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Distinct plasma biomarkers confirm the diagnosis of mastocytosis and identify increased risk of anaphylaxis

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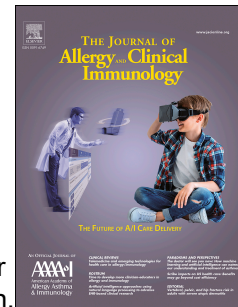
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Material and Methods

Study subjects and study design

For this prospective study, 19 patients (≥ 18 years), 15 with SM and four cases with monoclonal mast cell activation syndrome (MMAS) were recruited from the Outpatient Clinic of Respiratory Medicine and Allergy at Karolinska University Hospital Huddinge. All investigated subjects were in a clinically stable period of their condition during the visit and samples were taken at least one month after the last anaphylactic episode in patients with anaphylaxis. Additionally, two control groups were recruited for comparisons of plasma protein levels. The first group consisted of 13 healthy volunteers with no history of atopy and atopic diseases, whereas the other group included 11 subjects with mild allergic asthma treated with short-acting beta-agonists only. The Ethical Review Board in Stockholm approved the study (Dnr 2009/959-31-4), and subjects provided written informed consent.

Diagnosis of SM or MMAS was obtained after a comprehensive medical evaluation and bone marrow investigation following WHO criteria (1). Further, no SM patients had more than one B-findings or any C-findings, thereby obtained diagnoses of indolent SM (Please see Table E1). In patients with MMAS, the WHO criteria for SM are not fully met; however, these patients possess clonal MCs expressing CD25⁺, immunophenotypically aberrant MCs. Moreover, serum baseline tryptase (sBT) levels (Thermo Fisher) were measured. Anaphylactic reactions were diagnosed in accordance with WAO clinical criteria, when either reduced blood pressure or associated symptoms such as syncope/presyncope and/or respiratory compromise were present accompanied by the involvement of the skin/mucosal tissue and/or gastrointestinal symptoms (Please see Table E1) (2). Furthermore, all included patients and controls underwent a complete allergic work-up at Karolinska University Hospital Huddinge, Allergy Outpatient Clinic including medical history, skin prick testing (SPT) with commercial extracts (ALK-Abelló A/S, Horsholm, Denmark) of standard aeroallergens, and with food allergens and allergen of Hymenoptera venom (honeybee and common wasp) in relevant cases. Atopic subjects suffering attacks of dyspnea or wheezing upon contact with a particular allergen were considered to have allergic asthma when GINA criteria were fulfilled.

Protein analysis

Protein levels in plasma were analyzed by Protein Extension Assay (PEA) using Olink Proseek Multiplex Inflammation I^{96X96}, CVD I^{96X96} and Immune response panel kits and quantified by real-time PCR using the Fluidigm BioMark HD real-time PCR platform. In total 248 proteins were analyzed (3). Please see Table E2 for complete list of proteins measured. The sample analyses were performed at the Clinical Biomarker Facility, Science for Life Laboratory, Uppsala.

In brief, for each measured protein, a pair of oligonucleotide-labeled antibody probes binds to the targeted protein, and if the two probes are in close proximity, a PCR target sequence is

formed by a proximity-dependent DNA polymerization event and the resulting sequence is subsequently detected and quantified using real-time PCR. All assay characteristics including detection limits and measurements of assay performance and validations are available from the manufacturers' webpage (<http://www.olink.com/products/proseek-multiplex/downloads/data-packages>).

Data analysis and biostatistics

Statistical analyses were performed using GraphPad Prism 8, and differences were considered significant if $p < .05$. Categorical variables are presented as numbers and percentages and were compared using Fisher's exact test. Continuous variables are presented as mean values and standard deviation (SD), and group comparisons were analyzed using Kruskal-Wallis with Bonferroni correction and Mann-Whitney U-test. Protein levels of anaphylactic and non-anaphylactic CMD patients were compared using Mann-Whitney U-test and data is presented with means $\pm$ SD.

References to Methods:

1. Valent P, Akin C, Escribano L, Födinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. *Eur J Clin Invest.* 2007;37:435-53.
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3. Assarsson E, Lundberg M, Holmquist G, Björkstén J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PloS one.* 2014;9:1-11.

Table E1: Characteristics of patients with mast cell disorders

Pat. Nr, Sex	Age at Dx	Dx w/wo skin lesion	Hx of Ax	Elicitor of Ax	Clinical pattern of Ax	MC aggs	Atypical MCs	CD2 /25	D816V mutation	sBT (µg/L)	B (>1) /C-finding
1, F	46	ISMs ⁺	No	n/a	n/a	No	Yes	-/+	Positive	26	-/-
2, F	43	ISMs ⁺	Yes	Idiopathic	Flush, sync, dyspne,	Yes	Yes	-/+	Positive	15	-/-
3, M	53	ISMs ⁺	Yes	Idiopathic	Flush, sync, dyspne,	No	Yes	+/+	Positive	25	-/-
4, M	64	ISMs ⁺	Yes	Wasp, SPT ⁻ /CAP ⁺	Flush, sync, dyspne,	Yes	Yes	-/+	Negative	29	-/-
5, F	68	ISMs ⁺	Yes	Wasp, SPT ⁻ /CAP ⁺	Sync	No	Yes	+/+	Positive	15	-/-
6, F	21	ISMs ⁺	No	n/a	n/a	No	Yes	+/+	Positive	12	-/-
7, F	63	ISMs ⁺	Yes	Shrimp, SPT ⁻ /CAP ⁺	Flush, dyspne, sync, vomit	Yes	Yes	+/+	Positive	36	-/-
8, F	68	ISMs ⁺	No	n/a	n/a	Yes	Yes	-/+	Positive	81	-/-
9, F	69	ISMs ⁺	No	n/a	n/a	Yes	Yes	-/+	Positive	320	-/-
10, M	43	ISMs ⁻	Yes	Wasp, SPT ⁻ /CAP ⁺	BP-fall, dyspne	No	Yes	+/+	Positive	13	-/-
11, F	42	ISMs ⁺	No	n/a	n/a	Yes	Yes	+/+	Positive	217	-/-
12, F	49	ISMs ⁺	No	n/a	n/a	Yes	Yes	-/+	Positive	120	-/-
13, F	32	ISMs ⁺	No	n/a	n/a	No	Yes	-/+	Positive	18	-/-
14, M	45	ISMs ⁻	Yes	Idiopathic	Flush, sync	Yes	Yes	+/+	Positive	160	-/-
15, M	65	ISMs ⁺	Yes	Wasp, SPT ⁻ /CAP ⁺	BP-fall, sync	No	No	+/+	Positive	21	-/-
16, M	40	MMAS	Yes	Wasp, SPT ⁺ /CAP ⁺	BP-fall, sync, diarre	No	No	-/+	Negative	3.8	-/-
17, M	49	MMAS	Yes	Wasp, SPT ⁺ /CAP ⁺	BP-fall, sync	No	No	+/+	Negative	4.9	-/-
18, M	61	MMAS	Yes	Idiopathic	Flush, sync, dyspne	No	No	-/+	Negative	8.8	-/-
19, F	38	MMAS	Yes	Idiopathic	Flush, sync, diarre	No	No	+/+	Negative	5	-/-

ISM, indolent systemic mastocytosis; ISMs^{+/+}, indolent systemic mastocytosis with or without skin engagement; Dx, diagnosis; Hx, history; Ax, anaphylaxis; BP-fall, blood pressure fall; sync, syncope; MC, mast cell; aggs, aggregates; n/a, not applicable; sBT, serum baseline tryptase; SPT, skin prick test; M, male; F, female.

Table E2 – List of all measured proteins, means $\pm$ SD per group and indications of used Olink panel and excluded proteins.

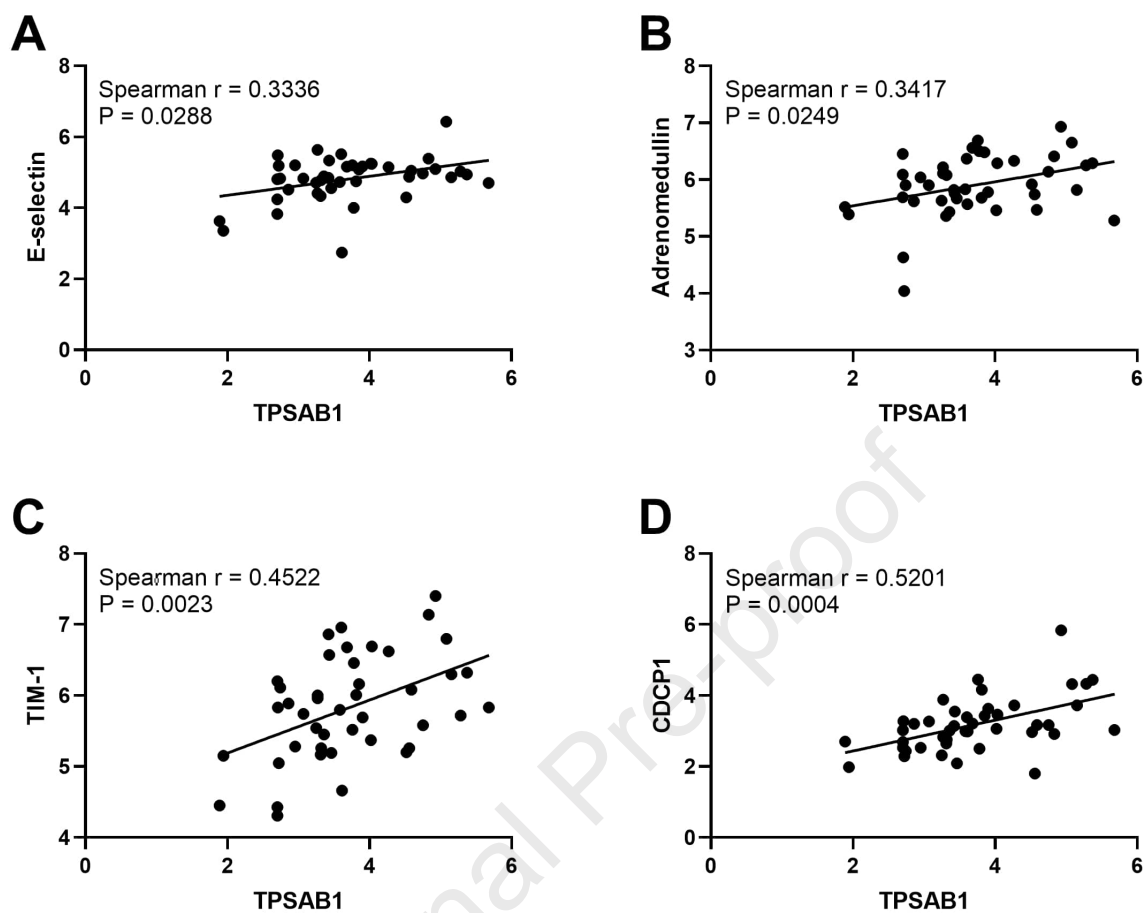
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	Full protein name	Protein abbreviation	UniProt No.	Healthy control (mean levels)	±SD Healthy control	Asthma (mean levels)	±SD Asthma	Mastocytosis (mean levels)	±SD Masto-cytosis	Olink panel	* > 5 measurements below detection limit were excluded from analysis
1	3-phosphotyrosine and 3-phosphoinositide (hDAPP1)	DAPP1	Q9UN19	6.90	1.33	6.60	2.01	7.24	0.45	Immune response	
2	Adenosine Deaminase	ADA	P00813	5.95	0.74	5.77	0.48	5.58	0.38	Inflammation	
3	Adrenomedullin	AM	P35318	5.79	0.38	5.57	0.66	6.17	0.39	CVD I	
4	Agouti-related protein	AGRP	O00253	2.82	0.49	2.58	0.51	2.89	0.38	CVD I	
5	Allergin-1	MILR1	Q726M3	2.45	0.25	2.61	0.36	2.94	0.96	Immune response	
6	Amphiregulin	AREG	P15514	1.25	0.32	0.99	0.38	1.24	0.50	Immune response	
7	Angiotensin-1 receptor	TEK/TIE2	Q02763	5.77	0.38	5.80	0.16	5.89	0.21	CVD I	
8	Artemin	ARTN	Q514W7	0.16	0.17	0.48	0.86	0.05	0.08	Inflammation	*
9	Aryl hydrocarbon receptor nuclear translocator (ARNT protein)	ARNT	P27540	1.63	1.98	0.74	1.27	0.08	0.38	Immune response	*
10	Axin-1	AXIN1	O15169	6.70	1.37	5.93	2.32	7.05	0.59	Inflammation	
11	Baculoviral IAP repeat-containing protein 2	BIRC2	Q19490	0.77	0.44	0.65	0.23	0.76	0.28	Immune response	
12	Beta-galactosidase	GLB1	P16278	1.58	1.03	1.69	0.65	1.81	0.60	Immune response	
13	Beta-nerve growth factor	Beta-NGF	P01138	1.32	0.16	1.26	0.17	1.20	0.13	Inflammation	
14	Butyrophilin subfamily 3 member A2	BTN3A2	P78410	1.55	0.45	1.57	0.28	1.63	0.21	Immune response	
15	C-C motif chemokine 3	MIP-1-alpha/CCL3	P10147	2.08	0.73	1.90	0.40	2.12	0.55	CVD I	
16	C-C motif chemokine 4	CCL4	P13236	5.47	0.80	5.19	0.42	5.51	0.63	Inflammation	
17	C-C motif chemokine 19	CCL19	Q99731	9.59	0.85	8.94	0.38	9.65	0.69	Inflammation	
18	C-C motif chemokine 20	CCL20	P78556	6.38	0.90	5.72	0.45	5.94	0.95	Inflammation	
19	C-C motif chemokine 23	CCL23	P55773	9.70	0.35	9.50	0.41	9.75	0.46	Inflammation	
20	C-C motif chemokine 25	CCL25	O15444	5.85	0.51	6.02	0.37	6.26	0.56	Inflammation	
21	C-C motif chemokine 28	CCL28	Q9NRJ3	1.57	0.39	1.28	0.15	1.40	0.27	Inflammation	
22	C-type lectin domain family 4 member A	CLEC4A	Q9UMR7	3.55	0.52	3.42	0.28	3.20	0.35	Immune response	
23	C-type lectin domain family 4 member C	CLEC4C	Q8WTT0	2.57	0.55	2.64	0.59	2.32	0.36	Immune response	
24	C-type lectin domain family 4 member D	CLEC4D	Q9BWN8	2.04	1.32	2.15	0.39	2.14	0.55	Immune response	
25	C-type lectin domain family 4 member G	CLEC4G	Q6LX84	2.76	0.46	2.94	0.36	2.77	0.38	Immune response	
26	C-type lectin domain family 6 member A	CLEC6A	Q6E1G7	1.81	0.91	1.60	0.53	1.53	0.51	Immune response	
27	C-type lectin domain family 7 member A	CLEC7A	Q9BXN2	2.97	0.52	2.55	0.49	2.94	0.44	Immune response	
28	C-X-C motif chemokine 1	CXCL1	P09341	9.19	0.98	9.21	0.69	9.72	0.46	Inflammation	
29	C-X-C motif chemokine 5	CXCL5	P42830	12.53	1.26	12.26	0.86	12.86	0.42	Inflammation	
30	C-X-C motif chemokine 6	CXCL6	P80162	8.62	1.30	8.45	0.73	9.04	0.61	Inflammation	
31	C-X-C motif chemokine 9	CXCL9	Q07325	6.92	0.69	6.54	0.48	7.03	0.78	Inflammation	
32	C-X-C motif chemokine 10	CXCL10	P02778	8.55	0.75	8.08	0.87	8.35	1.14	Inflammation	
33	C-X-C motif chemokine 11	CXCL11	Q14625	8.83	0.94	7.98	0.69	8.66	0.94	Inflammation	
34	C-X-C motif chemokine 16	CXCL16	Q9H2A7	2.69	0.13	2.46	0.18	2.76	0.26	CVD I	
35	Cathepsin D	CTSD	P07339	7.22	0.45	7.11	0.42	7.54	0.38	CVD I	
36	Cathepsin L1	CTSL1	P07711	5.16	0.27	5.21	0.26	5.31	0.36	CVD I	
37	Caspase 8	CASP-8	Q14790	3.90	1.40	3.82	0.69	4.29	0.51	Inflammation	
38	CD40 ligand	CD40L	P29965	7.51	1.48	7.56	1.05	8.12	0.45	CVD I	
39	CD40L receptor	CD40	P25942	10.27	0.70	10.09	0.77	10.47	0.38	Inflammation	
40	CD83 antigen (hCD83)	CD83	Q01151	2.38	0.22	2.30	0.27	2.32	0.27	Immune response	
41	Chitinase-3-like protein 1	CHI3L1	P36222	4.22	0.91	3.82	0.32	4.71	0.94	Immune response	
42	Contactin-associated protein-like 2	CNTNAP2	Q0UHC6	1.68	0.36	1.72	0.47	1.58	0.26	Immune response	
43	Corneodesmosin	CDSN	Q15517	3.21	0.53	2.89	0.21	3.02	0.62	Immune response	
44	Corticosteroid 11-beta-dehydrogenase isozyme 1	HSDB11B1	P28845	2.44	0.35	2.35	0.33	2.41	0.28	Immune response	
45	Coxsackievirus and adenovirus receptor (CAR)	CXADR	P78310	1.55	0.17	1.72	0.32	1.65	0.38	Immune response	
46	CUB domain-containing protein 1 (CDCP1)	CDCP1	Q9H5V8	2.98	0.43	2.56	0.43	3.68	0.76	Inflammation	
47	Cystatin-B	CSTB	P04080	4.73	1.00	4.28	0.61	4.63	0.51	CVD I	
48	Cystatin D	CST5	P28325	6.83	0.37	6.62	0.37	6.77	0.32	Inflammation	
49	Cytoskeleton-associated protein 4	OKAP4	Q07065	3.90	1.04	3.47	0.26	3.70	0.36	Immune response	
50	Delta and Notch-like epidermal growth factor related receptor	DNER	Q8NFT8	7.26	0.18	7.32	0.23	7.12	0.22	Inflammation	
51	Diacylglycerol kinase zeta (DAG kinase zeta)	DGKZ	Q13574	0.94	0.56	0.73	0.25	0.68	0.21	Immune response	*
52	Dickkopf-related protein 1	DKK-1	Q94907	6.77	0.71	6.84	0.39	6.95	0.30	CVD I	
53	Discordin, CUB and LCL domain-containing protein 2	DCBLD2	Q96F02	2.32	0.31	2.14	0.25	2.22	0.24	Immune response	
54	DNA fragmentation factor subunit alpha	DFFA	O00273	5.40	1.44	4.79	1.31	5.38	0.56	Immune response	
55	Dynactin subunit 1	DNCTN1	Q14203	5.57	0.96	5.35	1.08	6.03	0.36	Immune response	
56	E3 ubiquitin-protein ligase TRIM21	TRIM21	P19474	3.00	1.29	2.78	1.28	3.46	0.54	Immune response	
57	E-selectin	SELE	P16581	4.37	0.76	4.75	0.35	5.16	0.41	CVD I	
58	Egl nine homolog 1	EGLN1	Q9GZ79	1.95	1.85	1.77	0.35	1.54	0.59	Immune response	
59	Endothelial cell-specific molecule 1	ESM-1	Q9NQ30	2.81	0.34	2.52	0.41	3.00	0.39	CVD I	
60	Eosinophil cationic protein	ECP	P12724	3.10	0.80	3.77	1.00	3.27	0.86	CVD I	
61	Eotaxin	CCL11	P51671	7.05	0.47	6.81	0.35	6.85	0.41	Inflammation	
62	Eotaxin-1	CCL11	P51671	7.62	0.45	7.31	0.34	7.44	0.39	Immune response	
63	Epidermal growth factor	EGF	P01133	9.92	1.36	9.58	1.08	10.31	0.51	CVD I	
64	Eukaryotic translation initiation factor 4E-binding protein 1	4E-BP1	Q13574	7.63	1.52	7.00	1.52	7.46	0.50	Inflammation	
65	Eukaryotic translation initiation factor 4 gamma 1 (eIF-4-gamma 1)	EIF4G1	Q04637	6.55	0.95	5.81	2.21	6.92	0.25	Immune response	
66	Eukaryotic translation initiation factor 5A-1 (eIF-5A-1)	EIF5A	P63241	2.34	1.52	1.19	1.27	0.64	0.44	Immune response	*
67	Fatty acid-binding protein, adipocyte	FABP4	P15090	2.12	0.50	1.99	0.63	2.31	0.59	CVD I	
68	Fc receptor-like protein 3 (FcR-like protein 3)	FCRL3	Q96P31	0.77	0.22	0.89	0.22	0.74	0.22	Immune response	
69	Fc receptor-like protein 6 (FcR-like protein 6)	FCRL6	Q6DN72	1.98	0.42	2.19	0.62	2.12	0.48	Immune response	
70	Fibroblast growth factor 2	FGF2	P09038	1.89	0.72	2.20	0.39	1.97	0.68	Immune response	
71	Fibroblast growth factor 5	FGF-5	P12034	1.37	0.23	1.36	0.23	1.38	0.19	Inflammation	
72	Fibroblast growth factor 19	FGF-19	Q95750	8.47	1.12	7.79	0.65	7.91	0.76	Inflammation	
73	Fibroblast growth factor 21	FGF-21	Q9NSA1	3.79	1.41	3.51	1.42	4.67	1.04	Inflammation	
74	Fibroblast growth factor 23	FGF-23	Q9GZV9	2.65	0.75	2.19	0.59	2.62	0.47	Inflammation	
75	Fms-related tyrosine kinase 3 ligand	Flt3L	P49771	9.05	0.37	8.91	0.32	9.06	0.47	Inflammation	
76	Follistatin	FS	P19883	4.36	0.46	4.15	0.39	4.55	0.35	CVD I	
77	Fractalkine	CXCL3	P78423	6.16	0.49	5.82	0.22	5.86	0.23	CVD I	
78	FXR domain-containing ion transport regulator 5	FXR5	Q960B9	1.27	0.75	1.13	0.60	1.24	0.49	Immune response	
79	Galanin peptides	GAL	P22466	5.96	0.49	5.93	0.41	5.57	0.58	CVD I	
80	Galectin-3	Gal-3	P17931	5.12	0.46	4.69	0.30	4.79	0.27	CVD I	
81	Glial cell line-derived neurotrophic factor	GDNF	P39905	2.62	0.23	2.73	0.37	2.60	0.28	Inflammation	
82	Growth/differentiation factor 15	GDF-15	Q99988	7.34	0.43	7.05	0.40	7.75	0.54	CVD I	
83	Growth hormone	GH	P01241	6.49	2.02	5.73	2.16	6.70	2.38	CVD I	
84	Heat shock 27 kDa protein	HSP-27	P04792	7.65	1.33	6.99	2.60	8.24	0.31	CVD I	
85	Hematopoietic lineage cell-specific protein	HCLS1	P14317	6.09	1.11	5.85	0.91	6.32	0.40	Immune response	
86	Heparin-binding EGF-like growth factor	HB-EGF	Q99075	7.90	0.79	7.79	0.46	8.01	0.54	CVD I	
87	Hepatocyte growth factor	HGF	P14210	6.97	0.94	6.71	0.33	7.00	0.39	Inflammation	
88	Histamine N-methyltransferase	HNMT	P50135	0.58	0.56	0.51	0.14	0.48	0.23	Immune response	
89	Importin subunit alpha-5	KPNB1	P52294	2.17	3.03	1.53	2.20	0.69	1.11	Immune response	*
90	Inactive dipeptidyl peptidase 10	DPP10	Q8N608	1.00	0.44	1.07	0.60	0.83	0.26	Immune response	
91	Integral membrane protein 2A	ITGA2A	Q43736	1.42	0.63	1.32	0.41	1.47	0.61	Immune response	
92	Integrin alpha-6	ITGA6	P23229	1.79	0.98	1.56	0.94	2.09	0.67	Immune response	
93	Integrin alpha-11	ITGA11	Q9UKX5	2.24	0.31	2.25	0.41	2.03	0.34	Immune response	
94	Integrin beta-6	ITGB6	P18564	1.93	0.26	1.86	0.21	1.66	0.29	Immune response	
95	Interferon gamma	IFN-gamma	P01579	1.21	0.79	0.81	0.54	0.71	0.44	Inflammation	
96	Interferon lambda receptor 1 (IFN-lambda receptor 1)	IFNL1R1	Q8IU57	2.04	0.24	1.91	0.13	1.99	0.26	Immune response	
97	Interferon regulatory factor 9	IRF9	Q00978	1.86	1.26	1.66	0.39	2.07	0.42	Immune response	
98	Interleukin-1 alpha	IL-1 alpha	P01583	0.23	0.43	-0.02	0.04	-0.01	0.03	Inflammation	*
99	Interleukin-1 receptor antagonist protein	IL-1RA	P18510	4.60	1.03	4.27	0.62	4.55	0.70	CVD I	
100	Interleukin-1 receptor-associated kinase 1	IRAK1	P51617	2.52	0.90	2.39	0.67	2.62	0.43	Immune response	
101	Interleukin-1 receptor-associated kinase 4	IRAK4	Q9NW23	5.09	1.35	5.36	0.50	5.44	0.60	Immune response	
102	Interleukin-2	IL-2	P60568	0.09	0.31	0.00	0.00	0.30	0.42	Inflammation	*
103	Interleukin-2 receptor subunit beta	IL-2RB	P14784	0.77	0.57	0.32	0.43	0.14	0.32	Inflammation	*
104	Interleukin-12	IL-12	P05112	0.89	1.26	0.47	0.53	0.26	0.44	Inflammation	*
105	Interleukin-5	IL-5	P05113	0.90	0.86	0.88	1.22	0.53	0.79	Inflammation	*
106	Interleukin-5	IL-5	P05113	1.31	0.59	3.07	0.63	1.59	1.19	Immune response	*
107	Interleukin-6	IL-6	P05231	2.24	0.68	2.15	0.74	2.34	0.46	Inflammation	
108	Interleukin-6	IL-6	P05231	2.14	0.59	2.08	0.74	2.25	0.45	Immune response	
109	Interleukin-6 receptor subunit alpha	IL-6RA	P08887	6.73	0.27	6.76	0.31	6.77	0.32	CVD I	
110	Interleukin-7	IL-7	P13232	3.85	0.63	3.86	0.39	4.18	0.55	Inflammation	
111	Interleukin-8	IL-8	P01045	6.73	1.30	6.37	0.35	6.57	0.43	Inflammation	
112	Interleukin-10	IL-10	P22301	2.08	0.38	2.16	0.41	1.99	0.43	Immune response	
113	Interleukin-10	IL-10									

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	Full protein name	Protein abbreviation	UniProt No.	Healthy control (mean levels)	±SD Healthy control	Asthma (mean levels)	±SD Asthma	Mastocytosis (mean levels)	±SD Masto-cytosis	Olink panel	* > 5 measurements below detection limit were excluded from analysis
125	Interleukin-20	IL-20	Q9NY1	0.69	0.47	0.61	0.23	0.80	0.96	Inflammation	*
126	Interleukin-20 receptor subunit alpha	IL-20RA	Q9UHF4	0.76	1.27	0.51	0.78	0.25	0.36	Inflammation	*
127	Interleukin-22 receptor subunit alpha-1	IL-22RA1	Q9N8P7	0.28	0.66	0.19	0.59	0.13	0.56	Inflammation	*
128	Interleukin-24	IL-24	Q13007	0.57	0.93	0.21	0.45	0.12	0.34	Inflammation	*
129	Interleukin-27	IL-27	Q9NEV9; Q14213	3.58	0.33	3.44	0.35	3.72	0.33	CVD I	
130	Interleukin-33	IL-33	Q95760	0.10	0.35	0.00	0.00	0.00	0.00	Inflammation	*
131	Islet cell autoantigen 1	ICA1	Q05084	3.03	1.26	2.88	1.02	3.49	0.69	Immune response	
132	Kallikrein-6	KLK6	Q92876	4.52	0.27	4.44	0.30	4.50	0.33	CVD I	
133	Kallikrein-11	hK11	Q9U8X7	5.03	0.29	4.66	0.24	4.92	0.38	CVD I	
134	Keratin, type I cytoskeletal 19	KRT19	P08727	2.13	0.36	1.87	0.37	2.10	0.51	Immune response	
135	Latency-associated peptide transforming growth factor beta 1	LAP TGF-beta-1	P01137	7.64	0.68	7.58	0.30	7.73	0.36	Inflammation	
136	Lectin-like oxidized LDL receptor 1	LOX-1	P78380	5.82	0.81	6.03	0.88	5.83	0.62	CVD I	
137	Leptin	LEP	P41159	5.16	1.00	4.64	1.34	5.03	0.95	CVD I	
138	Leukemia inhibitory factor	LIF	P15018	0.21	0.50	0.06	0.20	0.08	0.23	Inflammation	*
139	Leukemia inhibitory factor receptor	LIF-R	P42702	2.67	0.28	2.59	0.22	2.74	0.26	Inflammation	
140	Leukocyte immunoglobulin-like receptor subfamily 8 member 4	LILRB4	Q9NHU6	2.55	0.38	2.36	0.35	2.69	0.33	Immune response	
141	Lymphocyte activation gene 3 protein	LAG3	P18627	1.84	0.32	1.75	0.27	1.75	0.41	Immune response	
142	Lymphocyte antigen 75	LY75	Q60449	1.99	0.46	1.99	0.25	1.84	0.26	Immune response	
143	Lysosome-associated membrane glycoprotein 3	LAMP3	Q9UQV4	2.73	0.40	2.84	0.31	2.94	0.67	Immune response	
144	Macrophage colony-stimulating factor 1	CSF-1	P09603	7.87	0.13	7.84	0.16	7.93	0.19	Inflammation	
145	Mannan-binding lectin serine protease 1	MASP1	P48740	1.23	0.31	1.40	0.22	1.39	0.25	Immune response	
146	Matrix metalloproteinase-1	MMP-1	P03956	2.83	0.59	2.94	0.87	3.66	0.84	Inflammation	
147	Matrix metalloproteinase-3	MMP-3	P08254	1.64	0.53	1.72	0.53	1.88	0.57	CVD I	
148	Matrix metalloproteinase-7	MMP-7	P09237	1.98	0.46	5.88	0.78	5.95	0.44	CVD I	
149	Matrix metalloproteinase-10	MMP-10	P09238	6.41	0.90	5.94	0.53	6.22	0.65	Inflammation	
150	Matrix metalloproteinase-12	MMP-12	P39900	6.44	0.57	5.79	0.37	5.90	0.37	CVD I	
151	Melusin/Integrin beta-1-binding protein 2	ITGB1BP2	Q9UKP3	5.48	1.54	4.75	2.31	5.96	1.04	CVD I	
152	Membrane-bound aminopeptidase P	mAmP	O43895	2.55	1.23	1.76	0.96	2.59	1.06	CVD I	
153	Merlin	NF2	P35240	2.28	0.78	2.08	0.55	2.18	0.61	Immune response	
154	Methylated-DNA-protein-cysteine methyltransferase	MGMT	P18655	5.84	1.18	5.38	1.73	6.11	0.22	Immune response	
155	Monocyte chemotactic protein 1	MCP-1	P13500	10.00	0.52	9.83	0.26	9.90	0.36	Inflammation	
156	Monocyte chemotactic protein 2	MCP-2	P80075	9.18	0.71	9.41	0.46	9.37	0.81	Inflammation	
157	Monocyte chemotactic protein 3	MCP-3	P80098	2.18	0.62	1.94	0.29	2.18	0.34	Inflammation	
158	Monocyte chemotactic protein 4	MCP-4	Q99616	3.75	0.72	3.43	0.51	3.73	0.52	Inflammation	
159	Myeloperoxidase	MPO	P05164	4.30	0.22	4.30	0.34	4.34	0.38	CVD I	
160	Myoglobin	MB	P02144	5.57	0.67	5.50	0.56	5.52	0.83	CVD I	
161	N-terminal pro-B-type natriuretic peptide	NT-pro-BNP	N/A	2.83	0.84	2.27	0.92	2.99	0.99	CVD I	
162	Natural cytotoxicity triggering receptor 1	NCR1	Q76036	1.33	0.28	1.25	0.29	1.27	0.33	Immune response	
163	Natural killer cell receptor 2B4	CD244	Q98ZW8	7.00	0.56	6.77	0.43	6.99	0.34	Inflammation	
164	Natural killer cells antigen CD94	KLRD1	Q13241	5.01	0.74	5.17	0.50	4.89	0.43	Immune response	
165	Natriuretic peptides B	BNP	P16860	0.08	0.23	-0.01	0.07	0.13	0.26	CVD I	*
166	Neurabin-2	PPP1R9B	Q96583	6.08	0.87	5.64	1.56	6.42	0.58	Immune response	
167	Neurotrophin-3	NT-3	P20763	2.74	0.30	2.47	0.32	2.47	0.26	Inflammation	
168	Neurotrophin-4 (NT-4)	NT4	P14130	1.17	0.39	0.97	0.32	1.03	0.37	Immune response	
169	Neurturin	NRTN	Q99748	0.46	0.61	0.35	0.50	0.24	0.39	Inflammation	*
170	NF-kappa-B essential modulator	NEMO	Q9YK93	6.61	1.19	6.35	1.26	6.99	0.44	CVD I	
171	Nuclear factor of activated T-cells, cytoplasmic 3	NFATC3	Q12968	1.03	1.19	0.60	0.74	0.61	0.71	Immune response	
172	Oncostatin-M	OSM	P13725	3.30	1.49	3.10	0.91	2.77	0.89	Inflammation	
173	Osteopontin	OPG	O00300	10.05	0.23	9.99	0.33	10.12	0.31	Inflammation	
174	Ovarian cancer-related tumor marker CA 125/Mucin-16	CA-125/MUC-16	Q8W0X7	2.78	0.40	2.65	0.68	2.30	0.63	CVD I	
175	P-selectin glycoprotein ligand 1	PSGL-1	Q14242	0.01	0.16	0.04	0.14	0.01	0.12	CVD I	
176	Pappalysin-1	PAPPA	Q13219	1.97	0.35	1.92	0.32	2.50	0.74	CVD I	
177	Parathyroid hormone/parathyroid hormone-related peptide receptor	PTH1R	Q03431	1.53	0.33	1.60	0.44	1.81	0.81	Immune response	
178	PC4 and SFRS1-interacting protein	PSIP1	O75475	2.80	1.72	2.41	0.65	2.75	0.75	Immune response	
179	Pentraxin-related protein PTX3	PTX3	P26022	2.47	1.29	1.88	0.26	2.40	0.89	CVD I	
180	Peroxiredoxin-1	PRDX1	Q06830	3.01	0.76	3.09	0.87	3.42	0.38	Immune response	
181	Peroxiredoxin-5, mitochondrial	PRDX5	Q98044	7.36	0.94	7.37	0.85	7.74	0.19	Immune response	
182	Phosphoinositide 3-kinase adapter protein 1	PIK3AP1	Q6Z0J8	4.33	1.26	4.11	0.75	4.00	0.72	Immune response	
183	Placenta growth factor	PGF	P49763	6.46	0.25	6.48	0.33	6.78	0.34	CVD I	
184	Platelet-derived growth factor subunit B	PDGF Subunit B	P01127	8.16	0.82	8.06	0.43	8.26	0.41	CVD I	
185	Platelet endothelial cell adhesion molecule	PECAM-1	P16284	5.64	0.64	5.50	0.63	5.96	0.20	CVD I	
186	Plexin-A4	PLXNA4	Q9H0C2	7.01	1.23	6.55	1.87	7.45	0.51	Immune response	
187	Polypeptide N-acetylgalactosaminyltransferase 3	GALNT3	Q14435	0.49	0.13	0.61	0.32	0.44	0.15	Immune response	*
188	Probable ATP-dependent RNA helicase DDX58	DDX58	Q95786	2.61	1.71	2.36	0.37	2.58	0.66	Immune response	
189	Programmed cell death 1 ligand 1	PD-L1	Q9N2Q7	0.75	0.98	0.82	0.90	1.31	0.80	Inflammation	
190	Prolactin	PRL	P01236	3.45	0.53	3.45	0.59	3.59	0.77	CVD I	
191	Proteinase-activated receptor 1	PAR1	P25116	7.61	0.58	6.92	2.02	7.85	0.18	CVD I	
192	Protein-arginine deiminase type-2	PADI2	Q9Y2I8	0.92	1.09	0.57	0.34	0.63	0.23	Immune response	*
193	Protein FAM38	FAM38	P58499	3.89	0.50	3.96	0.32	3.94	0.46	Immune response	
194	Protein HEXIM1	HEXIM1	Q98092	6.10	1.21	5.65	2.02	6.67	0.54	Immune response	
195	Protein kinase C theta type	PRKCO	Q4759	1.48	0.70	1.26	0.47	1.53	0.59	Immune response	
196	Protein S100-A12	EN-RAGE	P05111	2.07	1.08	2.76	1.58	1.87	0.44	Inflammation	
197	Protein sprouty homolog 2	SPRY2	O43597	3.50	1.54	3.99	0.71	3.86	0.98	Immune response	
198	Proto-oncogene tyrosine-protein kinase Src	SRC	P12931	6.76	0.29	6.13	1.34	6.63	0.24	CVD I	
199	Receptor for advanced glycosylation end products	RAGE	E15109	5.16	0.39	5.08	0.31	4.94	0.32	CVD I	
200	Renin	REN	P00797	5.75	0.61	5.98	0.32	5.74	0.65	CVD I	
201	Resistin	RETN	Q9H089	6.48	1.18	5.94	0.40	6.29	0.42	CVD I	
202	SH2B adaptor protein 3	SH2B3	Q9UQO2	6.42	1.55	6.75	0.68	6.71	0.69	Immune response	
203	SH2 domain-containing protein 1A	SH2D1A	O60880	1.91	1.76	1.88	0.68	1.76	0.81	Immune response	
204	Signaling lymphocytic activation molecule	SLAMF1	Q13291	2.44	0.39	2.54	0.37	2.55	0.39	Inflammation	
205	Signaling threshold-regulating transmembrane adapter 1	SIT1	Q9Y3P8	1.08	0.74	0.94	0.28	0.76	0.24	Immune response	
206	siR2-like protein 2	SIRT2	Q8X0U6	7.92	1.53	7.25	2.26	8.42	0.56	CVD I	
207	Spondin-1	SPON1	Q9H0N1	4.45	0.26	4.20	0.18	4.51	0.19	CVD I	
208	SRSF protein kinase 2	SRSF2	P78262	2.06	1.08	1.95	0.94	2.33	0.57	Immune response	
209	ST2 protein	ST2	Q01628	3.79	0.70	3.83	0.58	4.26	0.62	CVD I	
210	STAM-binding protein	STAMPB	O95630	7.74	1.35	7.07	1.97	8.02	0.51	Inflammation	
211	Stanniocalcin-1	STC1	P52823	4.83	0.48	4.84	0.38	4.91	0.45	Immune response	
212	Stem cell factor	SCF	P21583	8.07	0.25	7.82	0.40	7.88	0.31	CVD I	
213	Stromal cell-derived factor 1 (SDF-1)	CXCL12	P48061	1.25	0.23	1.18	0.26	1.32	0.17	Immune response	
214	Sulfotransferase 1A1	ST1A1	P50225	2.66	0.81	2.74	1.07	3.36	0.31	Inflammation	
215	T-cell immunoglobulin and mucin domain-containing protein 1	TIM-1	Q96042	5.66	0.62	5.33	0.56	6.24	0.65	CVD I	
216	T-cell-specific surface glycoprotein CD28	CD28	P10747	0.69	0.16	0.70	0.17	0.74	0.35	Immune response	
217	T-cell surface glycoprotein CD5	CD5	P06127	4.09	0.61	3.99	0.23	3.93	0.30	Inflammation	
218	T-cell surface glycoprotein CD6 isoform	CD6	P30203	4.27	0.93	4.38	0.37	3.97	0.34	Inflammation	
219	Thioredoxin-dependent peroxide reductase, mitochondrial	PRDX3	P30048	1.01	0.69	1.07	0.24	1.20	0.39	Immune response	
220	Thrombospondin	THM	P07204	8.93	0.25	8.78	0.16	8.88	0.17	CVD I	
221	Thymic stromal lymphopoietin	TSLP	Q96009	0.54	0.86	0.21	0.46	0.05	0.21	Inflammation	*
222	Tissue factor	TF	P13726	4.87	0.28	4.89	0.23	4.90	0.28	CVD I	
223	Tissue-type plasminogen activator	t-PA	P00750	7.49	0.65	7.83	0.59	7.57	0.58	CVD I	
224	TNF-beta	TNFB	P01374	3.61	0.31	3.66	0.31	3.48	0.42	Inflammation	
225	TNF receptor-associated factor 2	TRAF2	Q12933	3.48	1.11	3.60	1.07	4.02	0.49	CVD I	
226	TNF-related activation-induced cytokine	TRANCE	O14788	4.17	0.59	4.40	0.47	3.94	0.51	Inflammation	
227	TNF-related apoptosis-inducing ligand	TRAIL	P50591	8.80	0.37	8.75	0.15	8.77	0.22	Inflammation	
228	TNF-related apoptosis-inducing ligand receptor 2	TRAIL-R2	O14763	2.16	0.27	2.07	0.18	2.30	0.32	CVD I	
229	TRAF family member-associated NF-kappa-B activator	TANK	Q92844	2.83	0.87	2.74	0.61	2.68	0.65	Immune response	
230	Transcription factor AP-1	JUN	P05412	1.59	0.88	5.95	0.00	1.63	1.23	Immune response	*
231	Transcription regulator protein BACH1	BACH1	O14867	2.69	1.07	2.45	1.02	2.73	0.62	Immune response	
232	Transforming growth factor alpha	TGF-alpha	P01135	1.25	0.66	1.19	0.38	1.00	0.25	Inflammation	
233	Triggering receptor expressed on myeloid cells 1	TREM1	Q9N899	1.00	0.62	0.88	0.29	1.08	0.55	Immune response	
234	Tripartite motif-containing protein 5	TRIM5	Q03035	3.38	0.96	2.48	0.35	2.82	0.60	Immune response	
235	Tryptase alpha/beta-1	TPSAB1	Q15661	3.38	0.92	3.13	0.53	4.28	0.71	Immune response	
236	Tumor necrosis factor	TNF	P01375	0.27	0.38	0.					

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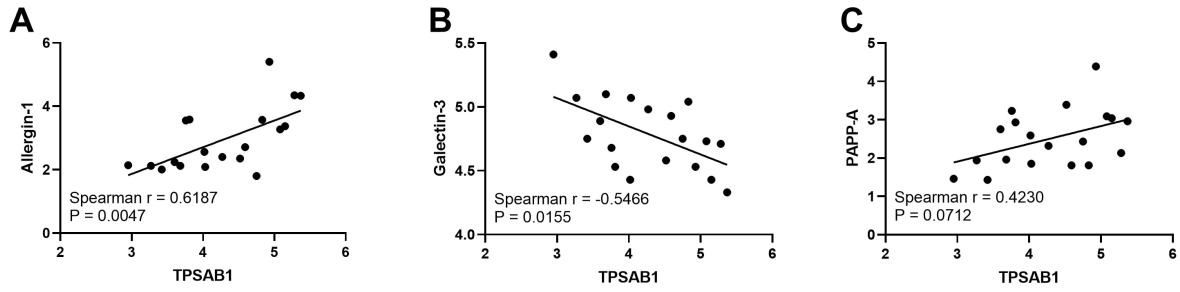


Figure 1

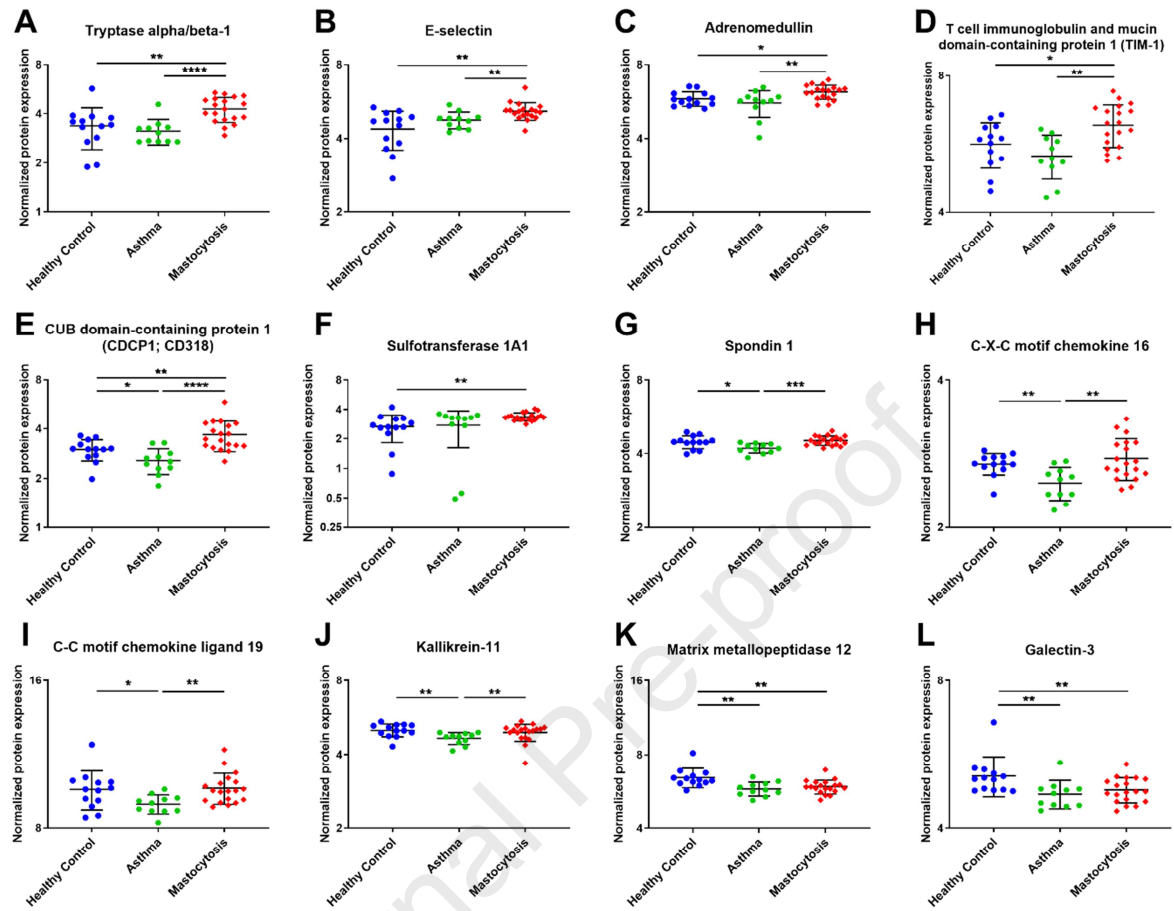
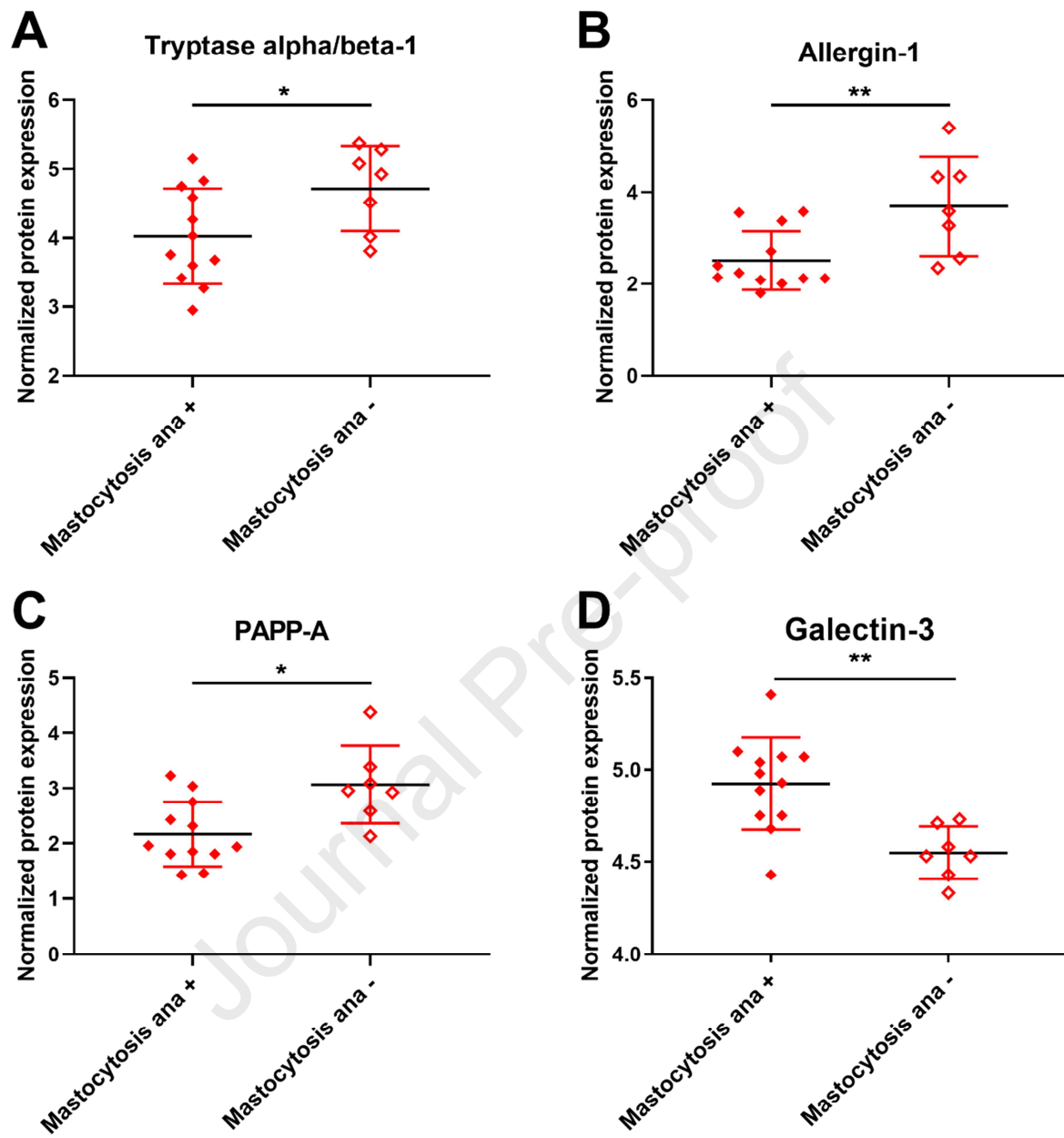


Figure 3



Distinct plasma biomarkers confirm the diagnosis of mastocytosis and identify increased risk of anaphylaxis

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Conflict of Interest

T. Gülen has received lecture fees from Thermo Fisher and Shire. G Nilsson has received honoraria (advisory board) from AstraZeneca and Novartis. B Dahlén reports personal fees from AstraZeneca, Teva, Sanofi, and grants from Novartis, GSK, outside the submitted work. S-E Dahlén reports personal fees from AZ, Cyaman Chemicals, GSK, Novartis, Sanofi-Regeneron, TEVA, outside the submitted work. Other authors declare no relevant conflicts of interest.

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ABSTRACT**Background**

Mastocytosis encompasses a heterogenous group of disorders characterized by accumulation of clonal mast cells (MCs) in the skin and/or internal organs. Patients typically present with a broad variety of recurrent mediator-related clinical symptoms, including severe anaphylaxis. However, not all patients with mastocytosis experience anaphylactic reactions.

Objective

We sought to identify disease specific biomarkers in plasma, that could be used to predict mastocytosis patients with increased risk of anaphylaxis.

Methods

Nineteen patients (≥ 18 years) and two control groups (11 subjects with allergic asthma, and 13 healthy volunteers without history of atopy) were recruited. In total, 248 plasma proteins were analyzed by Proximity Extension Assay (PEA) using Olink Proseek Multiplex panels.

Results

We identified four novel proteins, in addition to tryptase, E-selectin, Adrenomedullin, T cell immunoglobulin and mucin domain 1, and CUB domain-containing protein 1/CD138 to be significantly increased in mastocytosis compared to both asthma and healthy controls. Further, we investigated whether we could discriminate between mastocytosis patients with or without anaphylaxis. In addition to tryptase, we identified three novel proteins; i.e., Allergin-1, pregnancy-associated plasma protein-A and Galectin-3, with significantly different levels in mastocytosis patients with anaphylaxis compared to those without anaphylaxis.

Conclusion

Newly identified proteomic biomarkers may be used to predict mastocytosis patients with increased risk of anaphylaxis.

Clinical Implications

Newly detected proteomic biomarkers may have the potential to improve diagnosis of mastocytosis and increased risk of anaphylaxis.

Capsule Summary

This is the first study that unveils novel plasma biomarkers using a plasma proteomic approach that could be used to predict mastocytosis patients with increased risk of anaphylaxis.

Key words

Mastocytosis, anaphylaxis, tryptase, mast cell, plasma proteomic, PAPP-A, Galectin-3, Allergin-1, TPSAB1

Abbreviations

SM: Systemic mastocytosis

MMAS: Monoclonal mast cell activation syndrome

MC: Mast cell

PEA: Proximity Extension Assay

SELE: E-selectin

AM: Adrenomedullin

TIM-1: T cell immunoglobulin and mucin domain 1

CDCP1: CUB domain-containing protein 1

MMP12: Matrix metalloproteinase 12

Gal-3: Galectin-3

PAPP-A: Pregnancy-associated plasma protein-A

TPSAB1: Tryptase alpha/beta-1

117 Introduction

118 Mastocytosis refers to a heterogenous disorder characterized by excessive activation and
 119 accumulation of mast cells (MCs) in the skin and/or internal organs (1). By definition,
 120 patients with pathologic MCs limited to the skin will be classified as having cutaneous
 121 mastocytosis (CM), whereas in systemic mastocytosis (SM), at least one extracutaneous
 122 organ/tissue, most often the bone marrow, is involved (1). Furthermore, monoclonal mast cell
 123 activation syndrome (MMAS) is a rare variant of this disorder, where patients have clonal MCs
 124 but do not meet criteria for SM (1).

125 Patients with mastocytosis typically display elevated levels of MC mediators such as tryptase,
 126 histamine, and urinary metabolites of prostaglandin D₂ (2, 3). Accordingly, the patients
 127 exhibit a varying degree of “clinical state of hyperreactivity” and present with a range of
 128 recurrent mediator-related local or systemic clinical symptoms (4, 5). Indeed, a strong
 129 association between adult patients with various forms of mastocytosis and anaphylaxis has
 130 been described (6, 7). The most common trigger of anaphylaxis in mastocytosis appears to be
 131 Hymenoptera venom, followed by reactions without clear trigger, that is, idiopathic (8).
 132 However, not all patients with mastocytosis experience anaphylactic reactions. Therefore, a
 133 unique mastocytosis - anaphylaxis phenotype has been suggested (8). Nevertheless, there are
 134 currently no predictive/specific biomarker(s) to discriminate patients with mastocytosis at
 135 high risk of anaphylaxis. In the current study, we sought to search for disease specific
 136 biomarkers in plasma, which could be used to predict a mastocytosis-anaphylaxis phenotype
 137 at the point of care.

138 Methods

139 For detailed methods, please see the Methods section in this article’s Online Repository
 140 at www.jacionline.org.

141 Results and Discussion

142 In total 248 plasma proteins were analysed in 15 patients with indolent SM and four cases
 143 with MMAS and compared to 11 subjects with allergic asthma and 13 healthy volunteers
 144 without history of atopy. As expected, tryptase, a serine protease stored in mast cell granules
 145 and a well-established marker for mastocytosis and also one of the minor diagnostic criteria
 146 (1), was significantly increased in the mastocytosis group (Fig. 1). In addition to tryptase
 147 (measured as TPSAB1 products alpha and beta tryptase in the Olink platform (9)), we

identified four other proteins; E-selectin (SELE), Adrenomedullin (AM), T cell immunoglobulin and mucin domain 1 (TIM-1), and CUB domain-containing protein 1/CD138 (CDCP1), to be significantly increased in mastocytosis compared to both asthma and healthy controls (Fig. 1). Moreover, the plasma levels of these four proteins correlated with the levels of tryptase (Fig. 2). Sulfotransferase 1A1 was increased in mastocytosis patients compared to healthy controls, however, not significantly different to asthmatic patients (Fig. 1). Spondin 1, CXCL-16, CCL-19, and Kallikrein 11 were all decreased in asthmatics compared to healthy controls, and Matrix metalloproteinase 12 (MMP12) as well as Galectin-3 (Gal-3) were decreased in both mastocytosis and asthmatics compared to healthy subjects (Fig 1).

Aside from tryptase, none of the other four plasma proteins associated with mastocytosis have previously been described in this context. SELE is an adhesion molecule induced by TNF, of which MCs are one source. Adrenomedullin is a multifunctional peptide that is ubiquitously expressed. AM can be found in cardiac mast cell granules, and thus be released from MCs (10). In addition, AM can activate mast cells to degranulate, and thereby act in a self-activating mode. TIM-1 is predominantly expressed on Th2-cells and functions as a receptor for viruses and apoptotic cells, but it can also activate MCs through TIM-4 to release cytokines, e.g., IL-4 and IL-13 (11). CDCP1 is a transmembrane protein associated with src kinase family members (12). The increased levels of CDCP1 in the plasma of mastocytosis patients could hypothetically be explained by the increased tryptase levels seen in these patients, since the soluble form of CDCP1 is generated by cleavage of the extracellular domain of CDCP1 by various serine proteases (13). Furthermore, all four proteins showed significant correlation with tryptase levels (Fig 2). Thus, although we have not identified mechanisms for the increased plasma levels of E-selectin, AM, TIM-1 and CDCP1 in mastocytosis, there are plausible connections between MCs and these proteins that might explain their increased levels.

As anaphylaxis causes severe symptoms affecting approximately 50 % of those with mastocytosis (8), we next investigated if any of the 248 proteins analysed could discriminate between mastocytosis patients with or without anaphylaxis. One of the proteins identified was tryptase, which is a well-established marker of acute severe MC activation when compared to a baseline value (14). Interestingly, it is also included as a risk parameter in an algorithm that we recently described to determine mastocytosis patients at risk for anaphylaxis, since baseline tryptase levels have been shown to be relatively lower although above normal values in those with anaphylaxis (8) (Fig 3). Two additional proteins, Allergin-1 and pregnancy-

associated plasma protein-A (or pappalysin-1) (PAPP-A) were also found to be lower in patients with a history of anaphylaxis, whereas Galectin-3 was identified to be increased in those with anaphylaxis compared to those without anaphylaxis (Fig. 3). Furthermore, the plasma levels of all these 3 proteins correlated with the levels of tryptase (Fig 4). Interestingly, when we have compared patients with venom-induced anaphylaxis (n=6) vs. anaphylaxis patients without venom sensitivity (n=6), no statistically significant differences were found between the groups.

Allergin-1 is a membrane bound, immunoglobulin-like receptor which is predominantly expressed on mast cells and basophils (15). It is encoded by the *Mast Cell Immunoglobulin Like Receptor 1 (MILR1)* gene and its deficiency in mice causes enhanced passive cutaneous and systemic anaphylaxis (16,17). There are three known isoforms of human Allergin-1, of which the isoform Allergin-1S1 is predominantly expressed and inhibits FcεRI and consequently IgE-mediated mast cell activation (17). Interestingly, this notion is in line with our finding as mastocytosis patients with anaphylaxis showed lower plasma levels of Allergin-1 compared to patients without anaphylaxis.

PAPP-A is a metalloprotease expressed in, e.g., coronary artery endothelial cells and elevated serum levels have been identified as a biomarker for acute coronary syndrome, and also severe asthma (18, 19). There are observations indicating that plasma levels of PAPP-A might be connected to mast cell activation. In two studies where heparin, a proteoglycan stored in mast cell granules, was injected, increased levels of circulating PAPP-A was determined (20, 21). Another observation comes from patients with asthma who were treated with omalizumab. Although, initially elevated levels of PAPP-A were observed in severe asthmatics, the levels were significantly lowered after six months of omalizumab treatment (22). Since anti-IgE treatment has an inhibitory effect on mast cells, the effect of omalizumab on plasma levels of PAPP-A might be due to a direct or indirect effect on mast cells. In our study, we detected significantly lower levels of PAPP-A in mastocytosis patients with anaphylaxis than non-anaphylactic mastocytosis patients (Fig. 3), although levels were not significantly different compared to control groups. Nevertheless, our findings suggest PAPP-A and Allergin-1 may be predicting markers of anaphylaxis in mastocytosis.

Notably, Galectin-3, previously also called “IgE-binding protein” (23), was the only protein found to be increased in mastocytosis patients with anaphylaxis compared to those without anaphylaxis, although the levels were lower in mastocytosis patients compared to healthy controls. In a previous study including patients with persistent severe allergic asthma,

bronchial tissue Galectin-3 levels were suggested to be a predictive biomarker for therapy response to omalizumab, where responders had higher Galectin-3 levels (24). However, the effect of Galectin-3 on mast cell reactivity is not clear, as there are studies showing Galectin-3 to either promote or prevent mast cell activation. Hence, the potential role of Galectin-3 as a biomarker in mastocytosis remains elusive.

In conclusion, this is the first study that unveils novel plasma biomarkers using a plasma proteomic approach that have the potential to assist in predicting the risk of anaphylaxis in mastocytosis. Although several of the proteins identified in this study can be associated in one way or another to mast cells and their functions, it is still unclear why their plasma levels in mastocytosis differ to the control groups, and why other proteins can discriminate between mastocytosis patients with or without anaphylaxis. Hence, mechanistic studies are necessary to perform to address these questions. Furthermore, the generalization of our results needs to be validated in larger cohorts, before it can be used in clinical practise.

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Table 1 – Demography and characteristics of study subjects and controls

Characteristics	Mast cell disorders (n=19)	Allergic Asthma (n=11)	Healthy controls (n=13)
Age at diagnosis, Median (range)	47 (21-69)	30 (22-52)	33 (22-55)
Gender, male, n (%)	8/19 (42%)	8 (73)	5 (42)
Baseline tryptase ng/ml, median (range)	25 (3.8-320)	n/a	n/a
Total IgE kE/L, median (range)	16 (1-250)	85 (21-2500)	17 (6.2-36)
Presence of atopy, n (%)	7/17 (41%) (2 ND)	11 (100%)	0
Presence of atopic diseases, n (%)	4/17 (24%)	11 (100%)	0
Prevalence of anaphylaxis, n (%)	12 (63%)	n/a	n/a

n/a = not applicable; ND = not determined.

Figure Legends

Figure 1. Plasma biomarker levels in healthy controls, mild allergic asthma and mastocytosis identified with PEA proteomics. A - Tryptase. B - E-selectin. C - Adrenomedullin. D - T cell immunoglobulin and mucin domain 1. E - CUB domain-containing protein 1/CD138 (CDCP1). F - Sulfotransferase 1A1. G - Spondin 1. H - CXCL-16. I - CCL-19. J – Kallikrein. K - Matrix metalloproteinase 12. L – Galectin-3.

Statistical significance was determined by Kruskal-Wallis with Bonferroni correction ($p = 0.0167$), followed by Mann-Whitney U -test. Data is presented as mean $\pm$ SD. Statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure 2. Association between TPSAB1 and potential novel mastocytosis associated plasma protein biomarkers . A – E-selectin, B- Adrenomedullin, C- TIM-1, D – CDCP1. Correlations determined by Spearman's correlation test.

Figure 3. Plasma protein biomarkers that discriminates mastocytosis patients with or without anaphylaxis. A – Tryptase alpha/beta-1, B – Allergin-1, C – PAPP-A, D – Galectin-3 in differential diagnosis of anaphylaxis in patients with mastocytosis. Statistical significance was determined by Mann-Whitney U -test. Data is presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

Figure 4. Association between tryptase and potential anaphylaxis associated plasma protein biomarkers. A – TPSAB1 versus Allergin-1, B – TPSAB1 versus Galectin – 3, TPSAB1 versus PAPP-A. Correlations determined by Spearman's correlation test.