Title: Elevated basal serum typtase identifies a multisystem disorder associated with increased α tryptase copy number

Running Title: Hereditary α tryptasemia

Authors: Jonathan J. Lyons, MD1, Xiaomin Yu, Ph.D1, Jason D. Hughes, PhD2, Quang T. Le, PhD3, Ali Jamil BS1, Yun Bai, MS1, Nancy Ho, MD4, Ming Zhao, PhD5, Yihui Liu, PhD1, Michael P. O’Connell, PhD1, Neil N. Trivedi, MD6, Celeste Nelson, CRNP1, Thomas DiMaggio, ADN1, Nina Jones, RN, BSN7, Helen Matthews RN, BSN8, Katie L. Lewis, ScM9, Andrew J. Oler, PhD1, Ryan J. Carlson, BS1, Peter D. Arkwright, FRCPCH, D Phil10, Celine Hong, PhD9, Sherene Agama, BS1, Todd M. Wilson, DO1, Sofie Tucker, BA1, Yu Zhang, PhD11, Joshua J. McElwee, PhD2, Maryland Pao, MD12, Sarah C. Glover, DO13, Marc E. Rothenberg, MD, PhD14, Robert J. Hohman, PhD5, Kelly D. Stone, MD, PhD1, George H. Caughey, MD6, Theo Heller, MD4, Dean D. Metcalfe, MD1, Leslie G. Biesecker, MD9, Lawrence B. Schwartz, MD, PhD3, and Joshua D. Milner, MD1.

Affiliations: 1Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; 2Merck Research Laboratories, Merck & Co. Inc., Boston, MA; 3Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA; 4Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 5Research Technologies Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD; 6Cardiovascular Research Institute and Department of Medicine, University of California San Francisco, San Francisco, CA, and Veterans Affairs Medical Center, San Francisco, CA;
Correspondence: Joshua D. Milner

Building 10, Room 5W-3840
National Institutes of Health
Bethesda, MD 20892-1881
jdmilner@niaid.nih.gov
Tel: (301) 827-3662
Fax: (301) 480-8384

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Elevated basal serum tryptase is present in 4-6% of the general population but the cause and significance is unknown (1, 2). Previously we described a cohort with dominantly inherited basal serum tryptase elevations associated with multisystem complaints including cutaneous flushing and pruritus, dysautonomia, functional gastrointestinal symptoms, chronic pain, and connective tissue abnormalities including joint hypermobility. Herein we report identification of germline α tryptase gene duplications and triplications (at TPSAB1) that segregate with inherited basal serum tryptase elevations identified in 35 families presenting with associated multisystem complaints. Individuals harboring ααα alleles had higher basal serum tryptase levels and were more symptomatic than those with αα alleles, suggesting a gene-dose effect. Further, we found in two additional cohorts (172 individuals) that elevated basal serum tryptase was exclusively associated with α tryptase duplications and affected individuals reported symptom complexes seen in our initial familial cohort. α Tryptase duplications therefore link tryptase elevations with IBS, cutaneous complaints, connective tissue abnormalities, and dysautonomia.

Medically unexplained symptoms and symptom-complexes can be vexing for clinicians and patients alike. Manifestations such as cutaneous flushing, certain chronic pain disorders, autonomic dysfunction, and gastrointestinal dysmotility have been attributed to a number of disparate etiologies including neurologic, immunologic, physical, and psychological mechanisms (3-6). Despite a lack of diagnostic clinical findings, many of these symptoms are comorbid and often follow a dominant inheritance pattern in affected families (7-10). Furthermore, many of these features have been reported in association with genetic disorders or joint hypermobility syndromes such as Ehlers-Danlos Syndrome type III (hypermobility type, EDS III). For these
reasons, identifying genetic bases to characterize sub-groups of individuals with these disorders may substantially advance the field, but employing classic phenotypic ascertainment approaches in these individuals is extremely challenging.

Recently, we and others described family cohorts with symptom complexes conforming to these functional presentations, but uniquely, found them in association with elevated basal serum tryptase levels—a mast cell mediator commonly used to assist in the diagnosis of mast cell-associated diseases (7, 11). Mast cells have often been implicated in certain functional disorders, however our patients did not have evidence of clonal mast cell disease or evidence of mast cell activation, while many did have connective tissue manifestations overlapping with those seen in EDS III. Because elevated basal serum tryptase without mastocytosis in the general population is a relatively common trait, and in one report has been associated with functional symptoms (1, 2), we set out to identify the genetic cause for tryptase elevations and characterize associated clinical phenotypes in these families and in unselected individuals.

We approached this challenging problem by first mapping and identifying the genomic lesion associated with elevated basal serum tryptase and characterizing symptoms in affected families (Fig. S1A). A total of 96 subjects from 35 families were identified with a syndrome of elevated basal serum tryptase and complex clinical features following an autosomal dominant pattern of inheritance without evidence of mastocytosis (see Table S1 for demographics). Affected individuals had multiple comorbid symptoms including those often considered “functional” in nature due to the lack of pathological findings. Gastrointestinal dysmotility was common, most often manifest as irritable bowel syndrome (IBS) defined by Rome III criteria (49%) or symptoms of chronic gastroesophageal reflux (65%), both being present approximately 3-5 fold over the general population (12, 13). Connective tissue abnormalities were also
common; the overall prevalence of joint hypermobility (Beighton ≥4, ages 12-76 years) was 28% - approximately 2 times the general population prevalence (14) - while congenital skeletal abnormalities (26%) and retained primary dentition (21%) were also frequently identified. These findings were associated with chronic arthralgia (45%) and headache or body pain (47%). Complaints suggestive of autonomic dysfunction, including postural orthostatic tachycardia syndrome (POTS) were common. Forty-six percent of individuals had elevated composite autonomic symptom scores by validated measure (COMPASS 31), in whom 11 (34% of those with elevated scores) were validated by tilt-table testing (Fig. S2A). Additional symptoms included recurrent cutaneous flushing and pruritus (51%), which in some cases associated with urticaria, concomitant with significant complaints of sleep disruption (39%). Systemic reactions to stinging insects (e.g. hymenoptera), an occurrence known to be associated with elevated basal serum tryptase (15), were increased 2-3 fold over the general population (16%) (16) (Table 1).

Exome and genome sequencing (GS) of the first 12 families yielded no shared rare or common variants. However, linkage analysis identified a single 5.1 megabase peak on chromosome 16p13.3 (LOD = 4.46), a region containing the human tryptase locus, composed of four paralogous genes (TPSG1, TPSB2, TPSAB1, and TPSD1) (Fig. 1A). The primary secreted tryptase gene products at this locus include β tryptase encoded at TPSAB1 and TPSB2, and α tryptase, resulting from a series of variants within the TPSAB1 gene. A modified southern blot assay performed on 15 families (55 affected; 13 unaffected) identified elevated α/β tryptase ratios among affected family members, and when applied to pedigrees suggested multiple α copies were inherited together (Fig. S2B, C). Inspection of GS reads permitted in silico construction of a consensus reference sequence that was used to calculate α copy number and provided sequence to design a novel digital droplet PCR (ddPCR) assay to specifically target α
tryptase (Fig. S3A, B; S4). ddPCR analysis of all 35 families (96 affected; 41 unaffected) confirmed increased α tryptase copy number inherited on one or both alleles in all affected individuals. All individuals who inherited a single α tryptase copy on both alleles (thus also having a 2α genotype at TPSAB1) had normal basal serum tryptase levels (Fig. 1B, C; S5A-D). Having an α duplication on both alleles, or an α triplication on a single allele, was associated with significantly higher tryptase levels compared to having an α duplication on one allele. Furthermore, having an α triplication on one allele was associated with greater prevalence of associated clinical phenotypes than having an α duplication on one allele, demonstrating a gene-dose correlation (Table 1).

Mast cell cultures grown from CD34+ progenitors derived from whole blood of individuals with increased single-allele α tryptase gene copies did not have abnormal growth or morphology. Likewise, intracellular tryptase expression and IgE-mediated degranulation did not significantly differ from controls (Fig. S6A, B). However, increased total tryptase mRNA expression was identified both in PBMC-derived primary mast cells (n = 5 each group) and ex vivo total PBMCs (n = 10 each group) from individuals with α duplications or triplications (Fig. S6C). Furthermore, among individuals with increased mono-allelic α tryptase gene copies supernatants from primary mast cell cultures showed more spontaneous tryptase secretion compared to matched controls (n = 5 each group) (Fig. 1D).

To begin to determine whether extra α tryptase genes on a single allele might commonly be associated with basal serum tryptase elevations in the general population, we next applied our bioinformatic strategy to a large cohort of patients and healthy family members from NIAMS and NIAID programs in clinical genomics on whom GS was performed for reasons unrelated to mast cells or tryptase. This retrospective analysis was limited to those in whom coverage was
sufficient to call α tryptase copy number, and from whom sera were available to measure tryptase levels (Fig. S1B). ddPCR was performed on all individuals (n=17) with basal serum tryptase >8 ng/mL or ≥2 α tryptase gene copies identified using our bioinformatic strategy. Out of 98 individuals, eight (8.2%) were identified with α duplications on a single allele. This accounted for all individuals with basal serum tryptase elevations (>11.4 ng/ml) in the cohort (Fig. 2A). Moreover, a dominant inheritance pattern of elevated basal serum tryptase was observed in both families for whom there were samples available.

Finally to validate the observed association between α tryptase copy number and basal serum tryptase level, and to explore the effect of this genetic finding on clinical phenotypes in an unselected population, we interrogated the NHGRI ClinSeq® cohort, a group of healthy unrelated volunteers. First, 125 de-identified serum samples that were partially enriched for α tryptase duplications using a common haplotype, were screened for elevated basal serum tryptase (see Fig. S1C and accompanying legend for details of enrichment). All those above 8 ng/mL (n = 25) were genotyped by the bioinformatic algorithm (9 individuals were excluded due to a lack of genomic sequence coverage) and subsequently by our ddPCR assay (n=16). Attempts were then made to contact all available individuals for phone interview; interviewers were blinded to tryptase level and genotype (see Table S2 for demographics). Single allele α tryptase duplications were identified in 9 of these individuals, fully accounting for all elevated basal serum tryptase levels of those genotyped in this cohort (Fig. 2B). Three of the nine individuals were phenotypically indistinguishable from affected members of the initial referral cohort (Table S3) and α tryptase duplications were significantly associated with cutaneous flushing, itching or hives (P=0.014), systemic venom reactions (P=0.047), IBS (by Rome III criteria) (P=0.042), retained primary dentition (P=0.020) and elevated autonomic symptom scores (by COMPASS
Family histories suggestive of affected family members were present in 4/9, and elevated basal serum tryptase levels could be confirmed in first-degree relatives in two out of three available families.

The overall sensitivity of the α tryptase genotyping ddPCR assay for detecting individuals with elevated basal serum tryptase was 100% (95% CI 95.1% - 100%), and specificity was 90.0% (95% CI 85.1% - 93.7%).

Consistent with previous studies, our data indicate that elevated basal serum tryptase is a relatively common biochemical trait. We have found that it is most frequently inherited in an autosomal dominant manner and that when this occurs, it is exclusively associated with increased α tryptase copy number on a single allele, a genetic trait we have termed hereditary α tryptasemia. In turn, elevated basal serum tryptase is associated with an increased prevalence of multiple, predominantly functional, clinical phenotypes including recurrent cutaneous symptoms, symptoms of autonomic instability, functional GI disorders, as well as systemic venom reactions and connective tissue abnormalities. The families studied in our initial cohort likely represented the most severe phenotypes among individuals affected with hereditary α tryptasemia – due in part to the lack of α tryptase triplications detected in unselected populations – which we have tentatively designated as hereditary α tryptasemia syndrome.

*In vitro* experiments suggest that elevated α tryptase transcripts lead to increased α pro-tryptase translation and constitutive secretion, thereby accounting for the elevated basal serum tryptase levels seen *in vivo*. This may occur by a stoichiometric phenomenon, particularly if only a single allele of the locus is expressed as has been shown to commonly occur (17). The apparent α tryptase gene dose-effect manifested as total basal serum tryptase levels seems to
support this assertion. However, altered epigenetic regulation of the locus when additional $\alpha$ tryptase copies are present may serve as a contributing factor.

The genetics of the human tryptase locus are complex. It sits within a gene rich region at 16p13.3, which is a hotspot for genetic recombination (18, 19). It is hypothesized that the multiple tryptase genetic loci in humans evolved through duplication and inversion of this locus (20). Two adjacent genes, $TPSABI$ and $TPSB2$, encode the four major isoforms [$\beta I$, $\beta II$, $\beta III$, and $\alpha$ ($\alpha I$)] of what is believed to be biologically relevant soluble tryptase; the $\alpha$ isoform is only reported as being encoded at the $TPSABI$ locus. The high degree of sequence identity between $\alpha$ and $\beta$, and the presence of multiple paralogues in a single locus, makes detection of copy number variation difficult, likely precluding genome-wide association studies or quantitative arrays from detecting $\alpha$ tryptase CNV. Our digital droplet PCR assay provides indirect evidence that the gene duplications are occurring within the locus; duplicated or triplicated $\alpha$ tryptase did not randomly assort into droplets without restriction digestion, indicating that multiple copies were present within fragments of genomic DNA formed during extraction (maximum size is approximately 50 Kb) and therefore the duplicated copies are relatively tightly linked in the genome and may be subject to the same enhancers and other control elements.

Part of the clinical presentation in hereditary $\alpha$ tryptasemia syndrome includes symptoms that clinically may be associated with mast cell mediator release, and in the context of elevated basal serum tryptase can trigger an extensive work-up for clonal mast cell disease, including bone marrow biopsy. Because tryptase elevations are seen in a relatively large percentage of the general population, the decision to proceed with such a work-up can be challenging. Performing tryptase genotyping as part of this work-up may be warranted in light of our findings.
How elevated basal serum tryptase might contribute to the associated multisystem disorder we observed remains unclear. Based upon clinical phenotypes including pain and connective tissue abnormalities, a compelling case could be made for activation of protease activated receptor 2 (PAR2) dependent pathways. However, co-inheritance of a second functional genetic variant contributing to the complex clinical phenotype cannot be ruled out. While further work is required to determine the relationship between tryptase elevations and associated phenotypes, α tryptase remains an attractive future therapeutic candidate, since a significant percentage of the general population (>25%) are α tryptase deficient without known untoward effects.
METHODS

Subjects

αtryptasemia cohort

Informed consent was provided by all patients and their relatives on NIH IRB-approved research protocols designed to study mastocytosis (NCT00044122, NCT00001756) and/or atopy (NCT01164241, NCT00852943, NCT00557895). Over a 5-year period, family and personal medical histories were obtained and physical examinations were performed on all individuals able to travel to NIH. After recognizing that this familial presentation included a wide range of symptoms, histories and exams were expanded throughout family accrual (for demographics of this cohort see Table S1). When unavailable for a direct encounter, or if the patients were evaluated prior to establishing the full phenotype, a comprehensive history and assessment was performed using electronic media to characterize symptoms and reported physician diagnoses. Blood samples were collected for genetic testing and tryptase measurement. Reported clinical diagnoses were based upon patient report of physician diagnosis and/or a consistent clinical history and physical exam, as well as review of outside records and test results where available/applicable. Please see supplement for definitions and criteria for reported symptoms and diagnoses pertaining to all three cohorts (Supplementary Appendix, Section A). Two validated questionnaires, the Rome III questionnaire (21) to interrogate IBS, and the COMPASS 31 (22) questionnaire to interrogate dysautonomia, were also administered to a majority of the cohort.
NIAMS and NIAID clinical genomics cohort

Informed consent was provided by all patients and their relatives on NIH IRB-approved research protocols designed to study immunodeficiency and autoinflammation (NCT00246857, NCT00128973, and NCT00059748).

ClinSeq® cohort

Individuals were chosen (Fig. S1C) from the ClinSeq® study (NCT00410241), a project employing exome sequencing in a clinical research setting, to serve as an unselected study cohort. The majority of participants were healthy adult volunteers (for demographics of those included see Table S2), with approximately 25% having a personal history of coronary artery disease. Participants were broadly consented to genome sequencing and the return of individual sequencing results (23). Blinded phone interviews were conducted to identify clinical phenotypes and reported physician diagnoses among the ClinSeq® participants comporting with our defined criteria (Supplementary Appendix, Section A). Histories focused on symptoms and conditions we identified in association with inherited tryptase elevations (7), as well as those queried in a published questionnaire for the diagnosis of mast cell activation syndrome (MCAS) (24).

Standardized questionnaires to assess for IBS (Rome III) and autonomic dysfunction (COMPASS 31) were also administered to this population.

Individual controls

Volunteers, who did not have significant clinical allergic disease or connective tissue abnormalities and did not have elevated basal serum tryptase, were selected and provided
informed consent on NIH IRB-approved protocols and were recruited to act as experimental controls (NCT00806364).

**Genetic sequencing and analysis**

For the \( \alpha \) tryptasemia cohort, exome sequencing (ES) was performed on eight families using TruSeq (Illumina, San Diego, CA) capture kits and a custom analysis pipeline as described (25). Genome sequencing (GS) was performed as described (26) on nine families (five of which previously had ES) using the HiSeq platform (Illumina, San Diego, CA) with the Burrows-Wheeler Aligner and Picard (http://broadinstitute.github.io/picard/) used for basic alignment and sequence quality control. The same capture kits and strategies were employed for the GS performed in the NIAMS and NIAID clinical genomics cohorts. For the ClinSeq\textsuperscript® cohort, ES was performed as described (27).

**Linkage analysis**

GATK Unified Genotyper (parameters: -stand_call_conf 5.0, -stand_emit_conf 5.0, -dcov 500) and SAMtools were used to identify single nucleotide variants (SNVs) and insertions/deletions (Indels), and GATK VariantsToBinaryPed (parameters: -minGenotypeQuality 10) was used to produce binary pedigrees from variant call format (VCF) files. Plink (http://pngu.mgh.harvard.edu/purcell/plink/) was then used to convert the binary pedigree files to LINKAGE format files. PEDSTATS (ref. PEDSTATS: Descriptive statistics, graphics and quality assessment for gene mapping data) module in MERLIN (28) was used to check pedigree structure and MERLIN was used to perform parametric rare-dominant linkage analysis.
Bone Marrow Biopsy and KIT gene analysis

Bone marrow biopsies were performed on probands from seven families to exclude the diagnosis of systemic mastocytosis as described (7). An additional eight families were screened for the activating KIT mutation c.2447A>T p.(D816V) using allele-specific PCR, as described (29).

Tryptase protein quantification

Total basal serum tryptase levels were measured using a commercially available fluorescence enzyme immunoassay in Clinical Laboratory Improvement Amendments (CLIA) certified laboratories. Further fractionation and measurement of tryptase levels were performed as described (30), using the UniCAP immunofluorescent assay (ThermoFisher, Waltham, MA) for total (pro and mature forms of α/β tryptases) and an ELISA for mature α/β tryptase levels, in a CLIA-approved laboratory (LBS). The lower limits of detection for each tryptase assay was 1 ng/ml. Currently, the normal range in serum for total tryptase is 1-11.4 ng/mL and for mature tryptase is <1 ng/mL (31).

Tryptase genotyping

A unique reference consensus sequence for the tryptase locus was generated using GS data. A computer algorithm was then created to extract all reads originally mapped to the ~50 kilobase region containing the locus. These reads were then re-mapped to the deduced short consensus region (see Supplementary Appendix, Section B for complete description) to determine specific tryptase gene sequences and their relative abundance. Initial tryptase genotyping used a validated modified semi-quantitative Southern blot technique as described (32).
To directly quantitate allelic α and β tryptase copy number, a digital droplet PCR (ddPCR) assay was developed using custom primers (5’-TCCTGACCTGGCACCTGC-3’; 5’-GACTCTCAGGCTCACCTGCA-3’) and custom probes for α (5’-CTGCAGCAAGCGGGTATCGTC-3’) and β (5’-CTGCAGCGAGTGGGCATCGT-3’) tryptases based upon the published sequences (20, 33, 34) and consensus sequences derived in silico (Fig. S3A, B); the probes did not hybridize to γ or δ tryptases. The assay was performed on native or restriction endonuclease-treated genomic DNA using the PrimePCR ddPCR Copy Number reference AP3B1, according to the manufacturer’s specifications (Bio-Rad, Hercules, CA), allowing for accurate detection of multiple tryptase copies on a single allele (Supplementary Appendix, Section C; Fig. S5A-D).

**Code Availability**

The code generated for in silico tryptase genotyping is available in the Supplementary Appendix, Section B.

**Mast cell culture and analysis**

CD34+ cells were isolated from peripheral blood mononuclear cells (PBMCs) and cultured under conditions as described to yield primary mast cells (35). Cells were washed, stained with Live/Dead Fixable Aqua (Invitrogen), fixed with 4% paraformaldehyde, permeabilized with 5% saponin and stained intracellularly with anti-tryptase phycoerythrin (PE) (Novus Biologicals, Littleton, CO). Total mRNA was extracted from mast cells and real-time (RT)-PCR was performed as described (25) to quantitate total tryptase mRNA expression using the tryptase primer/probe set for TPSB2 (Life Technologies) that captures all α and β isoforms from TPSB2.
and $TPSAB1$, but not $\delta$ or $\gamma$ tryptases. Mast cell degranulation was assessed by measuring $\beta$-hexosaminidase release as described (36), and whole mast cell lysates were obtained as described (37). In order to characterize the size and quantity of tryptase molecules from these cultures, total tryptase in lysates and culture supernatants was determined by Western blotting, probed using rabbit anti-human tryptase (clone G3) (EMD Millipore, Billerica, MA).

**Statistical Analyses**

Mann-Whitney, Wilcoxon matched pairs, or Fisher’s exact test was employed to test significance of associations as indicated. In all populations examined basal serum tryptase levels did not follow a normal distribution (D’Agostino-Pearson test). A two-tailed F-test was used to determine whether the observed variances in populations were different. The standard deviations of basal serum tryptase values among individuals with hereditary $\alpha$ tryptasemia syndrome, regardless of genotype, were all significantly different than the standard deviation observed in unaffected individuals (F-value $>$7, $P < 0.0001$), indicating these populations were different than unaffected individuals. Among affected individuals the standard deviations of tryptase values were not significantly different when comparing between $\alpha\alpha$, $\alpha\alpha/\alpha\alpha$, and $\alpha\alpha\alpha$ individuals (F-value 1.2-2.1, $P = 0.14 – 0.9$).

To assess dysautonomia among individuals an expected scoring range was established. To accomplish this, the COMPASS 31 questionnaire was administered to 35 healthy family members from the $\alpha$ tryptasemia and NIAID clinical genomics cohorts, in whom tryptase levels were within the normal range and in whom $\alpha$ tryptase gene dose was confirmed both by bioinformatic algorithm and ddPCR assay to be $\leq 1$ copy per allele. The upper 95% confidence interval (CI) of the median was defined as the normal cut-off, with individuals scoring higher
than this number considered to be outliers and symptomatic. Fisher’s exact test was then applied
to test statistical significance.
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receives consulting fees from Genentech, Inc. LGB is an uncompensated advisor to the Illumina
Corp, receives royalties from Genentech, Inc., and Amgen, and honoraria from Wiley-Blackwell.
REFERENCES


FIGURE LEGENDS

Figure 1. Inherited α tryptase gene duplications and triplications are associated with elevated serum basal serum tryptase and enhanced mast cell tryptase secretion. (A) Linkage analysis performed on exome sequencing data from 8 families identified a single linkage region on chromosome 16p13.3 (Chr16: 556,104 – 5,653,182; GRCh37/hg19) (LOD=4.46) (top). The 36.6 kilobase (Kb) tryptase locus is present within this region and contains four tryptase genes TPSG1, TPSB2, TPSAB1, and TPSD1 (middle). TPSB2 and TPSAB1 both can encode for β tryptases, but α tryptase is only known to be present at TPSAB1. Patients with inherited basal tryptase elevations were found to have duplications or triplications of α tryptase on single alleles at TPSAB1; schematics of how this genetic change may manifest are shown (bottom). (B) Basal serum tryptase levels and corresponding TPSAB1 tryptase genotypes among families (n = 35) identified with inherited basal serum tryptase elevation. Upper limit of normal as defined by multiple clinical laboratories (>11.4 ng/mL) is indicated by the dashed line. Data shown with geometric mean, *P=0.0012; **P<0.0001; Mann-Whitney test. (C) Sample pedigrees from four families with hereditary α tryptasemia syndrome; numbers indicate basal serum tryptase levels (ng/mL). (D) Mast cells were cultured from peripheral CD34+ cells of individuals with single-allele α tryptase duplications or triplications (α tryptasemia) or from peripheral CD34+ cells of paired controls, and Western blots of media containing spontaneously released tryptase (10 uL, 3 uL, or 1 uL) were performed. Western blot from one of five independent culture experiments (left). Fold increase in total tryptase content of α tryptasemia supernatants relative to paired controls (n = 5) from five combined experiments is shown (right); mean ± SEM.
Figure 2. Single allele α tryptase gene duplications are associated with elevated serum basal serum tryptase in unselected populations. (A) Basal serum tryptase levels and corresponding TPSAB1 tryptase genotypes among individuals undergoing exome or genome sequencing for immune phenotypes unrelated to mast cell activation. (B) Basal serum tryptase levels and corresponding TPSAB1 tryptase gene expression among individuals genotyped from the ClinSeq® cohort. Upper limit of normal (>11.4 ng/mL) is indicated by the dashed line (A and B). Data shown with geometric mean, **P<0.0001; Mann-Whitney test.
Table 1. Clinical features and gene-dose effects in hereditary α tryptasemia syndrome.

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<td>Serum tryptase, ng/ mL median (interquartile range)</td>
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</tbody>
</table>

IBS – Irritable bowel syndrome; *Comparing 2α (ααα) to 3α (ααα) allele carriers; †Systemic immediate hypersensitivity reaction consistent with IgE-mediated to stinging insect, as described in the Supplement Appendix; §Defined as the presence of a congenital skeletal malformation (complete list of malformations identified is provided in the Supplement), or diagnosis of Ehler’s Danlos syndrome; ¶Only individuals over 12 years of age and who could be directly visualized were assessed and reported. ¶Number of individuals with a composite score above the upper 95% CI of median established in a healthy control cohort without increased α tryptase copy number.
Table 2. Self-reported clinical features among ClinSeq® participants with (αα) and without (WT) identified α tryptase duplications on a single allele.

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>αα N (%)</th>
<th>WT N (%)</th>
<th>OR</th>
<th>RR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic venom reaction*</td>
<td>2/9 (22)</td>
<td>2/82 (2)</td>
<td>11.4 (1.4-94.0)</td>
<td>9.1 (1.5-57.1)</td>
<td>0.047</td>
</tr>
<tr>
<td>Flushing/Pruritus</td>
<td>5/9 (55)</td>
<td>13/82 (16)</td>
<td>6.6 (1.6-28.1)</td>
<td>3.5 (1.6-7.6)</td>
<td>0.014</td>
</tr>
<tr>
<td>IBS (Rome III)</td>
<td>3/9 (33)</td>
<td>6/82 (7)</td>
<td>6.3 (1.3-31.9)</td>
<td>4.6 (1.4-15.2)</td>
<td>0.042</td>
</tr>
<tr>
<td>Chronic gastroesophageal reflux symptoms</td>
<td>7/9 (77)</td>
<td>39/82 (48)</td>
<td>3.9 (0.8-19.7)</td>
<td>1.6 (1.1-2.5)</td>
<td>0.158</td>
</tr>
<tr>
<td>Congenital skeletal abnormality†</td>
<td>1/9 (11)</td>
<td>3/82 (4)</td>
<td>3.3 (0.3-35.5)</td>
<td>3.0 (0.4-26.2)</td>
<td>0.346</td>
</tr>
<tr>
<td>Retained primary dentition</td>
<td>3/9 (33)</td>
<td>4/82 (5)</td>
<td>9.8 (1.8-54.0)</td>
<td>6.8 (1.8-25.8)</td>
<td>0.020</td>
</tr>
<tr>
<td>COMPASS 31§</td>
<td>4/9 (44)</td>
<td>11/82 (13)</td>
<td>5.2 (1.2-22.3)</td>
<td>3.3 (1.3-8.3)</td>
<td>0.038</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>4/9 (44)</td>
<td>25/82 (30)</td>
<td>1.8 (0.5-7.4)</td>
<td>1.5 (0.6-3.2)</td>
<td>0.459</td>
</tr>
<tr>
<td>Body Pain/Headache</td>
<td>3/9 (33)</td>
<td>12/82 (15)</td>
<td>2.9 (0.6-13.3)</td>
<td>2.3 (0.8-6.6)</td>
<td>0.165</td>
</tr>
<tr>
<td>Sleep disruption</td>
<td>2/9 (22)</td>
<td>21/82 (26)</td>
<td>0.8 (0.2-4.3)</td>
<td>0.9 (0.2-3.1)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

IBS – Irritable bowel syndrome; *Systemic immediate hypersensitivity reaction consistent with IgE-mediated to stinging insect, as described in the Supplement; † Spina bifida occulta, congenital absence of spinous process, pectus excavatum, and tibial torsion; § Number of individuals with a composite score above the upper 95% CI of median established in a healthy control cohort without increased α tryptase copy number; OR – odds ratio; RR – relative risk.
Figure 1.

a. LOD

b. Basal serum tryptase (ng/mL)

c. TPSG1  TPSB2  TPSAB1  TPSD1

b. Tryptase

~35 kDa
Figure 2.

a. TPSAB1

b. TPSAB1

basal serum trypase (ng/mL)

β/β α/β α/α αα/β αα/α

TPSAB1