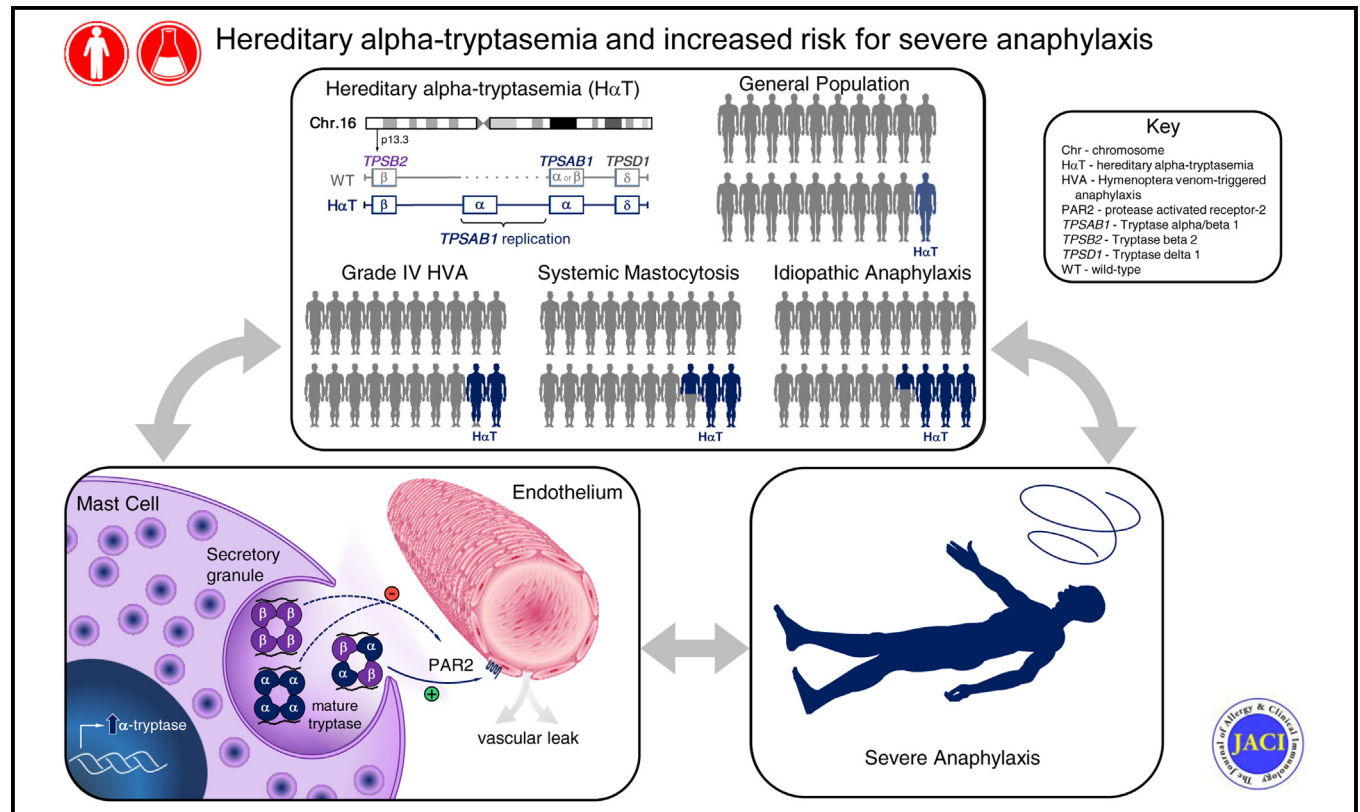


Heritable risk for severe anaphylaxis associated with increased α -tryptase–encoding germline copy number at *TPSAB1*

Jonathan J. Lyons, MD,^a Jack Chovanec, BS,^a Michael P. O'Connell, PhD,^a Yihui Liu, PhD,^a Julij Šelb, MD, PhD,^b Roberta Zanotti, MD,^c Yun Bai, PhD,^a Jiwon Kim, BS,^a Quang T. Le, PhD,^d Tom DiMaggio, ADN,^a Lawrence B. Schwartz, MD, PhD,^d Hirsh D. Komarow, MD,^a Matija Rijavec, PhD,^b Melody C. Carter, MD,^a Joshua D. Milner, MD,^{e,*} Patrizia Bonadonna, MD,^{f,*} Dean D. Metcalfe, MD,^{a,*} and Peter Korošec, PhD^{b,*} *Bethesda, Md, Golnik, Slovenia, Verona, Italy, Richmond, Va, and New York, New York*

GRAPHICAL ABSTRACT



From ^athe Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; ^bthe University Clinic of Respiratory and Allergic Diseases, Golnik; ^cthe Department of Medicine, Section of Hematology, Verona; ^dthe Department of Internal Medicine, Virginia Commonwealth University, Richmond; ^ethe Division of Allergy, Immunology and Rheumatology, Columbia University, New York; and ^fthe Allergy Unit, Verona University Hospital, Verona.

*These authors contributed equally.

Supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication January 23, 2020; revised June 10, 2020; accepted for publication June 15, 2020.

Corresponding author: Jonathan J. Lyons, MD, 9000 Rockville Pike, Building 29B, Room 5NN18, National Institutes of Health, Bethesda, MD 20892-1881. E-mail: jonathan.lyons@nih.gov.

0091-6749

Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaci.2020.06.035>

Background: An elevated basal serum tryptase level is associated with severe systemic anaphylaxis, most notably caused by Hymenoptera envenomation. Although clonal mast cell disease is the culprit in some individuals, it does not fully explain this clinical association.

Objective: Our aim was to determine the prevalence and associated impact of tryptase genotypes on anaphylaxis in humans.

Methods: Cohorts with systemic mastocytosis (SM) and venom as well as idiopathic anaphylaxis from referral centers in Italy, Slovenia, and the United States, underwent tryptase genotyping by droplet digital PCR. Associated anaphylaxis severity (Mueller scale) was subsequently examined. Healthy volunteers and controls with nonatopic disease were recruited and tryptase was genotyped by droplet digital PCR and *in silico* analysis of genome sequence, respectively. The effects of pooled and recombinant human tryptases, protease activated receptor 2 agonist and antagonist peptides, and a tryptase-neutralizing mAb on human umbilical vein endothelial cell permeability were assayed using a Transwell system.

Results: Hereditary α -tryptasemia (H α T)—a genetic trait caused by increased α -tryptase-encoding Tryptase- $\alpha/\beta 1$ (*TPSAB1*) copy number resulting in elevated BST level—was common in healthy individuals (5.6% [$n = 7$ of 125]) and controls with nonatopic disease (5.3% [$n = 21$ of 398]). H α T was associated with grade IV venom anaphylaxis (relative risk = 2.0; $P < .05$) and more prevalent in both idiopathic anaphylaxis ($n = 8$ of 47; [17%; $P = .006$]) and SM ($n = 10$ of 82 [12.2%; $P = .03$]) relative to the controls. Among patients with SM, concomitant H α T was associated with increased risk for systemic anaphylaxis (relative risk = 9.5; $P = .007$). *In vitro*, protease-activated receptor-2-dependent vascular permeability was induced by pooled mature tryptases but not α - or β -tryptase homotetramers.

Conclusions: Risk for severe anaphylaxis in humans is associated with inherited differences in α -tryptase-encoding copies at *TPSAB1*. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: Mastocytosis, venom, hypersensitivity, idiopathic anaphylaxis, mast cell activation, hereditary α -tryptasemia

Human tryptases are neutral serine proteases produced exclusively by tissue allergic effector mast cells and blood basophils in healthy individuals.¹ As mature enzymes, tryptases exist primarily in mast cell secretory granules as tetramers comprised of α and/or β isoforms.² Following mast cell activation and degranulation, tryptases contribute to the symptoms of immediate hypersensitivity via proteolytic cleavage of extracellular matrix components, fibrinogen, and protease activated receptors (PARs).²⁻⁵ Indeed, neutralization of human mature tryptases has been shown to reduce IgE-mediated anaphylaxis, as measured by core body temperature drop in a humanized mouse model.⁶ However, data demonstrating the clinical effects of tryptases on mast cell-mediated symptoms in humans are relatively lacking. In contrast to homotetrameric β -tryptases, mature α -tryptases have negligible proteolytic activity *in vitro*.⁷ However, α/β -tryptase heterotetramers have recently been shown to occur naturally,⁵ have enhanced stability and expanded proteolytic activity relative to β -tryptase homotetramers, and contribute to vibration-induced cutaneous symptoms via cleavage of the

Abbreviations used

ADGRE2:	Adhesion G protein-coupled receptor E2
BM:	Bone marrow
BST:	Basal serum tryptase
ddPCR:	Droplet digital PCR
EGM-2:	Endothelial growth medium-2
H α T:	Hereditary α -tryptasemia
HUVEC:	Human vascular endothelial cell
HVA:	Hymenoptera venom-triggered anaphylaxis
IA:	Idiopathic anaphylaxis
NIH:	National Institutes of Health
PAR:	Protease-activated receptor
PAR-2:	Protease-activated receptor-2
pNA:	<i>p</i> -Nitroanilide
RR:	Relative risk
SM:	Systemic mastocytosis
<i>TPSAB1</i> :	Tryptase- $\alpha/\beta 1$
<i>TPSB2</i> :	Tryptase- $\beta 2$
<i>TPSD1</i> :	Tryptase- $\delta 1$

mechanosensing adhesion GPCR, adhesion G protein-coupled receptor E2 (ADGRE2).

Whereas the proteolytic activity of tryptases appears to result predominantly from mature tetramers released acutely following mast cell degranulation, basal serum tryptase (BST) levels are dictated by constitutive secretion of α - and β -pro-tryptases.⁸ Elevated BST levels are associated most commonly with increased α -tryptase-encoding *TPSAB1* copy number, a genetic trait known as hereditary α -tryptasemia (H α T).⁹ Clonal mast cell diseases such as systemic mastocytosis (SM) also frequently result in elevated BST level¹⁰ but are estimated to be approximately 500 times less prevalent than H α T in the general population.^{9,11,12} H α T has been observed in patients with anaphylaxis,¹³ and in a small cohort study of unselected healthy volunteers, those found to have H α T were significantly more likely to report systemic Hymenoptera venom-induced anaphylaxis than were those who were not found to have H α T.⁹

Clonal mast cell disease and elevated BST levels due to unknown cause(s) have both been associated with more severe anaphylaxis.¹⁴⁻²² However, tryptase genotypes have not been prospectively evaluated in larger populations with a history of—or at high risk of—anaphylaxis to determine whether H α T, or tryptase genotype generally, may influence this potentially fatal clinical outcome. Herein, we have performed this analysis, demonstrated a positive association between α -tryptase overexpression and severe anaphylaxis, and characterized a putative mechanism by which α -tryptase may augment immediate hypersensitivity reactions in humans.

METHODS

Study subjects

All individuals in the Slovenian Hymenoptera venom-triggered anaphylaxis (HVA) cohort provided informed consent on a University Clinic Golnik institutional review board-approved research protocol (KME 150/09/13) designed to study anaphylaxis, and/or mastocytosis. Informed consent for the use of stored material and clinical data from the Italian HVA cohort was provided by all individuals in accordance with protocol no. 39620 approved by the Verona University Hospital ethics committee. For National Institutes of Health (NIH) Clinical Center study participants, informed consent was

provided by all patients and their relatives on National Institute of Allergy and Infectious Diseases institutional review board–approved research protocols designed to study severe allergic hypersensitivity, anaphylaxis, and/or mastocytosis (NCT00852943, NCT01164241, NCT00044122, NCT00001756, and NCT00719719). Healthy volunteers likewise provided informed consent on National Institute of Allergy and Infectious Diseases institutional review board–approved research protocols (NCT00806364 and NCT01164241). Controls with nonatopic disease included probands and unaffected family members being studied for immunologic phenotypes unrelated to allergic diseases or reactions such as immune dysregulation, deficiency, or autoinflammation as described.¹²

All study participants had BST levels obtained. Clinical histories were taken, and reaction grades were assigned according to the Mueller grading system.²³ Where clinically indicated, skin and blood testing to Hymenoptera species was performed as reported (see [Tables E1](#) and [E2](#) in this article's Online Repository at www.jacionline.org). In the NIH cohorts and Italian HVA cohort, all individuals underwent bone marrow (BM) biopsy, *KIT* mutational analysis of BM aspirates, and tryptase genotyping by droplet digital (ddPCR) as described later in this article. In the Slovenian HVA cohort, all individuals with grade IV HVA underwent tryptase ddPCR genotyping and *KIT* p.D816V mutational analysis, both in peripheral blood. For reaction grades I to III, all individuals with a BST level of 4 ng/mL or higher (a level approximately 2 SDs below the mean value reported among individuals with H α T) underwent tryptase genotyping by ddPCR; no individual with H α T and a BST level less than 8 ng/mL has ever been reported or observed. All individuals with a BST level of 8 ng/mL or higher and anyone found with H α T also had *KIT* mutational analysis performed. Additional samples were selected for analysis from each BST level quartile; in total, 56 of 171 samples selected for grade III and 14 of 118 selected for grades I and II were evaluated for *KIT* p.D816V by ddPCR.

Tryptase protein quantification

Total basal serum tryptase levels were measured by using a commercially available fluorescence enzyme immunoassay in Clinical Laboratory Improvement Amendments–certified laboratories. The lower limit of detection for this is 1 ng/mL. Currently, the normal range in serum for total tryptase is considered to be from 1 to 11.4 ng/mL.¹⁰

Tryptase genotyping

Genotyping of *TPSAB1* and *TPSB2* was accomplished as described.¹² Briefly, genomic DNA was extracted using the DNEasy blood and tissue kit (Qiagen, Germantown, Md) according to the manufacturer's instructions. ddPCR was performed on a QX200 (Bio-Rad, Hercules, Calif) using a manual droplet generator and custom primer/probe sets for α - and β -tryptase sequences at *TPSAB1* and *TPSB2* with use of the reference probe *AP3B1* (Bio-Rad). *In silico* tryptase genotyping was accomplished as described from existing data sets¹² by realignment of reads mapping to the tryptase locus at Chr 16: 1.25 Mb–1.35 Mb (GRCh37/hg19) to a consensus sequence. Mapping read depth was subsequently quantified and normalized to *TPSD1*, which demonstrates conserved 2n copy.

BM biopsy and *KIT* gene analysis

BM biopsies were performed to exclude the diagnosis of SM, as described elsewhere²⁴ and screened for the activating *KIT* c.2447A>T, p.D816V variant by using allele-specific PCR as described²⁵ or ddPCR on a research by competing wild-type and variant probes for position c.2447 as described.²⁶

Cell culture

Human umbilical vein vascular endothelial cells (HUVECs) were maintained as described²⁷ on fibronectin-coated flasks in endothelial growth medium-2 (EGM-2) containing supplements (Lonza, Rockland, Me) at 37°C in 5% CO₂.

Tryptases and activity assay

Recombinant human α - and β -tryptase homotetramers were generated as described and generously provided by Genentech (Tansheng Yi).⁶ Pooled tryptases purified from lung were obtained from a commercial source (Millipore Sigma, St Louis, Mo). Peptidolytic activity of recombinant human β -tryptase and pooled tryptases were confirmed by detection of *p*-nitroanilide (pNA) liberation from tosyl-gly-pro-lys-pNA (Millipore Sigma) as described,⁷ with absorbance of liberated pNA at 405 nm measured on a Spark Cyto microplate reader (Tecan, Switzerland).

In vitro permeability assays

Endothelial cell permeability was modeled by using HUVEC monolayers in a Transwell system as described.²⁷ Briefly, cells were seeded on fibronectin- or collagen-coated 6.5-mm 0.3- μ M polyethylene terephthalate membranes at 200,000 cells/insert and incubated overnight at 37°C in 5% CO₂. The inserts were washed in PBS, placed in EGM-2 containing 0.2% BSA (in an incubator with a 500- μ L lower chamber and a 50- μ L upper chamber), and incubated at 37°C in 5% CO₂ for 1 hour. The EGM-2/0.2% BSA in the upper chamber was removed from the insert and replaced with 200 μ L of fresh EGM-2 containing 1 mg of fluorescein isothiocyanate–dextran (Chondrex, Redmond, Wash) and histamine (100 μ M), tryptases (4 μ g/well), PAR-2 agonist (SLIGKV-NH₂, 50 nM), PAR-2 antagonist (FSLRLY-NH₂, 50 nM) (Cayman Chemical, Ann Arbor, Mich), and/or an anti-tryptase neutralizing antibody (clone B12) as specified. Next, 10 μ L of medium was serially collected from the lower chamber and transferred to a 96-well clear bottom black reading plate (Sigma) containing 50 μ L of PBS. After collection at all time points, fluorescent intensity was quantified at an Ex/Em value of 490/525 nm on a Spark Cyto microplate reader and the data were normalized as percentage of maximum permeability.

Statistics

Unpaired *t* tests and Fisher exact tests were used to test the significance of associations as indicated; in all cases, 2-tailed tests were used. The Katz method was used for contingency analysis when data sets contained null groups. CIs for prevalence data were determined by using binomial exact calculation.

RESULTS

Increased numbers of α -tryptase–encoding copies at *TPSAB1* are associated with severe Hymenoptera venom–triggered anaphylaxis

Among the 282 patients with Mueller grade IV²³ Hymenoptera venom–triggered anaphylaxis (HVA) in the cohort from a major referral center for mastocytosis and venom anaphylaxis in Italy, we identified 56 in whom no evidence of mastocytosis could be found after BM biopsy, BM aspirate smear, BM flow cytometric analysis, and allele-specific PCR of the BM aspirate for the gain-of-function *KIT* p.D816V missense variant ([Fig 1, A](#)). Of these patients, 26 had samples available for tryptase genotyping and 81% (21 of 26) had increased *TPSAB1* copy number ([Fig 1, A](#)). An additional 21 individuals of the 241 in this cohort with SM and grade IV HVA were also screened, 14.3% (*n* = 3) were found to have increased *TPSAB1* copy number, with all of them having a BST level higher than 8 ng/mL. None of the remaining individuals with grade IV HVA were available for tryptase genotyping.

Within this small sample, comprising only 16.7% of the total cohort with grade IV HVA (47 of 282), we identified an overall H α T prevalence in the entire cohort of at least 8.5% (see [Table E1](#)) compared with an estimated 5.5% prevalence in the general Caucasian population. However, this is likely an underestimate

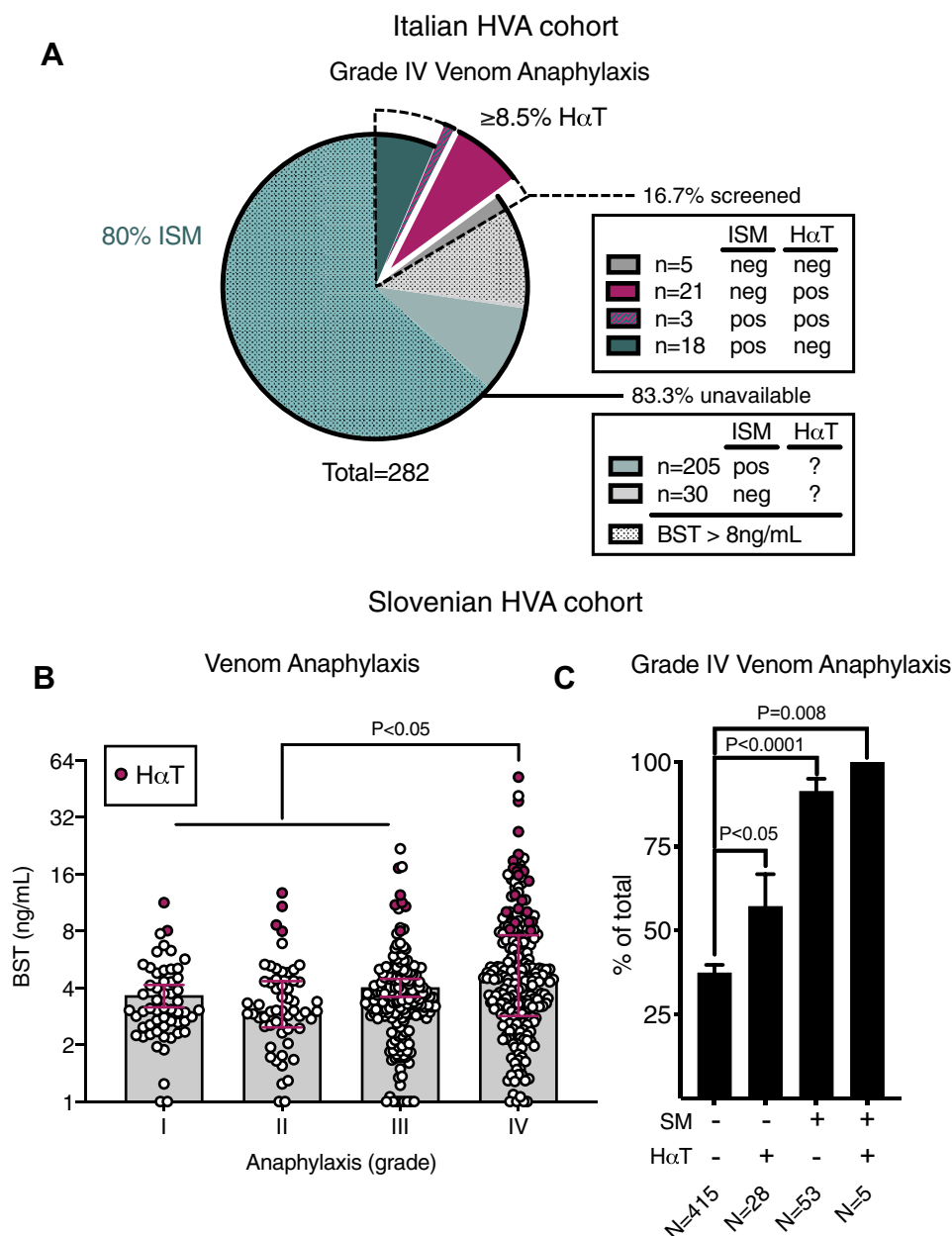


FIG 1. Severe anaphylaxis in response to HVA is associated with HαT. **A**, Proportion of the entire grade IV Italian HVA cohort with confirmed HαT ($n = 24$ of 282 [$\geq 8.5\%$]) after screening of available individuals ($n = 47$ of 282 [16.7%]). **B**, BST levels (medians with interquartile ranges) by severity of HVA (grade I-IV) in the Slovenian HVA cohort (individuals with HαT are indicated in red); 8.8% prevalence of HαT in grade IV compared with 4.3% in grades I to III; Fisher exact test. **C**, Prevalence of grade IV HVA depending on the concomitant presence of SM and/or HαT. Data are presented as means $\pm$ SEMs; Fisher exact test. neg, Negative; pos, positive.

given the high frequency (81%) of HαT among those with an elevated BST level and no evidence of clonal mast cell disease, and the fact that only 11% of those unavailable for testing (26 of 234) had a BST level less than 8 ng/mL (Fig 1, A). If this sample were representative, we would expect to observe up to 31% prevalence after testing the entire population, if samples were available for genotyping.

Because of the potential for bias and sampling error in this primary analysis, we next examined the prevalence of HαT in a second cohort of patients with HVA from Slovenia that is served

by a single referral center for all patients with HVA in a country of approximately 2 million people.

A total of 9.2% of all individuals with grade IV HVA (21 of 229) were found to have HαT versus 4.3% of those with grade I to III HVA (12 of 278) (Fig 1, B) ($P = .03$; odds ratio [OR] = 2.2 [95% CI = 1.1-4.5]; relative risk [RR] = 2.1 [95% CI = 1.1-4.2]). Among individuals without evidence of clonal disease (ie, wild-type *KIT* and BST level concordant with tryptase genotype), the rate of grade IV HVA was 57% (16 of 28) for individuals with HαT compared with 37% (155 of 415) for individuals with a

TABLE I. Tryptase genotypes of healthy volunteers and controls with nonatopic disease

Genotype, n (%)	$\alpha\alpha\beta,\beta\beta^*$	$\alpha\alpha\beta,\alpha\beta^*$	$\alpha\alpha\beta,\beta\beta^*$	$\alpha\beta,\alpha\beta$	$\alpha\beta,\beta\beta$	$\beta\beta,\beta\beta$	H α T, % (n)	95% CI†
Droplet digital PCR‡	—	3 (2.6)	4 (3)	30 (24)	55 (44)	33 (26)	5.6 (7 of 125)	2.3-11
Genome sequence analysis§	3 (1)	7 (1.8)	11 (3)	78 (20)	173 (43)	126 (32)	5.3 (21 of 398)	3.3-8.0
Total	3 (0.5)	10 (1.9)	15 (2.9)	108 (21)	228 (44)	159 (30)	5.4 (28 of 523)	3.6-7.7

*Genotypes diagnostic of H α T.

†95% CI values determined by using binomial exact calculation.

‡Unselected healthy individuals.

§Individuals sequenced for primary immunogenetic disorders unrelated to mast cell disorders or atopy as described (Lyons et al. 2016, Lyons 2018).

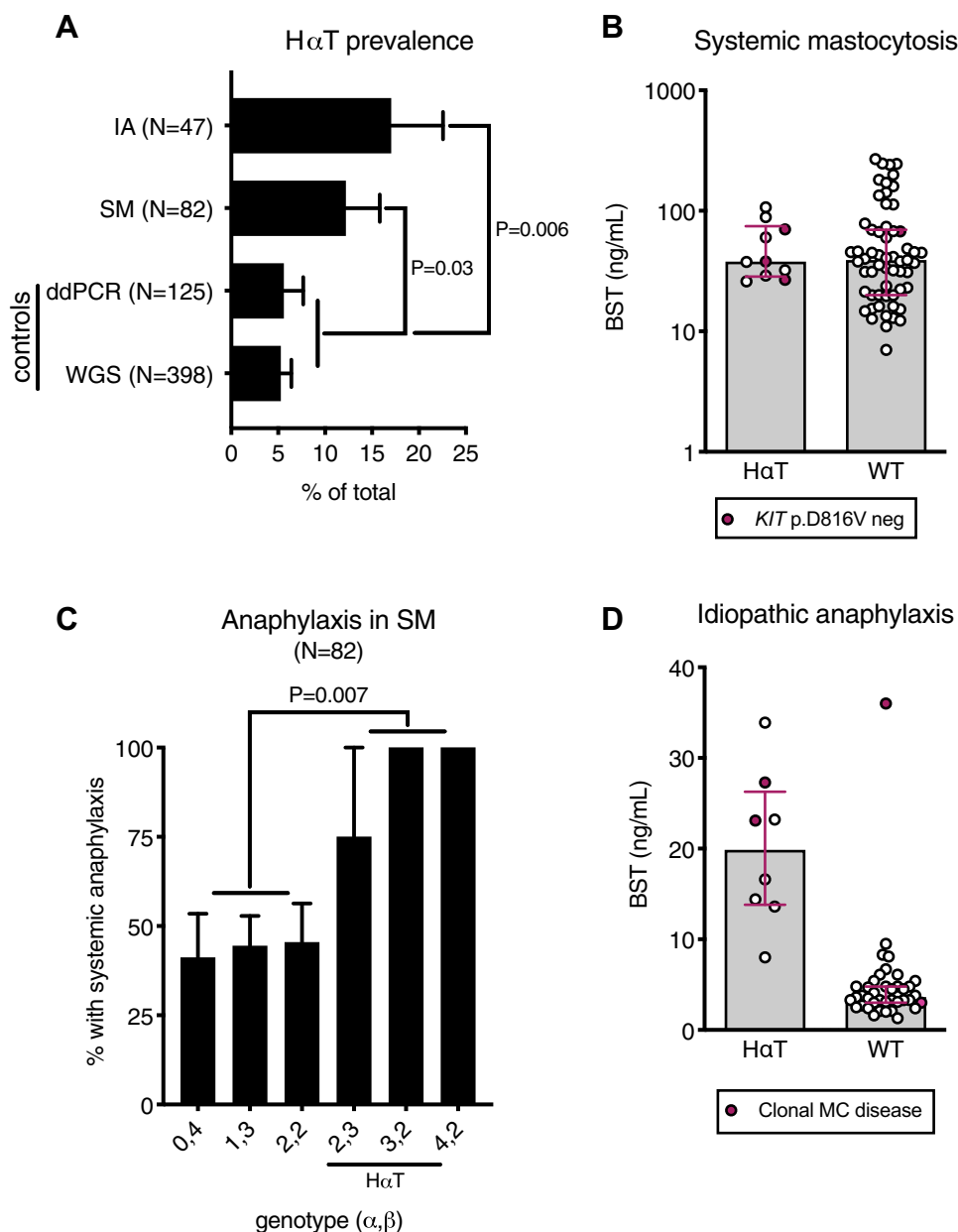


FIG 2. H α T is associated systemic anaphylaxis both with and without concomitant SM. **A**, Prevalence of H α T in patients with SM (n = 82) and IA (n = 47) compared with in controls with nonatopic disease (n = 398) (determined by analysis of whole genome sequencing [WGS] results) and healthy volunteers (n = 125) (genotyped by using ddPCR). **B**, BST levels (medians with interquartile ranges) in individuals with SM with and without H α T; absence of KIT p.D816V (indicated in red) was more common among individuals with H α T (P = .005); Fisher exact test. **C**, Prevalence of anaphylaxis by tryptase genotype among individuals with SM. **D**, BST levels (median with interquartile range) in individuals with IA with and without H α T; presence of clonal disease failing to meet the criteria for SM is indicated in red. In **(A and C)**, data are represented as means $\pm$ SEMs; the Fisher exact test was used for calculations. MC, Mast cell; WT, wild-type.

TABLE II. Clinical and laboratory characteristics of patients with SM

Age (y)	Sex	Diagnosis	BST level (ng/mL)	BM findings				Peripheral blood	Tryptase genotype		Anaphylaxis	
				MC aggregates	Spindle	KIT p.D816V	CD2/ CD25	KIT p.D816V	α	β	Y/N	Cause
21	M	ISM	107	+	+	+	+/+	+	4	2	Y	Drug
67	F	ISM	70	+	+	0	+/+	0	3	2	Y	IA, drug, food
34	F	ISM	37.9	+	0	0	0	0	3	2	Y	IA, exercise
68	F	ISM	88.7	+	>25%	+	+/+	+	3	2	Y	Drug
14	M	ISM	37.6	0	>25%	+	+/+	+	3	2	Y	IA
63	F	ISM	27.4	+	+	+	-/+	0	3	2	Y	Venom
25	F	ISM	26.8	+	0	0	0	0	2	3	N	—
12	F	ISM	60.1	+	>25%	+	+/+	+	2	3	Y	IA
58	F	ISM	32.2	0	+	+	-/+	+	2	3	Y	IA
60	M	ISM	24	—	+	+	+/+	+	2	3	Y	IA
74	M	ISM	23	+	>25%	+	+/+	+	2	2	Y	IA
47	M	ISM	12.9	0	>25%	+	+/+	+	2	2	Y	Drug
67	F	ISM	40	+	+	+	-/+	+	2	2	N	—
45	F	ISM	41.5	+	+	+	+/+	+	2	2	Y	Drug, AIT
32	M	ISM	48.5	+	>25%	+	+/+	+	2	2	N	—
44	M	ISM	245	+	>25%	+	+/+	+	2	2	Y	Drug
45	F	ISM	15.4	0	+	+	+/+	+	2	2	N	—
54	M	ISM	59.9	+	+	+	+/+	+	2	2	N	—
66	F	ISM	38.1	0	+	+	+/+	+	2	2	N	—
68	F	ISM	45.4	+	>25%	+	+/+	+	2	2	N	—
19	M	ISM	11	+	>25%	+	+/+	+	2	2	N	—
20	M	ISM	44.9	+	+	+	+/+	+	2	2	Y	IA, exercise
12	M	ISM	66.8	+	+	0	+/+	0	2	2	N	—
31	M	ISM	46	+	+	+	+/+	+	2	2	Y	IA
17	F	ISM	171	+	>25%	+	+/+	+	2	2	N	—
54	M	ISM	113	+	>25%	+	+/+	+	2	2	Y	Venom
74	F	ISM	114	+	>25%	+	+/+	+	2	2	Y	Food, IA
64	F	ISM/UP	13.6	ND	ND	ND	ND	+	2	2	N	—
57	F	SSM	96.2	+	+	+	+/+	+	2	2	Y	Drug, IA
76	M	SSM	328	+	+	+	+/+	+	2	2	N	—
33	F	ISM	12.3	0	+	+	-/+	+	1	3	Y	IA
57	F	ISM	42.9	+	>25%	+	+/+	0	1	3	Y	Drug
59	F	ISM	38	+	0	+	+/+	+	1	3	N	—
74	F	ISM	19.9	+	>25%	+	+/+	0	1	3	N	—
62	M	ISM	23.8	+	>25%	+	+/+	+	1	3	Y	IA
21	M	ISM	21.3	+	+	0	+/+	+	1	3	N	—
70	M	ISM	31.2	+	+	+	+/+	+	1	3	N	—
47	F	ISM	45	+	+	ND	-/+	+	1	3	N	—
64	M	ISM	13.4	0	>25%	+	ND	+	1	3	Y	Drug
64	F	ISM	22.3	+	>25%	+	+/+	+	1	3	Y	Drug, IA
36	F	ISM	12.7	+	+	—	+/+	+	1	3	Y	IA
62	F	ISM	31.2	+	+	+	-/+	+	1	3	Y	Drug
64	F	ISM	142	+	+	+	+/+	+	1	3	Y	Drug
55	F	ISM	19.6	+	+	ND	ND	+	1	3	N	—
28	F	ISM	14.7	0	0.5	+	+/+	+	1	3	Y	IA
76	M	ISM	134	+	>25%	+	+/+	+	1	3	Y	IA, exercise
40	M	ISM	16.1	0	>25%	+	+/+	+	1	3	Y	IA
26	M	ISM	241	+	>25%	+	+/+	+	1	3	N	—
18	F	ISM	67.7	+	>25%	+	+/+	+	1	3	N	—
7	F	ISM	247	0	>25%	+	+/+	+	1	3	N	—
8	M	ISM	269	+	>25%	+	+/+	+	1	3	Y	IA
12	M	ISM	32	+	+	+	+/+	+	1	3	N	—
15	M	ISM	181	+	0	0	+/+	+	1	3	N	—
13	M	ISM	78.4	+	>25%	+	+/+	+	1	3	N	—
18	M	ISM	15.2	0	0	+	+/+	0	1	3	N	—
64	M	ISM	39	+	+	+	+/+	+	1	3	N	—
58	F	ISM	19.9	+	>25%	+	+/+	+	1	3	Y	Venom, drug, food
41	F	ISM/UP	8.1	ND	ND	ND	ND	+	1	3	Y	Drug, IA
8	F	ISM/UP	25.4	ND	ND	ND	ND	+	1	3	N	—
19	F	ISM/UP	36.1	ND	ND	ND	ND	+	1	3	N	—

(Continued)

TABLE II. (Continued)

Age (y)	Sex	Diagnosis	BST level (ng/mL)	BM findings				Peripheral blood	Tryptase genotype		Anaphylaxis	
				MC aggregates	Spindle	KIT p.D816V	CD2/ CD25	KIT p.D816V	α	β	Y/N	Cause
59	M	ISM/UP	30.7	ND	ND	ND	ND	+	1	3	Y	Venom
33	F	ISM/UP	48.8	ND	ND	ND	ND	+	1	3	N	—
66	M	SSM	237	+	>25%	+	+/+	+	1	3	N	—
72	F	SSM	240	+	>25%	+	+/+	+	1	3	N	—
66	M	SSM	487	+	>25%	+	+/+	+	1	3	N	—
79	F	ISM	160	+	>25%	+	+/+	+	0	4	Y	IA
41	F	ISM	199	+	+	+	+/+	+	0	4	Y	Drug
66	M	ISM	37	+	+	+	-/+	+	0	4	N	—
45	F	ISM	31	+	+	ND	-/+	+	0	4	Y	IA
63	M	ISM	31.5	0	+	+	+/+	+	0	4	Y	Venom, drug
46	M	ISM	20	+	+	—	+/+	+	0	4	Y	Food
49	F	ISM	40.7	+	+	+	+/+	+	0	4	N	—
84	M	ISM	69.5	+	>25%	+	+/+	+	0	4	N	—
10	M	ISM	67.4	+	>25%	+	-/+	0	0	4	N	—
24	F	ISM	33.4	+	>25%	+	+/+	+	0	4	N	—
19	F	ISM	15.6	+	+	+	+/+	0	0	4	N	—
17	F	ISM	73.6	+	>25%	+	+/+	+	0	4	N	—
76	F	ISM	45.4	+	+	+	-/+	+	0	4	N	—
65	M	ISM	16.1	0	>25%	+	+/+	+	0	4	Y	Food
64	F	ISM	35.5	+	+	+	+/+	+	0	4	Y	Venom
7	M	ISM/DCM	38.5	ND	ND	ND	ND	+	0	4	N	—
9	F	ISM/UP	31.2	ND	ND	ND	ND	+	0	4	N	—

AIT, Allergen-specific immunotherapy; DCM, Diffuse cutaneous mastocytosis; F, female; ISM, indolent SM; M, male; MC, mast cell; ND, not determined; SSM, smoldering SM; UP, urticaria pigmentosa.

Boldface rows indicated the absence of KIT p.D816V; italicized rows indicate the presence of H α T.

normal tryptase copy number (Fig 1, C) ($P < .05$; OR = 2.2 [95% CI = 1.0-4.8]; RR = 2.1 [95% CI = 1.0-4.3]) (see Table E2).

It is worth noting that in this unselected population, 23% of individuals with grade IV HVA (53 of 229) were found to have the KIT p.D816V missense variant in their peripheral blood by ddPCR, whereas 8.9% of those individuals screened with grade III HVA (5 of 56) had detectable KIT p.D816V in their peripheral blood and none of the samples tested with grades I or II HVA ($n = 14$) had KIT p.D816V ($P = .003$, OR = 3.9 [95% CI = 1.6-9.4]; RR = 3.2 [95% CI = 1.4-7.7]). As only a limited number of patients could undergo extensive evaluation (that would include a BM biopsy) for clonal mast cell disease, we would expect an even greater prevalence of clonal disease or SM than was detectable in peripheral blood alone, particularly among those with an elevated BST level and wild-type *TPSAB1* copy number. Indeed, when prospectively examined, approximately one-third of individuals with SM do not have the KIT p.D816V missense variant detectable in their peripheral blood by allele-specific PCR.²⁸

Five individuals were identified as having both H α T and KIT p.D816V in their peripheral blood, and all 5 had grade IV HVA ($P = .008$) (Fig 1, C), whereas H α T together with KIT p.D816V was never observed in individuals with grades I to III HVA ($P = .02$). These data demonstrate that in addition to clonal mast cell disease, H α T accounts for a significant proportion of the observed association between elevated BST level and severe HVA and indicate that SM and H α T may have an additive effect on HVA severity.

H α T is associated with anaphylaxis among individuals with SM

Individuals with SM are at significantly increased risk overall for systemic anaphylaxis relative to the general population¹⁶ and thus represent a unique cohort to test the hypothesis that tryptase genotype may modify anaphylaxis risk with a relatively modest sample size. It is known that H α T and clonal mast cell disease may co-occur,¹³ and we found an unexpectedly high prevalence of concomitant H α T and clonal mast cell disease in both HVA cohorts that we examined herein; if H α T did not contribute to HVA, we would have expected a prevalence comparable to the general population estimates for H α T (ie, of 5.5%) rather than the prevalence of 14.3% of patients with SM in the Italian HVA cohort (3 of 21) or 8.6% of those identified with clonal mast cell disease in the Slovenian HVA cohort (5 of 58).

To examine the possibility that tryptase genotype affects the prevalence of anaphylaxis in high-risk patients with SM, we genotyped all available individuals from the cohort of patients with mastocytosis who were followed at the NIH Clinical Center irrespective of BST level or a history of atopy, venom allergy, or anaphylaxis. As comparators, we obtained genotypes from 398 control individuals with nonatopic disease *in silico* and also recruited 125 healthy volunteers to complement and validate the results of our bioinformatic prevalence data (Table I). We found that 12.2% of individuals with SM (10 of 82) also had H α T compared with 5.3% of controls with nonatopic disease (21 of 398) identified by whole genome sequence analysis and 5.6% of healthy volunteers genotyped using ddPCR (7 of 125).

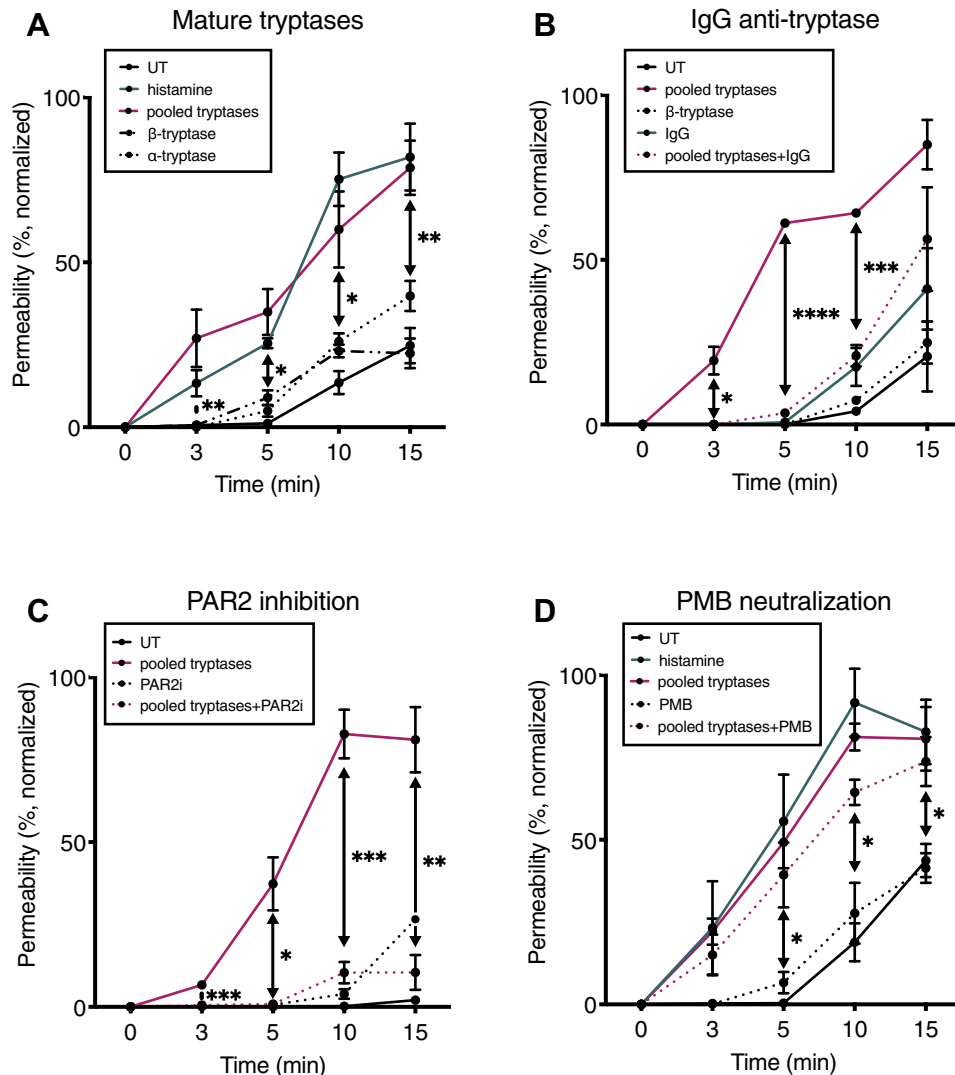


FIG 3. Pooled mature tryptases, but not α - or β -homotetrameric tryptases increase vascular endothelial permeability in a PAR-2-dependent manner. **A**, Pooled human tryptases deduced to contain α/β -tryptase heterotetramers and histamine acutely increase permeability of a monolayer of HUVECs *in vitro*, whereas recombinant α - or β -tryptase homotetramers do not. **B**, Inhibition of tryptase activity by disruption of proteolytically active tryptase tetramers abrogates the effect of pooled tryptases on HUVEC monolayer permeability. **C**, The PAR-2 inhibitory peptide FSLRY-NH₂ (PAR2i) likewise eliminates the activity of pooled tryptases on HUVEC monolayer permeability. **D**, Polymyxin-B (PMB), which neutralizes endotoxin, does not alter the effect of pooled tryptases on HUVEC permeability. Unpaired 2-tailed *t* test; **P* < .05, ***P* < .005, ****P* < .001, *****P* < .0001. UT, Untreated, media alone.

(Fig 2, A) (*P* = .03, OR = 2.5 [95% CI = 1.2-5.2]; RR = 2.3 [95% CI = 1.2-4.4]). Of the 10 individuals with SM and H α T, 3 lacked the *KIT* p.D816V missense variant; 2 of those with H α T had only round or well-differentiated mast cell infiltrates. By comparison, only 1 of the 72 individuals with SM alone lacked *KIT* p.D816V and had spindle-shaped mast cell infiltrates (Fig 2, B) (*P* = .005, OR = 30.4 [95% CI = 3.7-397]; RR = 8.4 [95% CI = 2.8-18.8]), suggesting that H α T may predispose to a unique molecular endotype of SM.

Secondary analysis of clinical phenotypes in the entire cohort revealed that 49% (40 of 82) had a physician-diagnosed history of anaphylaxis (Table II); this is a modestly increased

prevalence among individuals with SM relative to reports in the literature¹⁸ and may result from the concerted efforts to recruit patients to the NIH clinical center with severe anaphylaxis (NCT00719719, NCT01164241), in whom concomitant SM is often subsequently diagnosed. Remarkably, 90% of individuals with concomitant H α T and SM (9 of 10) had been diagnosed with systemic anaphylaxis, whereas 43% of those with SM and wild-type tryptase genotypes (31 of 72) had such a diagnosis (Fig 2, C) (*P* = .007, OR = 11.9 [95% CI = 1.7-133]; RR = 9.5 [95% CI = 1.7-56.5]). Causes for anaphylaxis in this cohort included foods, medications, HVA, and idiopathic presentations; no cause was selectively increased by the

concomitant presence of H α T (Table II). H α T is therefore associated with increased risk for anaphylaxis due to any cause among individuals with SM.

Severe IA is associated with H α T

To determine the potential effect of H α T on severe anaphylaxis among individuals who do not have SM, we next evaluated a cohort of individuals with idiopathic anaphylaxis (IA), none of whom met the World Health Organization diagnostic criteria for mastocytosis²⁹ after having undergone a BM biopsy and allele-specific PCR for the *KIT* p.D816V missense in BM and peripheral blood. Of these individuals, 17% (8 of 47) had H α T (Fig 2, A and D) ($P = .006$; OR = 3.6 [95% CI = 1.6-8.2]; RR = 3 [95% CI = 1.5-5.7]). Four of these individuals had evidence of clonal mast cell disease in which marrow findings revealed only 1 minor criterion each for the diagnosis of mastocytosis, and thus, they did not meet World Health Organization criteria for the diagnosis of SM; 2 individuals had more than 25% spindle-shaped mast cells, and 2 had the *KIT* p.D816V missense variant present at the lower limit of detection by allele-specific PCR. Two of these individuals also had H α T. If all 4 individuals with evidence of clonal mast cell disease were excluded, the association of IA with H α T remained significant ($P < .05$, OR = 2.9 [95% CI = 1.2-7.4]; RR = 2.5 [95% CI = 1.1-5.2]). Furthermore, H α T accounted for elevated BST levels in all of the individuals with IA in whom clonal disease was not identified (Fig 2, D). Thus, the association of H α T with increased risk for severe anaphylaxis is independent of the presence of concomitant clonal mast cell disease.

Pooled mature tryptases, but not α - or β -tryptase homotetramers, activate PAR-2 to promote endothelial permeability

To identify a potential mechanism contributing to the association between increased α -tryptase-encoding gene copies and severe anaphylaxis, we focused on recently described α/β -tryptase heterotetramers, which have been shown to be increased in mast cell secretory granules of individuals with H α T.⁵ Previously, we showed that tryptase genotype did not affect IgE-mediated degranulation of cultured mast cells as measured *in vitro* by β -hexosaminidase release.¹² However, it has been demonstrated that in contrast to β -tryptase homotetramers, heterotetrameric α/β -tryptases are able to cleave and activate PAR-2 on Jurkat cells. Given that PAR-1 has been implicated in vascular barrier function³⁰ and PAR-2 activation has been associated with paracellular permeability in the gut,³¹ we sought to determine whether α/β -tryptase heterotetramers could selectively promote human endothelial cell permeability in a PAR-2-dependent manner.

As expected, recombinant human α -tryptase tetramers *in vitro* had no proteolytic activity and no effect on HUVEC leak (Fig 3, A and see Fig E1, A in this article's Online Repository at www.jacionline.org). Recombinant human β -tryptase failed to acutely affect HUVEC barrier function despite having robust peptidolytic activity toward tosyl-gly-pro-lys-pNA (Fig 3, A and see Fig E1, A). As reported, overnight incubation of β -tryptase with endothelial cells did impair barrier function (see Fig E1, B), likely because of basement membrane degradation.³² However, in contrast to homotetrameric tryptases, tryptases pooled from large donor pools deduced to contain α/β -tryptase

heterotetramers were able to induce significant disruption of HUVEC barrier function, an effect comparable to that observed with 100 μ M histamine (Fig 3, A). This effect appeared to be dependent on the protease-activity of mature α/β -tryptases within the pooled tryptases on PAR-2, as it could be abrogated by both a neutralizing anti-tryptase antibody (clone B12) (Fig 3, B) and a PAR-2 inhibitory peptide (FSLLRY-NH2) (Fig 3, C). Furthermore, this effect could be recapitulated using a PAR-2 peptide agonist (SLIGKV-NH2) (see Fig E1, C), and it was not attributable to potential endotoxin activity, as polymyxin B (which neutralizes endotoxin) did not abrogate the activity of the pooled tryptases on HUVEC membrane permeability (Fig 1, D). Thus, in addition to the direct effects of mature tryptases on mast cells promoting degranulation and anaphylaxis in other model systems,^{5,6} mature heterotetrameric tryptases may promote anaphylaxis severity through unique activity on vascular endothelial cells, leading to enhanced permeability via PAR-2 activation.

DISCUSSION

Herein, we have demonstrated that increased germline copies of α -tryptase-encoding sequences at *TPSAB1* are associated with increased severity of venom anaphylaxis, are more prevalent among individuals with IA and SM, and are associated with an increased RR for anaphylaxis among patients with SM. Further, a putative contributing mechanism may exist among individuals with α -tryptase-encoding sequences that is lacking among individuals expressing only β -tryptase sequences, whereby heterotetramers selectively cleave PAR-2 on HUVEC monolayers, reflecting an analogous activity *in vivo* on vascular permeability and potentially increasing anaphylaxis severity.

To our knowledge, this is the first report of a common heritable genetic risk factor for anaphylaxis. Given the increased prevalence of H α T in both severe HVA and IA, the association between H α T and severe anaphylaxis appears to be independent of concomitant clonal mast cell disease. Furthermore, our data suggest that the RR for anaphylaxis imparted by either H α T or by the presence of clonal mast cell disease is additive. Thus, tryptase genotyping should be considered clinically when evaluating individuals who have a history of—or are at risk for—severe anaphylaxis (eg, individuals with HVA, SM, or IA).

It is important to note that in the Slovenian HVA cohort, which is the cohort least susceptible to potential biases in the present study, an increased prevalence of H α T was observed only among individuals with severe anaphylaxis (grade IV). The overall prevalence of H α T among the entire cohort (6.5% [32 of 507]) was not significantly higher than the expected prevalence in the general population. Thus, like SM, H α T does not appear to contribute to the development of sensitization to Hymenoptera venom but instead modifies the clinical outcome.

Clonal mast cell disease was identified in every individual (5 of 89) with an elevated BST level and wild-type *TPSAB1* copy number within the NIH cohorts; similar results have been reported previously.¹² Thus, tryptase genotyping may have clinical utility in screening patients when considering BM biopsy in general practice. Furthermore, in patients who have an elevated BST level because of H α T and do not have otherwise concerning signs or symptoms, deferring this invasive procedure may be reasonable. However, additional studies, including studies focusing on outcomes data, are needed to clarify the role of this testing on a more routine clinical basis.

A possible explanation for the increased prevalence of H α T among individuals with SM may be that clonal disease-associated symptoms in these individuals are augmented by this genetic trait—as we have reported here with regard to anaphylaxis—such that these individuals are more likely to have clinical manifestations for which they seek medical attention. Alternatively, given that well-differentiated and *KIT* p.D816V-negative SM were observed more frequently among those individuals with concomitant H α T, a second possibility is that α -tryptase expression alters mast cell proliferation or homeostasis through unidentified mechanisms. Additional mechanistic and population-based studies are necessary to evaluate these associations.

We have expanded on the unique activity of α/β -tryptase heterotetramers on PAR-2 that has been reported,⁵ confirming an absence of PAR-2 cleavage by homotetrameric β -tryptase. Using a pooled tryptase product, we have further demonstrated a PAR-2-specific effect on acute vascular membrane permeability. Although this *in vitro* result is provocative, additional studies are needed to determine whether this finding may potentiate anaphylaxis severity *in vivo*.

A role for tryptases in anaphylaxis is relevant to recent efforts toward developing a targeted mAb that neutralizes tryptase proteolytic activity and limits anaphylaxis severity in a humanized mouse model.⁶ Our data would suggest that patient outcomes in clinical trials targeting tryptases for neutralization or inhibition may be significantly affected by tryptase genetic variation and that tryptase genotyping may help identify individuals in whom these personalized therapies may be of most benefit.

Collectively, these findings expand our understanding of how tryptase genetic variation can affect mast cell-related disorders and phenotypes in humans and raise new questions about the potential effects of tryptase isoform expression on mast cell homeostasis.

We thank the patients, their families, and the healthy volunteers, as well as the clinical and administrative staff of the Laboratory of Allergic Diseases and NIH Clinical Center for their assistance. We also thank Dr Mihaela Zidarn, Dr Renato Erzen, Dr Peter Kopač, Dr Milan Zver, and Dr Mitja Košnik (Slovenia) for their clinical support. Finally, we thank Tansheng Yi and colleagues for generously providing recombinant human tryptases. Finally, the authors thank Drs Monica Lawrence, Thomas Platts-Mills, and Adnan Custovic for collectively providing the platform and introduction of collaborating authors on this project.

Key messages

- H α T is the common cause of elevated BST level in Western populations and the first common heritable genetic modifier of anaphylaxis to be described.
- Having concomitant clonal mast cell disease and H α T is associated with greater likelihood of severe anaphylaxis.
- α/β -Tryptase heterotetramers have unique activities that may potentiate immediate hypersensitivity reaction severity.

REFERENCES

- Schwartz LB. Tryptase: a mast cell serine protease. *Methods Enzymol* 1994;244:88-100.
- Caughey GH. Tryptase genetics and anaphylaxis. *J Allergy Clin Immunol* 2006;117:1411-4.
- Schwartz LB, Bradford TR, Littman BH, Wintroub BU. The fibrinolytic activity of purified tryptase from human lung mast cells. *J Immunol* 1985;135:2762-7.
- Schwartz LB, Kawahara MS, Hugli TE, Vik D, Fearon DT, Austen KF. Generation of C3a anaphylatoxin from human C3 by human mast cell tryptase. *J Immunol* 1983;130:1891-5.
- Le QT, Lyons JJ, Naranjo AN, Olivera A, Lazarus RA, Metcalfe DD, et al. Impact of naturally forming human alpha/beta-tryptase heterotetramers in the pathogenesis of hereditary alpha-tryptasemia. *J Exp Med* 2019;216:2348-61.
- Maun HR, Jackman JK, Choy DF, Loyet KM, Staton TL, Jia G, et al. An allosteric anti-tryptase antibody for the treatment of mast cell-mediated severe asthma. *Cell* 2019;179:417-31.e19.
- Huang C, Li L, Krilis SA, Chanasyk K, Tang Y, Li Z, et al. Human tryptases alpha and beta/II are functionally distinct due, in part, to a single amino acid difference in one of the surface loops that forms the substrate-binding cleft. *J Biol Chem* 1999;274:19670-6.
- Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, et al. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. *J Immunol* 2003;170:5667-73.
- Lyons JJ. Hereditary alpha tryptasemia: genotyping and associated clinical features. *Immunol Allergy Clin North Am* 2018;38:483-95.
- Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin North Am* 2006;26:451-63.
- Cohen SS, Skovbo S, Vestergaard H, Kristensen T, Moller M, Bindslev-Jensen C, et al. Epidemiology of systemic mastocytosis in Denmark. *Br J Haematol* 2014;166:521-8.
- Lyons JJ, Yu X, Hughes JD, Le QT, Jamil A, Bai Y, et al. Elevated basal serum tryptase identifies a multisystem disorder associated with increased TPSAB1 copy number. *Nat Genet* 2016;48:1564-9.
- Sabato V, Chovanec J, Faber M, Milner JD, Ebo D, Lyons JJ. First identification of an inherited TPSAB1 quintuplication in a patient with clonal mast cell disease. *J Clin Immunol* 2018;38:457-9.
- Aberer E, Savic S, Bretterklieber A, Reiter H, Berghold A, Aberer W. Disease spectrum in patients with elevated serum tryptase levels. *Australas J Dermatol* 2015;56:7-13.
- Aniceto V, Dias MM, Melo JML, Trevisan-Neto O, Aragon DC, Maia LSM, et al. Serum baseline tryptase level as a marker for the severity of anaphylaxis. *Int Arch Allergy Immunol* 2019;1-8.
- Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. *Allergy* 2008;63:226-32.
- Fellinger C, Hemmer W, Wohrl S, Sesztak-Greinecker G, Jarisch R, Wantke F. Clinical characteristics and risk profile of patients with elevated baseline serum tryptase. *Allergol Immunopathol (Madr)* 2014;42:544-52.
- Gulen T, Hagglund H, Dahlen B, Nilsson G. High prevalence of anaphylaxis in patients with systemic mastocytosis - a single-centre experience. *Clin Exp Allergy* 2014;44:121-9.
- Haeblerli G, Bronnimann M, Hunziker T, Muller U. Elevated basal serum tryptase and Hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. *Clin Exp Allergy* 2003;33:1216-20.
- Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M. Basal serum tryptase level correlates with severity of Hymenoptera sting and age. *J Investig Allergol Clin Immunol* 2007;17:65-9.
- Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase—a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. *J Allergy Clin Immunol* 2009;124:1047-54.
- Sahiner UM, Yavuz ST, Buyuktiryaki B, Cavkaytar O, Yilmaz EA, Tuncer A, et al. Serum basal tryptase may be a good marker for predicting the risk of anaphylaxis in children with food allergy. *Allergy* 2014;69:265-8.
- Mueller HL. Diagnosis and treatment of insect sensitivity. *J Asthma Res* 1966;3:331-3.
- Lyons JJ, Sun G, Stone KD, Nelson C, Wisch L, O'Brien M, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. *J Allergy Clin Immunol* 2014;133:1471-4.
- Kristensen T, Vestergaard H, Bindslev-Jensen C, Moller MB, Broesby-Olsen S. Mastocytosis Centre Oxford University Hospitals. Sensitive KIT D816V mutation analysis of blood as a diagnostic test in mastocytosis. *Am J Hematol* 2014;89:493-8.

26. Greiner G, Gurbisz M, Ratzinger F, Witzeneder N, Simonitsch-Klupp I, Mitterbauer-Hohendanner G, et al. Digital PCR: a sensitive and precise method for KIT D816V quantification in mastocytosis. *Clin Chem* 2018;64:547-55.
27. Hox V, O'Connell MP, Lyons JJ, Sackstein P, Dimaggio T, Jones N, et al. Diminution of signal transducer and activator of transcription 3 signaling inhibits vascular permeability and anaphylaxis. *J Allergy Clin Immunol* 2016;138:187-99.
28. Jara-Acevedo M, Teodosio C, Sanchez-Munoz L, Alvarez-Twose I, Mayado A, Caldas C, et al. Detection of the KIT D816V mutation in peripheral blood of systemic mastocytosis: diagnostic implications. *Mod Pathol* 2015;28:1138-49.
29. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. *Blood* 2017;129:1420-7.
30. Riewald M, Petrovan RJ, Donner A, Mueller BM, Ruf W. Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science* 2002;29:1880-2.
31. Jacob C, Yang PC, Darmoul D, Amadesi S, Saito T, Cottrell GS, et al. Mast cell tryptase controls paracellular permeability of the intestine. Role of protease-activated receptor 2 and beta-arrestins. *J Biol Chem* 2005;280:31936-48.
32. Rathore AP, Mantri CK, Aman SA, Syenina A, Ooi J, Jagaraj CJ, et al. Dengue virus-elicited tryptase induces endothelial permeability and shock. *J Clin Invest* 2019;130:4180-93.

Supplementary Appendix

Supplement to: Lyons et al. Heritable risk for severe anaphylaxis associated with increased α -tryptase-encoding germline copy number at *TPSAB1*

Table of Contents:

Fig. S1. Tryptase and PAR-2 agonist activity on HUVECs	2
Table S1. Italian Hymenoptera Venom Anaphylaxis (HVA) cohort characteristics	3
Table S2. Slovenian Hymenoptera Venom Anaphylaxis (HVA) cohort characteristics ..	5

Figure S1. Tryptase and PAR-2 agonist activity on HUVECs. (A) Absorbance at 405 nm following liberation of p-nitroanilide (pNA) from tosyl-gly-pro-lys-pNA by proteolysis by equivalent masses of indicated tryptases. **(B)** Overnight exposure of HUVEC monolayers to pooled human lung tryptase deduced to contain α/β -tryptase and recombinant human α -tryptase; UT – untreated, media alone. **(C)** Acute treatment of HUVECs with pooled tryptases, histamine, a PAR2 agonist, or media alone.

Table S1. Italian Hymenoptera Venom Anaphylaxis (HVA) cohort characteristics.

Age (years)	Gender	SM	HαT	Mueller (grade)	BST (ng/mL)	Serum IgE (kU/L)					Skin Testing*			
						Total	<i>Apis</i>	<i>Vespula</i>	<i>Polistes</i>	<i>Crabro</i>	<i>Apis</i>	<i>Vespula</i>	<i>Polistes</i>	<i>Crabro</i>
42	M	-	-	IV	58.9	ND	<0.35	2.36	ND	<0.35	-	+	-	-
70	F	+	+	IV	42	89	<0.35	25..2	26.8	1.6	ND	ND	ND	ND
75	M	-	+	IV	34.9	181	0.45	2.96	1.57	0.62	-	+	+	+
74	F	-	+	IV	34.0	127	50.2	2.5	3.51	7.05	+	+	+	+
61	M	-	+	IV	22.9	ND	ND	ND	1	ND	ND	ND	ND	ND
53	M	-	+	IV	21.9	ND	5.69	0.42	0.41	0.45	+	+	+	-
56	F	-	+	IV	20.7	34.3	0.31	2.14	1.15	1.52	ND	ND	ND	ND
55	M	-	+	IV	19.6	ND	0.12	2.23	3.23	0.59	-	+	+	ND
36	F	-	+	IV	18.0	ND	<0.35	4	3.35	<0.35	-	+	+	-
64	M	-	+	IV	18.0	482	4.46	13.9	10.9	4.35	ND	ND	ND	ND
71	M	+	+	IV	17.6	35	0.17	1.11	1.04	0.27	-	+	+	+
56	M	-	+	IV	17.4	2634	20.2	12.8	14.3	18.8	+	+	+	+
49	M	-	+	IV	17.1	ND	0.05	2.21	11.3	1.18	-	+	+	ND
53	M	-	+	IV	16.3	ND	0.04	0.06	0.04	0.01	-	-	-	-
50	M	+	-	IV	16.2	260	<0.35	11	10.9	2.35	-	+	+	-
50	M	+	-	IV	15	18	0.35	0.45	0.65	0.59	-	+	+	+
74	M	-	+	IV	14.7	ND	ND	1.73	1.74	ND	-	+	+	-
33	M	+	-	IV	13.6	28	<0.35	0.51	1.47	0.15	-	-	+	-
65	F	+	-	IV	13.4	93	4.21	19.3	3.83	0.41	+	+	+	+
56	M	+	-	IV	13.2	344	<0.35	2.74	2.98	0.15	-	+	+	+
63	F	-	+	IV	13.2	32	<0.35	<0.35	0.2	<0.35	-	-	-	-
72	M	-	+	IV	13.0	216	ND	0.7	0.73	0.22	-	+	-	ND
36	M	-	+	IV	12.6	461	0.43	5.08	6.45	3.93	ND	ND	ND	ND
26	M	-	+	IV	12.4	142	0.23	1.09	<0.35	<0.35	+	-	-	-
37	M	-	+	IV	12.2	55	<0.35	2.79	4.02	ND	-	+	+	ND
25	M	+	+	IV	11.9	ND	ND	0.67	3.79	ND	-	+	+	-
53	M	-	+	IV	11.8	ND	<0.35	11.9	6.94	1.41	-	+	+	+
52	M	+	-	IV	11.7	ND	ND	ND	ND	ND	-	+	+	+
20	M	-	+	IV	11.3	ND	0.02	0	0	0.03	+	-	-	-
51	M	-	+	IV	11.0	54	2.02	0.65	1.26	0.5	ND	ND	ND	ND
57	M	+	-	IV	10.6	418	0.33	9.09	0.93	1.81	-	+	+	+
74	M	-	-	IV	10.4	130	<0.35	1.51	5.47	0.21	-	-	-	+
61	M	-	-	IV	10.0	ND	4.12	0.38	2.48	0.55	ND	ND	ND	ND
69	F	-	-	IV	9.5	ND	1.42	12.3	2.29	0.63	ND	ND	ND	ND
80	M	+	-	IV	8.9	70	<0.35	2.70	<0.35	<0.35	-	+	+	+
49	M	+	-	IV	8.6	66	<0.35	6.99	5.61	0.26	-	+	+	+
54	F	-	-	IV	8.5	152	0.02	40.6	ND	0.13	-	+	-	-
66	M	+	-	IV	8.1	ND	1.18	7.59	5.34	0.80	-	+	+	-
76	F	-	+	IV	7.5	ND	3.1	0.34	0.11	0.36	+	-	-	-
66	M	+	-	IV	7.5	ND	<0.35	<0.35	<0.35	<0.35	-	-	-	-
52	M	+	-	IV	7.2	56	<0.35	0.74	1.69	0.14	-	+	+	+
56	M	+	-	IV	7	ND	ND	ND	ND	ND	+	+	+	+

51	M	+	-	IV	6.6	ND	<0.35	2.45	<0.35	0.17	-	+	-	+
56	M	+	-	IV	6.1	171	1.48	0.51	0.79	0.88	+	+	+	+
64	M	+	-	IV	5.5	28	<0.35	0.35	<0.35	<0.35	-	+	-	+
53	M	+	-	IV	5.2	28	0.23	0.21	<0.35	<0.35	ND	ND	ND	ND
63	M	+	-	IV	3.6	60	<0.35	0.61	<0.35	0.38	-	+	+	+

*Results for skin prick and where indicated intradermal skin testing. SM – systemic mastocytosis; HαT – Hereditary alpha-tryptasemia; ND – not determined.

Table S2. Slovenian Hymenoptera Venom Anaphylaxis (HVA) cohort characteristics.

Age (years)	Gender	K/I T p.D816V	HαT	Mueller (grade)	BST (ng/mL)	Serum IgE (kU/L)			Skin Testing*	
						Total	Apis	Vespula	Apis	Vespula
69	F	+	+	IV	52.2	13.1	1.2	<0.35	+	-
58	M	+	-	IV	39.2	14.6	0.46	<0.35	-	+
65	F	+	+	IV	38.8	17.2	<0.35	0.44	-	-
59	M	+	+	IV	26.8	19.9	3.76	2.48	-	-
61	F	+	-	III	21.8	19.3	7.34	2.2	+	+
57	M	+	+	IV	20.3	32.4	0.46	3.25	+	+
54	M	+	-	IV	19.4	1926	0.68	>100	-	+
65	M	-	-	IV	19.1	25.3	<0.35	11.4	-	+
66	F	+	+	IV	18.8	33.4	1.99	3.87	-	-
52	M	+	-	IV	17.9	28.9	<0.35	2.76	+	+
63	F	-	-	III	17.5	n.a.	4.31	10.7	ND	ND
50	M	-	+	III	17.2	55.9	0.53	5.35	-	-
41	M	-	+	IV	17.2	105	7.82	<0.35	+	-
82	F	-	+	IV	17	137	8.95	0.69	-	-
59	M	-	+	IV	16.6	78.7	<0.35	22.8	-	+
46	F	-	+	IV	16.5	62.3	<0.35	1.7	+	-
35	M	-	-	IV	15.9	34.7	<0.35	5.91	-	+
67	M	-	+	IV	15.8	18.9	<0.35	0.99	-	-
66	M	-	-	IV	15.4	18	1.74	<0.35	+	+
55	M	+	-	IV	15	11.3	<0.35	1.41	-	+
41	M	+	-	IV	14.7	44.6	<0.35	0.45	-	-
45	M	-	+	IV	14.7	100	2.26	31.7	-	+
58	F	+	-	IV	14.5	91.1	<0.35	2.67	-	+
35	F	+	-	IV	14.3	26.8	<0.35	22.4	-	+
65	M	+	-	IV	13.6	13.7	<0.35	0.65	ND	ND
42	M	-	-	IV	13.6	3.79	1.26	0.37	-	-
32	F	-	-	IV	12.8	31.6	0.69	1.11	+	+
36	M	-	+	II	12.7	168	31.6	8.73	+	+
56	F	-	+	III	12.4	271	4.7	7.35	+	+
76	M	-	+	IV	12.4	217	<0.35	50.8	-	+
68	M	-	-	IV	12.2	195	1.69	0.6	ND	ND
46	M	-	-	IV	11.7	26	2.25	2.24	+	+
51	M	-	+	IV	11.6	112	34.4	16.1	-	-
30	M	-	+	I	11.3	41.9	0.39	1.8	-	-
51	M	-	+	III	11.3	35.3	<0.35	<0.35	-	+
34	F	-	+	III	11	135	0.5	40.1	+	+
42	F	-	+	II	10.8	135	68	<0.35	+	-
29	F	-	+	III	10.8	18.1	<0.35	2.86	-	+
59	F	-	-	III	10.5	39.9	<0.35	0.89	+	+
65	M	-	+	IV	10.5	247	5.02	2.75	+	+
56	F	-	+	IV	10.1	24.5	1.24	1.76	-	+
53	M	-	+	IV	10.1	51.3	0.58	1.4	-	-

55	M	-	-	IV	10.1	56.9	1.83	1.26	+	+
57	M	+	-	IV	9.85	20.1	10.7	0.35	+	+
66	M	+	-	IV	9.05	n.a.	0.91	1.18	+	-
59	M	-	+	IV	8.95	158	0.56	10.9	+	+
56	M	-	+	IV	8.91	82	<0.35	14.1	+	+
44	F	-	+	IV	8.84	107	4.2	1.83	+	-
63	F	-	-	IV	8.83	59.4	23.1	<0.35	+	+
71	F	+	-	IV	8.68	12.9	5.27	<0.35	+	-
58	M	-	+	II	8.6	134	0.52	0.85	-	-
47	M	+	-	IV	8.45	112	<0.35	0.47	-	+
60	F	+	-	IV	8.44	29.6	0.49	<0.35	-	+
60	M	-	-	IV	8.44	47.9	<0.35	0.74	-	-
49	F	+	-	III	8.32	6.72	0.52	0.99	+	+
54	M	-	-	III	8.18	83.2	2.42	8.67	+	+
56	M	-	+	IV	8.18	29.1	1.4	11.8	-	+
59	M	-	-	IV	8.16	164	<0.35	10.2	ND	ND
38	M	-	-	IV	8.12	53.8	6.14	3.19	-	-
55	M	-	-	IV	8.12	46.5	0.54	6.17	+	+
64	F	-	+	I	8.03	66.6	2.05	4.6	-	+
60	M	-	+	IV	8.02	131	3.02	14.4	+	+
61	M	-	+	III	8	30.1	3.04	1.11	+	-
42	M	-	+	II	7.98	56.3	<0.35	11	-	+
64	F	+	-	IV	7.9	171	0.52	9.76	+	-
63	M	+	-	IV	7.82	157	2.22	3.75	-	+
51	M	-	-	I	7.74	132	4.92	2.49	-	-
59	M	-	-	III	7.73	35.4	1.38	0.39	+	-
57	M	-	-	IV	7.73	25.2	<0.35	5.51	-	+
48	M	+	-	IV	7.66	56.3	0.7	4.06	+	+
44	M	-	-	IV	7.53	140	0.35	1.52	-	-
50	M	-	-	IV	7.42	64.3	4.24	<0.35	+	-
73	F	-	-	IV	7.37	54	3.3	<0.35	+	-
56	M	+	-	IV	7.34	138	5.21	6.07	+	+
60	M	-	-	IV	7.32	139	3.03	0.94	+	+
62	M	-	-	IV	7.13	15.8	3.36	5.46	+	-
42	M	-	-	IV	7.1	1432	38.2	24.1	+	-
59	M	-	-	IV	7.07	17.3	<0.35	24.9	-	-
63	M	-	-	IV	6.99	13.9	<0.35	9.79	-	+
52	F	+	-	IV	6.92	86.1	0.62	0.88	-	-
65	F	-	-	III	6.89	59.1	<0.35	0.96	-	-
67	F	+	-	IV	6.89	12.3	<0.35	0.73	-	+
59	M	-	-	II	6.87	853	10.7	1.92	-	-
59	F	-	-	IV	6.8	53.3	20.9	4.42	-	-
68	F	+	-	III	6.77	19.6	<0.35	2.36	-	-
50	M	+	-	IV	6.76	24.9	<0.35	0.63	-	-
73	M	ND	-	I	6.71	136	3.19	22.7	-	-
78	F	-	-	III	6.6	44.4	2.42	<0.35	+	-

37	F	+	-	IV	6.51	44.7	<0.35	0.97	-	+
46	F	-	-	IV	6.49	69.2	0.39	18.8	-	+
62	M	-	-	III	6.47	134	6.78	1.11	+	+
64	M	ND	-	III	6.47	12.6	<0.35	<0.35	-	+
50	M	-	-	IV	6.42	76.4	3	1.4	+	+
61	F	-	-	III	6.41	76.2	2.97	2.09	+	+
71	F	ND	-	I	6.3	82.8	0.44	<0.35	-	-
55	M	-	-	I	6.21	38.3	12.2	<0.35	-	-
65	M	-	-	IV	6.15	50.3	0.87	1.29	-	-
46	M	-	-	IV	6.15	13.1	<0.35	1.07	ND	+
53	M	-	-	III	6	52.5	<0.35	6.38	ND	ND
41	M	-	-	IV	5.95	150	<0.35	17.7	-	+
66	F	ND	-	III	5.87	155	>100	<0.35	+	-
40	M	-	-	IV	5.83	93.9	9.26	0.49	-	+
67	F	+	-	IV	5.76	283	<0.35	6.4	+	+
48	M	-	-	IV	5.76	64.1	<0.35	5.84	-	-
69	F	+	-	IV	5.75	18.1	<0.35	3.71	-	+
47	F	ND	-	I	5.68	97.8	<0.35	5.32	-	-
36	F	ND	-	III	5.68	72.1	10	<0.35	+	-
53	F	-	-	IV	5.66	34.5	<0.35	1.2	-	+
69	F	-	-	III	5.59	232	<0.35	18.4	-	-
35	M	-	-	III	5.58	369	0.68	15	-	-
86	M	-	-	IV	5.47	287	53.8	2.61	+	+
55	M	-	-	III	5.46	539	4.32	3.53	+	+
74	F	-	-	IV	5.4	8.95	4.39	0.35	+	-
75	F	ND	-	I	5.32	45.8	2.53	2.29	-	-
57	F	ND	-	II	5.32	27.1	4.2	4.64	-	-
79	M	ND	-	II	5.29	97	0.63	25.8	+	+
77	M	-	-	IV	5.26	148	<0.35	15.6	-	-
50	F	-	-	III	5.23	34.5	0.71	4.33	+	-
55	M	ND	-	II	5.22	99.2	0.84	7.19	-	+
48	M	ND	ND	III	5.22	78.7	10.7	3.06	+	-
61	M	-	-	III	5.17	41.7	<0.35	0.74	-	-
54	F	-	-	IV	5.17	54.4	<0.35	3.81	-	+
49	F	-	-	IV	5.17	543	0.51	4.31	+	+
46	M	-	-	IV	5.15	133	5.92	61.8	ND	ND
65	F	-	-	III	5.14	41.3	0.35	9.25	ND	-
36	M	-	-	IV	5.14	54	3.45	1.07	+	+
55	M	ND	-	I	5.12	381	16.6	<0.35	+	-
33	M	-	-	III	5.12	95.2	2.45	3.2	+	-
55	F	-	-	III	5.12	318	13.5	12.6	+	+
60	M	-	-	IV	5.11	7.16	0.62	<0.35	-	-
65	M	-	-	IV	5.09	102	6.96	<0.35	+	+
53	F	-	-	III	5.08	78.5	<0.35	<0.35	+	+
69	M	-	-	IV	5.08	98.2	1.82	3.9	+	+
49	M	ND	-	I	5.07	237	5.2	7.52	-	-

75	M	ND	-	II	5.06	40.2	<0.35	1.78	-	-
63	F	ND	-	I	5.02	109	1.78	16.4	-	+
69	F	-	-	IV	5.02	58.4	15.4	1.12	+	+
34	M	-	-	IV	5.02	22	6.45	1.07	+	+
54	M	-	-	III	5.01	60.7	<0.35	17.6	-	-
52	M	-	-	IV	5	47.1	25.2	27.2	-	-
47	M	ND	-	II	4.99	155	3.56	1.97	+	+
68	M	-	-	IV	4.99	135	13.4	<0.35	+	+
36	M	-	-	III	4.97	43.1	7.61	2.26	-	-
59	F	-	-	IV	4.96	44.2	<0.35	19.3	-	+
73	M	ND	-	I	4.95	69.5	58.7	<0.35	-	-
28	M	-	-	III	4.95	n.a.	34.7	>100	+	+
31	M	+	-	IV	4.95	34	<0.35	2.97	-	-
56	F	-	-	IV	4.95	1023	5.87	<0.35	-	-
73	M	-	-	IV	4.93	37.3	0.72	4.07	-	+
40	F	+	-	IV	4.88	10.5	<0.35	1.83	ND	ND
68	M	ND	-	II	4.86	124	5.94	1.61	+	-
39	M	-	-	III	4.85	32.8	4.41	0.81	+	+
31	M	-	-	III	4.84	114	9.74	5	-	-
48	M	ND	-	II	4.82	203	<0.35	42.9	-	+
59	F	-	-	IV	4.82	89.1	23.3	<0.35	+	-
58	M	-	-	IV	4.8	307	2.41	16.8	-	-
67	F	ND	-	I	4.78	335	<0.35	20.7	-	-
54	F	-	-	III	4.75	95.3	0.35	8.97	-	+
24	M	-	-	III	4.71	72	3.48	<0.35	+	-
62	F	-	-	IV	4.7	107	0.41	0.77	-	+
52	M	ND	-	III	4.68	185	86.4	0.8	+	+
75	F	-	-	IV	4.68	490	0.75	24.1	-	-
53	F	-	-	III	4.67	16.9	0.82	3.38	-	+
43	M	-	-	IV	4.66	524	21.2	46.6	-	+
35	M	-	-	IV	4.64	112	<0.35	3.01	ND	ND
59	M	+	-	IV	4.63	7.6	<0.35	1.86	+	+
43	M	-	-	III	4.58	746	5.61	6.6	ND	ND
64	F	-	-	IV	4.57	30.1	3.93	0.88	-	-
73	M	ND	-	I	4.95	69.5	58.7	<0.35	-	-
67	F	-	-	IV	4.56	67.6	<0.35	31.5	-	-
58	M	-	-	IV	4.54	45.7	<0.35	24.5	+	+
52	M	-	-	IV	4.54	86.5	1.66	1.13	+	-
62	F	-	-	III	4.53	40.6	<0.35	1.99	-	+
68	M	-	-	IV	4.53	99.4	33.8	<0.35	+	+
45	F	ND	-	I	4.52	11.1	<0.35	3.2	-	-
76	M	-	-	IV	4.49	226	<0.35	21.3	ND	ND
62	M	-	-	IV	4.47	543	0.56	2.36	-	-
56	F	-	-	IV	4.47	228	1.64	53.5	-	-
39	M	ND	-	II	4.43	53	8.03	0.42	+	+
41	M	-	-	III	4.43	674	7.3	41.4	-	+

49	M	-	-	IV	4.43	324	1.69	1.54	-	-
39	M	-	-	IV	4.43	143	4.34	3.3	-	-
37	M	-	-	IV	4.43	431	1.66	11.8	-	+
44	M	-	-	III	4.42	87.9	<0.35	26.4	+	+
65	F	+	-	III	4.38	55	4.3	0.46	+	-
48	M	ND	-	II	4.36	293	<0.35	>100	-	-
41	F	-	-	III	4.36	42.3	0.864	<0.35	-	-
69	M	-	-	IV	4.36	81.5	0.6	2.23	-	-
67	F	-	-	III	4.35	105	7.44	0.46	+	-
60	M	-	-	IV	4.35	58.1	<0.35	10.3	-	+
39	M	ND	-	I	4.32	114	4.64	8.88	+	+
49	F	ND	-	II	4.32	17.6	0.36	<0.35	-	-
55	M	-	-	IV	4.31	37.9	0.46	2.56	ND	ND
63	F	ND	-	I	4.3	497	19.6	<0.35	+	-
63	M	-	-	IV	4.3	100	8.98	1.53	-	-
62	F	ND	-	II	4.29	897	100	4.1	+	+
64	M	+	-	IV	4.28	150	1.19	>100	+	+
50	M	ND	-	II	4.25	547	36.7	2.46	-	-
69	M	-	-	III	4.25	90.4	0.4	2.27	-	-
57	M	-	-	IV	4.22	58.4	0.61	51.6	+	+
84	F	-	-	IV	4.19	30.2	36.9	<0.35	+	-
49	F	-	-	III	4.18	736	0.48	18.9	+	+
39	F	ND	-	I	4.13	270	22.1	3.81	ND	ND
50	M	-	-	IV	4.13	104	1.87	22.1	+	+
44	M	ND	-	III	4.11	111	0.67	2.44	-	+
60	M	ND	-	II	4.09	17.4	1.02	0.46	-	-
49	M	+	-	IV	4.07	66.5	<0.35	1.17	-	+
38	M	-	-	III	4.05	202	13.4	10.3	-	-
56	M	-	-	III	4.03	103	6.55	0.91	+	-
45	M	-	-	III	4.02	133	7.56	1.42	-	-
33	M	-	-	III	4.02	61.5	12.1	<0.35	+	-
70	M	-	-	III	4.01	23.9	1.32	3.67	+	+
60	M	ND	ND	I	3.99	41.3	<0.35	0.74	-	-
36	F	ND	ND	III	3.99	48.4	6.13	18.8	-	-
73	M	-	-	IV	3.99	40.1	<0.35	0.84	-	+
58	M	+	-	IV	3.97	75.8	3.6	1.74	+	+
49	M	ND	ND	II	3.95	11.2	<0.35	4.69	-	+
51	M	ND	ND	II	3.95	33.8	11.2	<0.35	+	-
51	M	-	-	IV	3.94	17.3	22.5	2.31	+	-
69	F	-	-	IV	3.91	14.8	0.58	0.78	+	+
62	F	ND	ND	III	3.9	329	26.2	1.19	+	-
51	M	ND	ND	III	3.9	57.5	1.17	1.02	+	-
52	M	-	-	I	3.89	56	<0.35	2.2	-	+
40	M	ND	ND	III	3.88	79.4	<0.35	7.52	+	+
63	M	-	-	IV	3.88	21.3	4.59	0.7	-	+
68	M	-	-	III	3.86	40.3	17.8	0.62	+	+

60	M	ND	ND	III	3.85	2.61	0.93	<0.35	+	-
31	F	ND	ND	III	3.83	102	<0.35	1.62	-	-
44	F	ND	ND	III	3.8	57.9	3.45	<0.35	+	-
64	M	ND	ND	III	3.79	29.6	0.91	<0.35	-	-
44	M	-	-	III	3.78	232	7.01	5.97	-	+
42	M	ND	ND	III	3.78	376	3.23	9.69	+	ND
51	F	-	-	IV	3.78	126	1.86	<0.35	+	+
51	M	+	-	IV	3.75	158	4.43	2.35	-	-
67	F	ND	ND	I	3.74	18.7	2.81	48.5	-	-
73	M	-	-	III	3.74	22.3	<0.35	1.41	-	-
57	M	-	-	IV	3.74	204	25.6	1.3	+	+
52	F	ND	ND	I	3.73	111	2.06	4.93	+	-
63	M	ND	ND	III	3.73	68.8	3.84	3.57	+	-
39	M	ND	ND	III	3.73	41.7	0.54	1.65	+	+
31	F	ND	ND	III	3.71	21.3	0.72	0.65	-	-
65	M	ND	ND	III	3.71	113	8.05	<0.35	+	+
32	F	-	-	IV	3.71	35	2.55	1.59	-	-
36	M	ND	ND	I	3.7	20.8	<0.35	0.51	-	-
53	M	ND	ND	III	3.69	13.2	2.21	0.54	+	-
38	F	-	-	IV	3.69	69	13	1.83	-	-
56	M	ND	ND	III	3.66	515	9.34	14.3	+	+
49	M	-	-	IV	3.66	26.3	2.31	<0.35	+	-
32	F	ND	ND	III	3.65	38.8	60.4	0.95	-	-
66	F	-	-	IV	3.65	154	<0.35	66.3	-	+
54	M	-	-	IV	3.64	150	1.1	5.16	+	+
55	M	-	-	IV	3.64	695	7.54	>100	+	+
63	F	ND	ND	III	3.63	14.7	13.5	<0.35	-	-
52	M	ND	ND	III	3.6	51.1	10.7	11.5	+	+
44	F	ND	ND	III	3.59	564	0.95	8.22	-	-
64	F	ND	ND	II	3.58	21.4	3.5	0.36	-	+
48	F	ND	ND	III	3.58	40.3	28.6	<0.35	+	-
41	M	ND	ND	III	3.56	253	<0.35	22.3	-	+
45	M	-	-	IV	3.54	167	<0.35	2.59	-	+
59	M	-	-	IV	3.52	255	7.16	76.5	-	-
54	M	-	-	IV	3.52	46.3	13.5	1.63	-	-
59	F	ND	ND	II	3.51	23.3	37	8.53	+	+
66	M	-	-	IV	3.51	102	1.27	0.81	-	-
29	M	-	-	III	3.48	17.5	1.06	1.82	-	-
47	M	ND	ND	III	3.48	296	<0.35	79.7	-	-
52	M	-	-	IV	3.47	168	12.7	<0.35	+	-
58	M	ND	ND	I	3.45	254	0.36	0.94	-	-
38	M	-	-	IV	3.44	47.4	<0.35	0.58	ND	ND
54	M	ND	ND	III	3.43	22.5	10.1	12.8	-	-
51	M	ND	ND	III	3.43	24.8	1.96	0.56	+	-
59	M	-	-	IV	3.43	24.5	1.97	0.89	+	-
51	F	-	-	IV	3.41	23.3	<0.35	2.42	+	-

59	M	-	-	IV	3.4	56.9	<0.35	3.79	-	-
67	M	ND	ND	I	3.39	165	<0.35	1.33	-	+
55	M	ND	ND	II	3.39	182	1.39	1.5	-	+
62	M	ND	ND	III	3.39	309	<0.35	26.7	+	+
57	F	ND	ND	II	3.38	5.9	<0.35	1.12	-	-
28	M	ND	ND	III	3.37	120	17.7	2.6	+	-
53	F	ND	ND	III	3.37	53.4	1.55	0.71	+	+
39	F	+	-	IV	3.35	47.6	<0.35	2.03	-	+
27	M	-	-	IV	3.35	67.5	<0.35	18	-	+
55	M	ND	ND	III	3.34	35.6	4.56	<0.35	+	-
32	M	ND	ND	III	3.34	401	<0.35	<0.35	-	+
39	M	ND	ND	II	3.32	567	29.6	5.32	-	-
67	F	ND	ND	III	3.32	7.17	11.3	<0.35	+	-
33	M	-	-	IV	3.32	225	<0.35	3.35	ND	ND
57	M	-	-	IV	3.31	8.42	1.33	<0.35	+	-
39	F	ND	ND	III	3.3	226	25.8	7.85	+	+
64	M	-	-	IV	3.3	137	5.85	1.33	+	+
54	F	-	-	IV	3.3	251	43.8	17	+	+
66	M	-	-	IV	3.29	118	<0.35	7.36	-	+
55	M	-	-	IV	3.29	30.2	<0.35	2.65	-	+
31	F	ND	ND	III	3.28	167	<0.35	1.52	-	+
51	M	+	-	IV	3.28	11.9	0.36	<0.35	-	-
48	M	-	-	IV	3.28	133	16.2	0.68	+	-
66	M	ND	ND	III	3.27	42.3	<0.35	7.25	-	+
54	F	ND	ND	II	3.26	153	34.8	3.99	+	-
64	M	ND	ND	III	3.25	55.6	1.95	1.64	-	-
58	M	ND	ND	III	3.24	68.6	1.97	2.11	+	+
63	M	ND	ND	III	3.22	12.6	<0.35	<0.35	-	-
62	F	-	-	IV	3.2	245	14.7	19.1	+	+
65	F	ND	ND	III	3.19	14.8	0.625	7.88	-	+
42	F	-	-	IV	3.18	42.6	2.12	0.75	+	+
39	F	ND	ND	III	3.17	123	0.47	13.3	-	+
59	F	-	-	IV	3.17	92.6	<0.35	2.1	-	+
40	F	ND	ND	III	3.16	23.8	<0.35	3.91	-	+
51	M	-	-	IV	3.16	56.6	<0.35	1.67	-	+
30	F	ND	ND	III	3.15	115	<0.35	21.9	-	+
36	M	ND	ND	I	3.13	54.9	2.21	<0.35	+	-
64	M	ND	ND	III	3.12	392	2.9	5.36	+	+
65	M	ND	ND	I	3.11	66.7	0.9	13.8	-	-
48	M	-	-	IV	3.11	50.5	8.65	0.37	+	-
40	F	ND	ND	III	3.09	4.45	<0.35	4.51	-	+
44	F	-	-	IV	3.09	30.2	<0.35	6.6	-	+
52	F	-	-	III	3.08	45.6	<0.35	1.22	-	+
68	M	ND	ND	III	3.08	506	4.87	10.6	+	+
33	F	ND	ND	III	3.08	44.3	<0.35	2.3	-	+
43	F	ND	ND	I	3.07	146	<0.35	23.5	+	+

47	F	+	-	IV	3.07	71.1	<0.35	0.6	-	+
34	M	ND	ND	III	3.06	213	12.8	<0.35	-	-
65	F	ND	ND	I	3.05	99.6	17.7	9.87	+	+
30	F	ND	ND	I	3.04	47.8	<0.35	2.18	-	-
31	M	ND	ND	II	3.04	277	0.93	0.91	-	-
69	M	ND	ND	I	3.03	537	47.6	7.78	+	+
52	F	ND	ND	II	3.02	114	2.92	0.94	+	-
48	F	ND	-	II	3.02	52.6	1.44	0.43	-	-
67	M	ND	ND	II	3.01	209	2.16	<0.35	+	-
72	M	-	-	IV	3.01	73.6	0.53	2.07	-	+
40	M	ND	ND	I	3	13.1	<0.35	10.5	-	-
70	F	ND	ND	III	3	54.8	<0.35	57.8	-	+
37	M	ND	ND	II	2.99	21.8	0.56	3.41	-	-
54	M	ND	ND	I	2.97	21.1	2.92	<0.35	+	-
49	M	ND	ND	I	2.97	36.3	13.8	2.71	+	-
48	F	ND	ND	II	2.97	2.81	1.87	<0.35	+	-
31	M	ND	ND	III	2.97	45.4	1.53	5.27	-	-
35	M	ND	ND	III	2.95	138	5.24	2.23	-	-
54	M	ND	ND	III	2.95	476	77.8	12	+	+
68	M	-	-	IV	2.95	331	2.21	3.02	-	+
48	M	ND	ND	III	2.94	21.6	1.95	2.81	-	+
57	M	ND	ND	III	2.93	68	17.8	<0.35	+	-
25	M	ND	ND	III	2.93	72.7	1.4	0.98	+	+
44	M	ND	ND	II	2.92	128	0.86	41	-	-
50	M	ND	ND	III	2.92	54.8	1.74	20.1	-	+
72	M	ND	ND	II	2.91	69	1.58	<0.35	+	-
62	M	ND	ND	III	2.9	122	14	2.41	+	+
69	M	ND	ND	III	2.9	60.3	2.77	<0.35	+	-
58	M	-	-	IV	2.9	141	<0.35	4	ND	ND
59	F	ND	ND	III	2.89	69.3	<0.35	0.78	+	+
46	F	ND	ND	II	2.86	78.5	<0.35	16.2	-	+
63	F	+	-	III	2.86	26.2	<0.35	1.68	-	+
56	M	ND	ND	III	2.85	36.1	3.26	15.2	-	-
60	M	+	-	IV	2.84	63.5	0.35	11.3	-	+
40	F	ND	ND	I	2.83	719	19.8	>100	-	-
38	M	ND	ND	II	2.83	92.6	10.6	<0.35	ND	ND
56	M	ND	ND	III	2.83	35.8	<0.35	3.77	-	+
64	F	+	-	IV	2.83	7.87	1.12	0.59	-	-
65	F	ND	ND	I	2.81	67.7	7.82	3.09	-	+
50	M	ND	ND	II	2.8	140	<0.35	53.3	-	+
51	F	-	-	IV	2.8	363	5.78	13.1	-	+
55	M	-	-	IV	2.8	129	3.07	3.84	-	-
57	M	-	-	IV	2.8	29.7	0.81	4.53	-	-
60	M	-	-	IV	2.8	59.7	2.32	2.24	-	-
44	F	ND	ND	I	2.79	954	>100	2.23	+	+
56	F	ND	ND	II	2.78	54.6	<0.35	6.86	+	-

47	F	ND	ND	III	2.77	134	32.4	6.13	+	-
28	M	ND	ND	III	2.77	95.3	6.25	<0.35	+	-
51	F	+	-	IV	2.77	14.6	1.38	<0.35	+	-
53	F	ND	ND	III	2.76	24.4	6.5	<0.35	+	-
44	F	-	-	III	2.75	70.6	<0.35	14.1	-	+
45	M	ND	ND	II	2.74	469	9.71	13.2	+	+
30	M	ND	ND	I	2.7	197	7.96	0.39	+	-
31	F	+	-	IV	2.7	10.7	0.72	0.65	-	-
44	F	-	-	IV	2.69	244	2.96	3.56	-	+
46	M	-	-	IV	2.68	120	<0.35	6.03	-	+
40	F	-	-	I	2.67	17.6	<0.35	<0.35	-	-
51	M	ND	ND	III	2.67	39.8	3.35	12.3	-	-
52	F	ND	ND	I	2.66	41.8	12.4	<0.35	+	-
74	M	+	-	IV	2.66	63.3	0.42	3.56	-	+
50	F	ND	ND	II	2.65	23.7	7.3	1.14	+	+
62	M	-	-	IV	2.65	66.2	2.73	11	+	+
51	M	+	-	IV	2.63	72.6	1.74	3.36	-	+
58	M	ND	ND	I	2.62	177	5.03	3.23	-	-
59	M	ND	ND	III	2.6	813	4.3	21.6	-	-
66	F	-	-	IV	2.6	5.3	<0.35	0.46	-	+
45	M	ND	ND	II	2.58	5.47	35	0.63	+	+
38	F	-	-	IV	2.58	71.6	10	0.51	-	-
32	F	ND	ND	I	2.55	214	<0.35	5.81	-	-
65	M	-	-	IV	2.55	102	6.7	0.58	+	-
27	F	ND	ND	II	2.54	1736	26.1	12.1	+	+
61	M	-	-	IV	2.52	149	2.29	0.65	+	-
54	F	ND	ND	I	2.5	37.6	<0.35	2.7	ND	ND
73	F	ND	ND	II	2.5	91.4	<0.35	8.34	-	-
65	F	ND	ND	I	2.47	207	10.3	2.93	-	-
27	F	ND	ND	III	2.47	85.5	<0.35	13.4	-	+
66	M	ND	ND	II	2.44	29.3	<0.35	1.69	-	-
53	M	ND	ND	II	2.42	22.7	0.42	5.43	-	-
73	F	-	-	IV	2.42	184	21.2	12.3	-	-
27	F	-	-	IV	2.4	257	<0.35	6.86	-	+
51	M	ND	ND	III	2.37	47.9	<0.35	1.03	-	-
69	M	+	-	IV	2.36	522	2.46	12.8	+	+
45	F	-	-	IV	2.36	38.1	<0.35	3.79	-	+
36	M	ND	ND	I	2.35	38.9	0.35	22.9	-	-
41	F	-	-	IV	2.35	62.5	<0.35	3.57	-	+
50	F	ND	ND	I	2.34	17.8	9.22	7.23	-	-
52	M	-	-	IV	2.34	171	<0.35	0.98	-	+
53	F	ND	ND	III	2.33	3.08	0.43	<0.35	+	+
40	F	ND	ND	III	2.32	37.6	4.4	2.43	+	+
56	M	ND	ND	II	2.31	27.7	0.47	2.32	-	+
59	F	ND	ND	I	2.3	56.7	<0.35	1.17	-	-
47	F	ND	ND	III	2.3	344	48.7	12.2	+	+

30	M	ND	ND	I	2.27	50.2	12.3	1.04	+	ND
50	M	ND	ND	III	2.25	22.4	1.82	0.53	+	-
29	F	-	-	IV	2.25	154	<0.35	0.61	-	+
32	M	ND	ND	I	2.24	171	6.85	3.41	+	+
43	F	ND	ND	III	2.22	58.4	13.5	1.74	+	+
49	F	+	-	IV	2.22	24.9	3.53	<0.35	+	-
46	M	ND	ND	I	2.2	32.8	<0.35	14.5	+	+
44	F	ND	ND	I	2.2	59.6	<0.35	6.61	-	+
62	F	ND	ND	I	2.17	159	1.77	6.63	-	+
32	F	-	-	IV	2.17	18.2	<0.35	5.88	-	+
43	F	-	-	IV	2.15	37.5	<0.35	1.5	-	+
38	M	ND	ND	III	2.14	43.2	1.2	11.1	+	+
46	M	-	-	IV	2.14	237	6.52	0.97	+	+
59	F	-	-	IV	2.13	29	0.64	<0.35	+	-
83	M	-	-	IV	2.13	80	<0.35	3.47	-	+
59	M	ND	ND	III	2.11	14.9	<0.35	3.96	-	-
66	M	-	-	IV	2.11	230	5.02	2.05	-	-
66	M	+	-	IV	2.1	45.7	<0.35	20.8	-	+
32	M	ND	ND	II	2.03	61.4	<0.35	3.97	-	-
39	F	-	-	IV	2.03	387	14.5	5.14	-	-
61	F	-	-	III	2.02	31.8	<0.35	0.38	-	-
69	M	ND	ND	III	2.02	115	0.88	11.9	+	+
55	F	-	-	IV	2.02	1093	<0.35	6.52	-	-
44	F	ND	ND	III	1.97	36.1	16.3	5.09	-	-
47	M	-	-	IV	1.97	43.6	1.07	15.2	-	+
28	M	ND	ND	I	1.95	94	7.36	5.29	-	ND
51	F	ND	ND	III	1.95	43.1	<0.35	13.1	-	+
76	F	ND	ND	II	1.94	65.8	<0.35	4.77	-	-
35	M	-	-	IV	1.94	88.5	<0.35	3.48	+	+
48	F	-	-	IV	1.93	7.11	<0.35	1.95	-	+
48	M	ND	ND	I	1.88	103	0.89	4.45	-	+
46	F	ND	ND	III	1.86	74.4	<0.35	2.86	-	+
53	M	ND	ND	III	1.85	66.4	<0.35	75.1	-	-
43	M	ND	ND	III	1.85	111	3.55	10.1	+	+
55	F	ND	ND	III	1.84	1086	<0.35	45.7	+	+
42	F	ND	ND	III	1.82	101	<0.35	0.42	-	+
32	M	-	-	IV	1.78	17.3	1.24	4.83	-	+
22	F	ND	ND	III	1.77	108	<0.35	0.81	-	-
27	F	ND	ND	II	1.75	191	3.98	60.2	ND	ND
50	M	ND	ND	III	1.75	79.2	3.94	0.72	-	-
30	F	ND	ND	II	1.72	53.6	0.84	4.79	+	+
31	F	ND	ND	III	1.72	691	<0.35	4.38	ND	ND
32	M	ND	ND	III	1.72	129	1.52	0.51	-	-
63	M	ND	ND	III	1.72	112	<0.35	0.4	+	-
52	M	-	-	IV	1.72	1519	2.39	89	+	+
31	F	ND	ND	III	1.7	53.6	<0.35	<0.35	-	-

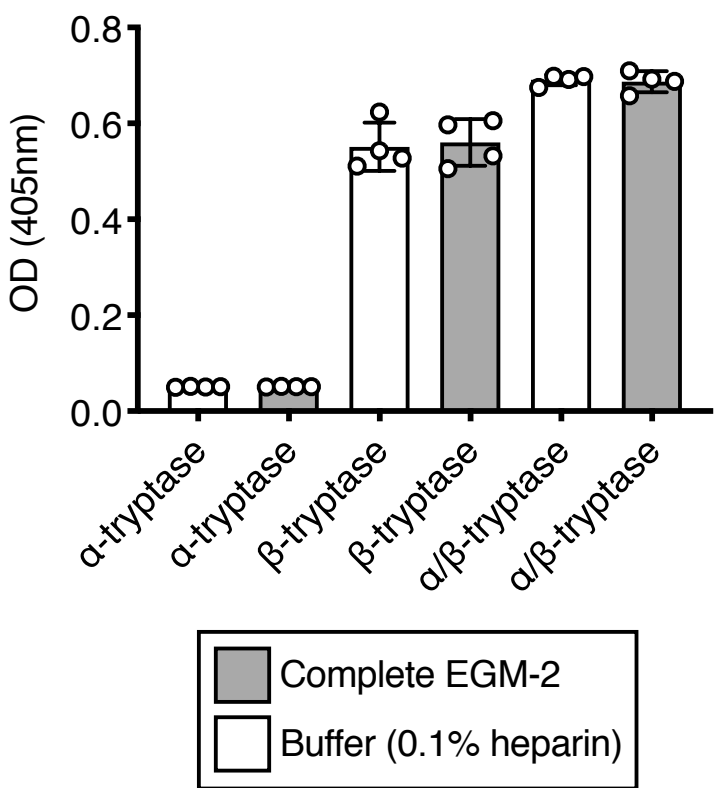
39	M	-	-	IV	1.7	81.8	0.5	3.54	-	-
29	M	ND	ND	II	1.67	286	3.41	11	+	+
28	M	ND	ND	III	1.66	116	9.86	19.8	+	+
55	F	-	-	IV	1.66	11.2	1.96	<0.35	+	-
51	F	ND	ND	II	1.64	42.7	<0.35	47.3	-	-
29	F	-	-	IV	1.63	8	<0.35	0.85	-	-
35	F	ND	ND	III	1.6	43.9	<0.35	9.67	-	-
49	M	-	-	IV	1.6	314	7.03	4.52	+	+
50	F	ND	ND	II	1.55	50.6	<0.35	14.4	-	+
53	M	ND	ND	III	1.49	144	0.44	45.6	+	+
59	M	-	-	IV	1.49	21	6.53	3.93	+	+
53	F	-	-	IV	1.48	740	0.4	>100	+	+
28	F	-	-	IV	1.39	25	<0.35	2.38	-	-
61	M	-	-	IV	1.39	111	<0.35	12.7	+	+
53	F	-	-	IV	1.38	44	<0.35	1.44	-	-
66	F	-	-	III	1.36	38	6.75	<0.35	+	-
57	F	ND	ND	III	1.34	387	4.71	1.11	+	+
52	F	-	-	IV	1.32	181	2.07	1.1	-	-
31	F	ND	ND	II	1.29	191	<0.35	9.48	-	-
45	F	+	-	IV	1.29	42.1	<0.35	13.9	-	+
40	M	-	-	IV	1.29	55.5	2.19	<0.35	+	+
55	M	-	-	IV	1.28	91.5	60.2	0.73	+	-
56	F	ND	ND	I	1.24	110	<0.35	14.8	+	+
31	M	ND	ND	II	1.24	331	28.7	0.97	+	-
45	M	ND	ND	III	1.22	171	<0.35	98.8	-	+
51	M	+	-	IV	1.09	138	0.37	36.2	-	+
38	F	ND	ND	III	1.06	47.7	0.91	1.17	-	-
54	F	-	-	IV	1.06	120	2.42	28.5	-	-
34	M	-	-	I	<1	175	22.6	5.57	-	+
47	F	ND	ND	I	<1	210	<0.35	14.8	-	+
29	F	ND	-	II	<1	7.22	8.91	0.92	+	-
46	F	ND	-	II	<1	29.8	6.01	<0.35	+	-
28	F	ND	ND	III	<1	1205	<0.35	13.3	-	-
67	M	ND	ND	III	<1	50	19.4	1.43	+	-
46	M	ND	ND	III	<1	138	2.03	9.83	-	+
32	F	ND	ND	III	<1	5.76	<0.35	4.6	-	-
57	M	ND	ND	III	<1	36.8	0.53	4.47	-	+
32	F	ND	ND	III	<1	35.8	4.13	<0.35	+	-
67	M	ND	ND	III	<1	358	33.6	14.1	+	-
57	F	ND	ND	III	<1	66.5	0.53	6.06	+	+
35	M	-	-	IV	<1	95.5	6.07	3.15	+	+
72	M	-	-	IV	<1	18.2	1	<0.35	+	-
30	M	-	-	IV	<1	93.1	6.97	11.7	+	+
25	M	-	-	IV	<1	277	<0.35	<0.35	-	+

*Results for skin prick and where indicated intradermal skin testing. H α T – Hereditary alpha-tryptasemia; ND – not determined.

Fig. S1

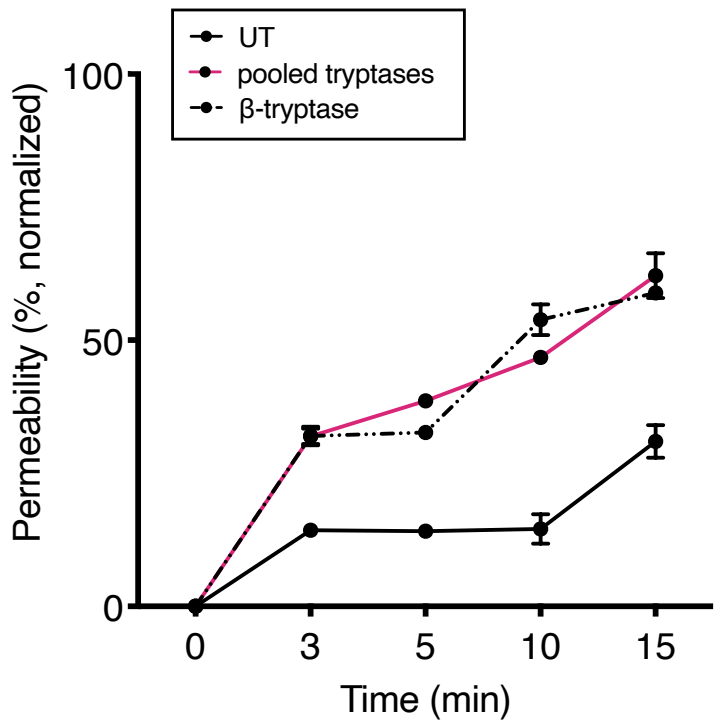
A.

Tryptase Enzymatic Activity



B.

Overnight tryptase exposure



C.

PAR2 agonist

