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

Running Title: Hereditary α tryptasemia

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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

72 reasons, identifying genetic bases to characterize sub-groups of individuals with these disorders
73 may substantially advance the field, but employing classic phenotypic ascertainment approaches
74 in these individuals is extremely challenging.

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

85 We approached this challenging problem by first mapping and identifying the genomic
86 lesion associated with elevated basal serum tryptase and characterizing symptoms in affected
87 families (Fig. S1A). A total of 96 subjects from 35 families were identified with a syndrome of
88 elevated basal serum tryptase and complex clinical features following an autosomal dominant
89 pattern of inheritance without evidence of mastocytosis (see Table S1 for demographics).
90 Affected individuals had multiple comorbid symptoms including those often considered
91 "functional" in nature due to the lack of pathological findings. Gastrointestinal dysmotility was
92 common, most often manifest as irritable bowel syndrome (IBS) defined by Rome III criteria
93 (49%) or symptoms of chronic gastroesophageal reflux (65%), both being present approximately
94 3-5 fold over the general population (12, 13). Connective tissue abnormalities were also

95 common; the overall prevalence of joint hypermobility (Beighton ≥4, ages 12-76 years) was 28%
96 - approximately 2 times the general population prevalence (14) - while congenital skeletal
97 abnormalities (26%) and retained primary dentition (21%) were also frequently identified. These
98 findings were associated with chronic arthralgia (45%) and headache or body pain (47%).
99 Complaints suggestive of autonomic dysfunction, including postural orthostatic tachycardia
100 syndrome (POTS) were common. Forty-six percent of individuals had elevated composite
101 autonomic symptom scores by validated measure (COMPASS 31), in whom 11 (34% of those
102 with elevated scores) were validated by tilt-table testing (Fig. S2A). Additional symptoms
103 included recurrent cutaneous flushing and pruritus (51%), which in some cases associated with
104 urticaria, concomitant with significant complaints of sleep disruption (39%). Systemic reactions
105 to stinging insects (e.g. hymenoptera), an occurrence known to be associated with elevated basal
106 serum tryptase (15), were increased 2-3 fold over the general population (16%) (16) (Table 1).
107 Exome and genome sequencing (GS) of the first 12 families yielded no shared rare or
108 common variants. However, linkage analysis identified a single 5.1 megabase peak on
109 chromosome 16p13.3 (LOD = 4.46), a region containing the human tryptase locus, composed of
110 four paralogous genes (*TPSG1, TPSB2*, *TPSAB1*, and *TPSD1*) (Fig. 1A). The primary secreted
111 tryptase gene products at this locus include β tryptase encoded at *TPSAB1* and *TPSB2*, and α
112 tryptase, resulting from a series of variants within the *TPSAB1* gene. A modified southern blot
113 assay performed on 15 families (55 affected; 13 unaffected) identified elevated α/β tryptase
114 ratios among affected family members, and when applied to pedigrees suggested multiple α
115 copies were inherited together (Fig. S2B, C). Inspection of GS reads permitted *in silico*
116 construction of a consensus reference sequence that was used to calculate α copy number and
117 provided sequence to design a novel digital droplet PCR (ddPCR) assay to specifically target α

118 tryptase (Fig. S3A, B; S4). ddPCR analysis of all 35 families (96 affected; 41 unaffected)
119 confirmed increased α tryptase copy number inherited on one or both alleles in all affected
120 individuals. All individuals who inherited a single α tryptase copy on both alleles (thus also
121 having a 2α genotype at *TPSAB1*) had normal basal serum tryptase levels (Fig. 1B, C; S5A-D).
122 Having an α duplication on both alleles, or an α triplication on a single allele, was associated
123 with significantly higher tryptase levels compared to having an α duplication on one allele.
124 Furthermore, having an α triplication on one allele was associated with greater prevalence of
125 associated clinical phenotypes than having an α duplication on one allele, demonstrating a gene-
126 dose correlation (Table 1).

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

136 To begin to determine whether extra α tryptase genes on a single allele might commonly
137 be associated with basal serum tryptase elevations in the general population, we next applied our
138 bioinformatic strategy to a large cohort of patients and healthy family members from NIAMS
139 and NIAID programs in clinical genomics on whom GS was performed for reasons unrelated to
140 mast cells or tryptase. This retrospective analysis was limited to those in whom coverage was

141 sufficient to call α tryptase copy number, and from whom sera were available to measure
142 tryptase levels (Fig. S1B). ddPCR was performed on all individuals (n=17) with basal serum
143 tryptase >8 ng/mL or ≥2 α tryptase gene copies identified using our bioinformatic strategy. Out
144 of 98 individuals, eight (8.2%) were identified with α duplications on a single allele. This
145 accounted for all individuals with basal serum tryptase elevations (>11.4 ng/ml) in the cohort
146 (Fig. 2A). Moreover, a dominant inheritance pattern of elevated basal serum tryptase was
147 observed in both families for whom there were samples available.

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

164 31; P=0.038) (Table 2). Family histories suggestive of affected family members were present in
165 4/9, and elevated basal serum tryptase levels could be confirmed in first-degree relatives in two
166 out of three available families.

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

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

181 *In vitro* experiments suggest that elevated α tryptase transcripts lead to increased α pro-
182 tryptase translation and constitutive secretion, thereby accounting for the elevated basal serum
183 tryptase levels seen *in vivo*. This may occur by a stoichiometric phenomenon, particularly if
184 only a single allele of the locus is expressed as has been shown to commonly occur (17). The
185 apparent α tryptase gene dose-effect manifested as total basal serum tryptase levels seems to

186 support this assertion. However, altered epigenetic regulation of the locus when additional α
187 tryptase copies are present may serve as a contributing factor.

188 The genetics of the human tryptase locus are complex. It sits within a gene rich region at
189 16p13.3, which is a hotspot for genetic recombination (18, 19). It is hypothesized that the
190 multiple tryptase genetic loci in humans evolved through duplication and inversion of this locus
191 (20). Two adjacent genes, *TPSAB1* and *TPSB2*, encode the four major isoforms [βI, βII, βIII, and
192 α (αI)] of what is believed to be biologically relevant soluble tryptase; the α isoform is only
193 reported as being encoded at the *TPSAB1* locus. The high degree of sequence identity between α
194 and β, and the presence of multiple paralogues in a single locus, makes detection of copy number
195 variation difficult, likely precluding genome-wide association studies or quantitative arrays from
196 detecting α tryptase CNV. Our digital droplet PCR assay provides indirect evidence that the gene
197 duplications are occurring within the locus; duplicated or triplicated α tryptase did not randomly
198 assort into droplets without restriction digestion, indicating that multiple copies were present
199 within fragments of genomic DNA formed during extraction (maximum size is approximately 50
200 Kb) and therefore the duplicated copies are relatively tightly linked in the genome and may be
201 subject to the same enhancers and other control elements.

202 Part of the clinical presentation in hereditary α tryptasemia syndrome includes symptoms
203 that clinically may be associated with mast cell mediator release, and in the context of elevated
204 basal serum tryptase can trigger an extensive work-up for clonal mast cell disease, including
205 bone marrow biopsy. Because tryptase elevations are seen in a relatively large percentage of the
206 general population, the decision to proceed with such a work-up can be challenging. Performing
207 tryptase genotyping as part of this work-up may be warranted in light of our findings.

208 How elevated basal serum tryptase might contribute to the associated multisystem
209 disorder we observed remains unclear. Based upon clinical phenotypes including pain and
210 connective tissue abnormalities, a compelling case could be made for activation of protease
211 activated receptor 2 (PAR2) dependent pathways. However, co-inheritance of a second
212 functional genetic variant contributing to the complex clinical phenotype cannot be ruled out.
213 While further work is required to determine the relationship between tryptase elevations and
214 associated phenotypes, α tryptase remains an attractive future therapeutic candidate, since a
215 significant percentage of the general population (>25%) are α tryptase deficient without known
216 untoward effects.
217

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, 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.

241 ***NIAMS and NIAID clinical genomics cohort***

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

245

246 ***ClinSeq® cohort***

247 Individuals were chosen (Fig. S1C) from the ClinSeq® study (NCT00410241), a project
248 employing exome sequencing in a clinical research setting, to serve as an unselected study
249 cohort. The majority of participants were healthy adult volunteers (for demographics of those
250 included see Table S2), with approximately 25% having a personal history of coronary artery
251 disease. Participants were broadly consented to genome sequencing and the return of individual
252 sequencing results (23). Blinded phone interviews were conducted to identify clinical phenotypes
253 and reported physician diagnoses among the ClinSeq® participants comporting with our defined
254 criteria (Supplementary Appendix, Section A). Histories focused on symptoms and conditions
255 we identified in association with inherited tryptase elevations (7), as well as those queried in a
256 published questionnaire for the diagnosis of mast cell activation syndrome (MCAS) (24).
257 Standardized questionnaires to assess for IBS (Rome III) and autonomic dysfunction
258 (COMPASS 31) were also administered to this population.

259

260 ***Individual controls***

261 Volunteers, who did not have significant clinical allergic disease or connective tissue
262 abnormalities and did not have elevated basal serum tryptase, were selected and provided

263 informed consent on NIH IRB-approved protocols and were recruited to act as experimental
264 controls (NCT00806364).
265
266 **Genetic sequencing and analysis**
267 For the α tryptasemia cohort, exome sequencing (ES) was performed on eight families using
268 TruSeq (Illumina, San Diego, CA) capture kits and a custom analysis pipeline as described (25).
269 Genome sequencing (GS) was performed as described (26) on nine families (five of which
270 previously had ES) using the HiSeq platform (Illumina, San Diego, CA) with the Burrows-
271 Wheeler Aligner and Picard (http://broadinstitute.github.io/picard/) used for basic alignment and
272 sequence quality control. The same capture kits and strategies were employed for the GS
273 performed in the NIAMS and NIAID clinical genomics cohorts. For the ClinSeq® cohort, ES was
274 performed as described (27).
275
276 **Linkage analysis**
277 GATK Unified Genotyper (parameters: -stand_call_conf 5.0, -stand_emit_conf 5.0, -dcov 500)
278 and SAMtools were used to identify single nucleotide variants (SNVs) and insertions/deletions
279 (Indels), and GATK VariantsToBinaryPed (parameters: -minGenotypeQuality 10) was used to
280 produce binary pedigrees from variant call format (VCF) files. Plink
281 (http://pngu.mgh.harvard.edu/purcell/plink/) was then used to convert the binary pedigree files to
282 LINKAGE format files. PEDSTATS (ref. PEDSTATS: Descriptive statistics, graphics and
283 quality assessment for gene mapping data) module in MERLIN (28) was used to check pedigree
284 structure and MERLIN was used to perform parametric rare-dominant linkage analysis.
285

286 **Bone Marrow Biopsy and *KIT* gene analysis**

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

290

291 **Tryptase protein quantification**

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

300

301 **Tryptase genotyping**

302 A unique reference consensus sequence for the tryptase locus was generated using GS data. A
303 computer algorithm was then created to extract all reads originally mapped to the ~50 kilobase
304 region containing the locus. These reads were then re-mapped to the deduced short consensus
305 region (see Supplementary Appendix, Section B for complete description) to determine specific
306 tryptase gene sequences and their relative abundance. Initial tryptase genotyping used a
307 validated modified semi-quantitative Southern blot technique as described (32).

308 To directly quantitate allelic α and β tryptase copy number, a digital droplet PCR (ddPCR) assay
309 was developed using custom primers (5'-TCCTGACCTGGCACCTGC-3'; 5'-
310 GACTCTCAGGCTCACCTGCCA-3') and custom probes for α (5'-
311 CTGCAGCAAGCGGGTATCGTC-3') and β (5'-CTGCAGCGAGTGGGCATCGT-3')
312 tryptases based upon the published sequences (20, 33, 34) and consensus sequences derived *in*
313 *silico* (Fig. S3A, B); the probes did not hybridize to γ or δ tryptases. The assay was performed
314 on native or restriction endonuclease-treated genomic DNA using the PrimePCR ddPCR Copy
315 Number reference *AP3B1*, according to the manufacturer's specifications (Bio-Rad, Hercules,
316 CA), allowing for accurate detection of multiple tryptase copies on a single allele
317 (Supplementary Appendix, Section C; Fig. S5A-D).
318
319 **Code Availability**
320 The code generated for in silico tryptase genotyping is available in the Supplementary Appendix,
321 Section B.
322
323 **Mast cell culture and analysis**
324 $CD34^+$ cells were isolated from peripheral blood mononuclear cells (PBMCs) and cultured under
325 conditions as described to yield primary mast cells (35). Cells were washed, stained with
326 Live/Dead Fixable Aqua (Invitrogen), fixed with 4% paraformaldehyde, permeabilized with 5%
327 saponin and stained intracellularly with anti-tryptase phycoerythrin (PE) (Novus Biologicals,
328 Littleton, CO). Total mRNA was extracted from mast cells and real-time (RT)-PCR was
329 performed as described (25) to quantitate total tryptase mRNA expression using the tryptase
330 primer/probe set for *TPSB2* (Life Technologies) that captures all α and β isoforms from *TPSB2*

331 and *TPSAB1*, but not δ or γ tryptases. Mast cell degranulation was assessed by measuring β-
332 hexosaminidase release as described (36), and whole mast cell lysates were obtained as described
333 (37). In order to characterize the size and quantity of tryptase molecules from these cultures, total
334 tryptase in lysates and culture supernatants was determined by Western blotting, probed using
335 rabbit anti-human tryptase (clone G3) (EMD Millipore, Billerica, MA).
336

337 **Statistical Analyses**

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

348 To assess dysautonomia among individuals an expected scoring range was established.
349 To accomplish this, the COMPASS 31 questionnaire was administered to 35 healthy family
350 members from the α tryptasemia and NIAID clinical genomics cohorts, in whom tryptase levels
351 were within the normal range and in whom α tryptase gene dose was confirmed both by
352 bioinformatic algorithm and ddPCR assay to be ≤1 copy per allele. The upper 95% confidence
353 interval (CI) of the median was defined as the normal cut-off, with individuals scoring higher

354 than this number considered to be outliers and symptomatic. Fisher's exact test was then applied

355 to test statistical significance.

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381
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386

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483

484 **FIGURE LEGENDS**

485 **Figure 1. Inherited α tryptase gene duplications and triplications are associated with**
486 **elevated serum basal serum tryptase and enhanced mast cell tryptase secretion. (A)** Linkage
487 analysis performed on exome sequencing data from 8 families identified a single linkage region
488 on chromosome 16p13.3 (Chr16: 556,104 – 5,653,182; GRCh37/hg19) (LOD=4.46) (top). The
489 36.6 kilobase (Kb) tryptase locus is present within this region and contains four tryptase genes
490 *TPSG1*, *TPSB2*, *TPSAB1*, and *TPSD1* (middle). *TPSB2* and *TPSAB1* both can encode for β
491 tryptases, but α tryptase is only known to be present at *TPSAB1*. Patients with inherited basal
492 tryptase elevations were found to have duplications or triplications of α tryptase on single alleles
493 at *TPSAB1*; schematics of how this genetic change may manifest are shown (bottom). **(B)** Basal
494 serum tryptase levels and corresponding *TPSAB1* tryptase genotypes among families (n = 35)
495 identified with inherited basal serum tryptase elevation. Upper limit of normal as defined by
496 multiple clinical laboratories (>11.4 ng/mL) is indicated by the dashed line. Data shown with
497 geometric mean, *P=0.0012; **P<0.0001; Mann-Whitney test. **(C)** Sample pedigrees from four
498 families with hereditary α tryptasemia syndrome; numbers indicate basal serum tryptase levels
499 (ng/mL). **(D)** Mast cells were cultured from peripheral $CD34^+$ cells of individuals with single-
500 allele α tryptase duplications or triplications (α tryptasemia) or from peripheral $CD34^+$ cells of
501 paired controls, and Western blots of media containing spontaneously released tryptase (10 uL, 3
502 uL, or 1 uL) were performed. Western blot from one of five independent culture experiments
503 (left). Fold increase in total tryptase content of α tryptasemia supernatants relative to paired
504 controls (n = 5) from five combined experiments is shown (right); mean ± SEM.
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507 **Figure 2. Single allele α tryptase gene duplications are associated with elevated serum basal**
508 **serum tryptase in unselected populations.** **(A)** Basal serum tryptase levels and corresponding
509 *TPSAB1* tryptase genotypes among individuals undergoing exome or genome sequencing for
510 immune phenotypes unrelated to mast cell activation. **(B)** Basal serum tryptase levels and
511 corresponding *TPSAB1* tryptase gene expression among individuals genotyped from the
512 ClinSeq® cohort. Upper limit of normal (>11.4 ng/mL) is indicated by the dashed line (A and B).
513 Data shown with geometric mean, **P<0.0001; Mann-Whitney test.
514

515 **TABLES**

Table 1. Clinical features and gene-dose effects in hereditary α tryptasemia syndrome.

	Hereditary α tryptasemia syndrome	αα	ααα	P-value*
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 (%)	
Systemic venom reaction†	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§	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)‖	14/50 (28)	11/30 (37)	3/13 (23)	*ns*
COMPASS 31¶	33/70 (47)	26/57 (46)	5/11 (45)	*ns*
Positive Tilt-table test	11 (≥11)	6 (≥8)	4 (≥26)	NA
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; *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.

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Table 2. Self-reported clinical features among ClinSeq® participants with (αα) and without (WT) identified α tryptase duplications on a single allele.

Manifestation	αα N (%)	WT N (%)	OR	RR	P-value
Systemic venom reaction*	**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†	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§	**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; *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 ration; RR – relative risk.

520

Figure 1.

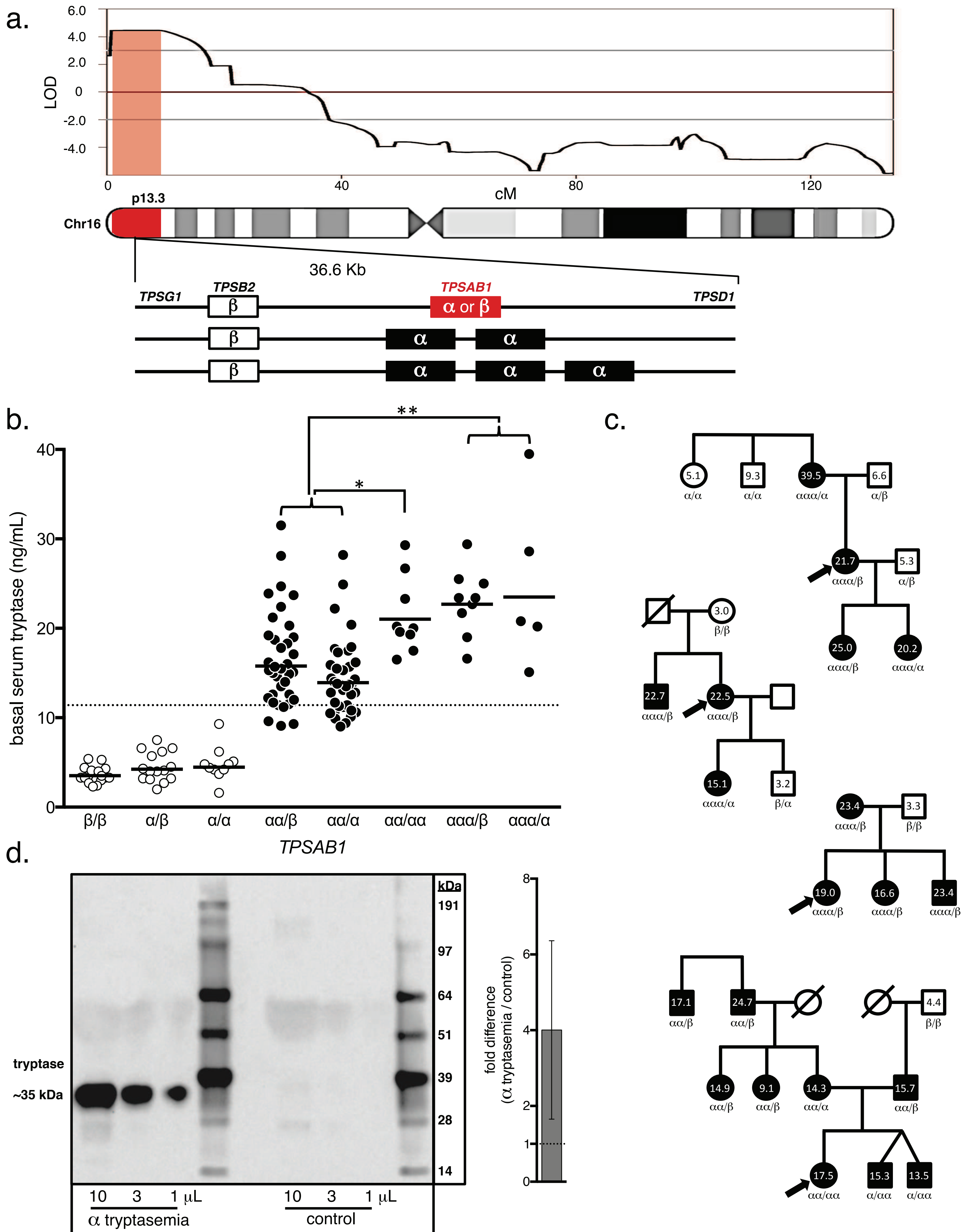
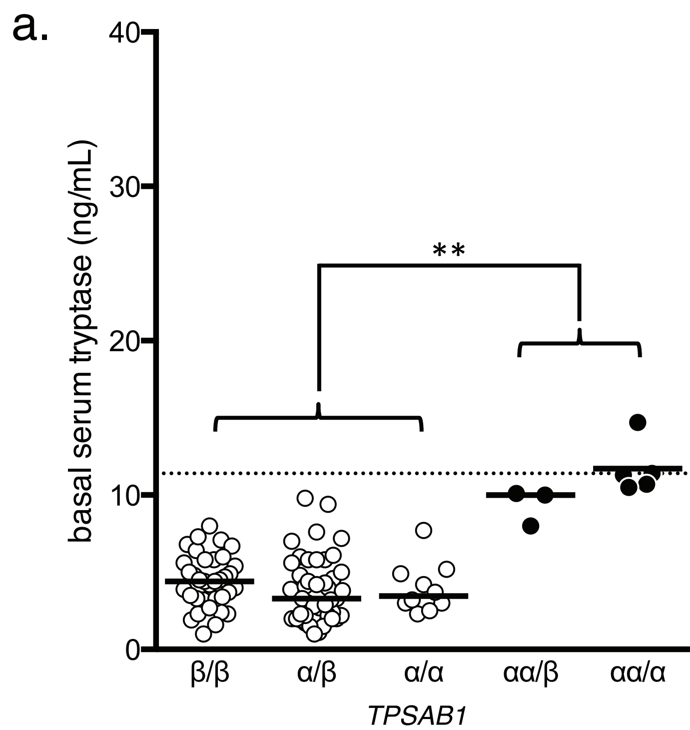


Figure 2.

a.



b.