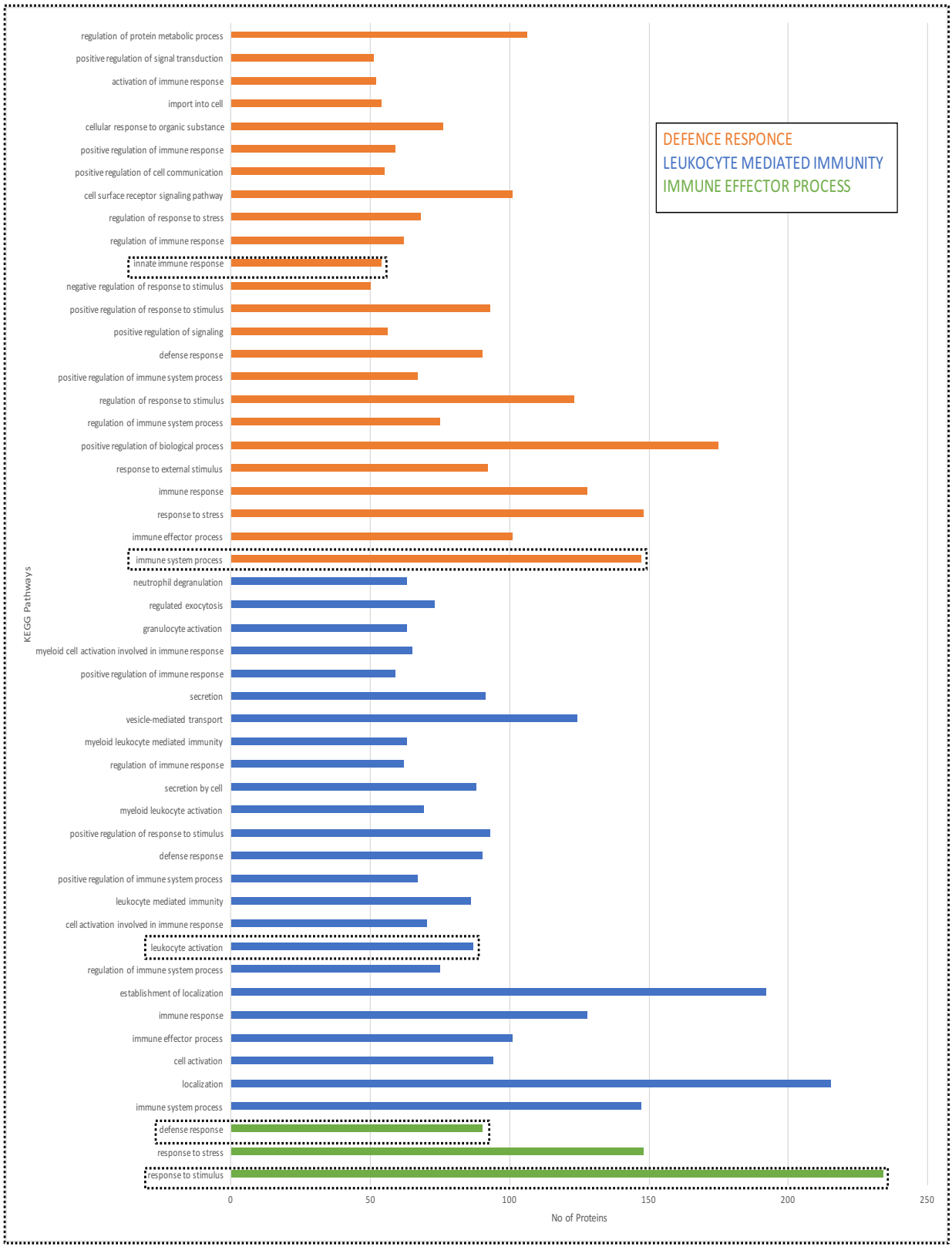


Figure 18: KEGG pathway analysis of significantly up regulated proteins found in the immune response. Many of the identified up regulated proteins exhibited biological functions in relation to immune system processes and response. The horizontal bars represent the number of differentially expressed proteins involved with various pathways (ClueGo, Version 3.6.1, a CytoScape plug-in).

Among the 360 significantly enriched proteins, 147 proteins were found to be involved with immune system process, 54 in the innate immune response, 234 in response to stimuli, 62 in the regulation of immune response, 90 in defence response, 148 in response to stress, 63 in neutrophil degranulation, 73 in regulated exocytosis and 87 in leukocyte activation (Figure 18). From these pathways, specific proteins were identified using KEGG pathway analysis and the names of the specific proteins may be found in table 8. The list of pathway specific proteins was interrogated by visual inspection to identify proteins common to the five pathways (immune system process, innate immune response, response to stimuli, defence response and leukocyte response) (Table 8). Proteins common to all pathways are highlighted in bold (Table 8) and proteins identified as upregulated by mass-spectrometry were verified by ELISA.

Table 8: KEGG pathways found in immune response along with the enriched proteins. These pathways include immune system process, innate immune response, response to stimulus, defence response and leukocyte activation. Proteins emboldened, such as Beta 2 Microglobulin (B2M), C-Reactive Protein (CRP), Transforming Growth Factor Beta 1 (TGFb1), Vascular Endothelial Growth Factor receptor 1 precursor (FLT1), Platelet basic protein (PPBP/CXCL7) and lipopolysaccharide binding protein (LBP) are consistently found throughout (ClueGo, Version 3.6.1, a CytoScape plug-in).

3.7 PANTHER – Descriptive Statistics

A statistical over-representation test was preformed between the 360 significantly enriched proteins identified and a relative reference list in PANTHER (Version 11.0) using a test type of Fisher's exact test with FDR multiple test correlations.

Statistically significant, overrepresented proteins were determined and grouped into biological process and reactome pathways, illustrated as bar charts, Figure 19 and Figure 20 ($P < 0.05$) (fold change >2.0).

Figure 19, illustrates the identified enriched proteins which are found to be significantly over represented in biological processes, with the greatest over-representation involved in, complement activation ($P < 0.001$, fold change 8.24), B cell mediated immunity ($P < 0.001$, fold change 8.24), cell recognition ($P < 0.001$, fold change 10.64), defence response to bacteria ($P < 0.001$, fold change 6.92), glycolysis ($P < 0.001$, fold change 10.64) and glycogen metabolic process ($P < 0.001$, fold change 6.81). Over- representation of other biological process included response to biotic stimulus ($P < 0.001$, fold change 5.38), fatty acid beta oxidation ($P < 0.001$, fold change 5.96), chromatin remodelling ($P < 0.001$, fold change 5.26), purine nucleobase metabolic process ($P < 0.001$, fold change 5.23), immune response (p value <0.001, fold change 3.42), immune system process ($P < 0.05$, fold change 2.49, protein metabolic processes ($P < 0.001$, fold change 2.33), endocytosis ($P < 0.001$, fold change 3.31), proteolysis ($P < 0.001$, fold change 2.53), proteins folding ($P < 0.001$, fold change 3.17) and angiogenesis ($P < 0.001$, fold change 3.31) .

The significantly over-represented proteins were mainly found to be in biological processes mainly involved with the immune response (immune system process, B cell mediated immunity, cell recognition and defence response to bacterium), inflammation (complement activation), angiogenesis, intra-cellular protein transport (endocytosis), energy metabolism (glycolysis, glycogen metabolic

process, fatty acid beta oxidation) and protein synthesis and breakdown (proteolysis, protein folding, protein metabolic process and chromatin remodelling) (Figure 19 and Table 9).

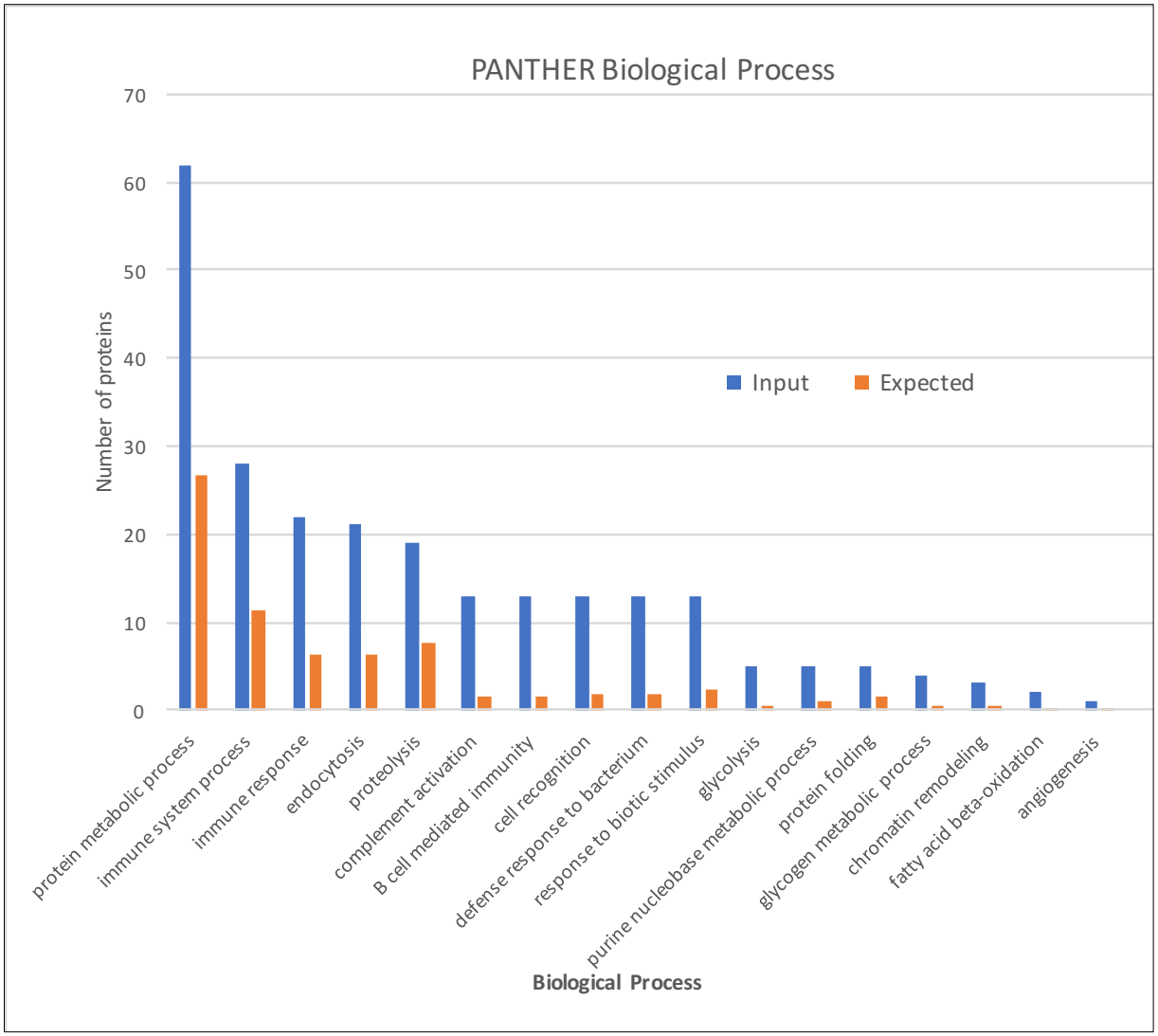


Figure 19: Bar Chart of over-representation of biological processes of the significantly enriched proteins when compared to expected values. The input values (blue) and expected values (orange) are mapped according to their biological process on the abscissa axis (x) and number of proteins involved on the ordinate axis (y). PANTHER (Version 11..0), (P <0.05, fold change >2.0).

Figure 20, illustrates the identified enriched proteins which are significantly over represented in reactome pathways, with the greatest over-representation involved in, complement cascade (P <0.001, fold change 8.86), classical antibody mediated compliment activation (P <0.001, fold change 15.69), initial triggering of complement (P <0.001, fold change 12.61), synthesis of prostaglandins and thromboxane(P <0.001, fold change 11.92), down regulation of TGFβ1 receptor signalling (P <0.001, fold change 9.54) and TGFβ receptor signalling activates SMADs (P <0.001, fold change 7.69). Over representation of other reactome pathways include the Innate immune system (P <0.001, fold change 3.57), VEGFA-VEGFR2 pathway (P <0.001, fold change 4.09), platelet activation, signalling and aggregation (P <0.001, fold change 4.13), platelet degranulation (P <0.001, fold change 6.68), signalling by SCF-KIT (P <0.001, fold change 3.46), class I MHC mediated antigen processing and presentation (P <0.001, fold change 2.42), MHC class II antigen presentation (P <0.001, fold change 3.54), T cell receptor signalling (P <0.001, fold change 6.35).

The analysis demonstrated that the reactome pathways that were shown to be significantly over-represented mainly involved pathways contributing to increased proliferation, migration survival and differentiation of hematopoietic progenitors (Signalling by SCF-KIT), complement (creation of C4 & C2 activators, initial triggering of complement, complement cascade, classical antibody mediated compliment activation) , T-cell proliferation, differentiation and activation (class I & II MHC mediated antigen processing and presentation, T-cell receptor signalling), cell proliferation, migration and survival, angiogenesis and tissue repair and

Inflammation (VEGFA-VEGFR2 pathway and signalling by FGR2) (Figure 20 and Table 10).

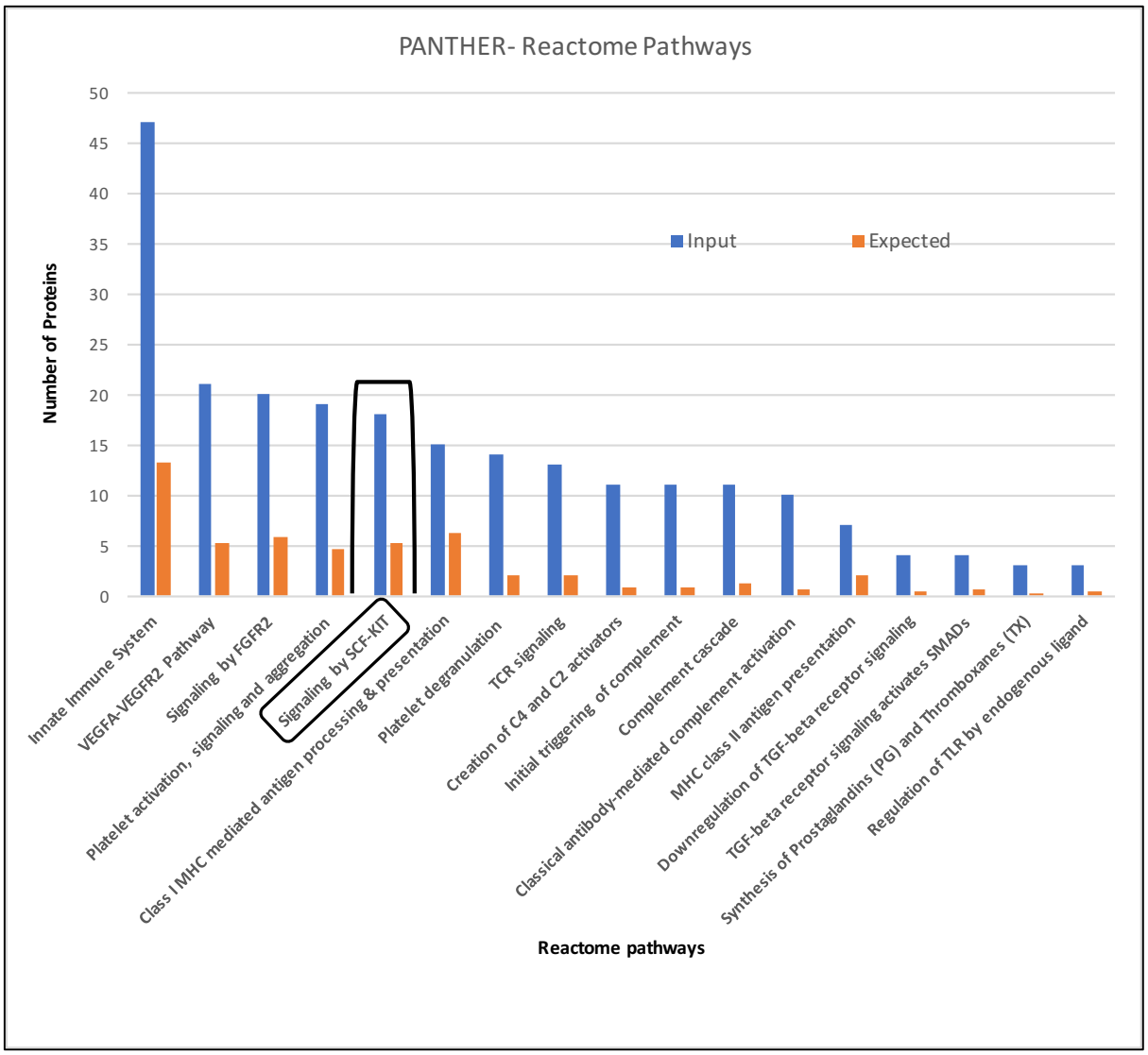


Figure 20: Bar Chart of over-representation of Reactome pathways of the significantly enriched proteins when compared to expected values. The input values (blue) and expected values (orange) are mapped according to their biological process on the abscissa axis (x) and number of proteins involved on the ordinate axis (y). PANTHER (Version 11..0), (P <0.05, fold change >2.0).

Significant over representation of proteins involved in these highlighted biological processes and reactome pathways, including P value and fold change, are shown in Tables 9 and 10. The input column is the number of the significantly upregulated

protein that are grouped based on the PANTHER classification - biological process. The PANTHER reference list column contains the number of proteins involved with the biological process. The expected value is the number of proteins that would be expected to be present in the input list for a specific biological process on the basis of the reference list. Therefore, if the biological process under investigation, more proteins are observed in the input list that expected, fold change >2.0, there is an over representation (+) of the proteins involved in that specific biological function. $P < 0.05$ determines if the over-representation is significant or not.

PANTHER analysis demonstrated many biological processes and reactome pathways shown to be over represented are central to pro-inflammation, immune response, increased cell survival and differentiation in the pathophysiology of systematic mastocytosis.

Table 9: Significant over representation of biological processes in PANTHER. Over-representation was determined by number of proteins in PANTHER reference list as well as number of Inputted significantly upregulated proteins. P <0.05 and fold change >2.0 was considered significantly over-represented.

Biological Process	*PANTHER-Ref List*	*Input*	*Expected*	*over/under representation*	*Fold Enrichment*	*P-value*
protein metabolic process	**1583**	62	26.56	+	2.33	$8.84E^{-10}$
immune system process	**669**	28	11.22	+	2.49	$2.26E^{-05}$
immune response	**383**	22	6.43	+	3.42	$1.18E^{-06}$
endocytosis	**378**	21	6.34	+	3.31	$3.40E^{-06}$
proteolysis	**448**	19	7.52	+	2.53	$3.05E^{-04}$
complement activation	**94**	13	1.58	+	8.24	$2.42E^{-08}$
B cell mediated immunity	**94**	13	1.58	+	8.24	$2.42E^{-08}$
cell recognition	**105**	13	1.76	+	7.38	$7.89E^{-08}$
defence response to bacterium	**112**	13	1.88	+	6.92	$1.57E^{-07}$
response to biotic stimulus	**144**	13	2.42	+	5.38	$2.16E^{-06}$
glycolysis	**28**	5	0.47	+	10.64	$1.93E^{-04}$
purine nucleobase metabolic process	**57**	5	0.96	+	5.23	$3.56E^{-03}$
protein folding	**94**	5	1.58	+	3.17	$2.41E^{-02}$
glycogen metabolic process	**35**	4	0.59	+	6.81	$3.80E^{-03}$
chromatin remodelling	**34**	3	0.57	+	5.26	$2.29E^{-02}$
fatty acid beta-oxidation	**20**	2	0.34	+	5.96	$5.05E^{-02}$
angiogenesis	**18**	1	0.3	+	3.31	$2.71E^{-01}$

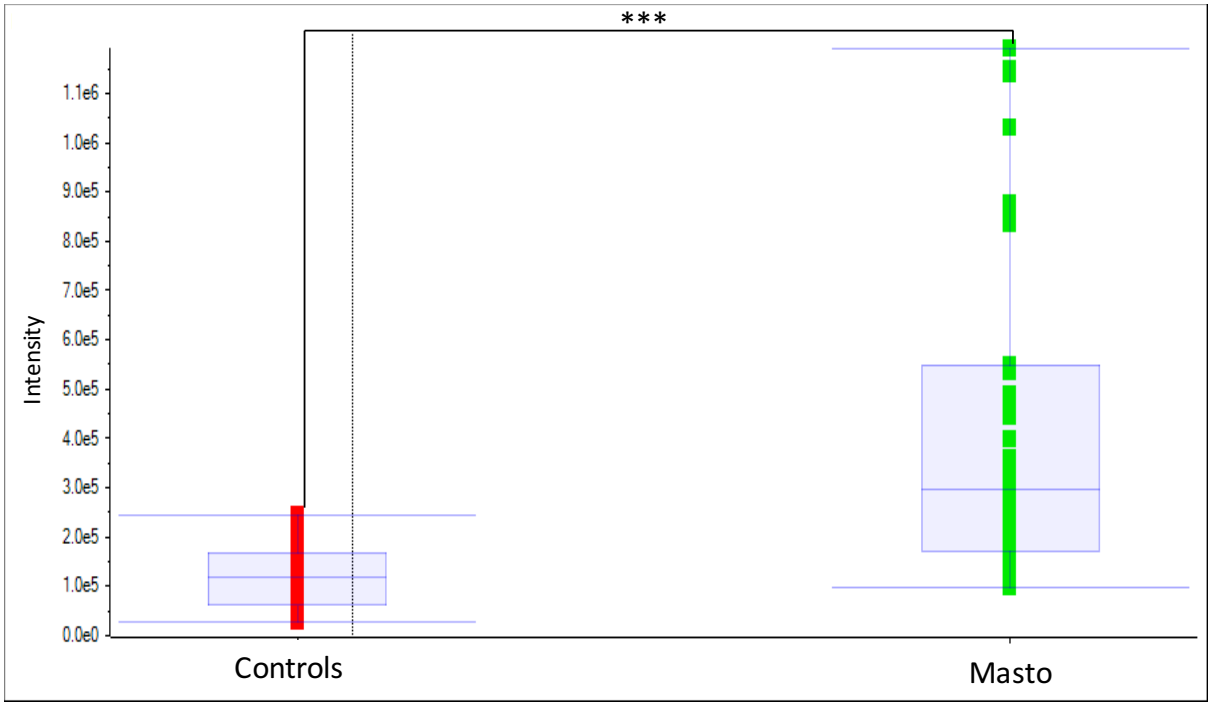
Table 10: Significant over representation of Reactome pathways in PANTHER. Over-representation was determined by number of proteins in PANTHER reference list as well as number of Inputted significantly upregulated proteins. P <0.05 and fold change >2.0 was considered significantly over-represented.

Reactome pathways	*PANTHER-Ref List*	*Input*	*Expected*	*Over/under representation*	*Fold Enrichment*	*P-value*
Innate Immune System	38	47	13.15	+	3.57	$1.57E^{-13}$
VEGFA-VEGFR2 Pathway	43	21	5.13	+	4.09	$1.35E^{-07}$
Signaling by FGFR2	52	20	5.8	+	3.45	$3.32E^{-06}$
Platelet activation, signaling and aggregation	15	19	4.6	+	4.13	$4.65E^{-07}$
Signaling by SCF-KIT	25	18	5.2	+	3.46	$9.70E^{-06}$
Class I MHC mediated antigen processing & presentation	19	15	6.19	+	2.42	$1.91E^{-03}$
Platelet degranulation	74	14	2.1	+	6.68	$7.81E^{-08}$
TCR signaling	31	13	2.05	+	6.35	$3.86E^{-07}$
Creation of C4 and C2 activators	125	11	0.72	+	15.25	$1.07E^{-09}$
Initial triggering of complement	122	11	0.87	+	12.61	$6.04E^{-09}$
Complement cascade	274	11	1.24	+	8.86	$1.52E^{-07}$
Classical antibody-mediated complement activation	306	10	0.64	+	15.69	$4.93E^{-09}$
MHC class II antigen presentation	784	7	1.98	+	3.54	$4.75E^{-03}$
Downregulation of TGF-beta receptor signaling	118	4	0.42	+	9.54	$1.25E^{-03}$
TGF-beta receptor signaling activates SMADs	310	4	0.52	+	7.69	$2.55E^{-03}$
Synthesis of Prostaglandins (PG) and Thromboxanes (TX)	346	3	0.25	+	11.92	$3.02E^{-03}$
Regulation of TLR by endogenous ligand	369	3	0.32	+	9.41	$5.43E^{-03}$

3.8 SWATH Ion intensity Graph and ELISA

To quantify proteins between patients and controls, SWATH-MS ion intensity graphs were plotted for key proteins that belong to immune response (Table 8) shown in Figure 21, 22, 23 and 24.

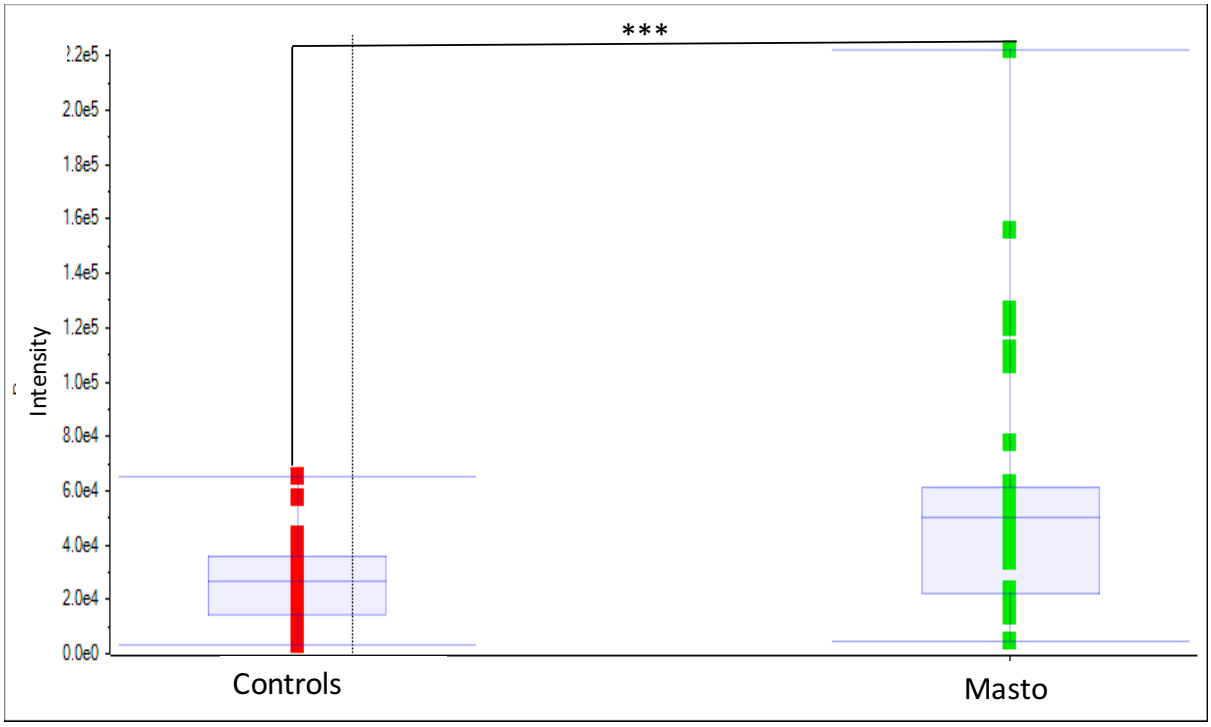
SWATH Data indicated that CRP was significantly up regulated in patients when compared to healthy control group as shown below in figure 21 (Fold Change 3.62 patients vs controls, P <0.001).



C- Reactive Protein

Figure 21: Box and whisker plot showing C-Reactive Protein (CRP) intensity of the peptide ions of SWATH-MS between patients and control groups (Mean and SD). Peptide ion intensity are plotted on the abscissa axis (x) and participant and control group on the ordinate axis (y) A P < 0.001.

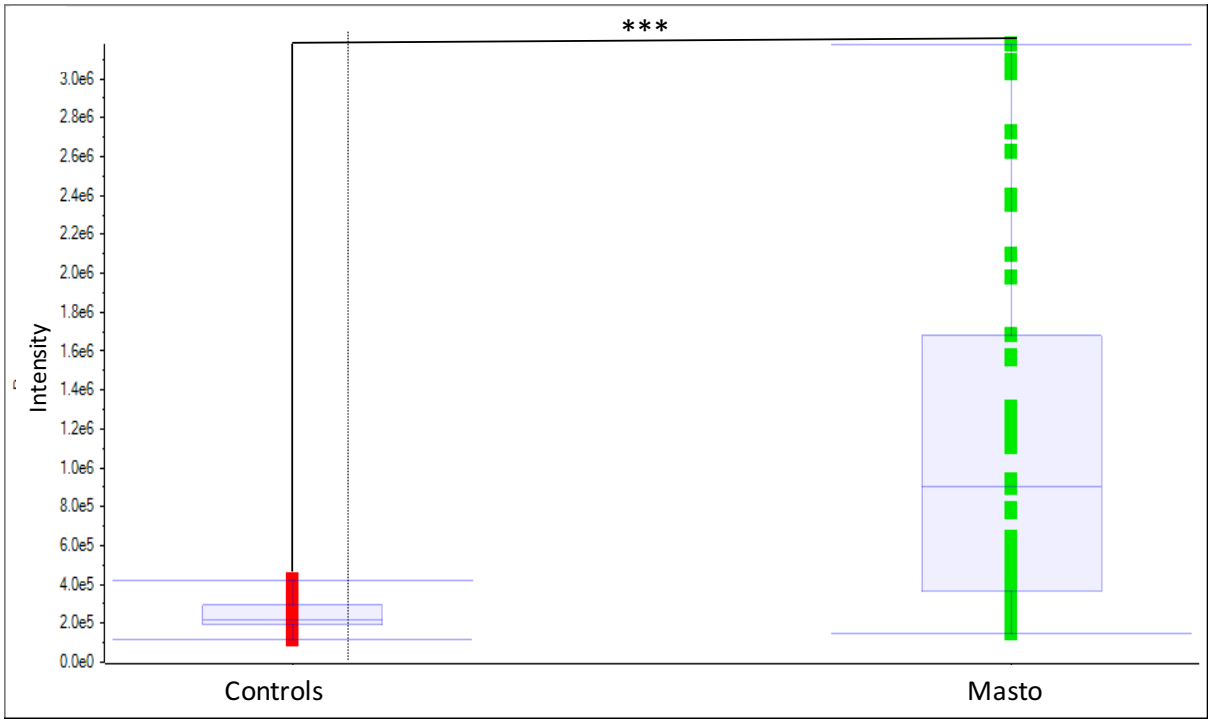
SWATH Data indicated that TGFβ1 was significantly up regulated in patients when compared to healthy control group as shown below in figure 22 (Fold change 2.06 patients vs controls, P<0.001).



Transforming Growth Factor Beta 1

Figure 22: Box and whisker plot showing Transforming growth factor beta 1 (TGFβ1) intensity of the peptide ions of SWATH-MS between patients and control groups (Mean and SD). Peptide ion intensity are plotted on the abscissa axis (x) and participant and control group on the ordinate axis (y). P <0.001

SWATH Data indicated that platelet basic protein (CXCL7) was significantly up regulated in patients when compared to heathy control group as shown below in figure 23 (Fold change 4.83 patients vs controls, P<0.001).



Platelet Basic Protein

Figure 23: Box and whisker plot showing Platelet basic protein (CXCL7) intensity of the peptide ions of SWATH-MS between patients and control groups (Mean and SD). Peptide ion intensity are plotted on the abscissa axis (x) and participant and control group on the ordinate axis (y). P <0.001)

SWATH Data indicated that beta 2 microglobulin (β2M) was significantly up regulated in patients when compared to heathy control group as shown below in figure 24 (Fold change 1.9 patients vs controls, P<0.001).

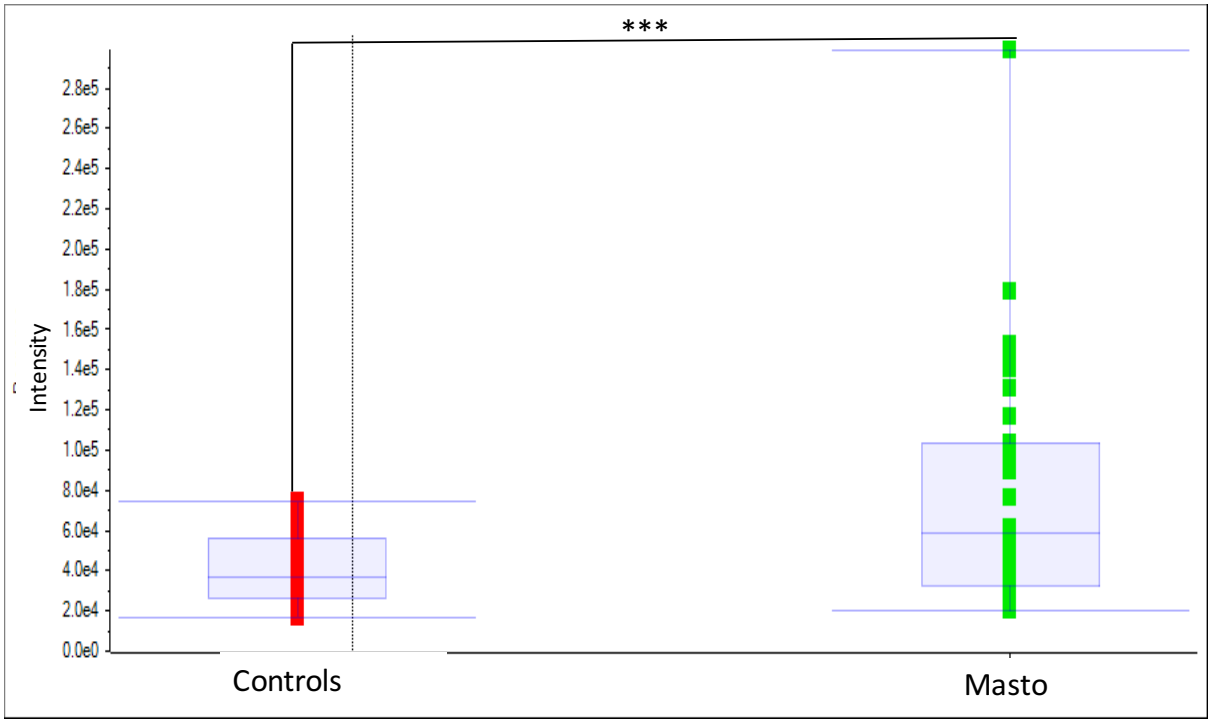


Figure 24: Box and whisker plot showing beta 2 microglobulin (β2M) intensity of the peptide ions of SWATH-MS between patients and control groups (Mean and SD). Peptide ion intensity are plotted on the abscissa axis (x) and participant and control group on the ordinate axis (y). A P <0.001)

Orthogonal confirmation of the SWATH-MS data was preformed using commercially available ELISA's in order to confirm the changes seen in the plasma proteome. C-reactive protein, beta 2 microglobulin, transforming growth factor beta 1, platelet basic protein, liposaccharide binding protein, and platelet derived growth factor receptor were measured, by commercially available ELISA, in the plasma of 7 patients and 7 controls.

Each of the plasma protein measurements were compared between patients and healthy control group, using independent t-tests, with statistical differences illustrated in figures 25,26,27,28,29 and 30, and values reported in table 11. A P<0.05 was considered statistically significant.

Figure 25 and Table 11 shows, patients with SM had a significantly increased mean circulating plasma concentration of CRP, compared to controls (417.6 pg/ml vs 76.14 pg/ml, P = 0.02)

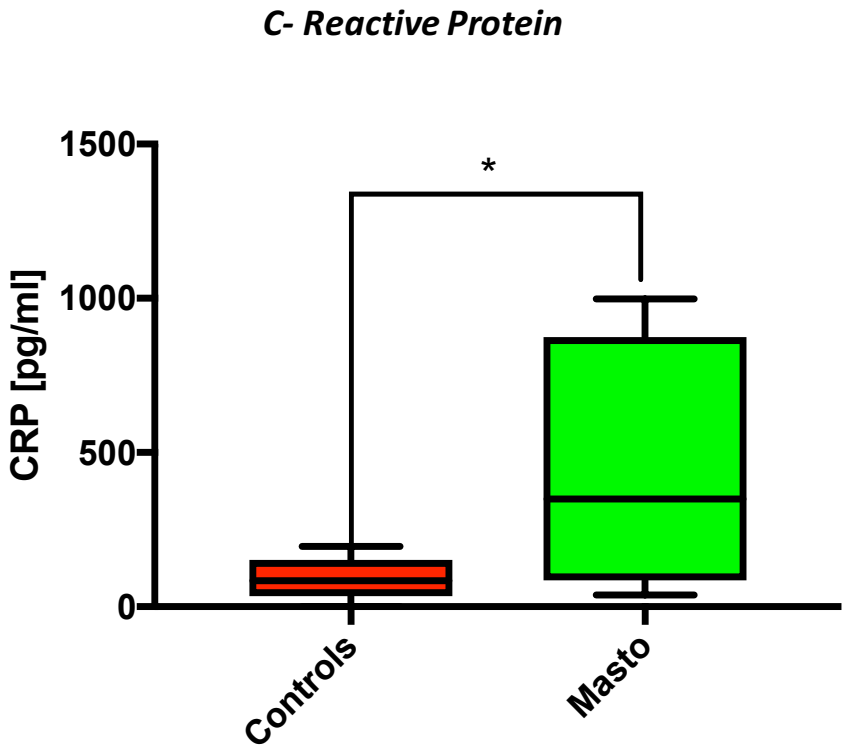


Figure 25: Box and Whiskers plot showing circulating levels of C-Reactive protein (CRP) (pg/ml) in patients with systematic mastocytosis and apparently heathy controls (Mean and SD). Plasma protein measurements were compared between systemic mastocytosis patients (Green) and healthy controls (Red) using Independent t-tests. P = 0.02. A P<0.05 was considered Statistically significant. (*<0.05, **<0.01,***<0.001).

Figure 26 and Table 11 shows, patients with SM had a significantly increased mean circulating plasma concentration of Platelet Derived Growth Factor Receptor Beta, compared to controls (538.8 pg/ml vs 293.3 pg/ml, P = 0.006)

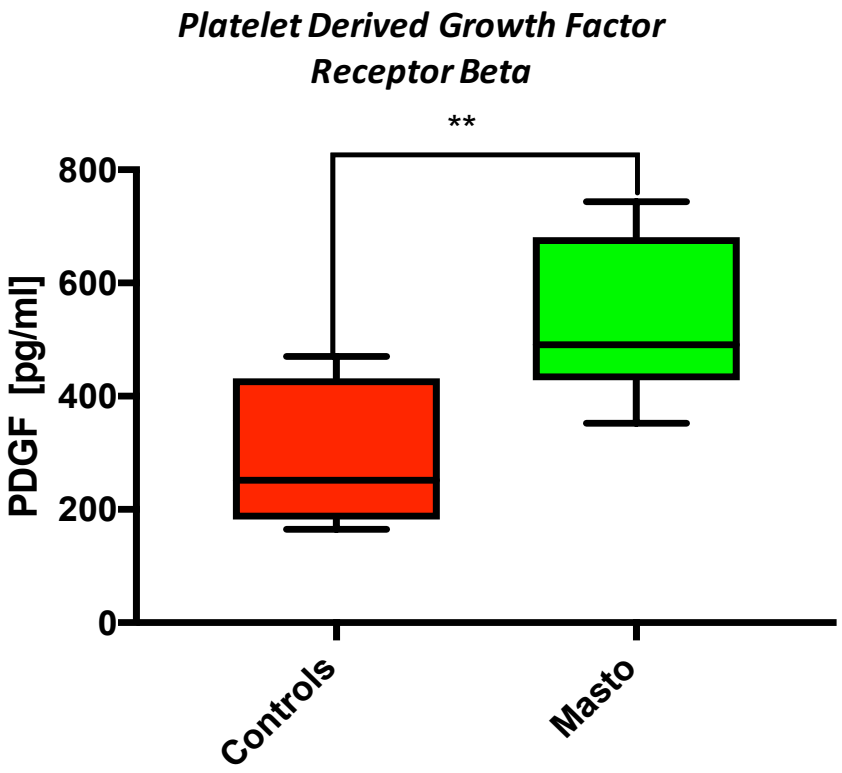


Figure 26: Box and Whiskers plot showing circulating levels of Platelet Derived Growth Factor Beta (PDGFrβ) (pg/ml) in patients with systematic mastocytosis and apparently heathy controls (Mean and SD). Plasma protein measurements were compared between systemic mastocytosis patients (Green) and healthy controls (Red) using Independent t-tests. P = 0.006. A P<0.05 was considered Statistically significant. (*<0.05, **<0.01,***<0.001).

Figure 27 and Table 11 shows, patients with SM had a significantly increased mean circulating plasma concentration of Platelet Basic Protein, compared to controls (167.2 pg/ml vs 59.78 pg/ml, P = 0.04)

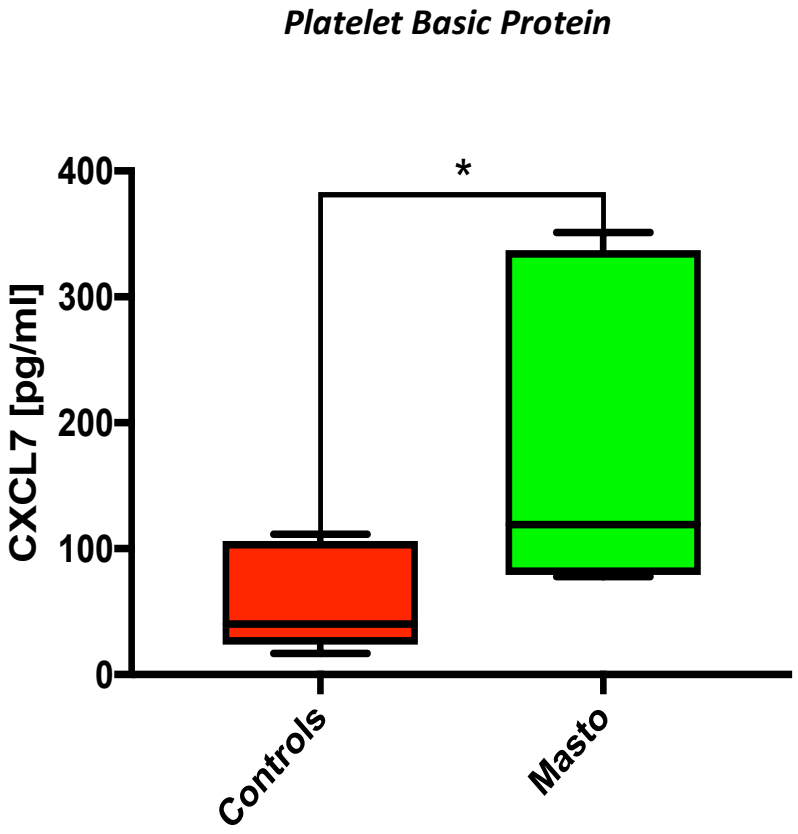


Figure 27: Box and Whiskers plot showing circulating levels of Platelet Basic Protein (CXCL7) (pg/ml) in patients with systematic mastocytosis and apparently heathy controls (Mean and SD). Plasma protein measurements were compared between systemic mastocytosis patients (Green) and healthy controls (Red) using Independent t-tests. P = 0.04. A P<0.05 was considered Statistically significant. (*<0.05, **<0.01,***<0.001).

Figure 28 and Table 11 shows, patients with SM had a significantly increased mean circulating plasma concentration of liposaccharide binding protein, compared to controls (93.54 ng/ml vs 80.67 ng/ml, P = 0.02)

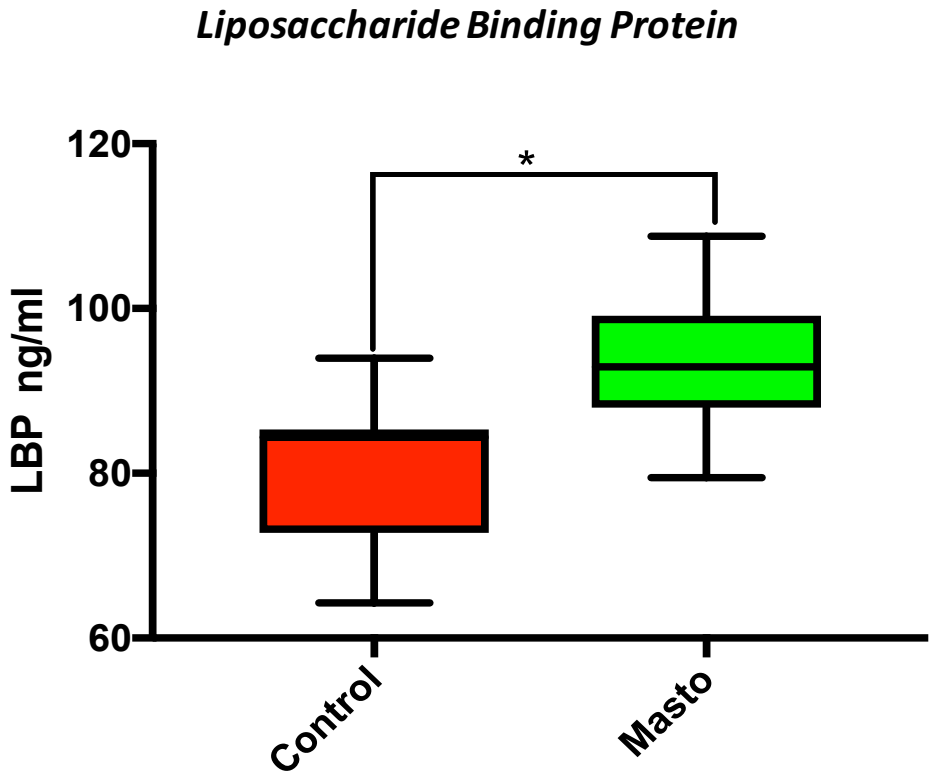


Figure 28: Box and Whiskers plot showing circulating levels of Liposaccharide binding protein (LBP) (ng/ml) in patients with systematic mastocytosis and apparently heathy controls (Mean and SD). Plasma protein measurements were compared between systemic mastocytosis patients (Green) and healthy controls (Red) using Independent t-tests. P= 0.02. A P<0.05 was considered Statistically significant. (*<0.05, **<0.01,***<0.001).

Figure 29 and Table 11 shows, patients with SM had a significantly increased mean circulating plasma concentration of Transforming Growth Factor Beta 1, compared to controls (1823 pg/ml vs 748.7 pg/ml, P = 0.02)

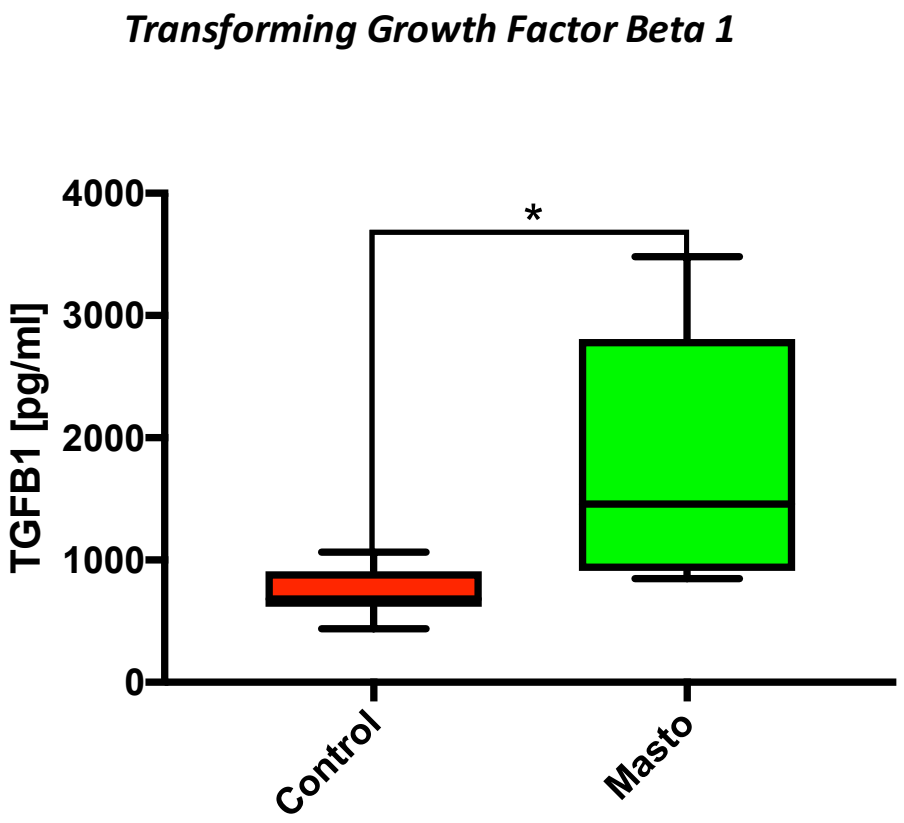


Figure 29: Box and Whiskers plot showing circulating levels of Transforming Growth Factor Beta 1 (TGFβ1) (pg/ml) in patients with systematic mastocytosis and apparently heathy controls (Mean and SD). Plasma protein measurements were compared between systemic mastocytosis patients (Green) and healthy controls (Red) using Independent t-tests. P= 0.02. A P<0.05 was considered Statistically significant. (*<0.05, **<0.01,***<0.001).

Figure 30 and Table 11 shows, patients with SM had non-statistically significant increased mean circulating plasma concentration of Beta 2 Microglobulin, compared to controls (437.8 pg/ml vs 180.2 pg/ml, P = 0.21)

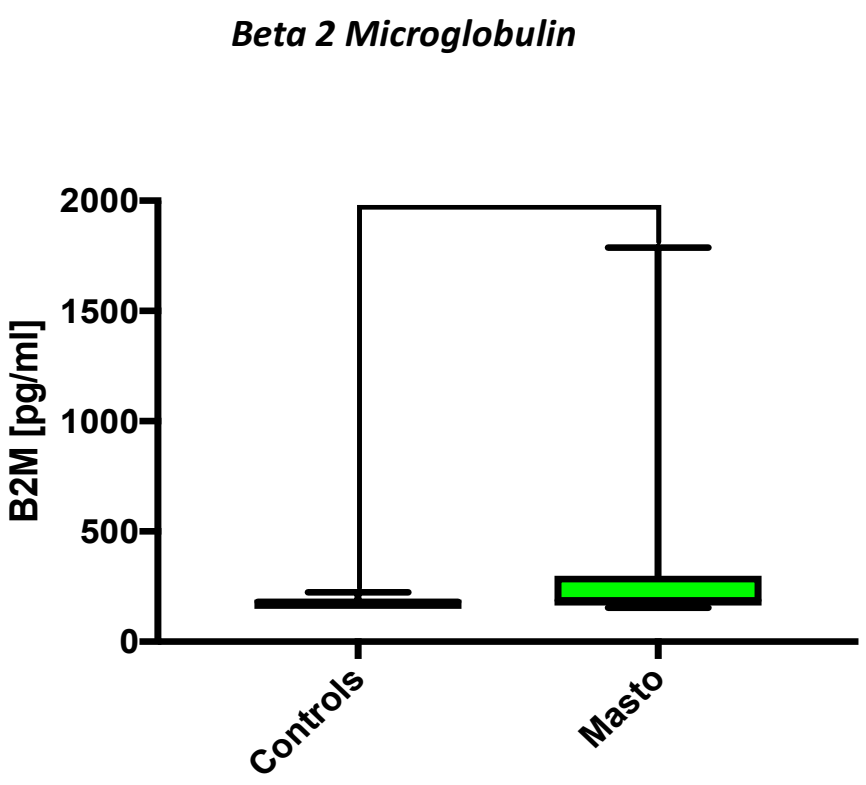


Figure 30: Box and Whiskers plot showing circulating levels of Beta 2 microglobulin (β2M) (pg/ml) in patients with systematic mastocytosis and apparently heathy controls (Mean and SD). Plasma protein measurements were compared between systemic mastocytosis patients (Green) and healthy controls (Red) using Independent t-tests. P= 0.21. A P<0.05 was considered Statistically significant. (*<0.05, **<0.01,***<0.001).

Table 11: Circulating plasma levels of C-Reactive Protein (CRP), Beta 2 Microglobulin (β2M), Transforming Growth Factor, Beta 1 (TGFβ1), Platelet Basic Protein (PPBP/CXCL7), Liposaccharide Binding Protein (LBP) and Platelet Derived Growth Factor Receptor (PDGFR). Plasma protein measurements were compared between systemic mastocytosis patients and healthy controls using Independent t-tests. A P<0.05 was considered statistically significant (*P <0.05, **<0.01, ***<0.001).

	Total	**Mastocytosis Patients**	**Control**
C-Reactive protein (pg/ml)	246.87 (225.12)	417.6 (375.5) *	76.14 (74.73)
Beta 2 Microglobulin (pg/ml)	309 (310.21)	437.8 (597.8)	180.2 (22.62)
Transforming Growth Factor, Beta 1 (pg/ml)	1286 (598.8)	1823 (982.6)*	748.7 (214.9)
Platelet Basic Protein (pg/ml)	113.49 (80.85)	167.2 (122.5) *	59.78 (39.2)
Liposaccharide Binding Protein (ng/ml)	87.105 (9.39)	93.54 (9.103)*	80.67 (9.682)
Platelet Derived Growth Factor Receptor (PDGFR) (pg/ml)	416.05 (137.35)	538.8 (149.4)**	293.3 (125.3)

Chapter 4: Discussion

4.1 Identification of Proteins

Screening for novel protein biomarkers from human plasma can provide essential clinical information. In the current project, SWATH-MS was used to identify proteins in patients with SM, compared to healthy controls and to assess any difference in concentration. A total of 1437 proteins were identified at a 1% FDR and 99% peptide confidence. The identification of 1437 proteins in the plasma samples of patients with SM is comparable to other reports in the literature. Recently, Miyauchi and colleagues (2018) report the identification of blood biomarkers in Glioblastoma using SWATH-MS. In this study, the authors reported the identification of 962 proteins with a 1% FDR and 99% protein confidence in samples derived from 14 patients (Miyauchi et al., 2018). In addition, a comparative proteomic analysis of five body fluids (plasma, urine, cerebrospinal fluid, amniotic fluid and saliva) reported the identification of 1189 proteins in plasma samples derived from 25 apparently healthy participants with a 1% FDR and 99% protein confidence (Zhao et al., 2018). It is worthy of note however, that Miyauchi and colleagues (2018) did not immunodeplete the high abundance proteins during pre-analytical sample preparation.

While SWATH-MS is not the only technique that could have been utilised for the identification of proteins it has a number of advantages when compared to the others. The run time for SWATH-MS is significantly less than other techniques. In their 2018 paper, Johnston (2018) and colleagues report a LC-MS/MS technique for proteomic profiling of CLL. The run time reported in that study was 210hours, compared to 2 hours for SWATH-MS. In addition, to a decrease in analytical run time, SWATH-MS has demonstrated enhanced sensitivity, reliability and precision

with lower sample volumes than isobaric tags for relative and absolute quantification (iTRAQ)-MS making it a superior technique for biomarker discovery in clinical samples (Jylha et al, 2018).

4.2 Summary of Results

The results of the current SWATH-MS study in patients with systematic mastocytosis have demonstrated the enrichment of 360 proteins involved in immune system process, innate immune response, response to stimulus, defence response and leucocyte activation. Further analysis has also demonstrated statistical over-representation of proteins involved in biological process and reactome pathways, supporting the results above. Formal immunoassay quantification by ELISA of proteins identified as common to all the above pathways has shown significantly increased levels of CRP, CXCL7, LBP, TGFβ1 and PDGF receptor-β in patients with systemic mastocytosis when compared to an apparently healthy control population. Levels of B2M were also increased in patients when compared to controls, however the difference did not reach statistical significance, most likely due to the large spread of results.

4.3 Sample Preparation

Sample preparation is considered an integral part of pre-analytical sample preparation and can significantly influence the overall analytical sensitivity of SWATH-MS. Due to the complexity of the plasma sample matrix and depth of proteome, a range of sample preparation strategies have been developed for targeted protein quantification. One of the most effective and most commonly

used techniques is the depletion of highly abundant proteins to enhance the analytical dynamic range and detection sensitivity. For the analysis of plasma samples, the depletion and tryptic digestion processes, as reported in Chapter 2, are therefore critically important in sampling processing, impacting the accuracy and precision of protein quantification overall (Pan, *et al* 2009).

In accordance with manufactures instructions (Pierce, Thermos Scientific, Rockford, USA), the top twelve most highly abundant proteins (>95%) (Table 12) were removed from 10 μl of peripheral blood plasma using depletion spin columns containing a resin of immobilised highly specific antibodies, whilst providing minimal nonspecific interactions with other proteins. Removing >95% of these top 12 abundant proteins from the plasma samples enabled the identification and quantification of low abundance proteins by mass spectrometry, removing the interference from the most abundant tryptic peptides which may mask the identification of the less abundant peptides. Previous reports have shown that the less abundant peptides are often derived from proteins of biological importance (Dayon, 2013).

4.4 SWATH-MS, Data Independent Analysis

MS can be either data dependent acquisition (DDA) or data independent acquisition (DIA). In DDA, a set of fixed rules and parameters must be set in advance. Peptides are ionised and analysed, and a fixed number of predefined peptides are selected at random for fragmentation. This produces a tandem MS/MS mass spectra that can be matched to spectra in an existing database. Although the selection is random, the

most abundant peptides are the ones selected based on having a much higher concentration in the sample, meaning it is difficult to reproducibly quantify and identify the lowest abundance peptides in the sample (Kang et al 2017).

In contrast, when utilising DIA all the peptides identified are subjected to fragmentation without being predefined enabling improved accuracy in peptide quantification and profiling.

SWATH MS is a DIA approach enabling a highly comprehensive interpretation of proteins and peptides in complex samples (Kang et al 2017). SWATH permits, in a single step, the identification and quantification of peptides eradicating the need for multiple scans. Consequently, SWATH has an improved turnaround time, accuracy and reduced error rate when compared with DDA. Only the DIA approach comprehensively detects and analyses every detectable compound within the sample under investigation (Kang et al 2017).

4.5 Enriched Protein Biological Process and Pathway Involvement

Clugo and DAVID databases were used to explore the role of the significantly enriched proteins identified by SWATH-MS in biological processes and KEGG pathways. Analysis revealed that proteins, identified in this study, are largely involved in metabolic process, immune response, regulation of development and cell adhesion, migration and cell surface receptor signalling pathways. Based on biological functional analysis, identified proteins largely play roles in the immune response. In addition, according to the KEGG pathway annotation, identified proteins involved within the immune response, were classified into different

pathways including immune system process, the innate immune response, response to stimulus, defence response and leukocytes activation. Further analysis, using PANTHER supported the above findings and identified that enriched proteins were significantly over represented in biological processes such as, immune response immune system process, compliment activation, B cell mediated immunity, glycolysis and cell recognition.

In addition, PANTHER identified enriched proteins found to be significantly over represented in Reactome pathways such as the Innate immune system, VEGFA-VEGFR2 pathway, platelet activation, signalling, aggregation and degranulation. Signalling by SCF-KIT, Class I MHC mediated antigen processing and presentation, MHC class II antigen presentation, T cell receptor signalling, compliment cascade, classical antibody mediated compliment activation, initial triggering of compliment, down regulation of TGFB1 receptor signalling and TGF-beta receptor signalling pathways were also identified.

The results of the current bioinformatics analysis identified up regulated proteins derived from immunoglobulins, acute phase reactants and binding proteins thought to play crucial roles in immune system regulation, inflammation and acute inflammatory response. These pathways are highly relevant to the present study since systemic mastocytosis is a disease caused by abnormalities which affect the immune system. To further support the results obtained from the SWATH-MS analysis, circulating plasma levels of C-reactive protein (CRP), Beta 2 Microglobulin (B2M), Transforming Growth Factor-β1 (TGFβ1), Liposaccharide Binding Protein

(LBP), Platelet Binding Protein (CXCL7/PBP) and Platelet Derived Growth Factor Receptor-β (PDGFRβ), were quantified by ELISA.

4.6 C- Reactive Protein

The results of the current investigation have demonstrated a significantly increased circulating plasma level of CRP in patients with systemic mastocytosis using both SWATH-MS and ELISA techniques.

CRP is predominantly synthesised by the liver, as a part of the acute-phase response, (Hurlimann *et al*., 1966). Extra-hepatic synthesis of CRP has also been reported in organs such as the kidneys suggesting local synthesis of CRP (Becker, *et al*., 1980 & Nakahara, *et al*., 2001). Irrespective of the site of synthesis, CRP is deposited at sites of acute inflammation (Du Clos & Mold., 2004). The main stimuli to CRP synthesis include interleukin (IL)-6 and IL-1 however, several different agents, such as corticosteroids, can alter synthesis of CRP. CRP is a classical acute phase protein with its blood concentration increasing from < 1μg/mL to as high as 600-1000μg/mL during at the maxima of an acute phase response (Du Clos & Mold., 2004). In myocardial infarction, levels may rise from < 2μg/mL to over 100μg/mL in approximately 24 hours (Kushner, *et al.,* 1978). In addition, levels >500μg/mL have been reported in patients with sepsis following burn injury.

CRP levels, in general, reflect circulating IL-6 levels and correlate with inflammation and other makers of the acute phase response, such as erythrocyte sedimentation rate. CRP levels, however Increase and decrease more rapidly than many other acute phase proteins, therefore making it a useful marker to follow clinical disease course and response to treatment (Du Clos & Mold., 2004). Despite a

comprehensive literature search, the author was unable to identify any clinical studies of the role of CRP in systematic mastocytosis. In multiple myeloma, however Devetzoglou and colleagues (2015), reported a correlation between mast cell density and IL-6 and CRP in 86 patients of varying degrees of disease severity. In this study, it was also reported an increase in mortality in patients with increased mast cell density, although the investigators evaluate this with IL-6 or CRP. It should be noted however that the number of patients was small and there was quite a spread of results for mast cell density, IL-6 and CRP on visual inspection of the data. Further a more recent study by Herishanu and colleagues (2017) reported that increased circulating CRP concentrations are associated with increased mortality and the development of future solid tumours in patients with chronic lymphocytic leukaemia. Again, however the number of patients was small (n=107). The exact role of CRP in the current study and studies reported above remains to be elucidated, however earlier work by Fujimoto and colleagues (2003) has shown that CRP can activate mast cells in a canine model.

4.7 Transforming Growth Factor Beta 1

The results of the current investigation have demonstrated a significantly increased circulating plasma level of TGFβ1 in patients with systemic mastocytosis using both SWATH-MS and ELISA techniques.

Transforming growth factor beta is a multipotent cytokine comprising of TGFβ1,2 and 3 (Derynck & Zhang., 2003). TGFβ1 is the most abundant isoform in most tissues, including the skin, and is secreted in a biologically latent form before

becoming activated when mature TGFβ1 dissociates from its latency associated peptide dimer (Han *et al.,* 2012). TGFβ1 stimulates migration of monocytes, lymphocytes, neutrophils and fibroblasts at low concentrations (McCartney & Wahl, 1994).

Physiologically, TGFβ signalling is essential for the normal regulation of cellular processes, including cell survival, migration, proliferation and differentiation. TGFβ has a negative impact on cellular proliferation while stimulating differentiation, mast cell activation and apoptosis in a concentration dependant manner during haematopoiesis and suggesting an important role during tumorigenesis (Dong & Blobe, 2006, Shi & Massague, 2003 and Ndaw, et al., 2017). Further, in haematological malignancy, the normal physiology of TGFβ can be modified by oncoproteins, suggesting that TGFβ may have a tumour suppressor function under normal physiology (Dong & Blobe, 2006).

Clinically, in patients with hairy cell leukaemia, Shehata and colleagues (2004) reported elevated circulating levels of TGFβ1 and confirmed that hairy cells were the predominant source of the elevated levels (Dong & Blode, 2006). Further the authors reported that TGFβ1 was also present, at increased concentration in bone marrow derived mononuclear cells (Shehata, et al., 2004). Moreover, the authors went on to demonstrate a correlation between the circulating levels of TGFβ1 and the extent of bone marrow fibrosis. (Shehata, et al., 2004). In patients with mastocytosis, bone marrow reticulin fibrosis is commonly observed (Li & Baek, 2002). Mast cells are a recognised source of pro-fibrotic cytokines, including TGFβ1.

In clinical studies, Li and colleagues (2002) have reported a correlation between the circulating plasma levels of TGFβ1 and bone marrow fibrosis.

4.8 Platelet derived Growth Factor Receptor Beta

The results of the current investigation have demonstrated a significantly increased circulating plasma level of PDGF Receptor-beta (PDGFrβ) in patients with systemic mastocytosis using both SWATH-MS and ELISA techniques.

Platelet derived growth factor is a potent mitogenic agent for fibroblasts and smooth muscle cells, and exerts its effect through the cell surface tyrosine kinase domain receptor designated the PDGF receptor (Raica & Cimpean, 2010). In addition, mast cells have been shown to be a source of PDGF (Li & Baek, 2002). In a recent study, Yang and colleagues (2018) reported increased expression, by immunohistochemistry, of PGDFRβ on leukemic large granular lymphocytes. In addition, the results demonstrated that those cells expressing PDGFRβ had a significant survival advantage. Similar results were also observed when PDGF was assayed directly by ELISA in the serum of patients with large granular lymphocyte leukaemia. The results from Yang and colleagues (2018) also support the earlier findings of Ulvestad and colleagues (2001) who reported PDGFRβ expression in patients with human acute myelongenous leukaemia.

The significance of PDGFRβ in patients with mastocytosis remains to be elucidated with no reports in the literature. The results of the current study however would suggest that PDGFRβ may have significance in this group of patients.

4.9 Platelet Basic Protein

The results of the current investigation have demonstrated a significantly increased circulating plasma level of platelet basic protein (CXCL7) in patients with systemic mastocytosis using both SWATH-MS and ELISA techniques.

In their recent review, Ntelis and colleagues (2017) discuss the potential significance of platelet derived growth factors in many autoimmune and vascular diseases, including a role in the development of fibrosis and is released following platelet activation (Ntelis, et al., 2017 & Li & Baek, 2002). The significance of platelet basic protein in the diagnosis and monitoring of mastocytosis remains to be elucidated.

4.10 Beta 2 Microglobulin

The results of the current investigation have demonstrated a significantly increased circulating plasma level of β2M in patients with systemic mastocytosis using SWATH-MS only. Results obtained using a specific ELISA showed increased circulating plasma levels of β2M although this did not reach statistical significance.

Beta 2 Microglobulin (β2M) is a non-glycosylated polypeptide of 100 amino acid residues. It is the invariant chain of the major histocompatibility (MHC) class 1 molecules on the cell surface of nearly all nucleated cells and is present in most biological fluids, including serum, urine and synovial fluid (Drueke., 2009), and functions to stabilise the tertiary structure of the MCH class 1 α-chain. Free β2M

can be measured in body fluids under physiological conditions as a result of shedding from cell surfaces or intracellular release. β2M is mainly catabolised within the kidney, with 95%-100% of circulating β2M being eliminated through glomerular filtration (Karlsson, *et al.,* 1980). In renal tubulopathies quantities of β2M in urine are increased, reflecting the degree of renal impairment. If the rate of glomerular filtration is reduced, the level of serum β2M is increased therefore, serum and urine concentrations of β2M are used to monitor glomerular and tubular nephropathies (Wilbell, *et al.,* 1978). In health, the serum concentration of β2M, is normally <2mg/L and the urinary excretion being < 400μg/24 hours (Xie, *et al.,* 2003). In addition, β2M is extensively involved in the functional regulation of cell survival, proliferation, apoptosis and metastasis in cancer cells (Li, *et al.,* 2016). Other groups have reported that increased serum β2M levels are a predictor of poor survival in several haematological malignancies including multiple myeloma, Hodgkin lymphoma, acute lymphoblastic leukaemia, chronic myeloid leukaemia, chronic lymphocytic leukaemia and myelodysplastic syndromes (Durie *et al.,* 1990, Chronowski *et al.,*2002, Kantarjian *et al.,* 1992, Molica *et al.,* 1999 and Gatto *et al.,* 2003).

Further, Durie and collugues (1990) described serum β2M measurements as having the highest prognostic significance in 612, previously untreated patients with acute multiple myeloma, then any other prognostic factor measured. Mean survival for patients, with pre-treatment serum β2M values of <6μg/ml, was reported, using radioimmunoassay, as 36 months, compared with a mean survival rate of 23 months for 225 patients with serum β2M ≥ 6μg /ml. Serum β2M was highly

correlated with stage and it was possible to stratify myeloma patient into low, intermediate and high risk categories based on serum β2M levels , albumin and age (Durie *et al.,* 1990). The authors concluded that serum β2M is the most powerful prognostic factor currently available for multiple myeloma and that it can be used for pre-treatment stratification (Durie *et al.,* 1990).

Further in their study of 86 patients with multiple myeloma, Devetzoglou and colleagues (2015) reported a correlation between mast cell density and β2M, although the data was widely dispersed on visual inspection. As previously reported increased mast cell density was associated with increased mortality, although a formal evaluation of β2M as a predictor of outcome was not included in their analysis.

The above results are also supported by results of earlier studies in haematological malignancies, which reported a correlation between levels of β2M and outcome including all-cause mortality (Campos., *et al.,* 1984 & Bataille *et al.,* 1984).

4.11 Mastocytosis and Inflammation

As outlined in Chapter 1, the mast cell plays a significant role in inflammation, secreting a number of biological mediators. The increases circulating plasma levels of CRP, Platelet Derived Growth Factor Receptor-β, Platelet Basic Protein, Liposaccharide Binding Proteins, Transforming Growth Factor-β1 and Beta-2 microglobulin all point to a low grade inflammatory state in patients with SM. The increased levels of CRP, a routinely measured marker of inflammation, while not clinically significant would also support the presence of a low grade inflammatory

state. Indeed, as discussed above, levels of CRP have been shown to be predictive of mortality in haematological malignancy.

Conclusion

In conclusion, the global aim of this investigation was to identify a circulating plasma proteome that may differentiate systemic mastocytosis from normal healthy individuals. The results reported here have demonstrated the utility of SWATH-MS for the discovery of potential biomarkers of systemic mastocytosis. The results obtained by mass spectrometry have demonstrated significantly increased levels of CRP, CXCL7, LBP, TGFβ1 and PDGF receptor-β in patients with systemic mastocytosis when compared to an apparently healthy population. Levels of β2M were also increased in patients when compared to controls, however the difference did not reach statistical significance, most likely due to the large spread of results. Results obtained by ELISA have confirmed the results obtained by mass-spectrometry, with one notable exception, that B2M was significantly increased in patients by SWATH-MS, but not when quantified by ELISA.

Limitations

The work reported here has a number of limitations:

- The sample numbers were small (13 patients and 7 controls), although the condition is relatively rare making it difficult to collect large numbers of participants from a single clinical site.

- The demographic data for some of the patients is missing and the c-KIT status and circulating tryptase levels of the control population is unknown, although it is assumed to be wild-type and normal respectively.
- The age of the patients is greater than the age of the controls however, this was not statistically significant. Likewise, the gender distribution between patients and controls is not exactly matched with a female predominance in the patient population
- The results reported here are for the plasma proteome only and does not include, for example the proteome of mononuclear cell and bone marrow fluid.

Further Work

The results of the current investigation would suggest a number of avenues for further work. The work presented here, details the results of the analysis of blood plasma derived from a peripheral blood sample collected at the antecubital fossa. As part of the current project, the author also isolated peripheral blood and bone marrow derived mononuclear cells together with bone marrow fluid, however time only permitted the completion of peripheral blood plasma sample. The next stage, would be to analyse the proteome of the intra-cellular lysate of mononuclear cells, derived from peripheral blood and bone marrow, in addition to bone marrow fluid.

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Appendices

1. Participant information sheet

Version 1.1 4th January 2017/18
Title of Study: Understanding the Molecular Mechanisms of Mastocytosis

Name of Researcher(s): Dr Ciaren Graham, Dr Robert Graham and Dr Bethan Myers
We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being carried out and what it would involve for you. Dr Myers will go through this information sheet with you and answer any questions you may have at your next hospital appointment. We expect that this will take about 15 minutes. If you wish to ask any questions about the study before your appointment, please feel free to use the contact details on page 4. 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. Take time to decide whether or not you wish to take part.

Part 1
What is the purpose of the study?
Mastocytosis is a term used to describe a blood disorder in which there are an increased number of mast cells. Mast cells are an important part of the immune system; they help fight infection and are part of the body's response to allergens. How mastocytosis develops is poorly understood. We would like to study this blood disorder from bone marrow and blood cells in patients with mastocytosis using a technique called mass spectrometry. We hope that by understanding mastocytosis development this will lead to better and improved therapies.
Why have I been invited?
You have been invited as you are patient affected by mastocytosis. We are hoping to include 10 patients currently affected by mastocytosis in this study, and we are inviting patients who currently attend the mastocytosis clinics to consider participating.
Do I have to take part?
It is up to you to decide whether you wish to take part in the study. You will have until your next routine clinic appointment, or as long as you need, to consider the information in this sheet and decide whether or not to take part. A member of the research team will go through all the information with you again on the day of your appointment and answer any questions you may have. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive. Version 1.1 4/01/2016 2

What will happen to me if I take part?
If you decide to take part in the study the only difference from when we routinely take blood and bone marrow from you is that three extra vials (approximately 12mls) of blood and two extra vials (approximately 8mls) of bone marrow will be taken. These additional samples will only be taken when you are having blood and/or bone marrow samples taken as part of your normal care. You will not need to have any extra procedures as part of the study.
What are the possible disadvantages and risks of taking part?
The main disadvantages to taking part are the possible discomfort associated with the blood test and the bone marrow aspiration, both of which you will have as part of your care, whether you chose to take part in the research study. You may experience a slight discomfort when blood is taken, and there is the potential for a small bruise to occur afterwards. You may also find the bone marrow aspiration uncomfortable both during and after the procedure. All staff performing these procedures are fully trained and experienced and will ensure that your needs are met during the process. Otherwise there are no known risks from taking part in this study. Taking part in the study will not affect your current treatment.
What are the possible benefits of taking part?
There is no immediate benefit to you taking part in this study. However, the information we get from this study may help to improve the treatment of yourself and others affected by mastocytosis
What if there is a problem?
Any complaint about the way you have been approached or treated during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.
Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.
This completes part 1.
If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2.
What will happen if I don't want to carry on with the study? Version 1.1 4/01/2016 3

You are free to withdraw from the study at any time. If you withdraw from the study, we will destroy all of your identifiable samples, but we will need to use the data collected up to your withdrawal.

What if there is a problem?

Complaints

If you have a concern about any aspect of this study, you should speak to Dr Myers who will do her best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by contacting [insert PALS details here]

Harm

In the unlikely event that something does go wrong and you are harmed during your participation in this research study there are no special compensation arrangements. However if you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against United Lincolnshire Hospitals NHS Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the organisations sponsoring and/or organising and conducting the research in order to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

What will happen to any samples I give?

As explained to you in Part 1 we will take three extra vials of your blood and bone marrow alongside routine samples taken during the course of your attendance at your routine clinical appointments. These samples will be transferred to the research team at University of Lincoln for processing and then to the University of Manchester for mass spectrometry analysis.

Only the authorised researcher's team will have access to your samples and will process them in respect of rules of confidentiality.

The remaining samples will be disposed of according to University of Lincoln regulations for biological samples.

The research will be conducted within United Lincolnshire Hospitals NHS Trust, the University of Lincoln and the University of Manchester; no materials will be sent outside of the UK.

What will happen to the results of the research study? Version 1.1 4/01/2016 4

The results of this study may be presented at meetings, conferences and submitted for publication in relevant medical journals. However, no personal data will be disclosed within the results and all samples will remain anonymous.

A webpage has been created for this study, where we will publish the studies finding: http://lincolnproteomics.blogs.lincoln.ac.uk/mastocytosis-digital-proteomic-maps-2/ In addition, a summary of the study findings will also be published on the ULHT Lincolnshire Clinical Research Facility webpage at www.ulh.nhs.uk/LCRF following completion of the study.

Who is organising and funding the research?

The research is being co-sponsored by the United Lincolnshire Hospitals NHS Trust and the University of Lincoln and is funded by the Mastocytosis Charity.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the NHS Research Ethics Committee.

Further Information and Contact Details

You will be offered a copy of this information sheet and your signed consent form (if applicable) to keep for future reference.

If you would like any further information regarding this study please contact:

Dr Bethan Myers	**Dr Ciaren Graham**	**Dr Robert Graham**
United Lincolnshire Hospitals NHS Trust	Manchester Metropolitan University	University of Manchester
Greetwell Road	John Dalton Building	Institute of Cancer Sciences
Lincoln	Chester Street	27 Palatine Road
Lincolnshire	Manchester	Manchester
LN2 5QY	M1 5GD	M20 3LJ
01522 512512	0161 2471146	0161 2750005
Bethan.Myers@ULH.nhs.uk	c.graham@mmu.ac.uk	Robert.graham@manchester.ac.uk

2. Patient Consent Form

UNIVERSITY OF LINCOLN

[Insert Trust header here]

CONSENT FORM

The Molecular Mechanisms of Mastocytosis

Name of Researcher: **Dr Bethan Myers – Consultant Haematologist**, United Lincolnshire Hospitals NHS Trust
Dr Ciaren Graham – Senior lecturer, Manchester Metropolitan University
Dr Robert Graham – Senior Lecturer, University of Manchester

Contact Details:
Dr Bethan Myers: ULHT, Greetwell Road, Lincoln, Lincolnshire, LN2 5QY, (01522) 512 512
Email: Bethan.Myers@ULH.nhs.uk
Dr Ciaren Graham: Manchester Metropolitan University, John Dalton Building Chester Street Manchester M1 5GD, 0161 247 1146 E-mail c.graham@mmu.ac.uk
University of Lincoln, Joseph Banks Laboratories, Email cgraham@lincoln.ac.uk
Dr Robert Graham: University of Manchester, 27 Palatine Road, Manchester, M20 3LJ, 0161 275 0005, robert.graham@manchester.ac.uk

Please initial box each box

1. I confirm that I have read and understand the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the regulatory authorities, the NHS Trust, or the Sponsor where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study. ☐

Name of Patient	Signature	Date
---	---	---
Name of Person taking consent	Signature	Date
---	---	---

3. Heathy Control Participant Consent Form

01/01/18
Amy McMullen
Department of Healthcare Science
John Dalton
Manchester Metropolitan University
Tel: 0161- 247-2000

Manchester Metropolitan University **Consent Form**

Title of Project:
Understanding the Molecular Mechanisms of Mastocytosis

Name of Researcher: Amy McMullen
Name of Supervisor: Dr C Graham

Participant Identification Code for this project:

1. I confirm that I have read and understood the information sheet dated …. for the above project and have had the opportunity to ask questions about the interview procedure. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason to the named researcher. ☐
3. I understand that my blood samples will be used for analysis for this research project. ☐
4. I give/do not give permission for any blood samples to be archived as part of this research project, making it available to future researchers. ☐
5. I agree to take part in the above research project. ☐

_____	_____
Name of Participant	Date Signature
_____	_____
Researcher	Date Signature

To be signed and dated in presence of the participant

Once this has been signed, you will receive a copy of your signed and dated consent form and information sheet by post.

4. NRES Letter

NHS
Health Research Authority

London - City & East Research Ethics Committee
Bristol Research Ethics Committee Centre
Whitefriars
Level 3, Block B
Lewins Mead
Bristol
BS1 2NT

Telephone: 02071048033/53

10 May 2016

Dr Ciaren Graham
Senior Lecture
Manchester Metropolitan University
John Dalton Building
Chester Street
Manchester
M1 5GD

Dear Dr Graham

Study title:	**Defining the Molecular Mechanisms of Mastocytosis using Mass Spectrometry**
REC reference:	**16/LO/0787**
IRAS project ID:	**192830**

Thank you for your response of 04 May 2016, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Mr Rajat Khullar, nrescommittee.london-cityandeast@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

5. List of Identified proteins

Row	Peak Name	Group	p-value	Fold Change
1	P51149	Ras-related protein Rab-7a	0.00	1.792977
2	Q9Y696	Chloride intracellular channel protein 4	0.00	1.681724
3	Q9Y490	Talin-1	0.00	1.47355
4	P02775	platelet basic protein	0.00	4.827426
5	P07996	Thrombospondin-1	0.00	3.010135
6	P00491	Purine nucleoside phosphorylase	0.00	2.556021
7	P02741	C-reactive protein	0.00	3.622838
8	O14980	Exportin-1	0.00	1.584915
9	Q05397	Focal adhesion kinase 1	0.00	2.161734
10	P01130	Low-density lipoprotein receptor	0.00	1.797412
11	Q12805	EGF-containing fibulin-like extracellular matrix protein 1	0.00	1.61988
12	P02750	Leucine-rich alpha-2-glycoprotein	0.00	1.726239
13	P63104	14-3-3 protein zeta/delta	0.00	1.871311
14	Q9UBX1	Cathepsin F	0.00	2.409348
15	P02786	Transferrin receptor protein 1	0.00	2.133517
16	P07942	Laminin subunit beta-1	0.00	1.621829
17	P30041	Peroxiredoxin-6	0.00	2.098338
18	P55072	Transitional endoplasmic reticulum ATPase	0.00	1.41768
19	P14780	Matrix metalloproteinase-9	0.00	1.948773
20	P46821	Microtubule-associated protein 1B	0.00	1.800807
21	P08567	Pleckstrin	0.00	1.902847
22	P12259	Coagulation factor V	0.00	1.465886
23	P18428	Lipopolysaccharide-binding protein	0.00	2.029301
24	P00736	Complement C1r subcomponent	0.00	1.417381
25	Q9BXR6	Complement factor H-related protein 5	0.00	1.670328
26	P06702	Protein S100-A9	0.00	1.939291
27	P11047	Laminin subunit gamma-1	0.00	1.470848
28	P07737	Profilin-1	0.00	1.63004
29	P08779	Keratin, type I cytoskeletal 16	0.00	2.077625
30	P60842	Eukaryotic initiation factor 4A-I	0.00	2.106019
31	Q13263	Transcription intermediary factor 1-beta	0.00	1.605154
32	P01024	Complement C3	0.00	0.79469
33	P43243	Matrin-3	0.00	1.650033
34	P22087	rRNA 2'-O-methyltransferase fibrillarin	0.00	1.881408
35	P12814	Alpha-actinin-1	0.00	1.449735
36	P62328	Thymosin beta-4	0.00	2.280595
37	P01714	Immunoglobulin lambda variable 3-19	0.00	2.737476
38	Q16555	Dihydropyrimidinase-related protein 2	0.00	1.752387
39	O75691	Small subunit processome component 20 homolog	0.00	1.967347
40	P26583	High mobility group protein B2	0.00	1.637314
41	O95568	Histidine protein methyltransferase 1 homolog	0.00	2.443121
42	P10643	Complement component C7	0.00	1.387812
43	Q13451	Peptidyl-prolyl cis-trans isomerase FKBP5	0.00	1.640447
44	P08582	Melanotransferrin	0.00	2.734
45	P24298	Alanine aminotransferase 1	0.00	2.04158
46	O43175	D-3-phosphoglycerate dehydrogenase	0.00	1.600545
47	P25398	40S ribosomal protein S12	0.00	2.487468
48	Q96HC4	PDZ and LIM domain protein 5	0.00	1.684346
49	P01877	Ig alpha-2 chain C region	0.00	3.011327
50	P02763	Alpha-1-acid glycoprotein 1	0.00	1.728418
51	P19320	Vascular cell adhesion protein 1	0.00	1.757248
52	Q09666	Neuroblast differentiation-associated protein AHNAK	0.00	1.44165
53	Q15819	Ubiquitin-conjugating enzyme E2 variant 2	0.00	2.197851
54	Q07912	Activated CDC42 kinase 1	0.00	1.60152
55	Q14146	Unhealthy ribosome biogenesis protein 2 homolog	0.00	1.817413
56	Q15746	Myosin light chain kinase, smooth muscle	0.00	1.471429
57	P02533	Keratin, type I cytoskeletal 14	0.00	1.467889
58	P37802	Transgelin-2	0.00	1.667529
59	Q7KZ85	Transcription elongation factor SPT6	0.00	1.434373
60	Q86VP6	Cullin-associated NEDD8-dissociated protein 1	0.00	1.640562

Row	Peak Name	Group	p-value	Fold Change
61	Q04721	Neurogenic locus notch homolog protein 2	0.00	1.5395024
62	P07203	Glutathione peroxidase 1	0.00	2.1700137
63	P62847	40s ribosomal protein s24	0.00	1.71698301
64	P00488	Coagulation factor XIII A chain	0.00	1.37525685
65	P0C0L5	Complement C4-B	0.00	1.61306168
66	P19174	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamm	0.00	1.57806644
67	Q92520	Protein FAM3C	0.00	1.68650716
68	P14625	Endoplasmin	0.00	1.38473788
69	P55259	Pancreatic secretory granule membrane major glycoprotein GP2	0.00	4.30583917
70	P11216	Glycogen phosphorylase, brain form	0.00	1.72564951
71	P50570	Dynamin-2	0.00	1.56225444
72	P55058	Phospholipid transfer protein	0.00	1.61063386
73	P17844	Probable ATP-dependent RNA helicase DDX5	0.00	1.86806585
74	Q13347	Eukaryotic translation initiation factor 3 subunit I	0.00	1.50496239
75	P60174	Triosephosphate isomerase	0.00	1.52797724
76	P29144	Tripeptidyl-peptidase 2	0.00	1.52518734
77	Q13554	Calcium/calmodulin-dependent protein kinase type II subunit beta	0.00	1.97911099
78	Q9H0E2	Toll-interacting protein	0.00	1.47605832
79	P51884	Lumican	0.00	1.42984105
80	P23528	Cofilin-1	0.00	1.74722144
81	Q13045	Protein flightless-1 homolog	0.00	1.58940031
82	P04746	Pancreatic alpha-amylase	0.00	2.87190931
83	P02748	Complement component C9	0.00	1.41056438
84	P12956	X-ray repair cross-complementing protein 6	0.00	1.54687762
85	P02042	Hemoglobin subunit delta	0.00	1.78150432
86	P49747	Cartilage oligomeric matrix protein	0.00	1.96730274
87	Q01082	Spectrin beta chain, non-erythrocytic 1	0.00	1.5430262
88	P32119	Peroxiredoxin-2	0.00	1.83732771
89	Q32MZ4	Leucine-rich repeat flightless-interacting protein 1	0.00	1.54332807
90	P01137	Transforming growth factor beta-1	0.00	2.05575147
91	P01834	Ig kappa chain C region	0.00	4.46540638
92	P48163	NADP-dependent malic enzyme	0.00	1.92661397
93	P05543	Thyroxine-binding globulin	0.00	1.34375244
94	P49913	Cathelicidin antimicrobial peptide	0.00	1.44030223
95	P00568	Adenylate kinase isoenzyme 1	0.00	1.84393339
96	P06733	Alpha-enolase	0.00	1.82830486
97	P13798	Acylamino-acid-releasing enzyme	0.00	1.73432413
98	Q14789	Golgin subfamily B member 1	0.00	1.41738194
99	P06737	Glycogen phosphorylase, liver form	0.00	1.53041341
100	Q99460	26S proteasome non-ATPase regulatory subunit 1	0.00	1.57729195
101	O75390	Citrate synthase, mitochondrial	0.00	1.42923475
102	P01771	Immunoglobulin heavy variable 3-33	0.00	4.05789101
103	P49321	Nuclear autoantigenic sperm protein	0.00	1.56574626
104	P01008	Antithrombin-III	0.00	0.71671832
105	P61626	Lysozyme C	0.00	1.42790352
106	P11021	78 kDa glucose-regulated protein	0.00	1.32546483
107	P52948	Nuclear pore complex protein Nup98-Nup96	0.00	1.45823639
108	Q02790	Peptidyl-prolyl cis-trans isomerase FKBP4	0.00	2.15659916
109	Q8NBS9	Thioredoxin domain-containing protein 5	0.00	1.9113134
110	Q9Y6R7	IgGFc-binding protein	0.00	1.37564999
111	Q8WVV4	Protein POF1B	0.00	1.99434341
112	P61769	Beta-2-microglobulin	0.00	1.89508639
113	O15511	Actin-related protein 2/3 complex subunit 5	0.00	1.70970174
114	P19021	Peptidyl-glycine alpha-amidating monooxygenase	0.00	1.61652039
115	P22314	Ubiquitin-like modifier-activating enzyme 1	0.00	1.45282084
116	Q86V48	Leucine zipper protein 1	0.00	1.71769392
117	P07237	Protein disulfide-isomerase	0.00	1.66054376
118	Q14204	Cytoplasmic dynein 1 heavy chain 1	0.00	1.40713006
119	Q70J99	Protein unc-13 homolog D	0.00	1.43671768
120	P07814	Bifunctional glutamate/proline--tRNA ligase	0.00	1.3445633

Row	Peak Name	Group	p-value	Fold Change
121	P08107	NA	0.00	1.69917077
122	P09871	Complement C1s subcomponent	0.00	1.19909897
123	Q96AB3	Isochorismatase domain-containing protein 2	0.00	1.91405138
124	P54577	Tyrosine--tRNA ligase, cytoplasmic	0.00	1.48301558
125	P00367	Glutamate dehydrogenase 1, mitochondrial	0.00	1.86798488
126	P17980	26S protease regulatory subunit 6A	0.00	1.75537501
127	P02792	Ferritin light chain	0.00	1.69278905
128	P28066	Proteasome subunit alpha type-5	0.00	1.57022548
129	Q14697	Neutral alpha-glucosidase AB	0.00	1.38313543
130	P22307	Non-specific lipid-transfer protein	0.00	1.4024583
131	P52272	Heterogeneous nuclear ribonucleoprotein M	0.00	1.49753696
132	Q9H4M9	EH domain-containing protein 1	0.00	1.79773417
133	P08865	40S ribosomal protein SA	0.00	1.86355556
134	Q15084	Protein disulfide-isomerase A6	0.00	1.5484223
135	P25788	Proteasome subunit alpha type-3	0.00	1.70390259
136	P04264	Keratin, type II cytoskeletal 1	0.00	0.56188372
137	P08246	Neutrophil elastase	0.00	1.80353985
138	P20290	Transcription factor BTF3	0.00	1.66504331
139	P43490	Nicotinamide phosphoribosyltransferase	0.00	1.45673054
140	Q13228	Selenium-binding protein 1	0.00	1.38733557
141	P21796	Voltage-dependant anion-selecive channel protein 1	0.00	2.43779507
142	Q92619	Minor histocompatibility protein HA-1	0.00	1.67453345
143	P01876	Ig alpha-1 chain C region	0.00	3.06439781
144	P05166	Propionyl-CoA carboxylase beta chain, mitochondrial	0.00	1.36120772
145	Q00796	Sorbitol dehydrogenase	0.00	1.8947924
146	P23381	Tryptophan--tRNA ligase, cytoplasmic	0.00	1.85987492
147	P16152	Carbonyl reductase [NADPH] 1	0.00	2.07455334
148	P80748	Immunoglobulin lambda variable 3-21	0.00	3.76170138
149	P10619	Lysosomal protective protein	0.00	1.77004316
150	P01763	Immunoglobulin heavy variable 3-48	0.00	2.18293985
151	P20061	Transcobalamin-1	0.00	1.87942504
152	Q14344	Guanine nucleotide-binding protein subunit alpha-13	0.00	1.52853381
153	P50440	Glycine amidinotransferase, mitochondrial	0.00	1.48654903
154	P13727	Bone marrow proteoglycan	0.00	1.54461065
155	Q9UIJ7	GTP:AMP phosphotransferase AK3, mitochondrial	0.00	1.64250467
156	P54108	Cystenine-rich secretory protein 3	0.00	1.36237208
157	Q9Y6I3	Epsin-1	0.00	1.81708475
158	Q96KN2	Beta-Ala-His dipeptidase	0.00	0.58255412
159	Q9UBQ0	Vacuolar protein sorting-associated protein 29	0.00	1.78508783
160	P15311	Ezrin	0.00	1.64379818
161	P48637	Glutathione synthetase	0.00	1.81392402
162	P29317	Ephrin type-A receptor 2	0.00	1.46277267
163	P52701	DNA mismatch repair protein Msh6	0.00	1.33554433
164	P00352	Retinal dehydrogenase 1	0.00	1.51632375
165	P08571	Monocyte differentiation antigen CD14	0.00	1.29991255
166	Q16401	26S proteasome non-ATPase regulatory subunit 5	0.00	1.67209716
167	P41222	Prostaglandin-H2 D-isomerase	0.00	1.66009491
168	P58107	Epiplakin	0.00	1.33030518
169	P01781	Ig heavy variable 3-7	0.00	2.1066067
170	P02652	Apolipoprotein A-II	0.00	0.56211596
171	P55285	Cadherin-6	0.00	1.85576234
172	P14618	Pyruvate kinase PKM	0.00	1.68873172
173	P0CG39	POTE ankyrin domain family member J	0.00	2.15434965
174	P31946	14-3-3 protein beta/alpha	0.00	1.68102498
175	O75608	Acyl-protein thioesterase 1	0.00	1.65406875
176	Q9H8L6	Multimerin-2	0.00	1.43662739
177	Q9UHD1	Cysteine and histidine-rich domain-containing protein 1	0.00	1.81506722
178	P07437	Tubulin beta chain	0.00	1.59606747
179	P62701	40S ribosomal protein S4, X isoform	0.00	1.87435635
180	Q9NWV4	UPF0587 protein C1orf123	0.00	3.14046968

Row	Peak Name	Group	p-value	Fold Change
181	Q14203	Dynactin subunit 1	0.00	1.46876293
182	O43776	Asparagine--tRNA ligase, cytoplasmic	0.00	1.48141237
183	P12111	Collagen alpha-3(VI) chain	0.00	1.33161029
184	P07355	Annexin A2	0.00	1.50862183
185	P10645	Chromogranin-A	0.00	1.43163979
186	P01857	Ig Heavy constant Gamma 1	0.00	2.80142066
187	Q92797	Symplekin	0.00	1.42206001
188	Q14956	Transmembrane glycoprotein NMB	0.00	1.51771858
189	Q9NXE4	Sphingomyelin phosphodiesterase 4	0.00	2.2795879
190	P09972	Fructose-bisphosphate aldolase C	0.00	1.42215329
191	Q07075	Glutamyl aminopeptidase	0.00	1.82640834
192	P02747	Complement C1q subcomponent subunit C	0.00	1.57103258
193	P21695	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	0.00	1.67612656
194	P52209	6-phosphogluconate dehydrogenase, decarboxylating	0.00	1.5123946
195	P08758	Annexin A5	0.00	1.34505287
196	P98095	Fibulin-2	0.00	1.52848235
197	P31151	Protein S100-A7	0.00	1.96941755
198	P06576	ATP synthase subunit beta, mitochondrial	0.00	1.43120446
199	P13671	Complement component C6	0.00	1.20230055
200	O60306	Intron-binding protein aquarius	0.00	1.59890077
201	Q15404	Ras suppressor protein 1	0.00	1.6031992
202	P18206	Vinculin	0.00	1.33897776
203	P07738	Bisphosphoglycerate mutase	0.00	1.9355109
204	P02745	Complement C1q subcomponent subunit A	0.00	1.85155124
205	Q9Y4L1	Hypoxia up-regulated protein 1	0.00	1.27775398
206	Q9UNF1	Melanoma-associated antigen D2	0.00	1.46939773
207	Q9NTJ3	Structural maintenance of chromosomes protein 4	0.00	1.32930792
208	P05090	Apolipoprotein D	0.00	0.72699178
209	P02686	Myelin basic protein	0.00	1.97787063
210	Q6VY07	Phosphofurin acidic cluster sorting protein 1	0.00	1.66535445
211	O94804	Serine/threonine-protein kinase 10	0.00	1.83335278
212	P14324	Farnesyl pyrophosphate synthase	0.00	1.56081262
213	Q16775	Hydroxyacylglutathione hydrolase, mitochondrial	0.00	1.59246379
214	Q9UI26	Importin-11	0.00	1.87737055
215	Q9BXK5	Bcl-2-like protein 13	0.00	2.10136896
216	Q00610	Clathrin heavy chain 1	0.00	1.24777273
217	P22061	Protein-L-isoaspartate(D-aspartate) O-methyltransferase	0.00	1.75035575
218	Q96KP4	Cytosolic non-specific dipeptidase	0.00	1.85759165
219	P01033	Metalloproteinase inhibitor 1	0.00	1.78051835
220	O14745	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	0.00	2.0855697
221	P36959	GMP reductase 1	0.00	2.18126207
222	P62937	Peptidyl-prolyl cis-trans isomerase A	0.00	1.59329131
223	Q15274	Nicotinate-nucleotide pyrophosphorylase [carboxylating]	0.00	2.15120725
224	Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	0.00	1.30305553
225	P09467	Fructose-1,6-bisphosphatase 1	0.00	1.39854687
226	P42785	Lysosomal Pro-X carboxypeptidase	0.00	1.95230786
227	P49454	Centromere protein F	0.00	1.54823534
228	Q7Z794	Keratin, type II cytoskeletal 1b	0.00	0.5833744
229	P05062	Fructose-bisphosphate aldolase B	0.00	1.41814995
230	P10809	60 kDa heat shock protein, mitochondrial	0.00	1.37486004
231	P35858	Insulin-like growth factor-binding protein complex acid labile subunit	0.00	0.78737957
232	P02760	Protein AMBP	0.00	1.24854281
233	Q9BUF5	Tubulin beta-6 chain	0.00	1.47833732
234	O75369	Filamin-B	0.00	1.5402354
235	Q8IUX7	Adipocyte enhancer-binding protein 1	0.00	2.38756404
236	P02647	Apolipoprotein A-I	0.00	0.84003239
237	Q9HDC9	Adipocyte plasma membrane-associated protein	0.00	1.51037559
238	Q99674	Cell growth regulator with EF hand domain protein 1	0.00	3.38186065
239	Q92841	Probable ATP-dependent RNA helicase DDX17	0.00	1.63459516
240	P04430	Immunoglobulin kappa variable 1-16	0.00	2.0228943

Row	Peak Name	Group	p-value	Fold Change
241	P35573	Glycogen debranching enzyme	0.00	1.654436
242	P20933	N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase	0.00	1.73746412
243	P30153	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	0.00	1.91153519
244	Q16706	Alpha-mannosidase 2	0.00	2.90597474
245	O75340	Programmed cell death protein 6	0.00	1.44959234
246	Q14258	E3 ubiquitin/ISG15 ligase TRIM25	0.00	1.54994305
247	P0DJI8	Serum amyloid A-1 protein	0.00	1.73104633
248	P21333	Filamin-A	0.00	1.32537021
249	Q6XQN6	Nicotinate phosphoribosyltransferase	0.00	1.50554835
250	P07333	Macrophage colony-stimulating factor 1 receptor	0.00	1.72735209
251	A5A3E0	Cytosolic non-specific dipeptidase	0.00	1.82826268
252	O15523	ATP- dependant RNA helicase DDx3y	0.00	2.02159187
253	P00746	Complement factor D	0.01	1.35508304
254	P02766	Transthyretin	0.01	0.76661676
255	P61160	Actin-related protein 2	0.01	1.67943571
256	P00390	Glutathione reductase, mitochondrial	0.01	1.42207053
257	O95479	GDH/6PGL endoplasmic bifunctional protein	0.01	1.79903869
258	Q9NQR4	Omega-amidase NIT2	0.01	1.48504224
259	P01903	HLA class II histocompatibility antigen, DR alpha chain	0.01	1.9466824
260	Q9H3U1	Protein unc-45 homolog A	0.01	1.36597287
261	P05060	Secretogranin-1	0.01	1.87944638
262	Q12913	Receptor-type tyrosine-protein phosphatase eta	0.01	1.74271745
263	P10599	Thioredoxin	0.01	1.70307271
264	Q9UQ35	Serine/arginine repetitive matrix protein 2	0.01	1.5970165
265	P39060	Collagen alpha-1(XVIII) chain	0.01	1.50059366
266	Q99829	Copine-1	0.01	1.68029484
267	Q9Y376	Calcium-binding protein 39	0.01	1.47787407
268	Q14914	Prostaglandin reductase 1	0.01	1.54053507
269	P14868	Aspartate--tRNA ligase, cytoplasmic	0.01	1.39079868
270	P21912	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	0.01	1.39114624
271	P63241	Eukaryotic translation initiation factor 5A-1	0.01	1.72330386
272	Q16853	Membrane primary amine oxidase	0.01	1.42262044
273	P19012	Keratin, type I cytoskeletal 15	0.01	1.65878684
274	O00462	Beta-mannosidase	0.01	1.714769
275	O15144	Actin-related protein 2/3 complex subunit 2	0.01	0.21129475
276	P29622	Kallistatin	0.01	0.80017677
277	Q8N2S1	Latent-transforming growth factor beta-binding protein 4	0.01	2.08591444
278	P07858	Cathepsin B	0.01	1.43391079
279	Q96PD5	N-acetylmuramoyl-L-alanine amidase	0.01	1.2055478
280	O00750	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta	0.01	1.92453891
281	Q15582	Transforming growth factor-beta-induced protein ig-h3	0.01	1.38429391
282	Q1KMD3	Heterogeneous nuclear ribonucleoprotein U-like protein 2	0.01	1.36317419
283	P36952	Serpin B5	0.01	1.70240337
284	O15347	High mobility group protein B3	0.01	1.68455957
285	P51888	Prolargin	0.01	1.28825229
286	P00450	Ceruloplasmin	0.01	1.25324077
287	Q12931	Heat shock protein 75 kDa, mitochondrial	0.01	1.28472912
288	Q8N392	Rho GTPase-activating protein 18	0.01	1.46782338
289	Q9HBB8	Cadherin-related family member 5	0.01	1.55058059
290	Q9Y5C1	Angiopoietin-related protein 3	0.01	1.81662386
291	Q13148	TAR DNA-binding protein 43	0.01	1.59515907
292	O75116	Rho-associated protein kinase 2	0.01	1.53746045
293	O75594	Peptidoglycan recognition protein 1	0.01	2.00180852
294	Q15185	Prostaglandin E synthase 3	0.01	1.44128209
295	Q6NZI2	Polymerase I and transcript release factor	0.01	1.43196194
296	P01861	Ig gamma-4 chain C region	0.01	4.55701856
297	P22392	Nucleoside diphosphate kinase B	0.01	2.6202471
298	Q16539	Mitogen-activated protein kinase 14	0.01	1.74953606
299	P78386	Keratin, type II cuticular Hb5	0.01	1.96374
300	O60610	Protein diaphanous homolog 1	0.01	1.32310126

Row	Peak Name	Group	p-value	Fold Change
301	Q99436	Proteasome subunit beta type-7	0.01	1.6431846
302	Q8N5P1	Zinc finger CCCH domain-containing protein 8	0.01	1.42350496
303	O00299	Chloride intracellular channel protein 1	0.01	1.4897075
304	P47897	Glutamine--tRNA ligase	0.01	1.2287075
305	P10768	S-formylglutathione hydrolase	0.01	1.48169039
306	Q9Y265	RuvB-like 1	0.01	1.32872198
307	P06396	Gelsolin	0.01	0.82661461
308	P04075	Fructose-bisphosphate aldolase A	0.01	1.27645916
309	P80108	Phosphatidylinositol-glycan-specific phospholipase D	0.01	0.75505491
310	O43488	Aflatoxin B1 aldehyde reductase member 2	0.01	1.47891792
311	P08473	Neprilysin	0.01	1.67814527
312	P30084	Enoyl-CoA hydratase, mitochondrial	0.01	1.50113742
313	P30740	Leukocyte elastase inhibitor	0.01	1.47169753
314	Q15485	NA	0.01	0.53295776
315	P38646	Stress-70 protein, mitochondrial	0.01	1.34705423
316	Q01459	Di-N-acetylchitobiase	0.01	1.66988783
317	P05109	Protein S100-A8	0.01	1.91112132
318	Q6YN16	Hydroxysteroid dehydrogenase-like protein 2	0.01	1.82780657
319	P05067	Amyloid beta A4 protein	0.01	1.83121201
320	P35613	Basigin	0.01	1.49148634
321	P12429	Annexin A3	0.01	1.70024371
322	P60903	Protein S100-A10	0.01	1.6028557
323	Q9Y2G3	Probable phospholipid-transporting ATPase IF	0.01	2.02216139
324	P14136	Glial fibrillary acidic protein	0.01	2.07483509
325	P48681	Nestin	0.01	1.32097205
326	P13611	Versican core protein	0.01	2.66819441
327	P01717	immunoglobulin lambda variable 3-25	0.01	1.62374402
328	O43837	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	0.01	1.37568678
329	P04275	von Willebrand factor	0.01	1.40688886
330	P15144	Aminopeptidase N	0.01	1.38385806
331	P69905	Hemoglobin subunit alpha	0.01	1.81987694
332	Q6P179	Endoplasmic reticulum aminopeptidase 2	0.01	1.68044876
333	P07911	Uromodulin	0.01	1.46604291
334	P13645	Keratin, type I cytoskeletal 10	0.01	0.70935053
335	P48426	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	0.01	1.64596851
336	Q5HYJ3	Protein FAM76B	0.01	2.25166839
337	P22626	Heterogeneous nuclear ribonucleoproteins A2/B1	0.01	1.37484689
338	Q9UKX7	Nuclear pore complex protein Nup50	0.01	1.83526308
339	Q16181	Septin-7	0.01	1.61457831
340	P12035	Keratin, type II cytoskeletal 3	0.01	1.93573182
341	Q92835	Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1	0.01	1.45789938
342	P31150	Rab GDP dissociation inhibitor alpha	0.01	1.46856534
343	Q562R1	Beta-actin-like protein 2	0.01	1.7796337
344	Q15080	Neutrophil cytosol factor 4	0.01	1.79339669
345	Q9BQE3	Tubulin alpha-1C chain	0.01	1.92604326
346	P02788	Lactotransferrin	0.01	1.31147504
347	Q12841	Follistatin-related protein 1	0.01	1.83002698
348	P68104	Elongation Factor 1 alpha 1	0.01	1.38699456
349	P35269	General transcription factor IIF subunit 1	0.01	1.70105668
350	Q13509	Tubulin beta-3 chain	0.01	1.39446991
351	O75822	Eukaryotic translation initiation factor 3 subunit J	0.01	1.55431659
352	P51610	Host cell factor 1	0.01	1.61177648
353	P54802	Alpha-N-acetylglucosaminidase	0.01	1.3394475
354	Q10567	AP-1 complex subunit beta-1	0.01	1.5608529
355	O60716	Catenin delta-1	0.01	1.34720916
356	Q92945	Far upstream element-binding protein 2	0.01	1.36056632
357	Q16877	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4	0.01	1.6048655
358	P04278	Sex hormone-binding globulin	0.01	1.61760905
359	Q5VUA4	Zinc finger protein 318	0.01	2.2087531
360	Q9NPD3	Exosome complex component RRP41	0.01	2.31453751

Row	Peak Name	Group	p-value	Fold Change
361	Q14141	Septin-6	0.01	1.72143138
362	O95373	Importin-7	0.01	1.57186662
363	Q12884	Prolyl endopeptidase FAP	0.01	2.5270793
364	P62277	protein YIBO	0.01	1.67121408
365	Q99714	3-hydroxyacyl-CoA dehydrogenase type-2	0.01	1.57606152
366	P01859	Ig gamma-2 chain C region	0.01	3.36408753
367	P36873	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	0.01	2.23793483
368	P35579	Myosin-9	0.01	1.25759595
369	Q9UHA4	Ragulator complex protein LAMTOR3	0.01	3.952388
370	P0DJI9	Serum amyloid A-2 protein	0.01	1.85132457
371	P38606	V-type proton ATPase catalytic subunit A	0.01	1.68850127
372	P04434	NA	0.01	2.37315789
373	Q14116	interleukin 18	0.01	1.41239281
374	P40939	Trifunctional enzyme subunit alpha, mitochondrial	0.01	1.37987231
375	P00813	Adenosine deaminase	0.01	1.57610827
376	P14550	Alcohol dehydrogenase [NADP(+)]	0.01	1.62432773
377	P04406	Glyceraldehyde-3-phosphate dehydrogenase	0.01	1.44890027
378	P50454	Serpin H1	0.01	0.65377843
379	P32754	4-hydroxyphenylpyruvate dioxygenase	0.01	1.63194816
380	Q9H0W9	Ester hydrolase C11orf54	0.01	1.7754397
381	P62888	60S ribosomal protein L30	0.01	1.67076381
382	Q9ULV4	Coronin-1C	0.01	1.47470186
383	P33176	Kinesin-1 heavy chain	0.01	1.31082617
384	P29279	Connective tissue growth factor	0.01	1.48998122
385	O75356	Ectonucleoside triphosphate diphosphohydrolase 5	0.01	1.63906712
386	O14786	neuropilin-1	0.02	1.34499946
387	Q9UI17	Dimethylglycine dehydrogenase, mitochondrial	0.02	1.57036953
388	P04216	Thy-1 membrane glycoprotein	0.02	1.77083579
389	O95445	Apolipoprotein M	0.02	0.71204381
390	P26373	60S ribosomal protein L13	0.02	2.35818082
391	P02746	Complement C1q subcomponent subunit B	0.02	1.34290768
392	P24592	Insulin-like growth factor-binding protein 6	0.02	1.62114865
393	Q9UL46	Proteasome activator complex subunit 2	0.02	1.53038662
394	P01833	Polymeric immunoglobulin receptor	0.02	2.46553942
395	Q7KZF4	Staphylococcal nuclease domain-containing protein 1	0.02	1.51327841
396	P33992	DNA replication licensing factor MCM5	0.02	1.25352774
397	Q15365	Poly(rC)-binding protein 1	0.02	1.78721113
398	P35241	Radixin	0.02	1.50906403
399	Q9H0P0	Cytosolic 5'-nucleotidase 3A	0.02	1.5667822
400	Q9UJJ9	N-acetylglucosamine-1-phosphotransferase subunit gamma	0.02	2.13470633
401	P09211	Glutathione S-transferase P	0.02	1.44436774
402	P30481	HLA class II histocompatibility antigen, B-44 alphachain	0.02	1.79024586
403	P68871	Hemoglobin subunit beta	0.02	1.88084569
404	Q9NZP8	Complement C1r subcomponent-like protein	0.02	1.1947061
405	P15880	40S ribosomal protein S2	0.02	1.5027788
406	Q9NQC3	Reticulon-4	0.02	2.20238867
407	Q96EY7	Pentatricopeptide repeat domain-containing protein 3, mitochondrial	0.02	1.58313366
408	P00338	L-lactate dehydrogenase A chain	0.02	1.54706373
409	Q07960	Rho GTPase-activating protein 1	0.02	1.4617796
410	O00743	Serine/threonine-protein phosphatase 6 catalytic subunit	0.02	1.51710009
411	P41250	Glycine--tRNA ligase	0.02	1.44373451
412	O14776	Transcription elongation regulator 1	0.02	1.3474195
413	P61158	Actin-related protein 3	0.02	1.24958076
414	P35442	Thrombospondin-2	0.02	2.19968106
415	P08195	4F2 cell surface Ag heavy chain	0.02	1.54844342
416	P08697	Alpha-2-antiplasmin	0.02	0.84069739
417	P07478	Trypsin-2	0.02	1.58575645
418	P11226	Mannose-binding protein C	0.02	1.50149235
419	O75131	Copine-3	0.02	1.26419073
420	P28072	Proteasome subunit beta type-6	0.02	1.79089542

Row	Peak Name	Group	p-value	Fold Change
421	P09496	Clathrin light chain A	0.02	1.32237807
422	Q06187	Tyrosine-protein kinase BTK	0.02	1.37147408
423	P37235	Hippocalcin-like protein 1	0.02	1.42737868
424	P29350	Tyrosine-protein phosphatase non-receptor type 6	0.02	1.45722542
425	Q9NYL9	Tropomodulin-3	0.02	1.73075638
426	P18669	Phosphoglycerate mutase 1	0.02	1.63963803
427	Q7LDG7	RAS guanyl-releasing protein 2	0.02	2.20790384
428	O15212	Prefoldin subunit 6	0.02	1.74749768
429	O75874	Isocitrate dehydrogenase [NADP] cytoplasmic	0.02	1.28518849
430	Q14525	Keratin, type I cuticular Ha3-II	0.02	1.61401297
431	P04040	Catalase	0.02	1.26996769
432	Q05707	Collagen alpha-1(XIV) chain	0.02	1.29675275
433	P01743	Immunoglobulin heavy variable 1-46	0.02	2.1440547
434	Q16658	Fascin	0.02	1.32480579
435	O43866	CD5 antigen-like	0.02	2.17135806
436	P01621	IG kappa variable 3-20	0.02	2.15358669
437	P11586	C-1-tetrahydrofolate synthase, cytoplasmic	0.02	1.26730637
438	Q9BVG4	Protein PBDC1	0.02	1.81641439
439	P26641	Elongation factor 1-gamma	0.02	1.68314987
440	Q96C90	Protein phosphatase 1 regulatory subunit 14B	0.02	1.52360231
441	Q5JSH3	WD repeat-containing protein 44	0.02	1.38180388
442	P04424	Argininosuccinate lyase	0.02	1.24021888
443	Q8NE71	ATP-binding cassette sub-family F member 1	0.02	1.48260343
444	P80303	Nucleobindin-2	0.02	1.48201019
445	P00918	Carbonic anhydrase 2	0.02	1.31064458
446	P11940	Polyadenylate-binding protein 1	0.02	1.60898055
447	P07339	Cathepsin D	0.02	1.30719367
448	Q9Y4E8	Ubiquitin carboxyl-terminal hydrolase 15	0.02	1.34549324
449	Q16663	C-C motif chemokine 15	0.02	1.7233837
450	P20742	Pregnancy zone protein	0.02	1.42272707
451	P54886	Delta-1-pyrroline-5-carboxylate synthase	0.02	1.32653133
452	Q92954	Proteoglycan 4	0.02	1.29081077
453	P02776	Platelet factor 4	0.02	1.83227294
454	P06310	Immunoglobulin kappa variable 2-30	0.02	1.93345476
455	P49327	Fatty acid synthase	0.02	1.19644204
456	P00747	Plasminogen	0.02	0.82060533
457	P98179	RNA-binding protein 3	0.02	1.65307788
458	O95678	Keratin, type II cytoskeletal 75	0.03	1.34108458
459	O15067	Phosphoribosylformylglycinamidine synthase	0.03	1.40355619
460	P48444	Coatomer subunit delta	0.03	1.22968325
461	Q15366	Poly(rC)-binding protein 2	0.03	1.43850429
462	Q5JRA6	Melanoma inhibitory activity protein 3	0.03	1.40202469
463	P42574	Caspase-3	0.03	1.46993424
464	Q3LXA3	Triokinase/FMN cyclase	0.03	1.41634594
465	Q13601	KRR1 small subunit processome component homolog	0.03	1.73240887
466	Q9HC38	Glyoxalase domain-containing protein 4	0.03	1.37044682
467	Q9UL25	Ras-related protein Rab-21	0.03	1.27465781
468	P22792	Carboxypeptidase N subunit 2	0.03	0.87249032
469	Q9BQE5	Apolipoprotein L2	0.03	1.4539766
470	O43505	Beta-1,4-glucuronyltransferase 1	0.03	5.3396123
471	P49753	Acyl-coenzyme A thioesterase 2, mitochondrial	0.03	1.93522226
472	P06703	Protein S100-A6	0.03	1.97406836
473	P02749	Beta-2-glycoprotein 1	0.03	1.16418899
474	Q9P260	LisH domain and HEAT repeat-containing protein KIAA1468	0.03	1.99307736
475	Q86WR0	Coiled-coil domain-containing protein 25	0.03	1.82970465
476	Q99729	Heterogeneous nuclear ribonucleoprotein A/B	0.03	1.48869188
477	Q86V81	THO complex subunit 4	0.03	1.78617922
478	P09455	Retinol-binding protein 1	0.03	1.59353156
479	Q9Y5K5	Ubiquitin carboxyl-terminal hydrolase isozyme L5	0.03	1.67359046
480	Q15063	Periostin	0.03	1.47116724

Row	Peak Name	Group	p-value	Fold Change
481	Q92626	Peroxidasin homolog	0.03	1.66745198
482	O60240	Perilipin-1	0.03	1.48483847
483	Q71F56	Mediator of RNA polymerase II transcription subunit 13-like	0.03	1.6505832
484	Q16719	Kynureninase	0.03	1.38440352
485	P36222	Chitinase-3-like protein 1	0.03	1.3373233
486	P08236	Beta-glucuronidase	0.03	1.84171223
487	P02671	Fibrinogen alpha chain	0.03	1.2926318
488	P18124	60S ribosomal protein L7	0.03	1.57525175
489	P13639	Elongation factor 2	0.03	1.36187487
490	Q06323	Proteasome activator complex subunit 1	0.03	1.39181124
491	Q13421	Mesothelin	0.03	1.55310182
492	P05362	Intercellular adhesion molecule 1	0.03	1.4494743
493	P05091	Aldehyde dehydrogenase, mitochondrial	0.03	1.66462751
494	P01011	Alpha-1-antichymotrypsin	0.03	1.19315129
495	Q06830	Peroxiredoxin-1	0.03	1.47866196
496	Q08495	Dematin	0.03	1.38982757
497	P04220	Ig heavy constant mu	0.03	6.38992935
498	P14923	Junction plakoglobin	0.03	1.60971763
499	P18065	Insulin-like growth factor-binding protein 2	0.03	1.41072555
500	P06730	Eukaryotic translation initiation factor 4E	0.03	3.46060463
501	Q9BTL3	RNMT-activating mini protein	0.03	3.98851825
502	O60664	Perilipin-3	0.03	1.466204
503	P29508	Serpin B3	0.03	1.63296994
504	P35527	Keratin, type I cytoskeletal 9	0.03	0.79651342
505	O75351	Vacuolar protein sorting-associated protein 4B	0.03	1.30177936
506	P23368	NAD-dependent malic enzyme, mitochondrial	0.03	1.26076813
507	Q14651	Plastin-1	0.03	1.68492948
508	O75223	Gamma-glutamylcyclotransferase	0.03	1.3773754
509	Q13231	Chitotriosidase-1	0.03	1.46458511
510	O95497	Pantetheinase	0.03	1.97242478
511	A8MPP1	Putative ATP-dependent RNA helicase DDX11-like protein 8	0.03	2.30568714
512	P48047	ATP synthase subunit O, mitochondrial	0.03	2.98664564
513	P50238	Cysteine-rich protein 1	0.03	5.16145292
514	P41226	Ubiquitin-like modifier-activating enzyme 7	0.03	1.31863004
515	Q15631	Translin	0.03	1.48285421
516	P52565	Rho GDP-dissociation inhibitor 1	0.03	1.42519241
517	P61604	10 kDa heat shock protein, mitochondrial	0.03	1.61056173
518	O15145	Actin-related protein 2/3 complex subunit 3	0.03	1.4388828
519	P31327	Carbamoyl-phosphate synthase [ammonia], mitochondrial	0.03	1.24166303
520	P00492	Hypoxanthine-guanine phosphoribosyltransferase	0.03	1.73724649
521	P22234	Multifunctional protein ADE2	0.03	1.22070562
522	P61978	Heterogeneous nuclear ribonucleoprotein K	0.04	1.64168569
523	O75347	Tubulin-specific chaperone A	0.04	1.5453698
524	P01871	Ig mu chain C region	0.04	4.10284808
525	P61457	Pterin-4-alpha-carbinolamine dehydratase	0.04	1.88947769
526	Q08380	Galectin-3-binding protein	0.04	1.28504512
527	P34896	Serine hydroxymethyltransferase, cytosolic	0.04	1.26239504
528	O00584	Ribonuclease T2	0.04	1.46893437
529	P27361	Mitogen-activated protein kinase 3	0.04	1.30865793
530	Q16822	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	0.04	1.41270368
531	Q13576	Ras GTPase-activating-like protein IQGAP2	0.04	1.53568947
532	Q9BWD1	Acetyl-CoA acetyltransferase, cytosolic	0.04	1.5706625
533	Q03252	Lamin-B2	0.04	1.2814089
534	P60520	Gamma-aminobutyric acid receptor-associated protein-like 2	0.04	1.62050124
535	O14773	Tripeptidyl-peptidase 1	0.04	2.31990416
536	P48059	LIM and senescent cell antigen-like-containing domain protein 1	0.04	1.67824877
537	Q8NCW5	NAD(P)H-hydrate epimerase	0.04	1.33939419
538	P25787	Proteasome subunit alpha type-2	0.04	1.63911788
539	P08493	Matrix Gla protein	0.04	2.14975428
540	P40227	T-complex protein 1 subunit zeta	0.04	1.29577069

Row	Peak Name	Group	p-value	Fold Change
541	P17948	Vascular endothelial growth factor receptor 1	0.04	1.7199928
542	O43143	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	0.04	1.28502267
543	P60981	Destrin	0.04	1.38718818
544	P04179	Superoxide dismutase [Mn], mitochondrial	0.04	1.28712849
545	Q14019	Coactosin-like protein	0.04	1.40970749
546	P31949	Protein S100-A11	0.04	1.51409215
547	P36980	Complement factor H-related protein 2	0.04	1.33117077
548	P08123	Collagen alpha-2(I) chain	0.04	1.37924687
549	P04217	Alpha-1B-glycoprotein	0.04	1.12032221
550	P03951	Coagulation factor XI	0.04	1.26765293
551	P05388	60S acidic ribosomal protein P0	0.04	1.59978049
552	P05787	Keratin, type II cytoskeletal 8	0.04	1.36827992
553	P39059	Collagen alpha-1(XV) chain	0.04	1.41819902
554	O43390	Heterogeneous nuclear ribonucleoprotein R	0.04	1.42357906
555	P06331	IGHv4-34	0.04	2.98982146
556	Q9UGM3	Deleted in malignant brain tumors 1 protein	0.04	1.64300574
557	P40926	Malate dehydrogenase, mitochondrial	0.04	1.36100826
558	P49588	Alanine--tRNA ligase, cytoplasmic	0.04	1.18771188
559	P51858	Hepatoma-derived growth factor	0.04	1.3922161
560	Q9GZT8	NIF3-like protein 1	0.04	1.81720856
561	O00391	Sulfhydryl oxidase 1	0.04	1.19620978
562	Q6IBS0	Twinfilin-2	0.04	1.26738472
563	P55209	Nucleosome assembly protein 1-like 1	0.04	1.37663701
564	Q14240	Eukaryotic initiation factor 4A-II	0.04	1.50325158
565	P17813	Endoglin	0.04	1.51346359
566	P60866	40S ribosomal protein S20	0.04	1.32173328
567	P78371	T-complex protein 1 subunit beta	0.04	1.30831137
568	P55786	Puromycin-sensitive aminopeptidase	0.05	1.45568433
569	P09651	Heterogeneous nuclear ribonucleoprotein A1	0.05	1.48406168
570	P11171	Protein 4.1	0.05	1.62382864
571	P84098	60S ribosomal protein L19	0.05	1.64938708
572	Q7Z3U7	Protein MON2 homolog	0.05	1.68644591
573	P02549	Spectrin alpha chain, erythrocytic 1	0.05	1.22604768
574	Q9H910	Hematological and neurological expressed 1-like protein	0.05	1.36537812
575	P61088	Ubiquitin-conjugating enzyme E2 N	0.05	1.62886598
576	Q96JB5	CDK5 regulatory subunit-associated protein 3	0.05	1.81421971
577	P36578	60S ribosomal protein L4	0.05	1.32688683
578	P54819	Adenylate kinase 2, mitochondrial	0.05	0.70019457
579	P05165	Propionyl-CoA carboxylase alpha chain, mitochondrial	0.05	1.25887601
580	O43451	Maltase-glucoamylase, intestinal	0.05	1.49712406
581	P02794	Ferritin heavy chain	0.05	2.9830966
582	Q8N163	Cell cycle and apoptosis regulator protein 2	0.05	1.46839138
583	P09619	Platelet-derived growth factor receptor beta	0.05	2.01902284
584	P50990	T-complex protein 1 subunit theta	0.05	1.36016703
585	P03950	Angiogenin	0.05	1.67209395
586	Q9UKY7	Protein CDV3 homolog	0.05	1.1736699
587	Q9UPT8	Zinc finger CCCH domain-containing protein 4	0.05	1.62952259
588	Q9HCY8	Protein S100-A14	0.05	1.3866531
589	P04003	C4b-binding protein alpha chain	0.05	1.23089306
590	P01591	Immunoglobulin J chain	0.05	2.94689053
591	Q9Y5L4	Mitochondrial import inner membrane translocase subunit Tim13	0.05	1.70614565
592	Q15075	Early endosome antigen 1	0.05	1.25272944
593	Q03154	Aminoacylase-1	0.05	1.81832248
594	P06454	Prothymosin alpha	0.05	13.6927402
595	Q9GZZ8	Extracellular glycoprotein lacritin	0.05	1.79881824
596	P99999	Cytochrome c	0.05	1.342661
597	Q9UK76	Hematological and neurological expressed 1 protein	0.05	1.5842073
598	Q969P0	Immunoglobulin superfamily member 8	0.05	1.36723243
599	P05156	Complement factor I	0.05	1.14732183
600	P01860	Immunoglobulin heavy constant gamma 3	0.05	2.77391458

Row	Peak Name	Group	p-value	Fold Change
601	P35542	Serum amyloid A-4 protein	0.05	0.5870771
602	P84243	Histone H3.3	0.05	2.82992906
603	P07451	Carbonic anhydrase 3	0.05	1.84259356
604	P09382	Galectin-1	0.05	1.29070683
605	Q08554	Desmocollin-1	0.05	1.54588171
606	O14672	Disintegrin and metalloproteinase domain-containing protein 10	0.05	1.45353891
607	P07148	Fatty acid-binding protein, liver	0.05	1.50179789
608	P07919	Cytochrome b-c1 complex subunit 6, mitochondrial	0.06	1.69956293
609	Q9H6X2	Anthrax toxin receptor 1	0.06	1.76847717
610	Q15113	Procollagen C-endopeptidase enhancer 1	0.06	1.36528414
611	P12277	Creatine kinase B-type	0.06	1.33368029
612	Q07954	Prolow-density lipoprotein receptor-related protein 1	0.06	1.21855187
613	Q9BRP8	Partner of Y14 and mago	0.06	1.47476839
614	P26196	Probable ATP-dependent RNA helicase DDX6	0.06	1.23755934
615	P07195	L-lactate dehydrogenase B chain	0.06	1.33220881
616	Q06481	Amyloid-like protein 2	0.06	1.34270288
617	P00325	Alcohol dehydrogenase 1B	0.06	1.79756039
618	P07358	Complement component C8 beta chain	0.06	1.15276932
619	P58546	Myotrophin	0.06	1.44843086
620	Q9UNZ2	NSFL1 cofactor p47	0.06	1.5146896
621	Q8IWV7	E3 ubiquitin-protein ligase UBR1	0.06	1.65240507
622	Q9BRX8	Redox-regulatory protein FAM213A	0.06	1.62545468
623	P02790	Hemopexin	0.06	1.14956461
624	Q9Y3I0	tRNA-splicing ligase RtcB homolog	0.06	1.32645352
625	O15394	Neural cell adhesion molecule 2	0.06	1.47681898
626	P50914	60S ribosomal protein L14	0.06	1.54656262
627	P20700	Lamin-B1	0.06	1.27312029
628	Q13177	Serine/threonine-protein kinase PAK 2	0.06	1.2509507
629	O15269	Serine palmitoyltransferase 1	0.06	1.24350049
630	P04156	Major prion protein	0.06	2.06807919
631	Q9NYL2	Mitogen-activated protein kinase kinase kinase MLT	0.06	1.49169981
632	Q12907	Vesicular integral-membrane protein VIP36	0.06	1.78879796
633	Q08257	Quinone oxidoreductase	0.06	1.29536918
634	Q15428	Splicing factor 3A subunit 2	0.06	1.77636229
635	P08575	Receptor-type tyrosine-protein phosphatase C	0.06	1.27518327
636	P01780	Immunoglobulin heavy variable 3-7	0.06	1.90528612
637	Q99733	Nucleosome assembly protein 1-like 4	0.06	1.30799729
638	O75531	Barrier-to-autointegration factor	0.06	1.54940086
639	O15231	Zinc finger protein 185	0.06	1.28296822
640	O95810	Serum deprivation-response protein	0.06	1.3458429
641	P80188	Neutrophil gelatinase-associated lipocalin	0.06	1.29434487
642	Q93088	Betaine--homocysteine S-methyltransferase 1	0.06	1.27233497
643	P51991	Heterogeneous nuclear ribonucleoprotein A3	0.07	1.76279012
644	Q9Y3C4	EKC/KEOPS complex subunit TPRKB	0.07	1.34809248
645	P17927	Complement receptor type 1	0.07	1.34955691
646	P19823	Inter-alpha-trypsin inhibitor heavy chain H2	0.07	0.88392049
647	P17050	Alpha-N-acetylgalactosaminidase	0.07	1.40669056
648	P01611	Immunoglobulin kappa variable 1D-12	0.07	1.28063523
649	P29401	Transketolase	0.07	1.27038538
650	Q9Y617	Phosphoserine aminotransferase	0.07	1.47845384
651	P01602	Immunoglobulin kappa variable 1-5	0.07	1.64091082
652	P49591	Serine--tRNA ligase, cytoplasmic	0.07	1.3576859
653	O43707	Alpha-actinin-4	0.07	1.28807068
654	P26038	Moesin	0.07	1.30303637
655	P09622	Dihydrolipoyl dehydrogenase, mitochondrial	0.07	1.37190335
656	Q86VB7	Scavenger receptor cysteine-rich type 1 protein M130	0.07	1.2932978
657	P00915	Carbonic anhydrase 1	0.07	1.28652557
658	P04206	Immunoglobulin kappa variable 3-20	0.07	1.36011891
659	P78417	Glutathione S-transferase omega-1	0.07	1.35326686
660	P62750	60S ribosomal protein L23a	0.07	1.23159821

Row	Peak Name	Group	p-value	Fold Change
661	P20618	Proteasome subunit beta type-1	0.07	1.29836364
662	P13667	Protein disulfide-isomerase A4	0.07	1.23017209
663	P54578	Ubiquitin carboxyl-terminal hydrolase 14	0.07	1.56810593
664	P28070	Proteasome subunit beta type-4	0.07	1.5569733
665	P08519	Apolipoprotein(a)	0.07	1.4372874
666	P01764	Immunoglobulin heavy variable 3-23	0.07	1.81258595
667	P46777	60S ribosomal protein L5	0.07	1.2452734
668	Q04695	Keratin, type I cytoskeletal 17	0.07	1.34268134
669	P02655	Apolipoprotein C-II	0.07	0.7310953
670	P00558	Phosphoglycerate kinase 1	0.07	1.28505808
671	P11279	Lysosome-associated membrane glycoprotein 1	0.07	1.28795349
672	P20774	Mimecan	0.07	1.81334967
673	O60294	tRNA wybutosine-synthesizing protein 4	0.07	1.87299689
674	P29966	Myristoylated alanine-rich C-kinase substrate	0.08	1.46386172
675	P53602	Diphosphomevalonate decarboxylase	0.08	1.4098579
676	Q13740	CD166 antigen	0.08	2.83136654
677	Q9UBX5	Fibulin-5	0.08	1.41240121
678	P43121	Cell surface glycoprotein MUC18	0.08	1.41319281
679	Q9Y446	Plakophilin-3	0.08	1.41489356
680	P19013	Keratin, type II cytoskeletal 4	0.08	1.29956486
681	Q86UX7	Fermitin family homolog 3	0.08	1.36557767
682	Q9Y263	Phospholipase A-2-activating protein	0.08	1.4051045
683	Q13103	Secreted phosphoprotein 24	0.08	0.70797077
684	Q6UX71	Plexin domain-containing protein 2	0.08	1.65853615
685	P06748	Nucleophosmin	0.08	1.41402611
686	P36969	Phospholipid hydroperoxide glutathione peroxidase, mitochondrial	0.08	1.32817104
687	P01034	Cystatin-C	0.08	1.27672039
688	P12931	Proto-oncogene tyrosine-protein kinase Src	0.08	1.38901536
689	P16435	NADPH--cytochrome P450 reductase	0.08	1.19087819
690	Q9HBR0	Putative sodium-coupled neutral amino acid transporter 10	0.08	1.47619269
691	P30613	Pyruvate kinase PKLR	0.08	1.53161969
692	P00740	Coagulation factor IX	0.08	1.11106375
693	Q9H3K6	BolA-like protein 2	0.08	1.32826449
694	P01009	Alpha-1-antitrypsin	0.08	1.33999672
695	P08709	Coagulation factor VII	0.08	1.28164435
696	O75122	CLIP-associating protein 2	0.08	1.25064852
697	P48740	Mannan-binding lectin serine protease 1	0.08	1.17570802
698	P02545	Prelamin-A/C	0.09	1.24204688
699	Q15102	Platelet-activating factor acetylhydrolase IB subunit gamma	0.09	1.50665049
700	Q9HCB6	Spondin-1	0.09	1.33344936
701	P05556	Integrin beta-1	0.09	1.25820043
702	P06309	Immunoglobulin kappa variable 2D-28	0.09	1.4614514
703	Q9NYU2	UDP-glucose:glycoprotein glucosyltransferase 1	0.09	1.22173365
704	P67936	Tropomyosin alpha-4 chain	0.09	1.33957217
705	P30044	Peroxiredoxin-5, mitochondrial	0.09	1.37026071
706	P55957	BH3-interacting domain death agonist	0.09	1.37870689
707	P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	0.09	1.17229581
708	P62314	Small nuclear ribonucleoprotein Sm D1	0.09	1.54215486
709	Q96HR3	Mediator of RNA polymerase II transcription subunit 30	0.09	1.71600614
710	Q96FV2	Secernin-2	0.09	1.59871139
711	Q9UBQ7	Glyoxylate reductase/hydroxypyruvate reductase	0.09	1.23578133
712	Q99497	Protein deglycase DJ-1	0.09	1.37813
713	P08637	Low affinity immunoglobulin gamma Fc region receptor III-A	0.09	1.80129617
714	P35232	Prohibitin	0.09	1.29508008
715	P23470	Receptor-type tyrosine-protein phosphatase gamma	0.09	1.93661495
716	P36542	ATP synthase subunit gamma, mitochondrial	0.09	1.27276146
717	Q9Y2T3	Guanine deaminase	0.09	1.57279212
718	O43852	Calumenin	0.09	1.23675465
719	P30050	60S ribosomal protein L12	0.09	1.48967162
720	Q8N423	Leukocyte immunoglobulin-like receptor subfamily B member 2	0.09	1.35922143

Row	Peak Name	Group	p-value	Fold Change
721	Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3	0.09	1.4748922
722	Q14103	Heterogeneous nuclear ribonucleoprotein D0	0.09	1.35864812
723	P28482	Mitogen-activated protein kinase 1	0.10	1.30567026
724	B9A064	Immunoglobulin lambda-like polypeptide 5	0.10	1.71338771
725	P09936	Ubiquitin carboxyl-terminal hydrolase isozyme L1	0.10	1.43255119
726	O75882	Attractin	0.10	1.12689634
727	P07954	Fumarate hydratase, mitochondrial	0.10	1.31657156
728	P04792	Heat shock protein beta-1	0.10	1.57568414
729	Q5TFE4	5'-nucleotidase domain-containing protein 1	0.10	1.37710428
730	P68036	Ubiquitin-conjugating enzyme E2 L3	0.10	1.47857438
731	P78347	General transcription factor II-I	0.10	1.22095421
732	Q7L8L6	FAST kinase domain-containing protein 5, mitochondrial	0.10	0.51446889
733	P49368	T-complex protein 1 subunit gamma	0.10	1.17643465
734	Q86VM9	Zinc finger CCCH domain-containing protein 18	0.10	1.29758564
735	O15264	Mitogen-activated protein kinase 13	0.10	1.32215595
736	P54136	Arginine--tRNA ligase, cytoplasmic	0.10	1.18379496
737	O15031	Plexin-B2	0.10	1.22967979
738	Q9Y244	Proteasome maturation protein	0.10	1.36308851
739	P42765	3-ketoacyl-CoA thiolase, mitochondrial	0.10	1.39985101
740	P49720	Proteasome subunit beta type-3	0.10	1.51383215
741	Q8IZA0	Dyslexia-associated protein KIAA0319-like protein	0.10	1.34679271
742	Q7Z4W1	L-xylulose reductase	0.10	1.45307937
743	P61764	Syntaxin-binding protein 1	0.10	1.4456273
744	Q9UBP6	tRNA (guanine-N(7)-)-methyltransferase	0.10	0.34872599
745	Q86SQ4	Adhesion G-protein coupled receptor G6	0.10	0.60326418
746	Q9Y2H5	Pleckstrin homology domain-containing family A member 6	0.11	1.49887795
747	P04632	Calpain small subunit 1	0.11	1.32539618
748	P30086	Phosphatidylethanolamine-binding protein 1	0.11	1.78520946
749	P25815	Protein S100-P	0.11	1.52918437
750	P17655	Calpain-2 catalytic subunit	0.11	1.20613706
751	P04066	Tissue alpha-L-fucosidase	0.11	0.79771614
752	Q9UJU6	Drebrin-like protein	0.11	1.26068347
753	O75083	WD repeat-containing protein 1	0.11	1.26974083
754	P62820	NA	0.11	1.25174897
755	P32004	Neural cell adhesion molecule L1	0.11	1.39510691
756	Q96CP6	GRAM domain-containing protein 1A	0.11	1.21210769
757	P25789	Proteasome subunit alpha type-4	0.11	1.51440535
758	Q8IZ83	Aldehyde dehydrogenase family 16 member A1	0.11	1.51022453
759	O43169	Cytochrome b5 type B	0.11	1.48117823
760	Q9Y2Z0	Protein SGT1 homolog	0.11	1.21558434
761	Q02809	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	0.11	1.33944486
762	P11766	Alcohol dehydrogenase class-3	0.11	1.30073326
763	Q9UBR2	Cathepsin Z	0.12	1.27545923
764	P50395	Rab GDP dissociation inhibitor beta	0.12	1.40703627
765	P02679	Fibrinogen gamma chain	0.12	1.17666774
766	Q53FA7	Quinone oxidoreductase PIG3	0.12	1.38786391
767	P33151	Cadherin-5	0.12	1.35369932
768	Q13283	Ras GTPase-activating protein-binding protein 1	0.12	1.27422936
769	Q9Y4Y9	U6 snRNA-associated Sm-like protein LSm5	0.12	1.37286964
770	Q99439	Calponin-2	0.12	1.37558535
771	Q13790	Apolipoprotein F	0.12	0.84258893
772	Q16363	Laminin subunit alpha-4	0.12	0.81269446
773	O00410	Importin-5	0.12	1.20437224
774	P16157	Ankyrin-1	0.12	1.52827502
775	P50281	Matrix metalloproteinase-14	0.12	1.33779133
776	P27169	Serum paraoxonase/arylesterase 1	0.12	0.86900068
777	P16930	Fumarylacetoacetase	0.12	1.29573299
778	P08514	Integrin alpha-IIb	0.12	1.42379688
779	Q9UGM5	Fetuin-B	0.12	0.80001025
780	P31025	Lipocalin-1	0.12	1.86011686

Row	Peak Name	Group	p-value	Fold Change
781	P02538	Keratin, type II cytoskeletal 6A	0.12	1.4990336
782	P81605	Dermcidin	0.12	1.27597197
783	Q8NBP7	Proprotein convertase subtilisin/kexin type 9	0.12	1.67067174
784	P23297	Protein S100-A1	0.12	1.58551445
785	P68133	Actin, alpha skeletal muscle	0.12	1.39929221
786	P50213	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	0.12	1.42275519
787	Q14353	Guanidinoacetate N-methyltransferase	0.12	1.41687086
788	P35555	Fibrillin-1	0.12	1.32430522
789	Q12906	Interleukin enhancer-binding factor 3	0.12	1.17660176
790	P52790	Hexokinase-3	0.12	1.56758872
791	P12110	Collagen alpha-2(VI) chain	0.12	1.75208422
792	P19022	Cadherin-2	0.13	1.37832952
793	Q9NY33	Dipeptidyl peptidase 3	0.13	0.53184188
794	O14818	Proteasome subunit alpha type-7	0.13	2.31779314
795	P09543	2',3'-cyclic-nucleotide 3'-phosphodiesterase	0.13	1.22164089
796	Q9BXX0	EMILIN-2	0.13	1.25409189
797	P04433	Immunoglobulin kappa variable 3-11	0.13	1.42670873
798	O00305	Voltage-dependent L-type calcium channel subunit beta-4	0.13	1.4152687
799	P02675	Fibrinogen beta chain	0.13	1.17109074
800	Q6ZVX7	F-box only protein 50	0.13	1.38493296
801	Q86VI3	Ras GTPase-activating-like protein IQGAP3	0.13	1.21863685
802	Q9H008	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	0.13	1.74735567
803	P50452	Serpin B8	0.13	1.6121926
804	Q04446	1,4-alpha-glucan-branching enzyme	0.13	1.28974142
805	Q9P0L0	Vesicle-associated membrane protein-associated protein A	0.13	1.24432734
806	P04259	Keratin, type II cytoskeletal 6B	0.13	1.40878222
807	P07602	Prosaposin	0.13	1.54460985
808	Q15375	Ephrin type-A receptor 7	0.13	1.46657134
809	Q9Y4I1	Unconventional myosin-Va	0.13	1.30610413
810	Q15833	Syntaxin-binding protein 2	0.13	1.17407067
811	P62910	60S ribosomal protein L32	0.13	1.18240741
812	O00592	Podocalyxin	0.13	1.32591264
813	P02787	Serotransferrin	0.14	0.71178191
814	Q8WXI9	Transcriptional repressor p66-beta	0.14	1.33914808
815	P04118	Colipase	0.14	1.41054351
816	Q5QJE6	Deoxynucleotidyltransferase terminal-interacting protein 2	0.14	1.38015934
817	Q9UIF8	Bromodomain adjacent to zinc finger domain protein 2B	0.14	2.95379398
818	Q9Y608	Leucine-rich repeat flightless-interacting protein 2	0.14	1.33591666
819	P41240	Tyrosine-protein kinase CSK	0.14	1.29094505
820	P23229	Integrin alpha-6	0.14	1.34232505
821	P06744	Glucose-6-phosphate isomerase	0.14	1.1876035
822	P15924	Desmoplakin	0.14	1.13130623
823	P06727	Apolipoprotein A-IV	0.14	0.82005432
824	P54289	Voltage-dependent calcium channel subunit alpha-2/delta-1	0.14	1.28979216
825	Q13200	26S proteasome non-ATPase regulatory subunit 2	0.14	1.24853175
826	P16035	Metalloproteinase inhibitor 2	0.14	1.39220856
827	Q15848	Adiponectin	0.14	1.42296167
828	Q14126	Desmoglein-2	0.14	1.27126442
829	Q96BZ4	Phospholipase D4	0.14	1.56780799
830	P07305	Histone H1.0	0.14	0.68576699
831	Q14515	SPARC-like protein 1	0.14	1.62214566
832	O94760	N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	0.14	1.51742645
833	P43251	Biotinidase	0.14	0.72091465
834	Q96FW1	Ubiquitin thioesterase OTUB1	0.14	1.75348058
835	P15104	Glutamine synthetase	0.14	1.44725451
836	P00441	Superoxide dismutase [Cu-Zn]	0.14	1.25778054
837	P13716	Delta-aminolevulinic acid dehydratase	0.15	1.21564773
838	Q9BS26	Endoplasmic reticulum resident protein 44	0.15	1.32025031
839	P13489	Ribonuclease inhibitor	0.15	1.31911698
840	P05160	Coagulation factor XIII B chain	0.15	1.19690683

Row	Peak Name	Group	p-value	Fold Change
841	P22352	Glutathione peroxidase 3	0.15	0.87072474
842	P23284	Peptidyl-prolyl cis-trans isomerase B	0.15	1.20815056
843	Q15370	Transcription elongation factor B polypeptide 2	0.15	1.63929652
844	O75376	Nuclear receptor corepressor 1	0.15	1.31598226
845	Q01995	Transgelin	0.15	1.4003257
846	Q92820	Gamma-glutamyl hydrolase	0.15	1.20181107
847	P27824	Calnexin	0.15	1.25973821
848	P25325	3-mercaptopyruvate sulfurtransferase	0.15	1.36261502
849	Q2PZI1	Probable C-mannosyltransferase DPY19L1	0.15	1.32690375
850	P32942	Intercellular adhesion molecule 3	0.15	1.32817922
851	Q9BTY2	Plasma alpha-L-fucosidase	0.15	1.360549
852	P20810	Calpastatin	0.15	0.58452593
853	P09493	Tropomyosin alpha-1 chain	0.15	1.51604185
854	O75144	ICOS ligand	0.16	0.67088538
855	Q10588	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2	0.16	1.47034091
856	P83731	60S ribosomal protein L24	0.16	1.34015566
857	Q9NZ08	Endoplasmic reticulum aminopeptidase 1	0.16	1.18370499
858	P17174	Aspartate aminotransferase, cytoplasmic	0.16	1.38970713
859	P28062	Proteasome subunit beta type-8	0.16	1.24631618
860	Q96D15	Reticulocalbin-3	0.16	1.29042991
861	P09104	Gamma-enolase	0.16	1.3346003
862	P16671	Platelet glycoprotein 4	0.16	1.22209669
863	O60234	Glia maturation factor gamma	0.16	1.50393964
864	P17931	Galectin-3	0.16	1.26102156
865	P12821	Angiotensin-converting enzyme	0.16	1.29220121
866	Q10471	Polypeptide N-acetylgalactosaminyltransferase 2	0.16	1.19658824
867	P09110	3-ketoacyl-CoA thiolase, peroxisomal	0.16	1.26243714
868	P53667	LIM domain kinase 1	0.16	1.71484649
869	P13591	Neural cell adhesion molecule 1	0.16	1.22851764
870	Q06278	Aldehyde oxidase	0.17	1.40707728
871	Q9BRX5	DNA replication complex GINS protein PSF3	0.17	1.51257777
872	P46939	Utrophin	0.17	1.12608394
873	P34932	Heat shock 70 kDa protein 4	0.17	1.20553068
874	Q96PK6	RNA-binding protein 14	0.17	1.21550453
875	P54760	Ephrin type-B receptor 4	0.17	1.26351334
876	Q02952	A-kinase anchor protein 12	0.17	1.18201363
877	P35908	Keratin, type II cytoskeletal 2 epidermal	0.17	0.8735928
878	P00326	Alcohol dehydrogenase 1C	0.17	1.24696379
879	P31944	Caspase-14	0.17	1.40413991
880	P47756	F-actin-capping protein subunit beta	0.17	1.37886237
881	P02765	Alpha-2-HS-glycoprotein	0.17	1.0975787
882	Q15942	Zyxin	0.17	1.58580563
883	O75475	PC4 and SFRS1-interacting protein	0.17	1.29781103
884	P42330	Aldo-keto reductase family 1 member C3	0.17	2.2540229
885	P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	0.17	1.3465336
886	Q9BRF8	Serine/threonine-protein phosphatase CPPED1	0.17	1.27659179
887	P16949	Stathmin	0.18	2.10327571
888	P31939	Bifunctional purine biosynthesis protein PURH	0.18	1.22164243
889	P30101	Protein disulfide-isomerase A3	0.18	1.14899027
890	P42166	Lamina-associated polypeptide 2, isoform alpha	0.18	1.16083047
891	P11142	Heat shock cognate 71 kDa protein	0.18	1.21796702
892	Q4VC31	Coiled-coil domain-containing protein 58	0.18	1.26954288
893	P04180	Phosphatidylcholine-sterol acyltransferase	0.18	0.87965857
894	P25311	Zinc-alpha-2-glycoprotein	0.18	1.09079465
895	P26639	Threonine--tRNA ligase, cytoplasmic	0.18	1.21667061
896	O43399	NA	0.18	1.34863041
897	P22692	Insulin-like growth factor-binding protein 4	0.18	1.3554942
898	P06865	Beta-hexosaminidase subunit alpha	0.18	1.18231058
899	Q9BY67	Cell adhesion molecule 1	0.18	1.24488485
900	P06753	Tropomyosin alpha-3 chain	0.19	1.26885413

Row	Peak Name	Group	p-value	Fold Change
901	P42680	Tyrosine-protein kinase Tec	0.19	1.65961279
902	Q9UMX5	Neudesin	0.19	1.47876591
903	Q15555	Microtubule-associated protein RP/EB family member 2	0.19	1.3255759
904	P23526	Adenosylhomocysteinase	0.19	1.20014267
905	P00742	Coagulation factor X	0.19	0.8998074
906	P48147	Prolyl endopeptidase	0.20	1.18075342
907	P52888	Thimet oligopeptidase	0.20	1.28909779
908	Q9Y2E5	Epididymis-specific alpha-mannosidase	0.20	1.28649434
909	P15291	Beta-1,4-galactosyltransferase 1	0.20	1.68355985
910	P53634	Dipeptidyl peptidase 1	0.20	0.78049796
911	P06400	Retinoblastoma-associated protein	0.20	1.22778889
912	Q6YHK3	CD109 antigen	0.20	1.46747723
913	O00533	Neural cell adhesion molecule L1-like protein	0.20	1.21812816
914	P21926	CD9 antigen	0.20	1.49370302
915	P05186	Alkaline phosphatase, tissue-nonspecific isozyme	0.20	1.27771651
916	P20851	C4b-binding protein beta chain	0.20	1.14694286
917	Q14314	Fibroleukin	0.20	1.33292214
918	Q8NC51	Plasminogen activator inhibitor 1 RNA-binding protein	0.20	0.7848439
919	P30043	Flavin reductase (NADPH)	0.20	0.86207534
920	P11532	Dystrophin	0.20	1.24000351
921	O95336	6-phosphogluconolactonase	0.21	1.4374501
922	P55290	Cadherin-13	0.21	1.25469545
923	Q9BRK5	45 kDa calcium-binding protein	0.21	1.32574247
924	Q15056	Eukaryotic translation initiation factor 4H	0.21	1.32099579
925	P00751	Complement factor B	0.21	1.12613799
926	Q15121	Astrocytic phosphoprotein PEA-15	0.21	1.30109906
927	Q02818	Nucleobindin-1	0.21	1.26216889
928	P16233	Pancreatic triacylglycerol lipase	0.21	0.66626849
929	P23142	Fibulin-1	0.21	1.14600253
930	P02654	Apolipoprotein C-I	0.21	0.83125917
931	Q13308	Inactive tyrosine-protein kinase 7	0.21	1.36432601
932	P27105	Erythrocyte band 7 integral membrane protein	0.21	1.29183095
933	Q15149	Plectin	0.21	1.11372435
934	Q27J81	Inverted formin-2	0.21	1.18214859
935	A6NL28	NA	0.21	1.58799552
936	P15169	Carboxypeptidase N catalytic chain	0.21	0.85651422
937	P52566	Rho GDP-dissociation inhibitor 2	0.21	0.65477748
938	P62805	Histone H4	0.22	1.36317395
939	Q7Z7G0	Target of Nesh-SH3	0.22	1.30345315
940	P01042	Kininogen-1	0.22	0.91959592
941	Q9H4B7	Tubulin beta-1 chain	0.22	1.30051249
942	Q03591	Complement factor H-related protein 1	0.22	1.24726959
943	Q9UHG3	Prenylcysteine oxidase 1	0.22	1.18988693
944	P22897	Macrophage mannose receptor 1	0.22	1.58846994
945	P00738	Haptoglobin	0.22	1.26258067
946	P26927	Hepatocyte growth factor-like protein	0.22	1.24859823
947	P53779	Mitogen-activated protein kinase 10	0.22	1.38155839
948	P40121	Macrophage-capping protein	0.22	1.44627125
949	P08134	Rho-related GTP-binding protein RhoC	0.22	1.69122597
950	Q8NHP8	Putative phospholipase B-like 2	0.23	1.38463889
951	P26572	Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	0.23	1.26403411
952	P31948	Stress-induced-phosphoprotein 1	0.23	1.15692476
953	P35237	Serpin B6	0.23	1.23666872
954	P07686	Beta-hexosaminidase subunit beta	0.23	1.21224114
955	Q15257	Serine/threonine-protein phosphatase 2A activator	0.23	1.37049223
956	P19367	Hexokinase-1	0.23	1.5055797
957	P49189	4-trimethylaminobutyraldehyde dehydrogenase	0.23	1.81105886
958	P98082	Disabled homolog 2	0.23	1.41527147
959	P00505	Aspartate aminotransferase, mitochondrial	0.24	1.13474522
960	P08397	Porphobilinogen deaminase	0.24	1.26203407

Row	Peak Name	Group	p-value	Fold Change
961	Q13630	GDP-L-fucose synthase	0.24	1.53248741
962	P61247	40S ribosomal protein S3a	0.24	1.18762771
963	P22105	Tenascin-X	0.24	1.09928373
964	P01040	Cystatin-A	0.24	1.28457047
965	P62263	40S ribosomal protein S14	0.24	1.17173002
966	P07711	Cathepsin L1	0.24	1.37134388
967	Q9UQ80	Proliferation-associated protein 2G4	0.24	1.20823331
968	P24158	Myeloblastin	0.24	1.46549503
969	Q13564	NEDD8-activating enzyme E1 regulatory subunit	0.24	1.28654408
970	Q04756	Hepatocyte growth factor activator	0.24	1.08959588
971	P78527	DNA-dependent protein kinase catalytic subunit	0.25	1.10627081
972	P63244	Receptor of activated protein C kinase 1	0.25	1.20526382
973	P19440	Gamma-glutamyltranspeptidase 1	0.25	2.06641718
974	P03952	Plasma kallikrein	0.25	0.91259924
975	Q8N1N4	Keratin, type II cytoskeletal 78	0.25	1.24473266
976	Q9H4A4	Aminopeptidase B	0.25	1.26116355
977	O43598	2'-deoxynucleoside 5'-phosphate N-hydrolase 1	0.25	1.23398784
978	P62633	Cellular nucleic acid-binding protein	0.25	1.32401177
979	Q9Y6G9	Cytoplasmic dynein 1 light intermediate chain 1	0.25	1.44211382
980	Q9UK55	Protein Z-dependent protease inhibitor	0.25	1.09675416
981	Q13825	Methylglutaconyl-CoA hydratase, mitochondrial	0.26	1.261039
982	P07910	Heterogeneous nuclear ribonucleoproteins C1/C2	0.26	1.19419822
983	P25786	Proteasome subunit alpha type-1	0.26	1.28959812
984	P02795	Metallothionein-2	0.26	1.38682006
985	Q92688	Acidic leucine-rich nuclear phosphoprotein 32 family member B	0.26	2.20614772
986	P62993	Growth factor receptor-bound protein 2	0.26	1.13244063
987	P49641	Alpha-mannosidase 2x	0.26	1.32048948
988	O15371	Eukaryotic translation initiation factor 3 subunit D	0.26	1.16321608
989	P01023	Alpha-2-macroglobulin	0.26	1.19697852
990	P18510	Interleukin-1 receptor antagonist protein	0.26	1.34550666
991	P07093	Glia-derived nexin	0.26	1.13278074
992	P60660	Myosin light polypeptide 6	0.26	0.83892886
993	P49773	Histidine triad nucleotide-binding protein 1	0.26	1.52326237
994	Q96Q06	Perilipin-4	0.26	1.3316494
995	P02730	Band 3 anion transport protein	0.27	1.1711094
996	P08648	Integrin alpha-5	0.27	1.22646052
997	P35754	Glutaredoxin-1	0.27	1.50177638
998	Q9NR12	PDZ and LIM domain protein 7	0.27	1.15561642
999	P62899	60S ribosomal protein L31	0.27	1.24801079
1000	Q5KU26	Collectin-12	0.27	1.17784739
1001	O15204	ADAM DEC1	0.28	1.41076234
1002	O00244	Copper transport protein ATOX1	0.28	1.22107779
1003	P06681	Complement C2	0.28	1.07594898
1004	P26640	Valine--tRNA ligase	0.28	1.11708384
1005	P05204	Non-histone chromosomal protein HMG-17	0.28	1.74208153
1006	P26447	Protein S100-A4	0.28	1.40918254
1007	Q53GG5	PDZ and LIM domain protein 3	0.28	1.52850697
1008	P13473	Lysosome-associated membrane glycoprotein 2	0.28	1.18753167
1009	Q9Y5Q8	General transcription factor 3C polypeptide 5	0.28	1.22077726
1010	P14543	Nidogen-1	0.28	0.59370112
1011	P11717	Cation-independent mannose-6-phosphate receptor	0.28	1.13950136
1012	Q76LX8	A disintegrin and metalloproteinase with thrombospondin motifs 13	0.28	2.94769625
1013	P21399	Cytoplasmic aconitate hydratase	0.28	1.26492308
1014	P05164	Myeloperoxidase	0.28	1.17504946
1015	P08294	Extracellular superoxide dismutase [Cu-Zn]	0.28	0.7781225
1016	P17936	Insulin-like growth factor-binding protein 3	0.29	0.90300199
1017	P07476	Involucrin	0.29	0.80735
1018	Q9HC84	Mucin-5B	0.29	1.2763872
1019	P62258	14-3-3 protein epsilon	0.29	1.16238912
1020	Q05682	Caldesmon	0.29	1.21196999

Row	Peak Name	Group	p-value	Fold Change
1021	P62424	60S ribosomal protein L7a	0.29	1.34731656
1022	P49643	DNA primase large subunit	0.29	1.17437588
1023	Q16851	UTP--glucose-1-phosphate uridylyltransferase	0.29	1.25594001
1024	Q9Y624	Junctional adhesion molecule A	0.29	1.93259792
1025	P08572	Collagen alpha-2(IV) chain	0.29	1.2788
1026	P52907	F-actin-capping protein subunit alpha-1	0.29	1.43734452
1027	Q9NZD4	Alpha-hemoglobin-stabilizing protein	0.29	1.6748403
1028	P09497	Clathrin light chain B	0.30	0.75859359
1029	P46783	40S ribosomal protein S10	0.30	1.2414097
1030	Q14508	WAP four-disulfide core domain protein 2	0.30	1.22397865
1031	Q6PKG0	La-related protein 1	0.30	0.91010508
1032	Q16819	Meprin A subunit alpha	0.30	1.57150189
1033	P07357	Complement component C8 alpha chain	0.30	1.10315797
1034	P46781	40S ribosomal protein S9	0.30	1.18543838
1035	P80511	Protein S100-A12	0.30	1.31883016
1036	P30040	Endoplasmic reticulum resident protein 29	0.31	1.15049063
1037	Q6PI48	Aspartate--tRNA ligase, mitochondrial	0.31	1.1681601
1038	P27487	Dipeptidyl peptidase 4	0.31	1.14248088
1039	O43396	Thioredoxin-like protein 1	0.32	1.40445031
1040	P08581	Hepatocyte growth factor receptor	0.32	1.19614356
1041	Q9Y646	Carboxypeptidase Q	0.32	1.14084465
1042	Q16610	Extracellular matrix protein 1	0.32	1.10218376
1043	P04114	Apolipoprotein B-100	0.32	0.93727441
1044	Q9NP97	Dynein light chain roadblock-type 1	0.32	1.30472956
1045	P07900	Heat shock protein HSP 90-alpha	0.33	1.16276279
1046	P16070	CD44 antigen	0.33	1.10076419
1047	P54727	UV excision repair protein RAD23 homolog B	0.33	1.20626169
1048	Q9UBG0	C-type mannose receptor 2	0.33	1.17924094
1049	Q86YZ3	Hornerin	0.33	1.34260663
1050	Q5D862	Filaggrin-2	0.33	1.49265754
1051	Q9HBI1	Beta-parvin	0.33	1.19932972
1052	Q15904	V-type proton ATPase subunit S1	0.33	1.51419846
1053	P05019	Insulin-like growth factor I	0.33	0.81941265
1054	P15328	Folate receptor alpha	0.33	1.61946803
1055	P12081	Histidine--tRNA ligase, cytoplasmic	0.33	1.24012091
1056	P00533	Epidermal growth factor receptor	0.33	1.23777558
1057	Q04760	Lactoylglutathione lyase	0.33	1.19548878
1058	P04054	Phospholipase A2	0.33	1.25663219
1059	P30679	Guanine nucleotide-binding protein subunit alpha-15	0.33	1.21976536
1060	Q10472	Polypeptide N-acetylgalactosaminyltransferase 1	0.33	1.12685952
1061	Q01581	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	0.34	1.18161222
1062	Q99969	Retinoic acid receptor responder protein 2	0.34	1.16624771
1063	Q01130	Serine/arginine-rich splicing factor 2	0.34	1.19769466
1064	Q6UWP8	Suprabasin	0.34	0.48385079
1065	P46926	Glucosamine-6-phosphate isomerase 1	0.34	1.13780617
1066	P26599	Polypyrimidine tract-binding protein 1	0.34	0.90227914
1067	P02743	Serum amyloid P-component	0.34	1.14536235
1068	P05387	60S acidic ribosomal protein P2	0.35	1.2606916
1069	P30405	Peptidyl-prolyl cis-trans isomerase F, mitochondrial	0.35	1.22032541
1070	P00966	Argininosuccinate synthase	0.35	1.2220819
1071	O75636	Ficolin-3	0.35	1.11316683
1072	Q05639	Elongation factor 1-alpha 2	0.35	1.14273054
1073	O95817	BAG family molecular chaperone regulator 3	0.35	0.79914817
1074	P16403	Histone H1.2	0.35	1.28963126
1075	P27348	14-3-3 protein theta	0.35	1.19686564
1076	P02649	Apolipoprotein E	0.35	0.92446686
1077	O15400	Syntaxin-7	0.35	0.79099719
1078	P13521	Secretogranin-2	0.36	1.16016807
1079	P06132	Uroporphyrinogen decarboxylase	0.36	1.28894221
1080	P21283	V-type proton ATPase subunit C 1	0.36	1.1680537

Row	Peak Name	Group	p-value	Fold Change
1081	Q13443	Disintegrin and metalloproteinase domain-containing protein 9	0.36	1.18698869
1082	P35052	Glypican-1	0.36	1.15567577
1083	P24928	DNA-directed RNA polymerase II subunit RPB1	0.36	0.8531162
1084	Q9HAT2	Sialate O-acetylesterase	0.36	1.1680588
1085	P28799	Granulins	0.36	1.35136448
1086	P09486	SPARC	0.36	0.76636844
1087	P39687	Acidic leucine-rich nuclear phosphoprotein 32 family member A	0.37	0.82307207
1088	Q9NZT1	Calmodulin-like protein 5	0.37	0.67005565
1089	Q07654	Trefoil factor 3	0.37	1.25814488
1090	O75832	26S proteasome non-ATPase regulatory subunit 10	0.37	0.12398223
1091	P80723	Brain acid soluble protein 1	0.37	1.33018025
1092	P01880	Ig delta chain C region	0.37	1.18879986
1093	P13688	Carcinoembryonic antigen-related cell adhesion molecule 1	0.38	1.3466987
1094	P35637	RNA-binding protein FUS	0.38	1.10245265
1095	O43639	Cytoplasmic protein NCK2	0.38	1.24870382
1096	P53597	Succinate--CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	0.38	1.1319942
1097	Q13185	Chromobox protein homolog 3	0.38	0.84356713
1098	Q9P1Y5	Calmodulin-regulated spectrin-associated protein 3	0.38	1.14254636
1099	P02774	Vitamin D-binding protein	0.38	1.05926698
1100	P00748	Coagulation factor XII	0.39	1.13607289
1101	Q10589	Bone marrow stromal antigen 2	0.39	1.40580078
1102	P04196	Histidine-rich glycoprotein	0.39	0.92250659
1103	P49593	Protein phosphatase 1F	0.39	1.17891439
1104	Q8TAD4	Zinc transporter 5	0.39	1.17967402
1105	P31997	Carcinoembryonic antigen-related cell adhesion molecule 8	0.39	0.83728274
1106	P02656	Apolipoprotein C-III	0.39	0.87217265
1107	Q99832	T-complex protein 1 subunit eta	0.40	1.115931
1108	P05452	Tetranectin	0.40	1.05780794
1109	Q9P2T1	GMP reductase 2	0.40	1.25394286
1110	Q969E8	Pre-rRNA-processing protein TSR2 homolog	0.40	1.26650952
1111	P41439	Folate receptor gamma	0.40	0.85748762
1112	Q96DA0	Zymogen granule protein 16 homolog B	0.40	0.77085903
1113	Q9UJC5	SH3 domain-binding glutamic acid-rich-like protein 2	0.40	1.17876146
1114	Q9BRA2	Thioredoxin domain-containing protein 17	0.40	1.17356104
1115	Q14520	Hyaluronan-binding protein 2	0.40	1.10449239
1116	P09960	Leukotriene A-4 hydrolase	0.40	1.13257488
1117	P01889	HLA class I histocompatibility antigen, B-7 alpha chain	0.41	1.22564829
1118	P23396	40S ribosomal protein S3	0.41	1.10573563
1119	Q6EMK4	Vasorin	0.41	1.08711546
1120	P61106	Ras-related protein Rab-14	0.41	1.16140009
1121	Q99627	COP9 signalosome complex subunit 8	0.41	0.83577494
1122	P21810	Biglycan	0.41	0.91244381
1123	P31937	3-hydroxyisobutyrate dehydrogenase, mitochondrial	0.41	1.15046931
1124	Q96G03	Phosphoglucomutase-2	0.41	1.1595146
1125	O95831	Apoptosis-inducing factor 1, mitochondrial	0.41	1.12868792
1126	O14907	Tax1-binding protein 3	0.41	0.35005347
1127	Q13867	Bleomycin hydrolase	0.41	1.2027304
1128	P14927	Cytochrome b-c1 complex subunit 7	0.41	1.22341229
1129	Q8N6Q3	CD177 antigen	0.41	0.28619317
1130	Q13557	Calcium/calmodulin-dependent protein kinase type II subunit delta	0.42	1.0985406
1131	Q9NZK5	Adenosine deaminase CECR1	0.42	0.80717185
1132	P30520	Adenylosuccinate synthetase isozyme 2	0.43	1.16273474
1133	O00233	26S proteasome non-ATPase regulatory subunit 9	0.43	1.32149041
1134	P13797	Plastin-3	0.43	1.14452288
1135	P50552	Vasodilator-stimulated phosphoprotein	0.43	1.17252236
1136	P36955	Pigment epithelium-derived factor	0.44	1.04781861
1137	P13598	Intercellular adhesion molecule 2	0.44	1.15123002
1138	Q7L1Q6	Basic leucine zipper and W2 domain-containing protein 1	0.44	1.13858706
1139	P02452	Collagen alpha-1(I) chain	0.44	1.15802212
1140	P37837	Transaldolase	0.44	1.09387794

Row	Peak Name	Group	p-value	Fold Change
1141	P27635	60S ribosomal protein L10	0.44	1.24581383
1142	Q15717	ELAV-like protein 1	0.44	0.70665287
1143	P46109	Crk-like protein	0.44	1.65360808
1144	Q9P1F3	Costars family protein ABRACL	0.44	1.4555348
1145	Q9Y6C2	EMILIN-1	0.45	1.1620145
1146	P48506	Glutamate--cysteine ligase catalytic subunit	0.45	1.12763817
1147	P50750	Cyclin-dependent kinase 9	0.45	1.27157741
1148	Q14315	Filamin-C	0.46	1.10601813
1149	P02753	Retinol-binding protein 4	0.47	0.92866253
1150	O75970	Multiple PDZ domain protein	0.47	1.28735694
1151	P23141	Liver carboxylesterase 1	0.47	1.23779482
1152	P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	0.47	0.86491533
1153	Q13884	Beta-1-syntrophin	0.47	1.22652166
1154	Q92686	Neurogranin	0.47	1.28328195
1155	P27918	Properdin	0.47	0.91345656
1156	P49736	DNA replication licensing factor MCM2	0.48	0.91290528
1157	P09417	Dihydropteridine reductase	0.48	1.15131495
1158	Q9NTX5	Ethylmalonyl-CoA decarboxylase	0.48	0.84378424
1159	P19957	Elafin	0.48	1.27179124
1160	P00739	Haptoglobin-related protein	0.48	0.88692568
1161	P08311	Cathepsin G	0.48	1.0806299
1162	Q99683	Mitogen-activated protein kinase kinase kinase 5	0.48	1.08460363
1163	P05106	Integrin beta-3	0.48	1.12702432
1164	P35900	Keratin, type I cytoskeletal 20	0.49	0.89760886
1165	Q9P0Z9	Peroxisomal sarcosine oxidase	0.49	1.17213717
1166	P62158	Calmodulin	0.49	1.15260298
1167	P07359	Platelet glycoprotein Ib alpha chain	0.49	0.92206253
1168	P30530	Tyrosine-protein kinase receptor UFO	0.49	1.12980891
1169	Q9BR76	Coronin-1B	0.50	1.11721596
1170	P62906	60S ribosomal protein L10a	0.50	1.14061521
1171	Q9BQ52	Zinc phosphodiesterase ELAC protein 2	0.50	1.14032733
1172	P20908	Collagen alpha-1(V) chain	0.50	1.18492748
1173	P55145	Mesencephalic astrocyte-derived neurotrophic factor	0.50	1.10734024
1174	P05121	Plasminogen activator inhibitor 1	0.50	0.86546963
1175	Q9NQ79	Cartilage acidic protein 1	0.51	1.09238751
1176	P19827	Inter-alpha-trypsin inhibitor heavy chain H1	0.51	0.9494489
1177	Q96BY6	Dedicator of cytokinesis protein 10	0.51	0.88616172
1178	Q7Z7M0	Multiple epidermal growth factor-like domains protein 8	0.51	1.21291202
1179	P17661	Desmin	0.51	0.92832467
1180	P62081	40S ribosomal protein S7	0.51	0.85703032
1181	P62269	40S ribosomal protein S18	0.52	1.19598685
1182	Q9Y3D6	Mitochondrial fission 1 protein	0.52	0.86438419
1183	P04083	Annexin A1	0.52	1.08288169
1184	P62280	40S ribosomal protein S11	0.52	1.21978828
1185	P31146	Coronin-1A	0.52	1.14064732
1186	Q14247	Src substrate cortactin	0.53	0.89167898
1187	P00734	Prothrombin	0.53	0.96660178
1188	P02751	Fibronectin	0.53	0.81664509
1189	P08727	Keratin, type I cytoskeletal 19	0.53	1.07506135
1190	Q9Y5K8	V-type proton ATPase subunit D	0.53	1.14606903
1191	P07585	Decorin	0.53	1.27198436
1192	P35659	Protein DEK	0.53	1.24999581
1193	Q07820	Induced myeloid leukemia cell differentiation protein Mcl-1	0.53	1.25345058
1194	[RT-Cal prote	Retention time calibration protein	0.53	0.95046426
1195	P62857	40S ribosomal protein S28	0.53	1.26475494
1196	Q9Y613	FH1/FH2 domain-containing protein 1	0.54	1.08479153
1197	P05089	Arginase-1	0.54	1.16166143
1198	P27797	Calreticulin	0.54	1.09022779
1199	Q969H8	Myeloid-derived growth factor	0.54	1.26371334
1200	Q99784	Noelin	0.54	1.14923024

Row	Peak Name	Group	p-value	Fold Change
1201	P30048	Thioredoxin-dependent peroxide reductase, mitochondrial	0.54	1.117318793
1202	P50991	T-complex protein 1 subunit delta	0.54	0.897546103
1203	O14618	Copper chaperone for superoxide dismutase	0.54	0.851774014
1204	P01031	Complement C5	0.54	1.032629566
1205	Q14767	Latent-transforming growth factor beta-binding protein 2	0.54	0.631647967
1206	P25705	ATP synthase subunit alpha, mitochondrial	0.55	1.186949276
1207	P68366	Tubulin alpha-4A chain	0.55	1.159706315
1208	P24821	Tenascin	0.55	1.06935679
1209	Q14974	Importin subunit beta-1	0.55	1.073743414
1210	P09429	High mobility group protein B1	0.55	1.105638878
1211	P62241	40S ribosomal protein S8	0.56	1.193602857
1212	Q92496	Complement factor H-related protein 4	0.56	1.118827229
1213	P42126	Enoyl-CoA delta isomerase 1, mitochondrial	0.56	1.10235189
1214	O14791	Apolipoprotein L1	0.57	0.909554663
1215	O15143	Actin-related protein 2/3 complex subunit 1B	0.57	1.08526464
1216	Q9H479	Fructosamine-3-kinase	0.57	0.748103241
1217	Q14289	Protein-tyrosine kinase 2-beta	0.58	1.057419195
1218	Q14012	Calcium/calmodulin-dependent protein kinase type 1	0.59	0.905285158
1219	Q5T013	Putative hydroxypyruvate isomerase	0.59	1.19268924
1220	Q9Y2B0	Protein canopy homolog 2	0.59	1.146401679
1221	Q6UX04	Peptidyl-prolyl cis-trans isomerase CWC27 homolog	0.59	1.109287983
1222	P62244	40S ribosomal protein S15a	0.59	1.096757606
1223	P13796	Plastin-2	0.60	1.050420811
1224	P51570	Galactokinase	0.60	1.196714915
1225	P35611	Alpha-adducin	0.60	1.095370538
1226	Q14847	LIM and SH3 domain protein 1	0.60	1.13594057
1227	Q9UHG2	ProSAAS	0.60	0.615716263
1228	Q9H1R3	Myosin light chain kinase 2, skeletal/cardiac muscle	0.60	1.129675022
1229	O94985	Calsyntenin-1	0.60	1.115231328
1230	Q04917	14-3-3 protein eta	0.60	0.854602762
1231	P36871	Phosphoglucomutase-1	0.60	1.084051398
1232	Q9NPH3	Interleukin-1 receptor accessory protein	0.61	1.052249388
1233	P02814	Submaxillary gland androgen-regulated protein 3B	0.61	1.149413345
1234	Q15223	Nectin-1	0.61	0.865697447
1235	P49247	Ribose-5-phosphate isomerase	0.62	1.109918852
1236	O00203	AP-3 complex subunit beta-1	0.62	1.18567444
1237	P11166	Solute carrier family 2, facilitated glucose transporter member 1	0.63	1.099630441
1238	Q0PNE2	Elongator complex protein 6	0.63	1.108064806
1239	O95633	Follistatin-related protein 3	0.63	0.868095911
1240	Q9UBW5	Bridging integrator 2	0.63	1.113507923
1241	P08133	Annexin A6	0.64	0.91338975
1242	P41567	Eukaryotic translation initiation factor 1	0.64	1.199833864
1243	P12830	Cadherin-1	0.64	0.898713872
1244	Q15843	NEDD8	0.64	1.081386404
1245	P40925	Malate dehydrogenase, cytoplasmic	0.65	1.112044679
1246	P10412	Histone H1.4	0.65	1.186201853
1247	P60900	Proteasome subunit alpha type-6	0.65	0.908625225
1248	P16109	P-selectin	0.65	0.806593363
1249	Q9NX63	MICOS complex subunit MIC19	0.65	0.877298218
1250	O60888	Protein CutA	0.65	1.091352968
1251	P08185	Corticosteroid-binding globulin	0.65	0.968175046
1252	P12109	Collagen alpha-1(VI) chain	0.65	0.956141647
1253	P13647	Keratin, type II cytoskeletal 5	0.65	1.069165306
1254	Q8WZ74	Cortactin-binding protein 2	0.65	1.123545952
1255	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	0.66	1.028033672
1256	Q5TH69	Brefeldin A-inhibited guanine nucleotide-exchange protein 3	0.66	0.812379392
1257	P62249	40S ribosomal protein S16	0.66	1.152364637
1258	P03973	Antileukoproteinase	0.66	1.103278856
1259	P02768	Serum albumin	0.66	0.894898614
1260	P18827	Syndecan-1	0.66	1.119945907

Row	Peak Name	Group	p-value	Fold Change
1261	Q9BYE9	Cadherin-related family member 2	0.67	0.90638789
1262	P80404	4-aminobutyrate aminotransferase, mitochondrial	0.67	1.07642719
1263	Q02487	Desmocollin-2	0.67	1.07637936
1264	Q93099	Homogentisate 1,2-dioxygenase	0.67	1.15210352
1265	O60462	Neuropilin-2	0.68	1.26799616
1266	P10586	Receptor-type tyrosine-protein phosphatase F	0.68	0.92498024
1267	Q15828	Cystatin-M	0.68	1.09415829
1268	P10909	Clusterin	0.68	1.02693889
1269	Q9UNW1	Multiple inositol polyphosphate phosphatase 1	0.68	1.0880514
1270	Q9HAB8	Phosphopantothenate--cysteine ligase	0.69	1.12074578
1271	P53396	ATP-citrate synthase	0.69	0.80487804
1272	P23083	Immunoglobulin heavy variable 1-2	0.69	0.92777149
1273	Q6P9B6	TLD domain-containing protein 1	0.69	0.90457527
1274	P11413	Glucose-6-phosphate 1-dehydrogenase	0.69	1.09068431
1275	Q9Y6U3	Adseverin	0.69	1.06471508
1276	Q9UBP9	PTB domain-containing engulfment adapter protein 1	0.70	0.88111861
1277	Q92673	Sortilin-related receptor	0.70	1.07302612
1278	P24666	Low molecular weight phosphotyrosine protein phosphatase	0.70	0.92739301
1279	A5YKK6	CCR4-NOT transcription complex subunit 1	0.70	1.05369553
1280	Q15691	Microtubule-associated protein RP/EB family member 1	0.71	1.04926762
1281	Q93063	Exostosin-2	0.71	0.91934233
1282	Q9NUQ6	SPATS2-like protein	0.71	1.06917757
1283	P20674	Cytochrome c oxidase subunit 5A, mitochondrial	0.72	1.07726762
1284	P28074	Proteasome subunit beta type-5	0.72	1.07727707
1285	P30085	UMP-CMP kinase	0.72	0.94869836
1286	P54652	Heat shock-related 70 kDa protein 2	0.72	1.06764337
1287	O00468	Agrin	0.72	1.05056979
1288	P49908	Selenoprotein P	0.72	0.95751493
1289	Q9H4A9	Dipeptidase 2	0.73	1.11583432
1290	P49005	DNA polymerase delta subunit 2	0.73	1.0503018
1291	P53778	Mitogen-activated protein kinase 12	0.73	1.06627299
1292	P43487	Ran-specific GTPase-activating protein	0.74	0.93275486
1293	P0C0L4	Complement C4-A	0.74	0.96946382
1294	P10155	60 kDa SS-A/Ro ribonucleoprotein	0.74	1.05213855
1295	O00461	Golgi integral membrane protein 4	0.74	1.10391982
1296	P35268	60S ribosomal protein L22	0.75	0.94240826
1297	P28838	Cytosol aminopeptidase	0.75	0.96030431
1298	Q96IY4	Carboxypeptidase B2	0.75	0.98095761
1299	Q9Y3B2	Exosome complex component CSL4	0.75	0.93770547
1300	Q8NBJ4	Golgi membrane protein 1	0.75	0.93378225
1301	Q00688	Peptidyl-prolyl cis-trans isomerase FKBP3	0.75	1.07739866
1302	P61981	14-3-3 protein gamma	0.75	1.05315264
1303	Q9UDT6	CAP-Gly domain-containing linker protein 2	0.75	1.08927388
1304	P31943	Heterogeneous nuclear ribonucleoprotein H	0.75	1.0463125
1305	P40763	Signal transducer and activator of transcription 3	0.76	1.05712858
1306	P14384	Carboxypeptidase M	0.76	0.94035618
1307	P82979	SAP domain-containing ribonucleoprotein	0.76	1.05637464
1308	Q15029	116 kDa U5 small nuclear ribonucleoprotein component	0.76	0.96790341
1309	Q13442	28 kDa heat- and acid-stable phosphoprotein	0.76	1.04470271
1310	P62942	Peptidyl-prolyl cis-trans isomerase FKBP1A	0.76	0.91941469
1311	Q9NNW7	Thioredoxin reductase 2, mitochondrial	0.76	1.11591376
1312	P05154	Plasma serine protease inhibitor	0.76	1.03175318
1313	P14151	L-selectin	0.76	1.04360943
1314	Q5SSJ5	Heterochromatin protein 1-binding protein 3	0.76	0.95219294
1315	O00429	Dynamin-1-like protein	0.76	0.95581859
1316	Q9Y2S2	Lambda-crystallin homolog	0.76	1.04688213
1317	Q15369	Transcription elongation factor B polypeptide 1	0.76	1.06124539
1318	P62913	60S ribosomal protein L11	0.77	1.04640188
1319	P15151	Poliovirus receptor	0.77	0.95244342
1320	P19971	Thymidine phosphorylase	0.78	1.07211735

Row	Peak Name	Group	p-value	Fold Change
1321	P45974	Ubiquitin carboxyl-terminal hydrolase 5	0.78	1.06681269
1322	Q9BYX7	Putative beta-actin-like protein 3	0.78	1.09399711
1323	P10153	Non-secretory ribonuclease	0.78	1.05486992
1324	P08603	Complement factor H	0.78	1.02228946
1325	Q00839	Heterogeneous nuclear ribonucleoprotein U	0.78	1.09142303
1326	Q16531	DNA damage-binding protein 1	0.78	1.04025282
1327	Q92859	Neogenin	0.78	0.93253461
1328	P63279	SUMO-conjugating enzyme UBC9	0.78	1.04330457
1329	P12955	Xaa-Pro dipeptidase	0.78	1.04132511
1330	Q14690	Protein RRP5 homolog	0.79	1.07818246
1331	P22891	Vitamin K-dependent protein Z	0.79	0.9641095
1332	P01019	Angiotensinogen	0.79	1.02511555
1333	P12532	Creatine kinase U-type, mitochondrial	0.79	1.04324419
1334	P18621	60S ribosomal protein L17	0.79	1.04462162
1335	O15240	Neurosecretory protein VGF	0.79	1.10974484
1336	P34913	Bifunctional epoxide hydrolase 2	0.79	1.05710831
1337	P60983	Glia maturation factor beta	0.79	1.0999946
1338	P04070	Vitamin K-dependent protein C	0.79	1.02579993
1339	Q14112	Nidogen-2	0.79	0.94257762
1340	Q9Y230	RuvB-like 2	0.80	1.17173377
1341	P21291	Cysteine and glycine-rich protein 1	0.80	1.09518241
1342	Q14766	Latent-transforming growth factor beta-binding protein 1	0.80	0.96519215
1343	Q5ZPR3	CD276 antigen	0.80	1.07875254
1344	Q9UBF2	Coatomer subunit gamma-2	0.80	0.95482894
1345	P02461	Collagen alpha-1(III) chain	0.81	0.9319815
1346	P61313	60S ribosomal protein L15	0.81	1.04060391
1347	O00151	PDZ and LIM domain protein 1	0.81	1.06848138
1348	Q9BXN1	Asporin	0.81	1.04940678
1349	A0PJW6	Transmembrane protein 223	0.81	0.95057544
1350	Q9BXS5	AP-1 complex subunit mu-1	0.82	1.02688586
1351	P06732	Creatine kinase M-type	0.82	1.04221095
1352	Q9NZN3	EH domain-containing protein 3	0.82	1.05685944
1353	P08238	Heat shock protein HSP 90-beta	0.82	1.03826266
1354	P14314	Glucosidase 2 subunit beta	0.82	1.0214911
1355	Q96RQ1	Endoplasmic reticulum-Golgi intermediate compartment protein 2	0.82	0.93740403
1356	Q13418	Integrin-linked protein kinase	0.83	0.94166984
1357	O75368	SH3 domain-binding glutamic acid-rich-like protein	0.83	0.93823688
1358	P07360	Complement component C8 gamma chain	0.83	1.0142577
1359	Q9UKV8	Protein argonaute-2	0.84	1.0354466
1360	P04211	Immunoglobulin lambda variable 7-43	0.84	0.90909305
1361	P19652	Alpha-1-acid glycoprotein 2	0.84	1.02333071
1362	P62851	40S ribosomal protein S25	0.84	0.90762193
1363	P67809	Nuclease-sensitive element-binding protein 1	0.84	0.9226196
1364	Q02880	DNA topoisomerase 2-beta	0.84	0.97106259
1365	P43652	Afamin	0.84	1.01588512
1366	Q01469	Fatty acid-binding protein, epidermal	0.84	0.931747
1367	P16401	Histone H1.5	0.85	1.050597
1368	P09172	Dopamine beta-hydroxylase	0.85	0.97977291
1369	P01344	Insulin-like growth factor II	0.85	1.02023596
1370	Q6IQ49	Protein SDE2 homolog	0.86	1.04990247
1371	P61224	Ras-related protein Rap-1b	0.86	0.95725391
1372	P63313	Thymosin beta-10	0.86	1.1137774
1373	P08670	Vimentin	0.86	1.02234385
1374	Q9UNN8	Endothelial protein C receptor	0.86	1.04681181
1375	O75348	V-type proton ATPase subunit G 1	0.87	0.97895835
1376	P46782	40S ribosomal protein S5	0.87	1.10676481
1377	Q9ULA0	Aspartyl aminopeptidase	0.87	0.96712686
1378	Q13201	Multimerin-1	0.88	0.96549834
1379	P49721	Proteasome subunit beta type-2	0.88	0.96806122
1380	O43583	Density-regulated protein	0.88	0.95055002

Row	Peak Name	Group	p-value	Fold Change
1381	Q9BWP8	Collectin-11	0.88	0.96853415
1382	P51452	Dual specificity protein phosphatase 3	0.88	1.02943116
1383	Q9Y2X3	Nucleolar protein 58	0.88	1.03139517
1384	Q9BV36	Melanophilin	0.88	0.97829399
1385	P35520	Cystathionine beta-synthase	0.88	0.97786827
1386	P13646	Keratin, type I cytoskeletal 13	0.88	1.02687874
1387	P06276	Cholinesterase	0.89	1.0111515
1388	P62826	GTP-binding nuclear protein Ran	0.89	0.96583779
1389	P39019	40S ribosomal protein S19	0.89	1.0199926
1390	P46060	Ran GTPase-activating protein 1	0.89	0.97443292
1391	P11277	Spectrin beta chain, erythrocytic	0.89	1.01812802
1392	Q16643	Drebrin	0.90	0.98481654
1393	P09668	Pro-cathepsin H	0.90	0.97319718
1394	P05546	Heparin cofactor 2	0.91	0.99209169
1395	O75380	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial	0.91	1.01630476
1396	P05155	Plasma protease C1 inhibitor	0.92	1.00791269
1397	P07108	Acyl-CoA-binding protein	0.92	0.97775078
1398	P01767	Immunoglobulin heavy variable 3-53	0.92	1.0339914
1399	Q12860	Contactin-1	0.92	0.9721696
1400	Q9NTK5	Obg-like ATPase 1	0.92	1.01781761
1401	P16870	Carboxypeptidase E	0.92	1.02534323
1402	P16284	Platelet endothelial cell adhesion molecule	0.92	0.97453335
1403	P33908	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	0.93	0.9838285
1404	Q13642	Four and a half LIM domains protein 1	0.94	0.98870917
1405	P0CG05	Ig lambda-2 chain C regions	0.94	1.01984359
1406	Q8IXQ3	Uncharacterized protein C9orf40	0.95	0.95896787
1407	Q99459	Cell division cycle 5-like protein	0.95	0.99069724
1408	P39023	60S ribosomal protein L3	0.95	1.01314406
1409	P31947	14-3-3 protein sigma	0.95	0.99041986
1410	P27695	DNA-(apurinic or apyrimidinic site) lyase	0.95	1.00997099
1411	Q02413	Desmoglein-1	0.95	0.99242197
1412	Q96T51	RUN and FYVE domain-containing protein 1	0.96	0.9924836
1413	P34096	NRibonuclease 4	0.96	1.03411328
1414	P17900	Ganglioside GM2 activator	0.96	0.98392873
1415	P07225	Vitamin K-dependent protein S	0.97	0.99711173
1416	Q9P2E9	Ribosome-binding protein 1	0.97	1.00565689
1417	O94776	Metastasis-associated protein MTA2	0.97	1.00578632
1418	O15230	Laminin subunit alpha-5	0.98	0.99683391
1419	P50895	Basal cell adhesion molecule	0.98	1.00530055
1420	Q9NP79	Vacuolar protein sorting-associated protein VTA1 homolog	0.98	0.99358341
1421	P07384	Calpain-1 catalytic subunit	0.98	0.99689744
1422	P04004	Vitronectin	0.98	0.99837881
1423	P07951	Tropomyosin beta chain	0.98	1.00440404
1424	Q9NQP4	Prefoldin subunit 4	0.99	1.00376216
1425	P56537	Eukaryotic translation initiation factor 6	0.99	1.00242418
1426	P20930	Filaggrin	0.99	1.00772795
1427	P09326	CD48 antigen	0.99	1.00453579
1428	Q15363	Transmembrane emp24 domain-containing protein 2	0.99	1.00213132
1429	P63220	40S ribosomal protein S21	0.99	0.99641031
1430	Q8IVL1	Neuron navigator 2	0.99	1.00523025
1431	P15121	Aldose reductase	0.99	0.99689338
1432	P22102	Trifunctional purine biosynthetic protein adenosine-3	0.99	1.00108835
1433	Q15019	Septin-2	0.99	1.00079971
1434	P55268	Laminin subunit beta-2	1.00	0.99888233
1435	Q01518	Adenylyl cyclase-associated protein 1	1.00	1.00113803
1436	Q8IUI8	Cytokine receptor-like factor 3	1.00	1.00101581
1437	Q86UD1	Out at first protein homolog	1.00	0.99976131

6. List of Significantly Up Regulated proteins

PROTEIN	p-value	Fold Change
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1	0.00	1.58
10 kDa heat shock protein, mitochondrial	0.03	1.61
14-3-3 protein beta/alpha	0.00	1.68
14-3-3 protein zeta/delta	0.00	1.87
26S protease regulatory subunit 6A	0.00	1.76
26S proteasome non-ATPase regulatory subunit 1	0.00	1.58
26S proteasome non-ATPase regulatory subunit 5	0.00	1.67
3-hydroxyacyl-CoA dehydrogenase type-2	0.01	1.58
4-hydroxyphenylpyruvate dioxygenase	0.01	1.63
40S ribosomal protein S12	0.00	2.49
40S ribosomal protein S13	0.01	1.67
40S ribosomal protein S2	0.02	1.50
40S ribosomal protein S24	0.00	1.72
40S ribosomal protein S4, X isoform	0.00	1.87
40S ribosomal protein SA	0.00	1.86
4F2 cell-surface antigen heavy chain	0.02	1.55
6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4	0.01	1.60
6-phosphogluconate dehydrogenase, decarboxylating	0.00	1.51
60S acidic ribosomal protein P0	0.04	1.60
60S ribosomal protein L13	0.02	2.36
60S ribosomal protein L19	0.05	1.65
60S ribosomal protein L30	0.01	1.67
60S ribosomal protein L7	0.03	1.58
Acetyl-CoA acetyltransferase, cytosolic	0.04	1.57
Actin-related protein 2	0.01	1.68
Actin-related protein 2/3 complex subunit 5	0.00	1.71
Activated CDC42 kinase 1	0.00	1.60
Acyl-coenzyme A thioesterase 2, mitochondrial	0.03	1.94
Acyl-protein thioesterase 1	0.00	1.65
Acylamino-acid-releasing enzyme	0.00	1.73
Adenosine deaminase	0.01	1.58
Adenylate kinase isoenzyme 1	0.00	1.84
Adipocyte enhancer-binding protein 1	0.00	2.39
Adipocyte plasma membrane-associated protein	0.00	1.51
Alanine aminotransferase 1	0.00	2.04
Alcohol dehydrogenase [NADP(+)]	0.01	1.62
Aldehyde dehydrogenase, mitochondrial	0.03	1.66
Alpha-1-acid glycoprotein 1	0.00	1.73
Alpha-enolase	0.00	1.83
Alpha-mannosidase 2	0.00	2.91
Aminoacylase-1	0.05	1.82
Amyloid beta A4 protein	0.01	1.83

Angiogenin	0.05	1.67
Angiopoietin-related protein 3	0.01	1.82
Annexin A2	0.00	1.51
Annexin A3	0.01	1.70
AP-1 complex subunit beta-1	0.01	1.56
ATP synthase subunit O, mitochondrial	0.03	2.99
ATP-dependent RNA helicase DDX3Y	0.00	2.02
Bcl-2-like protein 13	0.00	2.10
Beta-1,4-glucuronyltransferase 1	0.03	5.34
Beta-2-microglobulin	0.00	1.90
Beta-actin-like protein 2	0.01	1.78
Beta-glucuronidase	0.03	1.84
Beta-mannosidase	0.01	1.71
Bisphosphoglycerate mutase	0.00	1.94
Bone marrow proteoglycan	0.00	1.54
C-C motif chemokine 15	0.02	1.72
C-reactive protein	0.00	3.62
Cadherin-6	0.00	1.86
Cadherin-related family member 5	0.01	1.55
Calcium/calmodulin-dependent protein kinase type II subunit beta	0.00	1.98
Carbonic anhydrase 3	0.05	1.84
Carbonyl reductase [NADPH] 1	0.00	2.07
Cartilage oligomeric matrix protein	0.00	1.97
Cathepsin F	0.00	2.41
CD5 antigen-like	0.02	2.17
CDK5 regulatory subunit-associated protein 3	0.05	1.81
Cell growth regulator with EF hand domain protein 1	0.00	3.38
Centromere protein F	0.00	1.55
Chloride intracellular channel protein 4	0.00	1.68
Cofilin-1	0.00	1.75
Coiled-coil domain-containing protein 25	0.03	1.83
Collagen alpha-1(XVIII) chain	0.01	1.50
Complement C1q subcomponent subunit A	0.00	1.85
Complement C1q subcomponent subunit C	0.00	1.57
Complement C4-B	0.00	1.61
Complement factor H-related protein 5	0.00	1.67
Copine-1	0.01	1.68
Cullin-associated NEDD8-dissociated protein 1	0.00	1.64
Cysteine and histidine-rich domain-containing protein 1	0.00	1.82
Cysteine-rich protein 1	0.03	5.16
Cytosolic 5'-nucleotidase 3A	0.02	1.57
Cytosolic non-specific dipeptidase	0.00	1.86
D-3-phosphoglycerate dehydrogenase	0.00	1.60
Deleted in malignant brain tumors 1 protein	0.04	1.64
Desmocollin-1	0.05	1.55
Di-N-acetylchitobiase	0.01	1.67

Dihydropyrimidinase-related protein 2	0.00	1.75
Dimethylglycine dehydrogenase, mitochondrial	0.02	1.57
Dynamin-2	0.00	1.56
E3 ubiquitin/ISG15 ligase TRIM25	0.00	1.55
Ectonucleoside triphosphate diphosphohydrolase 5	0.01	1.64
EGF-containing fibulin-like extracellular matrix protein 1	0.00	1.62
EH domain-containing protein 1	0.00	1.80
Elongation factor 1-gamma	0.02	1.68
Endoglin	0.04	1.51
Endoplasmic reticulum aminopeptidase 2	0.01	1.68
Enoyl-CoA hydratase, mitochondrial	0.01	1.50
Epsin-1	0.00	1.82
Ester hydrolase C11orf54	0.01	1.78
Eukaryotic initiation factor 4A-I	0.00	2.11
Eukaryotic initiation factor 4A-II	0.04	1.50
Eukaryotic translation initiation factor 3 subunit I	0.00	1.50
Eukaryotic translation initiation factor 3 subunit J	0.01	1.55
Eukaryotic translation initiation factor 4E	0.03	3.46
Eukaryotic translation initiation factor 5A-1	0.01	1.72
Exosome complex component RRP41	0.01	2.31
Exportin-1	0.00	1.58
Extracellular glycoprotein lacritin	0.05	1.80
Ezrin	0.00	1.64
Farnesyl pyrophosphate synthase	0.00	1.56
Fatty acid-binding protein, liver	0.05	1.50
Ferritin heavy chain	0.05	2.98
Ferritin light chain	0.00	1.69
Fibulin-2	0.00	1.53
Filamin-B	0.00	1.54
Focal adhesion kinase 1	0.00	2.16
Follistatin-related protein 1	0.01	1.83
Gamma-aminobutyric acid receptor-associated protein-like 2	0.04	1.62
GDH/6PGL endoplasmic bifunctional protein	0.01	1.80
General transcription factor IIF subunit 1	0.01	1.70
Glial fibrillary acidic protein	0.01	2.07
Glutamate dehydrogenase 1, mitochondrial	0.00	1.87
Glutamyl aminopeptidase	0.00	1.83
Glutathione peroxidase 1	0.00	2.17
Glutathione synthetase	0.00	1.81
Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	0.00	1.68
Glycogen debranching enzyme	0.00	1.65
Glycogen phosphorylase, brain form	0.00	1.73
Glycogen phosphorylase, liver form	0.00	1.53
GMP reductase 1	0.00	2.18
GTP:AMP phosphotransferase AK3, mitochondrial	0.00	1.64
Guanine nucleotide-binding protein subunit alpha-13	0.00	1.53

Heat shock 70 kDa protein 1A	0.00	1.70
Hematological and neurological expressed 1 protein	0.05	1.58
Hemoglobin subunit alpha	0.01	1.82
Hemoglobin subunit beta	0.02	1.88
Hemoglobin subunit delta	0.00	1.78
Heterogeneous nuclear ribonucleoprotein K	0.04	1.64
Heterogeneous nuclear ribonucleoprotein M	0.00	1.50
High mobility group protein B2	0.00	1.64
High mobility group protein B3	0.01	1.68
Histidine protein methyltransferase 1 homolog	0.00	2.44
Histone H3.3	0.05	2.83
HLA class I histocompatibility antigen, B44 alfa chain	0.02	1.79
HLA class II histocompatibility antigen, DR alpha chain	0.01	1.95
Host cell factor 1	0.01	1.61
Hydroxyacylglutathione hydrolase, mitochondrial	0.00	1.59
Hydroxysteroid dehydrogenase-like protein 2	0.01	1.83
Hypoxanthine-guanine phosphoribosyltransferase	0.03	1.74
Ig alpha-1 chain C region	0.00	3.06
Ig alpha-2 chain C region	0.00	3.01
Ig gamma-2 chain C region	0.01	3.36
Ig gamma-4 chain C region	0.01	4.56
Ig heavy chain constant	0.03	6.39
Ig heavy constant gamma 1	0.00	2.80
Ig heavy constant gamma 3	0.05	2.77
Ig Heavy Variable 3-33	0.00	4.06
Ig heavy variable 3-7	0.00	2.11
Ig HV4-34	0.04	2.99
Ig kappa chain C region	0.00	4.47
Ig kappa variable 3-20	0.02	2.15
Ig mu chain C region	0.04	4.10
Immunoglobulin heavy variable 1-46	0.02	2.14
Immunoglobulin heavy variable 3-48	0.00	2.18
Immunoglobulin J chain	0.05	2.95
Immunoglobulin kappa variable 1-16	0.00	2.02
Immunoglobulin kappa variable 2-30	0.02	1.93
Immunoglobulin lambda variable 3-19	0.00	2.74
Immunoglobulin lambda variable 3-21	0.00	3.76
Immunoglobulin lambda variable 3-25	0.01	1.62
Importin-11	0.00	1.88
Importin-7	0.01	1.57
Insulin-like growth factor-binding protein 6	0.02	1.62
Intron-binding protein aquarius	0.00	1.60
Isochorismatase domain-containing protein 2	0.00	1.91
Junction plakoglobin	0.03	1.61
Keratin, type I cuticular Ha3-II	0.02	1.61
Keratin, type I cytoskeletal 15	0.01	1.66

Keratin, type I cytoskeletal 16	0.00	2.08
Keratin, type II cuticular Hb5	0.01	1.96
Keratin, type II cytoskeletal 3	0.01	1.94
KRR1 small subunit processome component homolog	0.03	1.73
L-lactate dehydrogenase A chain	0.02	1.55
Laminin subunit beta-1	0.00	1.62
Latent-transforming growth factor beta-binding protein 4	0.01	2.09
Leucine zipper protein 1	0.00	1.72
Leucine-rich alpha-2-glycoprotein	0.00	1.73
Leucine-rich repeat flightless-interacting protein 1	0.00	1.54
LIM and senescent cell antigen-like-containing domain protein 1	0.04	1.68
Lipopolysaccharide-binding protein	0.00	2.03
LisH domain and HEAT repeat-containing protein KIAA1468	0.03	1.99
Low-density lipoprotein receptor	0.00	1.80
Lysosomal Pro-X carboxypeptidase	0.00	1.95
Lysosomal protective protein	0.00	1.77
Macrophage colony-stimulating factor 1 receptor	0.00	1.73
Maltase-glucoamylase, intestinal	0.05	1.50
Mannose-binding protein C	0.02	1.50
Matrin-3	0.00	1.65
Matrix Gla protein	0.04	2.15
Matrix metalloproteinase-9	0.00	1.95
Mediator of RNA polymerase II transcription subunit 13-like	0.03	1.65
Melanotransferrin	0.00	2.73
Mesothelin	0.03	1.55
Metalloproteinase inhibitor 1	0.00	1.78
Microtubule-associated protein 1B	0.00	1.80
Minor histocompatibility protein HA-1	0.00	1.67
Mitochondrial import inner membrane translocase subunit Tim13	0.05	1.71
Mitogen-activated protein kinase 14	0.01	1.75
Myelin basic protein	0.00	1.98
N-acetylglucosamine-1-phosphotransferase subunit gamma	0.02	2.13
N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase	0.00	1.74
Na(+)/H(+) exchange regulatory cofactor NHE-RF1	0.00	2.09
NADP-dependent malic enzyme	0.00	1.93
Neprilysin	0.01	1.68
Neurogenic locus notch homolog protein 2	0.00	1.54
Neutrophil cytosol factor 4	0.01	1.79
Neutrophil elastase	0.00	1.80
Nicotinate phosphoribosyltransferase	0.00	1.51
Nicotinate-nucleotide pyrophosphorylase [carboxylating]	0.00	2.15
NIF3-like protein 1	0.04	1.82
Nuclear autoantigenic sperm protein	0.00	1.57
Nuclear pore complex protein Nup50	0.01	1.84
Nucleoside diphosphate kinase B	0.01	2.62
obsolete	0.01	2.37

Pancreatic alpha-amylase	0.00	2.87
Pancreatic secretory granule membrane major glycoprotein GP2	0.00	4.31
Pantetheinase	0.03	1.97
PDZ and LIM domain protein 5	0.00	1.68
Pentatricopeptide repeat domain-containing protein 3, mitochondrial	0.02	1.58
Peptidoglycan recognition protein 1	0.01	2.00
Peptidyl-glycine alpha-amidating monooxygenase	0.00	1.62
Peptidyl-prolyl cis-trans isomerase A	0.00	1.59
Peptidyl-prolyl cis-trans isomerase FKBP4	0.00	2.16
Peptidyl-prolyl cis-trans isomerase FKBP5	0.00	1.64
Peroxidasin homolog	0.03	1.67
Peroxiredoxin-2	0.00	1.84
Peroxiredoxin-6	0.00	2.10
Phosphatidylinositol 4-phosp 3-kinase C2 domain-containing subunit beta	0.01	1.92
Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	0.01	1.65
Phosphofurin acidic cluster sorting protein 1	0.00	1.67
Phosphoglycerate mutase 1	0.02	1.64
Phospholipid transfer protein	0.00	1.61
Plastin-1	0.03	1.68
Platelet basic protein	0.00	4.83
Platelet factor 4	0.02	1.83
Platelet-derived growth factor receptor beta	0.05	2.02
Pleckstrin	0.00	1.90
Poly(rC)-binding protein 1	0.02	1.79
Polyadenylate-binding protein 1	0.02	1.61
Polymeric immunoglobulin receptor	0.02	2.47
POTE ankyrin domain family member F	0.00	1.83
POTE ankyrin domain family member J	0.00	2.15
Prefoldin subunit 6	0.02	1.75
Probable ATP-dependent RNA helicase DDX17	0.00	1.63
Probable ATP-dependent RNA helicase DDX5	0.00	1.87
Probable phospholipid-transporting ATPase IF	0.01	2.02
Profilin-1	0.00	1.63
Prolyl endopeptidase FAP	0.01	2.53
Prostaglandin reductase 1	0.01	1.54
Prostaglandin-H2 D-isomerase	0.00	1.66
Proteasome activator complex subunit 2	0.02	1.53
Proteasome subunit alpha type-2	0.04	1.64
Proteasome subunit alpha type-3	0.00	1.70
Proteasome subunit alpha type-5	0.00	1.57
Proteasome subunit beta type-6	0.02	1.79
Proteasome subunit beta type-7	0.01	1.64
Protein 4.1	0.05	1.62
Protein disulfide-isomerase	0.00	1.66
Protein disulfide-isomerase A6	0.00	1.55

Protein FAM3C	0.00	1.69
Protein FAM76B	0.01	2.25
Protein flightless-1 homolog	0.00	1.59
Protein MON2 homolog	0.05	1.69
Protein PBDC1	0.02	1.82
Protein phosphatase 1 regulatory subunit 14B	0.02	1.52
Protein POF1B	0.00	1.99
Protein S100-A10	0.01	1.60
Protein S100-A11	0.04	1.51
Protein S100-A6	0.03	1.97
Protein S100-A7	0.00	1.97
Protein S100-A8	0.01	1.91
Protein S100-A9	0.00	1.94
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	0.00	1.75
Prothymosin alpha	0.05	13.69
Pterin-4-alpha-carbinolamine dehydratase	0.04	1.89
Purine nucleoside phosphorylase	0.00	2.56
Putative ATP-dependent RNA helicase DDX11-like protein 8	0.03	2.31
Pyruvate kinase PKM	0.00	1.69
Radixin	0.02	1.51
Ragulator complex protein LAMTOR3	0.01	3.95
Ras GTPase-activating-like protein IQGAP2	0.04	1.54
RAS guanyl-releasing protein 2	0.02	2.21
Ras suppressor protein 1	0.00	1.60
Ras-related protein Rab-7a	0.00	1.79
Receptor-type tyrosine-protein phosphatase eta	0.01	1.74
Reticulon-4	0.02	2.20
Retinal dehydrogenase 1	0.00	1.52
Retinol-binding protein 1	0.03	1.59
Rho-associated protein kinase 2	0.01	1.54
RNA-binding protein 3	0.02	1.65
RNMT-activating mini protein	0.03	3.99
rRNA 2'-O-methyltransferase fibrillarin	0.00	1.88
Secretogranin-1	0.01	1.88
Septin-6	0.01	1.72
Septin-7	0.01	1.61
Serine/arginine repetitive matrix protein 2	0.01	1.60
Serine/threonine-protein kinase 10	0.00	1.83
Serine/threonine-protein phosp 2A 65 kDa regulatory subunit A alpha isoform	0.00	1.91
Serine/threonine-protein phosp 6 catalytic subunit	0.02	1.52
Serine/threonine-protein phosp PP1-gamma catalytic subunit	0.01	2.24
Serpin B3	0.03	1.63
Serpin B5	0.01	1.70
Serum amyloid A-1 protein	0.00	1.73
Serum amyloid A-2 protein	0.01	1.85

Sex hormone-binding globulin	0.01	1.62
Small subunit processome component 20 homolog	0.00	1.97
Sorbitol dehydrogenase	0.00	1.89
Spectrin beta chain, non-erythrocytic 1	0.00	1.54
Sphingomyelin phosphodiesterase 4	0.00	2.28
Staphylococcal nuclease domain-containing protein 1	0.02	1.51
TAR DNA-binding protein 43	0.01	1.60
Thioredoxin	0.01	1.70
Thioredoxin domain-containing protein 5	0.00	1.91
THO complex subunit 4	0.03	1.79
Thrombospondin-1	0.00	3.01
Thrombospondin-2	0.02	2.20
Thy-1 membrane glycoprotein	0.02	1.77
Thymosin beta-4	0.00	2.28
Transcobalamin-1	0.00	1.88
Transcription factor BTF3	0.00	1.67
Transcription intermediary factor 1-beta	0.00	1.61
Transferrin receptor protein 1	0.00	2.13
Transforming growth factor beta-1	0.00	2.06
Transgelin-2	0.00	1.67
Transmembrane glycoprotein NMB	0.00	1.52
Triosephosphate isomerase	0.00	1.53
Tripeptidyl-peptidase 1	0.04	2.32
Tripeptidyl-peptidase 2	0.00	1.53
Tropomodulin-3	0.02	1.73
Trypsin-2	0.02	1.59
Tryptophan--tRNA ligase, cytoplasmic	0.00	1.86
Tubulin alpha-1C chain	0.01	1.93
Tubulin beta chain	0.00	1.60
Tubulin-specific chaperone A	0.04	1.55
Ubiquitin carboxyl-terminal hydrolase isozyme L5	0.03	1.67
Ubiquitin-conjugating enzyme E2 N	0.05	1.63
Ubiquitin-conjugating enzyme E2 variant 2	0.00	2.20
Unhealthy ribosome biogenesis protein 2 homolog	0.00	1.82
UPF0587 protein C1orf123	0.00	3.14
V-type proton ATPase catalytic subunit A	0.01	1.69
Vacuolar protein sorting-associated protein 29	0.00	1.79
Vascular cell adhesion protein 1	0.00	1.76
Vascular endothelial growth factor receptor 1	0.04	1.72
Versican core protein	0.01	2.67
Voltage-dependent anion-selective channel protein 1	0.00	2.44
X-ray repair cross-complementing protein 6	0.00	1.55
Zinc finger CCCH domain-containing protein 4	0.05	1.63
Zinc finger protein 318	0.01	2.21

7. List of Significantly Down Regulated proteins

Group	p-value	Fold Change
Actin-related protein 2/3 complex subunit 2	0.01	0.21
Apolipoprotein A-II	0.00	0.56
Beta-Ala-His dipeptidase	0.00	0.58
Ficolin-2	0.01	0.53
Keratin, type II cytoskeletal 1	0.00	0.56
Keratin, type II cytoskeletal 1b	0.00	0.58
Serpin H1	0.01	0.65
Serum amyloid A-4 protein	0.05	0.59

8. Abstract from The British Society of Haematology Conference 2018- Liverpool

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Quantitative Proteomic Studies in Mastocytosis

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Please indicate your preferred method of presentation: Poster
Has this abstract been presented at a British Haematology meeting before?: No
Has this abstract been presented at an overseas meeting?: No
Please select your position from the list: Scientist

Abstract Content: Mastocytosis is a myeloproliferative disease that is characterised by the accumulation of neoplastic mast cells in one or several organs resulting in mediator release symptoms, tissue damage and, in aggressive cases, organ failure. The vast majority of adult patients with mastocytosis present with systemic mastocytosis (SM) and it is defined as mast cell accumulation in one or more visceral organs. Despite advances in the understanding of myeloid neoplasia the aetiology of mastocytosis is poorly understood. Around 80-90% of patients with SM harbour a somatic activating mutation in the *c-KIT* gene (*D816V).* The *D816V* mutation results in the constitutive activation of the c-Kit receptor causing the activation of multiple signalling pathways resulting in an increased proliferative and survival advantage of the mast cell lineage. However, the presence of the *c-KIT* mutation does not explain the heterogeneous clinical behaviour of the disease, and the molecular mechanisms underlying the different subtypes of SM remain largely elusive.There is a clear need to develop strategies that will aid in the understanding of the molecular pathology of SM. We carried-out a global discovery proteome analysis of the plasma of SM patients (n=3) and compared these to healthy control plasma samples(n=3); using the new high resolution mass spectrometry protocol Sequential Window Acquisition of all Theoretical fragment-ion spectra mass spectrometry (SWATH MS). SM patients were chosen for eligibility using the WHO selection criteria. Samples were immunodepleted and SWATH MS permanent digital proteomic maps were generated for all samples on an 6600 TripleTOF mass spectrometer (AB Sciex, Warrington, UK) and an Eksigent 1D+ Nano LC systems (Eksigent, Dublin, CA) all samples were run with triplicate mass spectrometry injections per sample. We identified ~1000 proteins in each of the samples at a 5% FDR and they were mapped on to their relevant pathways using KEGG. Heatmaps and volcano plots were generated using the MSstats program in the R environment. Bioinformatic analysis and pathway mapping demonstrated that a number of immunological and metabolic pathways were significantly upregulated in the plasma of the mastocytosis patients including B cell proliferation, phagocytosis response and calcium signalling. Full characterisation of key regulatory pathways in relation to mastocytosis disease aetiology will identify new direct cellular targets potentially paving the way for new bespoke treatment strategies in this blood disorder.

Disclosure of Interest: None Declared

Keywords: None