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Elevated basal serum tryptase identifies a multisystem disorder associated with increased *TPSAB1* copy number

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URLs. Burrows–Wheeler aligner and Picard, http://broadinstitute.github.io/picard/; PLINK, http://pngu.mgh.harvard.edu/purcell/plink/; Genome Analysis Toolkit (GATK), https://software.broadinstitute.org/gatk/; SAMtools, http://www.htslib.org/.

Accession codes. Exome and genome sequencing data have been deposited in the Sequence Read Archive (SRA) under accession PRJNA342304.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

AUTHOR CONTRIBUTIONS

J.J.L. and J.D.M. designed the study. J.J.L., J.D.M., C.N., T.D., N.J., H.M., T.M.W., K.D.S., D.D.M., S.C.G., P.D.A., and M.E.R. all recruited subjects to the study. J.J.L., J.D.M., T.H., C.N., N.J., T.D., N.H., M.P., S.C.G., R.J.C., S.A., S.T., T.M.W., and A.J. collected and/or analyzed clinical data. X.Y., J.D.H., C.H., Y.Z., A.J.O., J.J.M., L.G.B., and J.D.M. performed and supported genomic sequencing. X.Y., J.D.H., C.H., Y.Z., and A.J.O. performed the bioinformatic analyses. J.J.L., N.N.T., G.H.C., and L.B.S. designed and J.J.L. performed the ddPCR assay. J.J.L., Q.T.L., Y.B., M.Z., Y.L., M.P.O'C., R.J.H., L.B.S., and J.D.M. designed and performed the functional studies. K.L.L., C.H., and L.G.B. facilitated all ClinSeq-related studies. J.J.L. and J.D.M. prepared the draft manuscript. All authors contributed to discussion of the results and to manuscript preparation.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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Abstract

Elevated basal serum tryptase levels are present in 4–6% of the general population, but the cause and relevance of such increases are unknown[1, 2]. Previously, we described subjects with dominantly inherited elevated basal serum tryptase levels associated with multisystem complaints including cutaneous flushing and pruritus, dysautonomia, functional gastrointestinal symptoms, chronic pain, and connective tissue abnormalities, including joint hypermobility. Here we report the identification of germline duplications and triplications in the *TPSAB1* gene encoding α-tryptase that segregate with inherited increases in basal serum tryptase levels in 35 families presenting with associated multisystem complaints. Individuals harboring alleles encoding three copies of α-tryptase had higher basal serum levels of tryptase and were more symptomatic than those with alleles encoding two copies, suggesting a gene-dose effect. Further, we found in two additional cohorts (172 individuals) that elevated basal serum tryptase levels were exclusively associated with duplication of α-tryptase–encoding sequence in *TPSAB1*, and affected individuals reported symptom complexes seen in our initial familial cohort. Thus, our findings link duplications in *TPSAB1* with irritable bowel syndrome, 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 neurological, immunological, 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 subgroups of individuals with these disorders may substantially advance the field; however, employing classical 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 found them in association uniquely with elevated basal serum levels of tryptase—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, whereas many did have connective tissue manifestations overlapping with those seen in EDS III. Because elevated basal serum tryptase levels without mastocytosis is a relatively common trait in the general population and in one report has been associated with functional symptoms[1,2], we set out to identify the genetic cause for elevated tryptase levels and to 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 levels and characterizing symptoms in affected families (Supplementary Fig. 1). We identified 96 subjects from 35 families with a syndrome of elevated basal serum tryptase levels and complex clinical features following an autosomal dominant pattern of inheritance without evidence of mastocytosis (see Supplementary Table 1 for demographics). Affected individuals had multiple comorbid symptoms, including those often considered ‘functional’ in nature because of the lack of pathological findings. Gastrointestinal dysmotility was common, most often manifesting as irritable bowel syndrome (IBS), defined by Rome III criteria (49%) or symptoms of chronic gastroesophageal reflux (65%), with both present at a prevalence approximately three- to fivefold greater than that in 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 twice the prevalence for the general population[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), of whom 11 (34% of those with elevated scores) were validated by tilt-table testing (Supplementary Fig. 2a). Additional symptoms included recurrent cutaneous flushing and pruritus (51%), which in some cases associated with urticaria, concomitant with complaints of sleep disruption (39%). Systemic reaction to stinging insects (for example, Hymenoptera), an occurrence known to be associated with elevated basal serum tryptase levels[15], was increased by two- to threefold (16%) over the frequency of such reactions in the general population[16] (Table 1).

Exome and genome sequencing of the first 12 families yielded no shared rare or common variants. However, linkage analysis identified a single 5.1-Mb peak on chromosome 16p13.3 (logarithm of odds (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 by *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 and 13 unaffected individuals) identified elevated α–tryptase/β-tryptase ratios among affected family members and, when applied to pedigrees, suggested that multiple copies of α-tryptase–encoding sequence were inherited together (Supplementary Fig. 2b,c). Inspection of genome sequencing reads permitted *in silico* construction of a consensus reference sequence that was used to calculate copy number for the sequence encoding α-tryptase and for design of a digital droplet PCR (ddPCR) assay to specifically target α-tryptase (Supplementary Figs. 3 and 4). ddPCR analysis of all 35 families (96 affected and 41 unaffected individuals) confirmed increased copy number for α-tryptase–encoding sequence in *TPSAB1* inherited on one or both alleles in all affected individuals. All individuals who inherited a single copy of α-tryptase–encoding sequence on both alleles (thus, also having a 2α genotype at *TPSAB1*) had normal basal serum tryptase levels (Fig. 1b,c and Supplementary Fig. 5). Having duplication of α-tryptase–encoding sequence on both alleles or a triplication of the sequence on a single allele was associated with significantly higher tryptase levels than having duplication on one allele ($P = 0.0012$ and $P < 0.0001$, respectively). Furthermore, having triplication of α-tryptase–encoding sequence on one allele was associated with greater prevalence of associated clinical phenotypes than having duplication on one allele, demonstrating a correlation of phenotype with gene dose (Table 1).

Mast cells grown from $CD34^+$ progenitors derived from the whole blood of individuals with increased copy number on a single allele of α-tryptase–encoding sequence in *TPSAB1* did not have abnormal growth or morphology. Likewise, intracellular tryptase expression and IgE-mediated degranulation activity did not significantly differ in comparison to controls (Supplementary Fig. 6a,b). However, we identified increased total *TPSAB1* and *TPSB2* mRNA levels both in peripheral blood mononuclear cell (PBMC)-derived primary mast cells ($n = 5$ for each group) and *ex vivo* total PBMCs ($n = 10$ for each group) from individuals with duplication or triplication of α-tryptase–encoding sequence in *TPSAB1* (Supplementary Fig. 6c). Furthermore, among individuals with a single-allele increase in the number of copies of α-tryptase–encoding sequence, supernatants from primary mast cell cultures showed more spontaneous tryptase secretion than comparable cells from matched controls ($n = 5$ individuals for each group) (Fig. 1d).

To begin to determine whether additional copies on a single allele of α-tryptase–encoding sequence in *TPSAB1* might commonly be associated with elevated basal serum tryptase levels in the general population, we next applied our bioinformatic strategy to a large cohort of patients and healthy family members from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and National Institute of Allergy and Infectious Diseases (NIAID) programs in clinical genomics on whom genome sequencing was performed for reasons unrelated to mast cells or tryptase. This retrospective analysis was limited to individuals in whom sequencing coverage was sufficient to call *TPSAB1* copy number and from whom serum samples were available to measure tryptase levels (Supplementary Fig. 1b). We performed ddPCR on all individuals ($n = 17$) with basal serum tryptase concentration >8 ng/ml or ≥2 copies of α-tryptase–encoding sequence in *TPSAB1*

identified using our bioinformatic strategy. Among 98 individuals, we identified 8 (8.2%) with duplications on a single allele of α-tryptase–encoding sequence. This accounted for all individuals with elevated basal serum tryptase concentration (>11.4 ng/ml) in the cohort (Fig. 2a). Moreover, we observed a dominant inheritance pattern for elevated basal serum tryptase levels in both families for which samples were available.

Finally, to validate the observed association between copy number of α-tryptase–encoding sequence in *TPSAB1* and basal serum tryptase levels, and to explore the effect of this genetic finding on clinical phenotypes in an unselected population, we interrogated the National Human Genome Research Institute (NHGRI) ClinSeq cohort, a group of healthy unrelated volunteers. First, 125 deidentified serum samples were found to be partially enriched for duplication of α-tryptase–encoding sequence using a common haplotype and were screened for elevated basal serum tryptase levels (see Supplementary Fig. 1b for details of enrichment). Sixteen of the individuals with a concentration above 8 ng/ml ($n = 25$) were genotyped by the bioinformatic algorithm (9 individuals were excluded because of a lack of genomic sequence coverage) and subsequently by our ddPCR assay. Attempts were then made to contact all available individuals for phone interview; interviewers were blinded to tryptase levels and genotype (see Supplementary Table 2 for demographics). Single-allele duplications of α-tryptase–encoding sequence were identified in nine of these individuals, fully accounting for all individuals with 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 (Supplementary Table 3), and duplication of α-tryptase–encoding sequence was significantly associated with cutaneous flushing, itching, or hives ($P = 0.014$), systemic venom reactions ($P = 0.047$), IBS (defined by Rome III criteria; $P = 0.042$), retained primary dentition ($P = 0.020$), and elevated autonomic symptom scores (defined by COMPASS 31; $P = 0.038$) (Table 2). Family histories suggestive of affected family members were present for four of the nine, and elevated basal serum tryptase levels could be confirmed in first-degree relatives for two of the three families for which these measures were available.

The overall sensitivity of the ddPCR *TPSAB1* genotyping assay in detecting individuals with elevated basal serum tryptase levels was 100% (95% confidence interval (CI) = 95.1–100%), and the specificity was 90.0% (95% CI = 85.1–93.7%).

Consistent with previous studies, our data indicate that elevated basal serum tryptase concentration is a relatively common biochemical trait. We have found that this phenotype is most frequently inherited in an autosomal dominant manner and that, when this occurs, it is exclusively associated with increased copy number on a single allele of α-tryptase–encoding sequence in the *TPSAB1* gene, a genetic trait we have termed hereditary α-tryptasemia. In turn, elevated basal serum tryptase concentration is associated with increased prevalence of multiple predominantly functional and clinical phenotypes, including recurrent cutaneous symptoms, symptoms of autonomic instability, and functional gastrointestinal disorders, as well as systemic venom reactions and connective tissue abnormalities. The families studied in our initial cohort likely represent the most severe phenotypes among individuals affected with hereditary α-tryptasemia, owing in part to the lack of detection of triplication of α-

tryptase–encoding sequence in unselected populations, which we have tentatively designated as hereditary α-tryptasemia syndrome.

In vitro experiments suggest that elevated *TPSAB1* transcript levels lead to increased translation and constitutive secretion of α-protryptase, 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 *TPSAB1* locus is expressed, as has commonly been shown to occur for other genes[17]. The apparent gene-dose effect manifested as total basal serum tryptase levels seems to support this assertion. However, altered epigenetic regulation of the locus when additional copies of α-tryptase–encoding sequence are present may serve as a contributing factor.

The genetics of the human tryptase locus are complex. This locus sits within a gene-rich region at 16p13.3 that 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, *TPSAB1* and *TPSB2*, encode the four major isoforms (βI, βII, βIII, and α (αI)) of what is believed to be biologically relevant soluble tryptase; the α-tryptase isoform is only reported as being encoded at the *TPSAB1* locus. The high degree of identity between sequences encoding the α-tryptase and β-tryptase isoforms, and the presence of multiple paralogs in a single locus, makes detection of copy number variation difficult, likely precluding genome-wide association studies or quantitative arrays from detecting *TPSAB1* copy number variation. Our ddPCR assay provides indirect evidence that gene duplications are occurring within the locus; duplicated or triplicated α-tryptase–encoding sequence in *TPSAB1* did not randomly assort into droplets without restriction digestion, indicating that multiple copies were present within fragments of genomic DNA formed during extraction (maximum fragment size is approximately 50 kb) and that the duplicate copies are therefore 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 α-tryptasemia syndrome includes symptoms that may be associated clinically with mast cell mediator release and, in the context of elevated basal serum tryptase levels, can trigger an extensive work-up for clonal mast cell disease, including bone marrow biopsy. Because elevated tryptase levels are seen in a relatively large proportion 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 levels might contribute to the associated multisystem disorder we observed remains unclear. On the basis of 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, coinheritance of a second functional genetic variant contributing to the complex clinical phenotype cannot be ruled out. Although further work is required to determine the relationship between elevated tryptase levels and associated phenotypes, α-tryptase remains an attractive future therapeutic candidate, as a substantial proportion of the general population (>25%) is deficient in α-tryptase without known untoward effects.

METHODS

Methods and any associated references are available in the online version of the paper.

ONLINE METHODS

Subjects

α-Tryptasemia cohort—Informed consent was provided by all patients and their relatives on NIH IRB-approved research protocols designed to study mastocytosis (NCT00044122 and NCT00001756) and/or atopy (NCT01164241, NCT00852943, and 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 the 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 Supplementary Table 1). When patients were unavailable for a direct encounter or if the patients were evaluated before 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. For definitions and criteria for reported symptoms and diagnoses pertaining to all three cohorts, see the Supplementary Note. Two validated questionnaires, the Rome III questionnaire to interrogate IBS[21] and the COMPASS 31 questionnaire to interrogate dysautonomia[22], were also administered to a majority of individuals in 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 (Supplementary Fig. 1c) 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 Supplementary Table 2), 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 Note). Histories focused on symptoms and conditions we identified in association with inherited elevation of tryptase levels[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 levels were selected and provided informed consent on NIH IRB-approved protocols. They were recruited to act as experimental controls (NCT00806364).

Genetic sequencing and analysis

For the α-tryptasemia cohort, exome sequencing was performed on eight families using TruSeq (Illumina) capture kits and a custom analysis pipeline as described[25]. Genome sequencing was performed as described[26] on nine families (five of which had previously undergone exome sequencing) using the HiSeq platform (Illumina) with the Burrows–Wheeler aligner, and Picard was used for basic alignment and sequence quality control. The same capture kits and strategies were employed for the genome sequencing performed in the NIAMS and NIAID clinical genomics cohorts. For the ClinSeq cohort, exome sequencing was performed as described[27].

Linkage analysis

The GATK UnifiedGenotyper (with parameters -stand_call_conf 5.0, -stand_emit_conf 5.0, -dcov 500) and SAMtools were used to identify single-nucleotide variants (SNVs) and indels, and GATK VariantsToBinaryPed (with parameter -minGenotypeQuality 10) was used to produce binary pedigrees from variant call format (VCF) files. PLINK was then used to convert the binary pedigree files to LINKAGE format files. The PEDSTATS 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.Asp816Val) 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 (Thermo Fisher) for total protein (precursor and mature forms of α- and β-tryptase) and an ELISA for mature α- and β-tryptase levels, in a CLIA-approved laboratory (L.B.S.). The lower limit 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 (ref. 31).

Tryptase genotyping

A unique reference consensus sequence for the tryptase locus was generated using genome sequencing data. A computer algorithm was then created to extract all reads originally mapped to the ~50-kb region containing the locus. These reads were then remapped to the

deduced short consensus region (see the Supplementary Note for a complete description) to determine specific tryptase gene sequences and their relative abundance. Initial tryptase genotyping used a validated modified semiquantitative Southern blot technique as described[32].

To directly quantify allelic α- and β-tryptase copy number, a ddPCR assay was developed using custom primers and probes for α- and β-tryptase based on published sequences[20,33,34] and consensus sequences derived *in silico* (Supplementary Fig. 3a,b and Supplementary Table 4); the probes did not hybridize to γ- or δ-tryptase. 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), allowing for accurate detection of multiple *TPSAB1* copies on a single allele (Supplementary Fig. 5a–d and Supplementary Note).

Code availability

The code generated for *in silico* tryptase genotyping is provided in the Supplementary Note.

Referenced accession codes

GRCh37/hg19 NCBI assembly accession, GCF_ 000001405.13.

Mast cell culture and analysis

$CD34^+$ cells were isolated from 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 antibody to tryptase (clone AA1) conjugated to phycoerythrin (Novus Biologicals). Total mRNA was extracted from mast cells and real-time PCR was performed as described[25] to quantify 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 δ- or γ-tryptase. Mast cell degranulation was assessed by measuring β-hexosaminidase release as described[36], and whole-cell lysates from mast cells were obtained as described[37]. To characterize the size and quantity of tryptase molecules from these cultures, total tryptase levels in lysates and culture supernatants were determined by immunoblotting, using rabbit antibody to human tryptase at a 1:1,000 dilution (clone G3, EMD Millipore).

Statistical analyses

Mann–Whitney, Wilcoxon matched-pairs, or Fisher's exact tests were employed to test the significance of associations as indicated; in all cases, two-tailed tests were used. In all populations examined, basal serum tryptase levels did not follow a normal distribution (D'Agostino–Pearson test): unaffected individuals ($n = 196$, $K^2 = 16.84$, $P = 0.0002$), individuals with α-tryptasemia ($n = 113$, $K^2 = 18.38$, $P = 0.0001$), αα tryptase allele carriers ($n = 91$, $K^2 = 15.85$, $P = 0.0004$), and ααα tryptase allele carriers ($n = 17$, $K^2 = 8.28$, $P = 0.0159$). A two-tailed F test was used to determine whether the observed variances in populations were different. The standard deviation of basal serum tryptase values among individuals with hereditary α-tryptasemia ($n = 113$), regardless of genotype, was significantly different than the standard deviation observed in unaffected individuals ($n =$

196) (F value = 11, DFn = 112, Dfd = 195, $P < 0.0001$), indicating that this population distribution is distinct from that of unaffected individuals. Among affected individuals, the standard deviation of tryptase values was not significantly different when comparing between αα ($n = 91$) and ααα ($n = 17$) individuals (F value = 1.144, DFn = 16, Dfd = 90, $P = 0.65$).

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 α-tryptasemia and NIAID clinical genomics cohorts, in whom tryptase levels were within the normal range and in whom α-tryptase gene dose was confirmed both by bioinformatic algorithm and ddPCR assay to be ≤1 copy per allele. The upper 95% confidence interval of the median was defined as the normal cutoff, with individuals scoring higher than this number considered to be outliers and symptomatic. Fisher's exact test was then applied to test statistical significance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Fellinger C, et al. Clinical characteristics and risk profile of patients with elevated baseline serum tryptase. Allergol Immunopathol (Madr). 2014; 42:544–552. [PubMed: 25224360]
2. Gonzalez-Quintela A, et al. Factors influencing serum total tryptase concentrations in a general adult population. Clin Chem Lab Med. 2010; 48:701–706. [PubMed: 20178448]
3. Henningsen P, Zimmermann T, Sattel H. Medically unexplained physical symptoms, anxiety, and depression: a meta-analytic review. Psychosom Med. 2003; 65:528–533. [PubMed: 12883101]
4. Cardet JC, Castells MC, Hamilton MJ. Immunology and clinical manifestations of non-clonal mast cell activation syndrome. Curr Allergy Asthma Rep. 2013; 13:10–18. [PubMed: 23212667]
5. Li H, et al. Autoimmune basis for postural tachycardia syndrome. J Am Heart Assoc. 2014; 3:e000755. [PubMed: 24572257]
6. Zarate N, et al. Unexplained gastrointestinal symptoms and joint hypermobility: is connective tissue the missing link? Neurogastroenterol Motil. 2010; 22:252–e78. [PubMed: 19840271]
7. Lyons JJ, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. J Allergy Clin Immunol. 2014; 133:1471–1474. [PubMed: 24472624]

8. Ross J, Grahame R. Joint hypermobility syndrome. Br Med J. 2011; 342:c7167. [PubMed: 21252103]
9. Buskila D, Sarzi-Puttini P. Biology and therapy of fibromyalgia. Genetic aspects of fibromyalgia syndrome. Arthritis Res Ther. 2006; 8:218. [PubMed: 16887010]
10. Castori M. Ehlers–Danlos syndrome, hypermobility type: an underdiagnosed hereditary connective tissue disorder with mucocutaneous, articular, and systemic manifestations. ISRN Dermatol. 2012; 2012:751768. [PubMed: 23227356]
11. Sabato V, et al. Familial hypertryptasemia with associated mast cell activation syndrome. J Allergy Clin Immunol. 2014; 134:1448–1450.e3. [PubMed: 25086867]
12. Saito YA, Schoenfeld P, Locke GR III. The epidemiology of irritable bowel syndrome in North America: a systematic review. Am J Gastroenterol. 2002; 97:1910–1915. [PubMed: 12190153]
13. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2014; 63:871–880. [PubMed: 23853213]
14. Remvig L, Jensen DV, Ward RC. Epidemiology of general joint hypermobility and basis for the proposed criteria for benign joint hypermobility syndrome: review of the literature. J Rheumatol. 2007; 34:804–809. [PubMed: 17407233]
15. Ruëff F, 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; 124:1047–1054. [PubMed: 19895993]
16. Sturm GJ, et al. Sensitization to Hymenoptera venoms is common, but systemic sting reactions are rare. J Allergy Clin Immunol. 2014; 133:1635–43 e1. [PubMed: 24365141]
17. Savova V, et al. Genes with monoallelic expression contribute disproportionately to genetic diversity in humans. Nat Genet. 2016; 48:231–237. [PubMed: 26808112]
18. Bailey JA, et al. Recent segmental duplications in the human genome. Science. 2002; 297:1003–1007. [PubMed: 12169732]
19. Chiang PW, et al. Somatic and germ-line mosaicism in Rubinstein–Taybi syndrome. Am J Med Genet A. 2009; 149A:1463–1467. [PubMed: 19533794]
20. Trivedi NN, Tong Q, Raman K, Bhagwandin VJ, Caughey GH. Mast cell α and β tryptases changed rapidly during primate speciation and evolved from γ-like transmembrane peptidases in ancestral vertebrates. J Immunol. 2007; 179:6072–6079. [PubMed: 17947681]
21. Drossman DA, Dumitrascu DL. Rome III: new standard for functional gastrointestinal disorders. J Gastrointestin Liver Dis. 2006; 15:237–241. [PubMed: 17013448]
22. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: a refined and abbreviated Composite Autonomic Symptom Score. Mayo Clin Proc. 2012; 87:1196–1201. [PubMed: 23218087]
23. Biesecker LG, et al. The ClinSeq Project: piloting large-scale genome sequencing for research in genomic medicine. Genome Res. 2009; 19:1665–1674. [PubMed: 19602640]
24. Alfter K, et al. New aspects of liver abnormalities as part of the systemic mast cell activation syndrome. Liver Int. 2009; 29:181–186. [PubMed: 18662284]
25. Zhang Y, et al. Autosomal recessive phosphoglucomutase 3 (GM3) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment. J Allergy Clin Immunol. 2014; 133:1400–1409. 1409.e1–1409.e5. [PubMed: 24589341]
26. Brennan CW, et al. The somatic genomic landscape of glioblastoma. Cell. 2013; 155:462–477. [PubMed: 24120142]
27. Johnston JJ, et al. Individualized iterative phenotyping for genome-wide analysis of loss-of-function mutations. Am J Hum Genet. 2015; 96:913–925. [PubMed: 26046366]
28. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet. 2002; 30:97–101. [PubMed: 11731797]
29. Kristensen T, Vestergaard H, Bindslev-Jensen C, Møller MB, Broesby-Olsen S. Sensitive *KIT* D816V mutation analysis of blood as a diagnostic test in mastocytosis. Am J Hematol. 2014; 89:493–498. [PubMed: 24443360]

30. Ferrer M, et al. Serum total tryptase levels are increased in patients with active chronic urticaria. Clin Exp Allergy. 2010; 40:1760–1766. [PubMed: 20718781]

31. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin North Am. 2006; 26:451–463. [PubMed: 16931288]

32. Le QT, Lotfi-Emran S, Min HK, Schwartz LB. A simple, sensitive and safe method to determine the human α/β-tryptase genotype. PLoS One. 2014; 9:e114944. [PubMed: 25545679]

33. Miller JS, Moxley G, Schwartz LB. Cloning and characterization of a second complementary DNA for human tryptase. J Clin Invest. 1990; 86:864–870. [PubMed: 2203827]

34. Trivedi NN, Tamraz B, Chu C, Kwok PY, Caughey GH. Human subjects are protected from mast cell tryptase deficiency despite frequent inheritance of loss-of-function mutations. J Allergy Clin Immunol. 2009; 124:1099–105.e1. 4. [PubMed: 19748655]

35. Kirshenbaum AS, et al. Demonstration that human mast cells arise from a progenitor cell population that is $CD34^+$, c-kit^+, and expresses aminopeptidase N (CD13). Blood. 1999; 94:2333–2342. [PubMed: 10498605]

36. Schwartz LB, Lewis RA, Seldin D, Austen KF. Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. J Immunol. 1981; 126:1290–1294. [PubMed: 7009736]

37. Tkaczyk C, Metcalfe DD, Gilfillan AM. Determination of protein phosphorylation in FcεRI-activated human mast cells by immunoblot analysis requires protein extraction under denaturing conditions. J Immunol Methods. 2002; 268:239–243. [PubMed: 12215392]

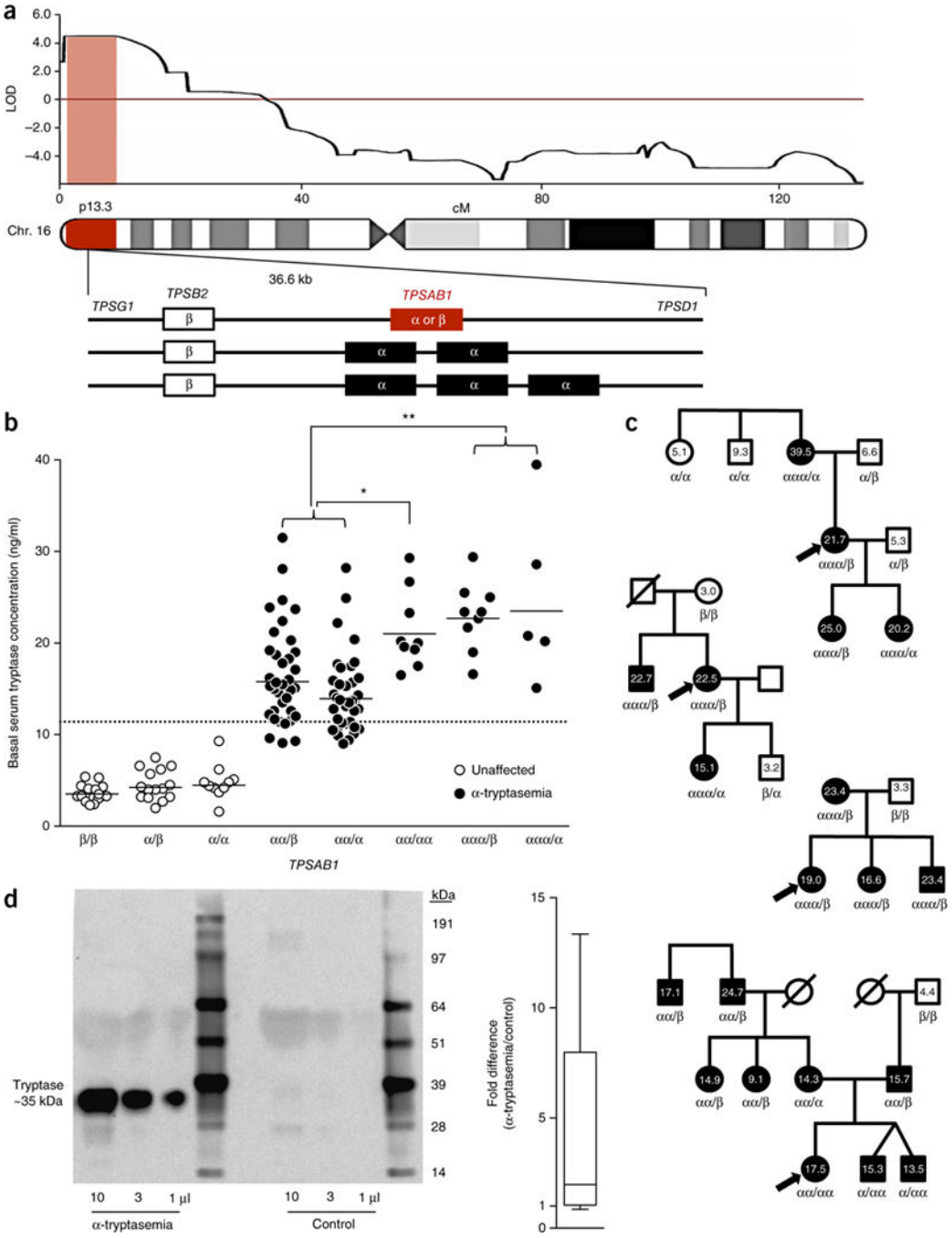

Figure 1.

Inherited duplications and triplications of the α-tryptase–encoding sequence in the *TPSAB1* gene are associated with elevated basal serum tryptase levels and enhanced mast cell secretion of tryptase. (**a**) Top, linkage analysis of exome sequence data from eight families showing the single linkage region on chromosome 16p13.3 (556,104–5,653,182, GRCh37/hg19) (LOD = 4.46). Middle, the 36.6-kb tryptase locus present within this region contains four tryptase-encoding genes: *TPSG1*, *TPSB2*, *TPSAB1*, and *TPSD1*. *TPSB2* and *TPSAB1* can both encode β-tryptase, but only *TPSAB1* encodes α-tryptase. Individuals with inherited

elevated basal serum tryptase levels had duplication or triplication on single alleles of the α-tryptase–encoding sequence in *TPSAB1*; schematics of how this genetic change may manifest are shown at the bottom. (**b**) Basal serum tryptase levels and corresponding *TPSAB1* tryptase genotypes (where α represents one copy of α-tryptase and β represents one copy of β-tryptase) among families ($n = 35$) identified with hereditary α-tryptasemia syndrome. The upper limit of normal, as defined by multiple clinical laboratories (>11.4 ng/ml), is indicated by the dashed line. Data are shown as geometric means; $*P = 0.0012$, $**P < 0.0001$, Mann–Whitney test. (**c**) Sample pedigrees from four families with hereditary α-tryptasemia syndrome; numbers indicate basal serum tryptase concentration (in ng/ml). (**d**) Left, immunoblot of culture medium (10, 3, or 1 μl) from one of five paired mast cell cultures derived from the peripheral CD34+ cells of individuals with single-allele duplication or triplication of α-tryptase–encoding sequence in *TPSAB1* (α-tryptasemia) or controls. Right, fold increase in the total tryptase content of α-tryptasemic supernatants ($n = 5$) shown relative to supernatants from paired controls ($n = 5$) for five independent mast cell culture experiments. Each box shows the median, 25th percentile, and 75th percentile, and whiskers extend to minimum and maximum values.

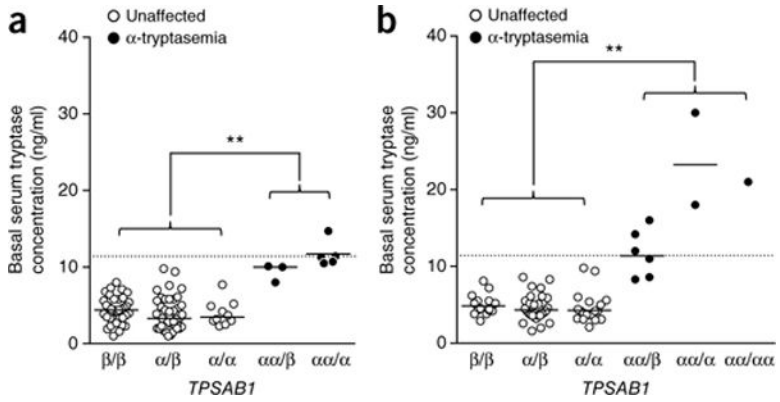

Figure 2.

Single-allele duplication of α-tryptase–encoding sequence in *TPSAB1* is associated with elevated basal serum tryptase levels in unselected populations. (**a**) Basal serum tryptase levels and corresponding *TPSAB1* genotypes among individuals who underwent exome or genome sequencing for immune phenotypes unrelated to mast cell activation. (**b**) Basal serum tryptase levels and corresponding *TPSAB1* genotypes among studied individuals from the ClinSeq cohort. The upper limit of normal (>11.4 ng/ml) is indicated by the dashed line in **a** and **b**. Data are shown as geometric means; $^{**}P < 0.0001$, Mann–Whitney test.

Table 1

Clinical features and gene-dose effects in hereditary α-tryptasemia syndrome

	Hereditary α-tryptasemia syndrome (αα)		*TPSAB1* duplication (ααα)		*TPSAB1* triplication (αααα)		*P* value[a]
Serum tryptase, ng/ml Median Interquartile range	**15.9 12.6–20.7**		**14.3 11.6–17.8**		**23.4 19.8–26.4**		**<0.0001**
Manifestation	***n***	**%**	***n***	**%**	***n***	**%**	***P* value[a]**
Systemic venom reaction[b]	15/96	16	11/73	15	4/15	27	NS
Flushing/pruritus	49/96	51	**33/73**	**45**	**12/15**	**80**	**0.022**
IBS (Rome III)	34/70	49	26/53	49	7/12	58	NS
Chronic gastroesophageal reflux symptoms	62/96	65	**42/73**	**49**	**15/15**	**100**	**0.001**
Congenital skeletal abnormality[c]	25/96	26	**14/73**	**19**	**8/15**	**53**	**0.009**
Retained primary dentition	20/96	21	**12/73**	**16**	**7/15**	**47**	**0.016**
Hypermobility (Beighton score ≥4)[d]	14/50	28	11/30	37	3/13	23	NS
COMPASS 31[e]	33/70	47	26/57	46	5/11	45	NS
Positive tilt-table test	11	≥11	6	≥8	4	≥26	ND
Arthralgia	43/96	45	**31/73**	**42**	**11/15**	**73**	**0.045**
Body pain/headache	45/96	47	**32/73**	**44**	**11/15**	**73**	**0.049**
Sleep disruption	37/96	39	**23/73**	**32**	**11/15**	**73**	**0.004**

IBS, irritable bowel syndrome; ND, not able to determine. Statistically significant differences are marked in bold. NS, not significant.

[a] Comparison of duplication (αα) and triplication (ααα) carriers at *TPSAB1*.

[b] Systemic immediate hypersensitivity reaction consistent with IgE-mediated response to stinging insects, as described in the Supplementary Note.

[c] Presence of a congenital skeletal malformation (the complete list of malformations identified is provided in the Supplementary Note) or diagnosis of EDS.

[d] Only individuals over 12 years of age and who could be directly visualized were assessed and reported.

[e] Number of individuals with a composite score above the upper 95% confidence interval of the median established in a healthy control cohort without increased copy number at *TPSAB1*.

Table 2

Self-reported clinical features among ClinSeq participants with and without identified *TPSAB1* duplication on a single allele

Manifestation	*TPSAB1* duplication (αα)		WT *TPSAB1*		OR		RR		
	n	%	*n*	%	Value	Range	Value	Range	*P* value
Systemic venom reaction[a]	**2/9**	**22**	**2/82**	**2**	**11.4**	**1.4–94.0**	**9.1**	**1.5–57.1**	**0.047**
Flushing/pruritus	**5/9**	**55**	**13/82**	**16**	**6.6**	**1.6–28.1**	**3.5**	**1.6–7.6**	**0.014**
IBS (Rome III)	**3/9**	**33**	**6/82**	**7**	**6.3**	**1.3–31.9**	**4.6**	**1.4–15.2**	**0.042**
Chronic gastroesophageal reflux symptoms	7/9	77	39/82	48	3.9	0.8–19.7	1.6	1.1–2.5	0.158
Congenital skeletal abnormality[b]	1/9	11	3/82	4	3.3	0.3–35.5	3.0	0.4–26.2	0.346
Retained primary dentition	**3/9**	**33**	**4/82**	**5**	**9.8**	**1.8–54.0**	**6.8**	**1.8–25.8**	**0.020**
COMPASS 31[c]	**4/9**	**44**	**11/82**	**13**	**5.2**	**1.2–22.3**	**3.3**	**1.3–8.3**	**0.038**
Arthralgia	4/9	44	25/82	30	1.8	0.5–7.4	1.5	0.6–3.2	0.459
Body pain/headache	3/9	33	12/82	15	2.9	0.6–13.3	2.3	0.8–6.6	0.165
Sleep disruption	2/9	22	21/82	26	0.8	0.2–4.3	0.9	0.2–3.1	1.000

IBS, irritable bowel syndrome; OR, odds ratio; RR, relative risk. Statistically significant differences are marked in bold.

[a] Systemic immediate hypersensitivity reaction consistent with IgE-mediated response to stinging insects, as described in the Supplementary Note.

[b] Spina bifida occulta, congenital absence of spinous process, pectus excavatum, and tibial torsion.

[c] Number of individuals with a composite score above the upper 95% confidence interval of the median established in a healthy control cohort without increased copy number of TPSAB1.