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

Document Version
Accepted author manuscript

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1 **Title:** Elevated basal serum typtase identifies a multisystem disorder associated with increased α

2 tryptase copy number

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4 **Running Title:** Hereditary α tryptasemia

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49 **Elevated basal serum tryptase is present in 4-6% of the general population but the cause**

50 **and significance is unknown (1, 2). Previously we described a cohort with dominantly**

51 **inherited basal serum tryptase elevations associated with multisystem complaints including**

52 **cutaneous flushing and pruritus, dysautonomia, functional gastrointestinal symptoms,**

53 **chronic pain, and connective tissue abnormalities including joint hypermobility. Herein we**

54 **report identification of germline α tryptase gene duplications and triplications (at *TPSAB1*)**

55 **that segregate with inherited basal serum tryptase elevations identified in 35 families**

56 **presenting with associated multisystem complaints. Individuals harboring $\alpha\alpha\alpha$ alleles had**

57 **higher basal serum tryptase levels and were more symptomatic than those with $\alpha\alpha$ alleles,**

58 **suggesting a gene-dose effect. Further, we found in two additional cohorts (172**

59 **individuals) that elevated basal serum tryptase was exclusively associated with α tryptase**

60 **duplications and affected individuals reported symptom complexes seen in our initial**

61 **familial cohort. α Tryptase duplications therefore link tryptase elevations with IBS,**

62 **cutaneous complaints, connective tissue abnormalities, and dysautonomia.**

63

64 Medically unexplained symptoms and symptom-complexes can be vexing for clinicians and

65 patients alike. Manifestations such as cutaneous flushing, certain chronic pain disorders,

66 autonomic dysfunction, and gastrointestinal dysmotility have been attributed to a number of

67 disparate etiologies including neurologic, immunologic, physical, and psychological mechanisms

68 (3-6). Despite a lack of diagnostic clinical findings, many of these symptoms are comorbid and

69 often follow a dominant inheritance pattern in affected families (7-10). Furthermore, many of

70 these features have been reported in association with genetic disorders or joint hypermobility

71 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

218 **METHODS**

219 **Subjects**

220 *αtryptasemia cohort*

221 Informed consent was provided by all patients and their relatives on NIH IRB-approved research

222 protocols designed to study mastocytosis (NCT00044122, NCT00001756) and/or atopy

223 (NCT01164241, NCT00852943, NCT00557895). Over a 5-year period, family and personal

224 medical histories were obtained and physical examinations were performed on all individuals

225 able to travel to NIH. After recognizing that this familial presentation included a wide range of

226 symptoms, histories and exams were expanded throughout family accrual (for demographics of

227 this cohort see Table S1). When unavailable for a direct encounter, or if the patients were

228 evaluated prior to establishing the full phenotype, a comprehensive history and assessment was

229 performed using electronic media to characterize symptoms and reported physician diagnoses.

230 Blood samples were collected for genetic testing and tryptase measurement. Reported clinical

231 diagnoses were based upon patient report of physician diagnosis and/or a consistent clinical

232 history and physical exam, as well as review of outside records and test results where

233 available/applicable. Please see supplement for definitions and criteria for reported symptoms

234 and diagnoses pertaining to all three cohorts (Supplementary Appendix, Section A). Two

235 validated questionnaires, the Rome III questionnaire (21) to interrogate IBS, and the COMPASS

236 31 (22) questionnaire to interrogate dysautonomia, were also administered to a majority of the

237 cohort.

238

239

240

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

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

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.

370

371   **Author Contributions:** J.J.L. and J.D.M. designed the study. J.J.L., J.D.M, C.N., T.D., N.J.,

372   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.

373   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.,

374   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

375   J.D.M. performed and supported genomic sequencing and X.Y., J.D.H., C.H., Y.Z., and A.J.O.

376   performed the bioinformatics analyses. J.J.L., N.T., G.H.C., and L.B.S. designed and J.J.L.

377   performed the ddPCR assay. J.J.L., Q.L., Y.B., M.Z., Y.L., M.P.O., R.J.H., L.B.S., and J.D.M.

378   designed and performed the functional studies. K.L.L., C.H., and L.G.B. facilitated all ClinSeq[®]

379 related studies. J.J.L. and J.D.M. prepared the draft manuscript. All authors contributed to

380 discussion of the results and to manuscript preparation.

381

382 **Competing Financial Interests**: The NIH authors declare no conflicts of interest. LBS receives

383 royalties from VCU that are collected from ThermoFisher for the tryptase UniCAP assay and

384 receives consulting fees from Genentech, Inc. LGB is an uncompensated advisor to the Illumina

385 Corp, receives royalties from Genentech, Inc., and Amgen, and honoraria from Wiley-Blackwell.

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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506

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.
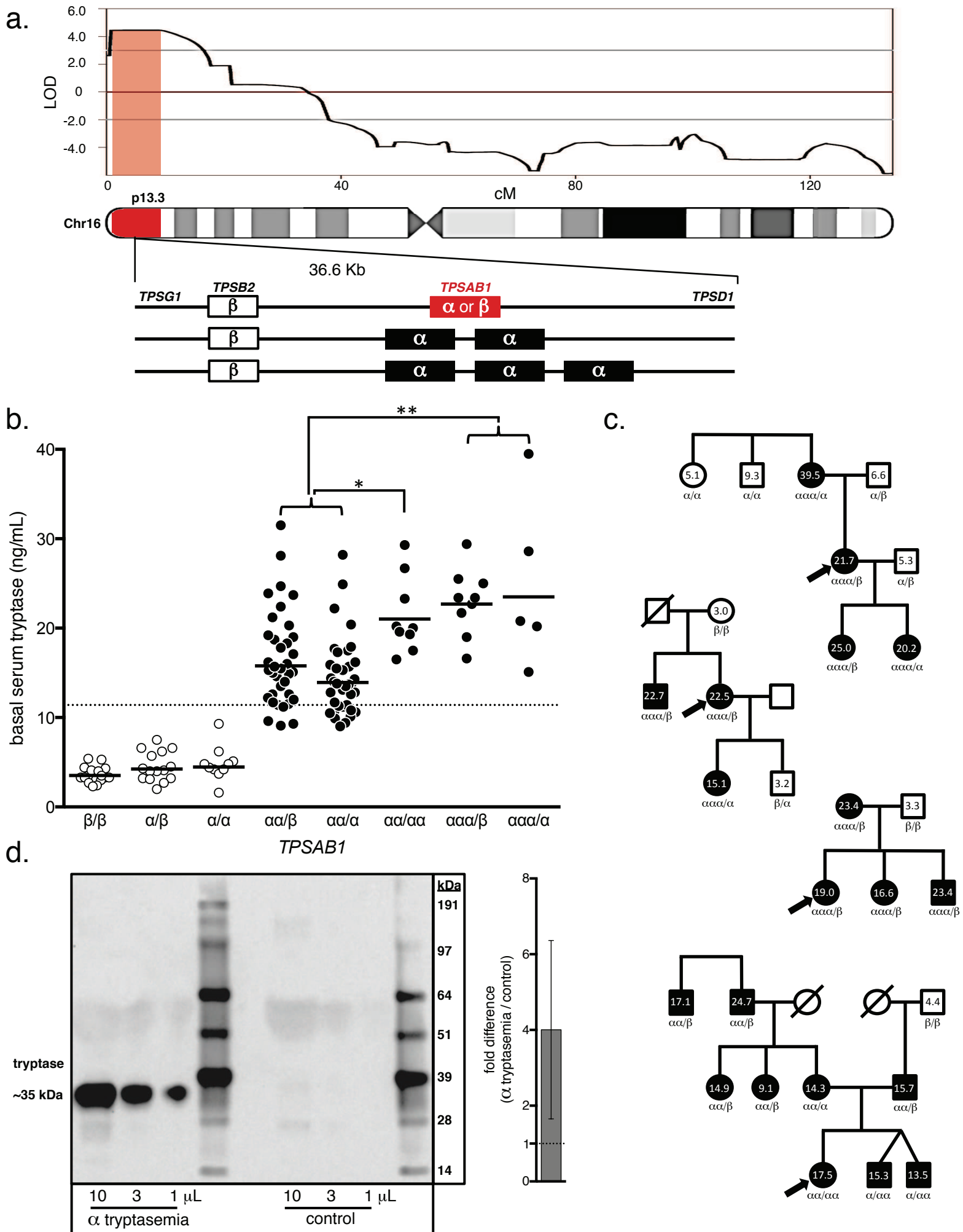
520

Figure 1.

a.

b.

c.

d.

tryptase
~35 kDa

10   3   1 µL   10   3   1 µL
α tryptasemia   control

Figure 2.

a.

b.