

# Human subjects are protected from mast cell tryptase deficiency despite frequent inheritance of loss-of-function mutations

Neil N. Trivedi, MD,<sup>a,b,f</sup> Bani Tamraz, PharmD,<sup>a,d</sup> Catherine Chu, BS,<sup>c,d</sup> Pui-Yan Kwok, MD, PhD,<sup>a,c,d</sup> and George H. Caughey, MD<sup>a,b,e,f</sup> San Francisco, Calif

**Background:** Mast cell tryptases have proposed roles in allergic inflammation and host defense against infection. Tryptase gene loci *TPSAB1* and *TPSB2* are known to be polymorphic, but the nature and extent of diversity at these loci have not been fully explored. **Objective:** We sought to compare functional and nonfunctional tryptase allele frequencies and establish haplotypes in human populations.

**Methods:** Tryptase allele frequencies were determined by means of direct sequencing in 270 individuals from HapMap populations of European, African, Chinese, and Japanese ancestry. Haplotypes were predicted, validated in parent-child trios, and compared between populations.

**Results:** We identify a new frame-shifted tryptase allele ( $\beta\text{III}^{\text{FS}}$ ) carried by 23% and 19% of individuals of European and African ancestry but 0% of Asian subjects. Homology models predict that  $\beta\text{III}^{\text{FS}}$  is functionless. Our genotyping assay shows that allele and haplotype distributions in each population are unique. Strong linkage disequilibrium between *TPSAB1* and *TPSB2* ( $r^2=0.83$ ,  $D'=0.85$ ) yields 2 major and 5 minor tryptase haplotypes.

**Conclusions:** Tryptase deficiency alleles ( $\alpha$  and the newly discovered  $\beta\text{III}^{\text{FS}}$ ) are common, causing the number of inherited active genes to range from a minimum of 2 to a maximum of 4, with major differences between populations in the proportion of individuals inheriting 2 versus 4 active alleles. African and Asian populations are especially enriched in genes encoding functional and nonfunctional tryptases, respectively. Strong linkage of *TPSAB1* and *TPSB2* and pairing of deficiency alleles with functional alleles in observed haplotypes protect human subjects from “knockout” genomes and indeed from inheritance of fewer than 2 active alleles. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Tryptase, mast cells, human genetics

## Abbreviations used

CEU: Centre d'Etude du Polymorphisme Humain study collection from Utah  
CHB: Han Chinese individuals in Beijing  
JPT: Japanese individuals in Tokyo  
SNP: Single nucleotide polymorphism  
YRI: Yoruba in Ibadan, Nigeria

Tryptases are a family of serine peptidases that are the major component of mast cell secretory granules.<sup>1-4</sup> They are stored as heparin-bound tetramers that resist inactivation by plasma inhibitors after secretion. As a group, tryptases are implicated in asthma and other inflammatory disorders. They might promote bronchoconstriction by cleaving bronchodilating peptides and enhancing airway smooth muscle contractility and might increase airway remodeling by stimulating growth of subepithelial fibroblasts and airway smooth muscle.<sup>5-9</sup> In isolated airways tryptases augment histamine-induced bronchoconstriction, and in asthmatic subjects tryptase inhibitors reduce late-phase antigen-induced bronchoconstriction.<sup>10-12</sup> In animal models of asthma, tryptase inhibitors block allergen-induced airway inflammation, hyperresponsiveness, and goblet cell hyperplasia.<sup>13-16</sup> Mice lacking tryptase genes are more likely to die from *Klebsiella pneumoniae* and have diminished eosinophil recruitment and helminth containment after *Trichinella spiralis* infection.<sup>17,18</sup> Tryptases might contribute to antibacterial defense by recruiting neutrophils to sites of infection.<sup>19,20</sup> Thus they are implicated in allergic inflammation and host defense.

Human soluble tryptases are produced by 3 closely spaced genes: *TPSD1*, *TPSAB1*, and *TPSB2* (Fig 1). *TPSD1* ( $\delta$ -tryptase) genes developed inactivating truncation mutations in the great ape lineage.<sup>21</sup> Thus enzymatically active soluble tryptases in human subjects are products of *TPSAB1* and *TPSB2*, which are polymorphic. These 2 loci produce 4 major tryptase isoforms:  $\alpha$ ,  $\beta\text{I}$ ,  $\beta\text{II}$ , and  $\beta\text{III}$ . Work from our laboratory suggests that  $\alpha$  and  $\beta\text{I}$  are restricted to *TPSAB1* and that  $\beta\text{II}$  and  $\beta\text{III}$  are restricted to *TPSB2*.<sup>22,23</sup>  $\alpha$ -Tryptases have minimal to no catalytic activity and are genetically absent in many human subjects.<sup>24-28</sup> In contrast,  $\beta\text{I}$  tryptase is an active peptidase that might recruit neutrophils.<sup>29</sup>  $\beta\text{II}$  tryptase substrate specificity and heparin-dependent activation are equivalent to those of  $\beta\text{I}$ .<sup>30,31</sup> Less is known of  $\beta\text{III}$  tryptases, although they are predicted to be active.<sup>32</sup> The present study evaluates ethnically diverse backgrounds to explore tryptase gene prevalence and variation. The results reveal greater

From <sup>a</sup>the Cardiovascular Research Institute, the Departments of <sup>b</sup>Medicine and <sup>c</sup>Dermatology, and <sup>d</sup>the Institute for Human Genetics, University of California at San Francisco; <sup>e</sup>the Veterans Health Research Institute; and <sup>f</sup>the Veterans Affairs Medical Center.

Supported by National Institutes of Health grant HL024136 and the Veterans Health Research Institute.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication June 1, 2009; revised July 13, 2009; accepted for publication July 16, 2009.

Reprint requests: George H. Caughey, MD, Veterans Affairs Medical Center 111D, 4150 Clement St, San Francisco, CA 94121. E-mail: George.Caughey@ucsf.edu.

0091-6749/\$00.00

© 2009 by Elsevier, Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.07.026

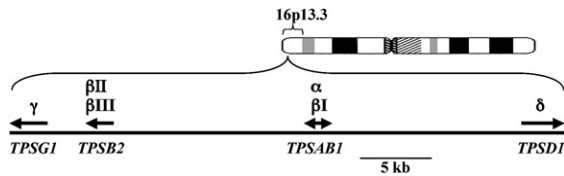


**FIG 1.** The tryptase gene cluster on chromosome 16p13.3. *TPSG1*, which encodes transmembrane tryptase  $\gamma$ , anchors the telomeric end. At adjacent *TPSB2*,  $\beta$ II and  $\beta$ III tryptase genes compete as alleles. Similarly,  $\alpha$  and  $\beta$ I genes compete as alleles at adjacent locus *TPSAB1*. *TPSD1* is the  $\delta$  locus, which encodes a truncated, largely inactive peptidase. Arrows depict transcriptional orientation.

diversity than previously described and suggest that linkage disequilibrium and haplotype variations are strong determinants of individual and population-specific differences in tryptase genotype.

## METHODS

### Sequencing of LAD2 and HMC-1 tryptase genes and transcripts

LAD2 and HMC-1 cells were obtained from Dr Dean Metcalfe and Dr Joseph Butterfield, respectively. Sequences of LAD2-derived  $\alpha$  and  $\beta$ II tryptase and of HMC-1-derived  $\beta$ I and  $\beta$ III tryptase were determined *de novo*. Briefly, LAD2 genomic DNA was extracted by using GenElute Miniprep kits (Sigma, St Louis, Mo). HMC-1 mRNA was extracted with RNeasy kits (Qiagen, Germantown, Md) and converted to cDNA by using Superscript III (Invitrogen, Carlsbad, Calif). Tryptase genes and transcripts were PCR amplified with the Advantage 2 system (Clontech, Mountain View, Calif). Primers were based on conserved parts of published tryptase gene sequences. Individual amplimers were ligated into pCR2.1-TOPO (Invitrogen) for sequencing.

### Molecular modeling

Protein databank files representing HMC-1 frame-shifted  $\beta$ III ( $\beta$ III<sup>FS</sup>) tetramers were generated with Swiss-Model (<http://swissmodel.expasy.org>) by threading the deduced amino acid sequence onto each monomer of crystal-based  $\beta$ II tetramer 1AOL.10. Images were created from resulting files by using Chimera ([www.cgl.ucsf.edu/chimera](http://www.cgl.ucsf.edu/chimera)). HMC-1 Glu<sup>121</sup>Lys  $\beta$ I and Thr<sup>141</sup>Ala  $\beta$ III tryptase were analyzed similarly.

### Population samples

Tryptase alleles were analyzed in 270 samples from the HapMap project ([www.hapmap.org](http://www.hapmap.org)), representing individuals from 4 populations: 30 trios from the Yoruba in Ibadan, Nigeria (YRI); 30 trios from the Centre d'Etude du Polymorphisme Humain study collection from Utah residents with ancestry from Northern and Western Europe (CEU); 45 unrelated Japanese subjects in Tokyo (JPT); and 45 unrelated Han Chinese subjects in Beijing (CHB), which were represented as African, European, Japanese, and Chinese, respectively, in this work. Pooled results from JPT and CHB are represented as Asian.

### Genotyping

We used PCR amplification and direct sequencing to screen for allele-specific single nucleotide polymorphisms (SNPs) in coding regions (see the [Methods](#) section in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Sequencing was performed with Big Dye Terminator kits and an ABI 3100 sequencer (Applied Biosystems, Foster City, Calif). Primers were designed with Primer3.<sup>33</sup>

### Statistical analysis

Allele frequencies were compared by using 2-tailed Fisher exact tests. Hardy-Weinberg equilibrium, linkage disequilibrium, and haplotype frequencies were estimated with HelixTree software (Golden Helix, Inc, Bozeman, Mont). Haplotype frequencies were compared by using 2-tailed Fisher exact tests.

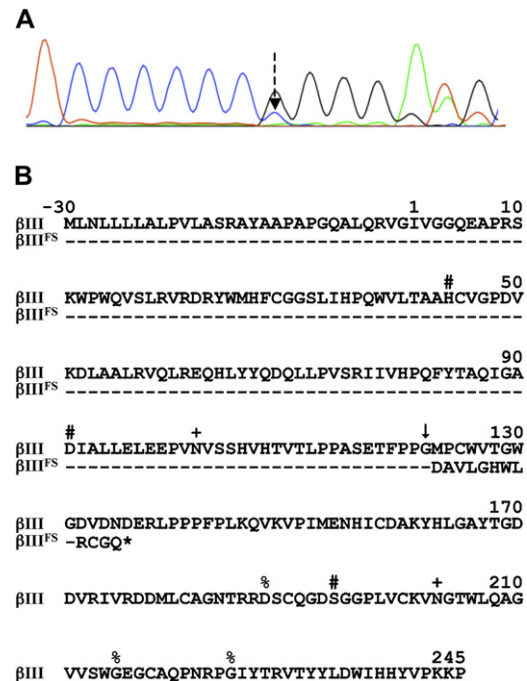


**FIG 2.** Alignment of frame-shifted and full-length  $\beta$ III tryptases. **A**, The chromatogram identifies the cytosine insertion (arrow) in  $\beta$ III<sup>FS</sup>. Red, blue, black, and green signify thymine, cytosine, guanine, and adenine, respectively. **B**, Comparison of the amino acid sequence of  $\beta$ III<sup>FS</sup> and full-length  $\beta$ III: -, identical residue; #, conserved "catalytic triad" residue; %, conserved "specificity triad" residue; +, N-glycosylation site. The arrow identifies the residue with a codon containing the frame-shifting insertion. Mature enzyme begins with residue 1.

## RESULTS

### Identification of $\alpha$ and $\beta$ tryptase genes in human mast cell lines

LAD2 cells, which are monozygous for chromosome 16, possess 1  $\alpha$  and 1  $\beta$ II gene.<sup>34</sup> Compared with  $\alpha$  tryptase cDNA sequence M30038.1, LAD2  $\alpha$  gene (accession no. FJ931116) exons contain the synonymous SNPs c.309A>G and c.333A>C and the nonsynonymous SNPs c.44-46GCG>CGG, c.253A>G, and c.508C>T, which lead to Arg<sup>15</sup>Pro, Thr<sup>85</sup>Ala, and Pro<sup>170</sup>Ser, respectively.<sup>35</sup> LAD-2  $\beta$ II gene (accession no. FJ931117) exons are identical to M33492.<sup>32</sup> HMC-1 cells lack both tryptases identified in LAD2 cells, instead having 2  $\beta$ I and 2  $\beta$ III genes. One HMC-1  $\beta$ I cDNA is identical to M33494, whereas the other (accession no. FJ931118) contains SNP c.382 G>A, which results in an amino acid change (Glu<sup>121</sup>Lys).<sup>32</sup> Of 2 HMC-1  $\beta$ III cDNAs identified, one matches AF099143, except for a 1-base insertion (c.980\_981insC, accession no. FJ9311119).<sup>22</sup> The other (accession no. FJ9311120) contains the synonymous SNP c.420C>T and the nonsynonymous SNPs c.421A>G (Thr<sup>141</sup>Ala) and c.719 G>A (Gly<sup>240</sup>Ala).

### Discovery and characterization of a frame-shifted $\beta$ III tryptase allele

The c.980\_981insC mutation identified in an HMC-1  $\beta$ III cDNA results in a frame-shifted tryptase ( $\beta$ III<sup>FS</sup>) with truncation of 109 amino acids (Fig 2) and creates a *Bs*II RFLP, which we confirmed using PCR of genomic DNA followed by *Bs*II digestion (data not shown). Databank mining revealed a small intestine

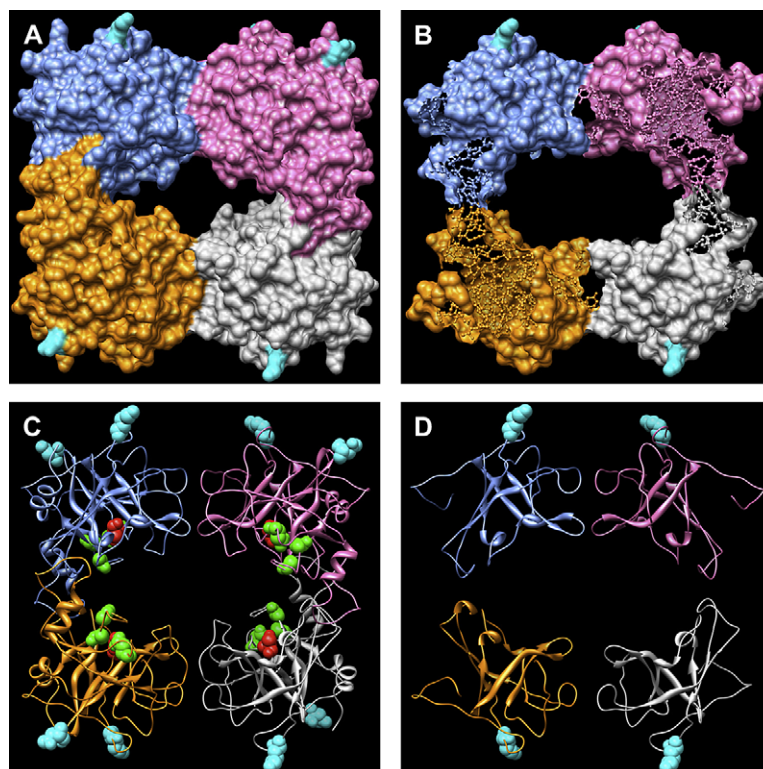


**FIG 3.** Models of full-length and frame-shifted  $\beta$ III tryptases. Full-length (**A** and **C**) and frame-shifted (**B** and **D**) models are shown with the “specificity triad” Asp<sup>188</sup>, Gly<sup>215</sup>, and Gly<sup>225</sup> in green, catalytic Ser<sup>195</sup> in red, and the N-glycosylation sites Asn<sup>102</sup> and Asn<sup>203</sup> in cyan. Residues truncated in  $\beta$ III<sup>FS</sup> are ball-and-stick models in Fig 3, **B**. Blue, pink, grey, and orange residues, respectively, identify monomers 1 to 4 of the tryptase tetramer.

partial cDNA (DC387615) that is identical to  $\beta$ III<sup>FS</sup> in regions of overlap. To establish whether  $\beta$ III<sup>FS</sup> genes are common, we determined carrier frequency in several populations using PCR-based genotyping, which revealed that  $\beta$ III<sup>FS</sup> prevalence varies strikingly, being present in 23% and 19%, respectively, of individuals of European and African ancestry but in 0% of surveyed Chinese and Japanese subjects.

### Modeling of $\beta$ III and $\beta$ III<sup>FS</sup> tryptases

Homology models of full-length  $\beta$ III and  $\beta$ III<sup>FS</sup> (Fig 3) show that the 109-residue truncation deletes  $\beta$ III<sup>FS</sup> residues crucial for substrate specificity and catalysis. Specifically, the deleted segment includes “specificity triad” residues Asp<sup>188</sup>, Gly<sup>215</sup>, and Gly<sup>225</sup>, which shape the pocket accommodating the substrate P1 side chain at the site of hydrolysis.<sup>36</sup> Truncation removes nearly half of the catalytic domain, including the catalytic Ser<sup>195</sup>, which is universally present in active serine peptidases. Therefore translated  $\beta$ III<sup>FS</sup> likely lacks enzyme activity.

### Tracking additional $\beta$ tryptase polymorphisms

The HMC-1 Glu<sup>121</sup>Lys variant of  $\beta$ I tryptase is detected solely in the European subjects, in whom it is present in 7% of genomes. Indeed, every subject with this  $\beta$ I SNP also possesses the  $\beta$ III<sup>FS</sup> mutation, comprising a haplotype perhaps limited to subjects of European ancestry. Only 22% of genomes with a  $\beta$ III<sup>FS</sup> mutation

also possess the  $\beta$ I Glu<sup>121</sup>Lys mutation, suggesting that the  $\beta$ III<sup>FS</sup> mutation, which is also present in African subjects, occurred earlier in tryptase evolution. Homology modeling of this  $\beta$ I variant does not predict a functional change (data not shown). By contrast, the c.421A>G (Thr<sup>141</sup>Ala)  $\beta$ III SNP is present in all 4 populations and, indeed, is the dominant form, representing 90%, 91%, and 70% of African, Asian, and European  $\beta$ III genes, respectively. Modeling of Thr<sup>141</sup>Ala  $\beta$ III tryptase does not predict altered protease function. Based on the likely functional significance of its truncation, we prioritized  $\beta$ III<sup>FS</sup> for genotyping in population screens.

### $\alpha/\beta$ Tryptase genotyping assay

We developed an assay that simultaneously identifies  $\alpha$  and  $\beta$  tryptase alleles, including  $\alpha$ ,  $\beta$ I,  $\beta$ II,  $\beta$ III, and  $\beta$ III<sup>FS</sup> in individual samples. Because there are 2 relevant loci (*TPSAB1* and *TPSB2*), each subject inherits 4 alleles. All known  $\alpha$  tryptases uniquely feature an SNP (c.733 G>C) that results in a Gly<sup>215</sup>Asp mutation that distorts the substrate binding site and limits peptidase activity.<sup>25,26</sup>  $\beta$ II tryptases are defined by another SNP (c.396C>G), which results in an Asn<sup>102</sup>Lys mutation that eliminates a highly conserved glycosylation site.<sup>37</sup>  $\beta$ I and  $\beta$ III genes are detected by allele-specific SNPs c.158C and c.158 G, respectively. This assay accurately genotypes 2 bacterial artificial chromosomes (324 and 48 of established tryptase content) and 2 cell lines (LAD2 and HMC-1), with tryptase gene contents determined here by means of sequencing individual cDNAs and genes.<sup>23</sup>

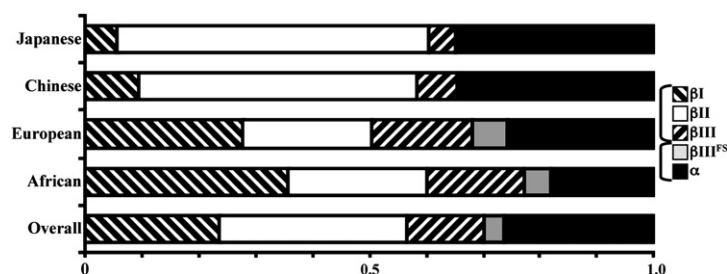


**FIG 4.** Tryptase allele frequencies in HapMap project populations from Yoruba in Ibadan, Nigeria (African); Centre d'Etude du Polymorphisme Humain collection from Utah (European); Beijing Han (Chinese); and Tokyo Japanese. Overall allele frequencies are from pooled populations. Large and small brackets mark functional and dysfunctional alleles, respectively.

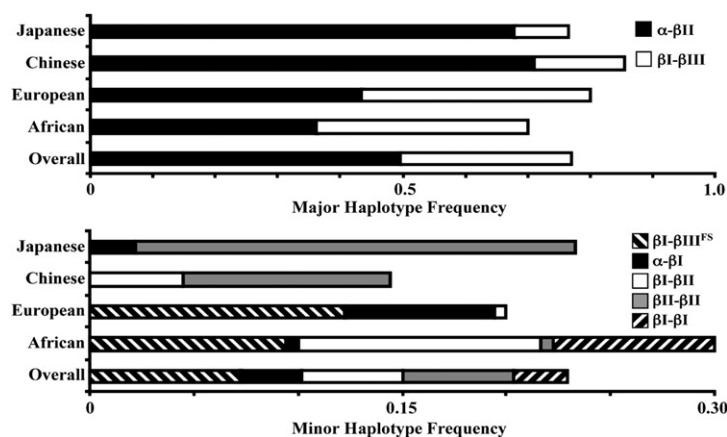


**FIG 5.** Tryptase haplotype frequencies in 4 populations. Haplotypes with overall frequencies of  $\geq 0.15$  and  $< 0.15$  are defined as major and minor, respectively.

### Distribution of alleles in populations of African, Asian, and European ancestry

Using this assay, we identified alleles in 270 subjects with a range of ancestries. Overall, as revealed in Fig 4 and Table E1 (available in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)),  $\beta II$  is the most common allele (frequency of 0.33). The least abundant allele type is  $\beta III$  (including  $\beta III^{FS}$ ), being half as common (frequency of 0.17) as  $\beta II$  ( $P < .0001$ ). Nonfunctional  $\alpha$  frequency (0.26) does not differ substantially from that of functional  $\beta I$  (0.23). Further analysis by subgroups reveals striking population-specific differences. For example,  $\alpha$  tryptase is nearly twice as common in Asian subjects as in African subjects (JPT/CHB=0.35/0.34 vs YRI=0.18;  $P < .0001$ ) and is slightly more common in Asian subjects than in European subjects (JPT/CHB=0.35/0.34 vs CEU=0.26;  $P=.01$ ). Similarly,  $\beta II$  is over twice as prevalent in Asian subjects as in African or European subjects (JPT/CHB=0.55/0.49, YRI=0.18, and CEU=0.26;  $P < .0001$ ). Conversely,  $\beta I$  and  $\beta III$  are strikingly enriched in African and European subjects (0.36 and 0.17 in YRI and 0.27 and 0.18 in CEU, respectively) compared with Asian subjects (JPT/CHB=0.06/0.09 and 0.04/0.07, respectively; all  $P < .0001$ ). African and European subjects differ in the frequency of  $\alpha$  (0.18 vs 0.26, respectively;  $P=.01$ ) and  $\beta I$  (0.36 and 0.27, respectively;  $P=.02$ ). Japanese and Chinese subjects do not differ significantly with respect to any allele. Individual populations are in Hardy-Weinberg equilibrium; however, pooled populations depart

from equilibrium ( $P < .001$  and  $P < .04$  for *TPSAB1* and *TPSB2*, respectively), as expected of polymorphic genes in reproductively separated populations.

### Estimation of linkage disequilibrium

To explore linkage between specific alleles at *TPSAB1* and *TPSB2*, we calculated 2 measures of linkage disequilibrium ( $r^2$  and  $D'$ ) that are strongly positive when populations are pooled ( $r^2=0.83$ ,  $D'=0.85$ ,  $P < .0001$ ) or considered individually ( $r^2/D'$  is 0.94/0.99, 0.63/0.96, 0.80/0.99, and 0.90/0.91 in CEU, YRI, CHB, and JPT, respectively; all  $P < .0001$ ).

### Haplotype predictions

Tryptase haplotypes and their corresponding frequencies in each population are shown in Fig 5 and Table E2 (available in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Given 5 alleles and 2 loci, 15 haplotypes are possible, but only 7 are encountered. The 2 major haplotypes (Fig 5), defined as having a frequency of 15% or greater, are  $\alpha$ - $\beta II$  and  $\beta I$ - $\beta III$ , which together represent 77% of all chromosomes. Indeed,  $\alpha$ - $\beta II$  and  $\beta I$ - $\beta III$  are the only haplotypes present in all populations. Consistent with observed allele frequencies,  $\alpha$ - $\beta II$  is much more common in Asian subjects than in African and European subjects (JPT/CHB=0.68/0.71, YRI=0.36, and CEU=0.44;

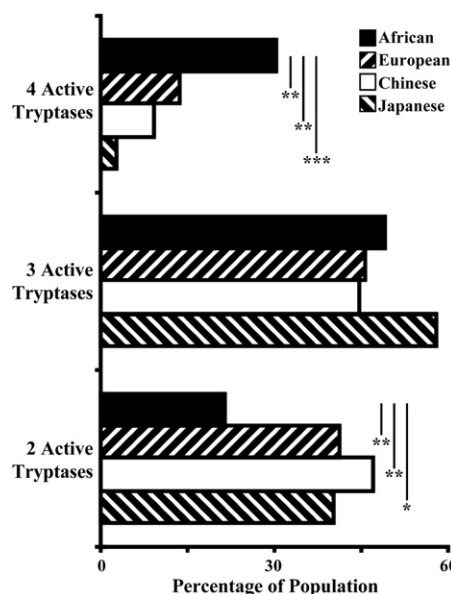


**FIG 6.** Active tryptase gene dosage in 4 populations. \* $P < .05$ , \*\* $P < .001$ , and \*\*\* $P < .0001$ , respectively.

$P < .0001$ ), whereas  $\beta$ I- $\beta$ III exhibits the opposite trend (JPT/CHB=0.09/0.14, YRI=0.34, and CEU=0.36;  $P < .0001$ ). African and European subjects do not differ in frequency of the 2 major haplotypes, as is also true of Japanese versus Chinese populations.

In addition to the 2 major haplotypes, we found 5 minor haplotypes (Fig 5) with frequencies of less than 15%, some of which are enriched in specific populations. For example,  $\beta$ II- $\beta$ II is much more common in Asian subjects than in others. Indeed, in the Japanese subjects  $\beta$ II- $\beta$ II is more prevalent than  $\beta$ I- $\beta$ III ( $P = .04$ ), which is a major haplotype in the overall survey but is present in just 9% of Japanese chromosomes. The results also suggest that minor haplotype  $\alpha$ - $\beta$ I is “European,” whereas  $\beta$ I- $\beta$ II and  $\beta$ I- $\beta$ I are “African.” The  $\beta$ I- $\beta$ III<sup>FS</sup> haplotype is found solely in the African and European subjects and does not differ between them.

The haplotype data also indicate that  $\alpha$  and  $\beta$ III (including  $\beta$ III<sup>FS</sup>) are restricted to *TPSAB1* and *TPSB2*, respectively, which is consistent with maps of tryptase loci cloned into bacterial artificial chromosomes.<sup>22,23</sup> Thus no  $\alpha$ - $\alpha$  or  $\beta$ III- $\beta$ III chromosomes are detected. Furthermore, the  $\alpha$  tryptase gene is almost always coinherited with  $\beta$ II at the neighboring locus (linkage frequency, 0.94; 95% CI, 0.91-0.98). More strikingly,  $\beta$ III and  $\beta$ III<sup>FS</sup> are 100% linked to  $\beta$ I at the neighboring locus. On most chromosomes,  $\beta$ I and  $\beta$ II are restricted to *TPSAB1* and *TPSB2*, respectively, but in 16% of chromosomes,  $\beta$ II and  $\beta$ I apparently occupy the adjacent locus, and both can form haplotypes with themselves (ie,  $\beta$ II- $\beta$ II and  $\beta$ I- $\beta$ I).

### Testing of haplotype predictions in family trios

Modes of inheritance of alleles based on the 7 predicted haplotypes are fully Mendelian in all YRI and CEU trios, thereby validating the haplotype predictions made on the basis of statistical association of alleles (see Fig E1 in this article’s Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Population-selective differences in active tryptase gene dosage

Strikingly, not a single Asian (JPT and CHB) sample possesses the  $\beta$ III<sup>FS</sup> deficiency allele (Fig 4). However, Asian subjects are enriched for the other known deficiency allele,  $\alpha$ . Consequently, overall frequency of dysfunctional alleles ( $\alpha + \beta$ III<sup>FS</sup>) in European, Chinese, and Japanese subjects is not statistically different, and the African subjects have the lowest frequency (Fig 4;  $P = .007$  YRI vs CEU;  $P < .0001$ , YRI vs JPT or CHB). Analysis of individuals with respect to the number of inherited active alleles reveals dramatic skewing between populations (Fig 6). Specifically, 30% of African subjects inherit 4 active alleles compared with just 2% of Japanese subjects ( $P < .0001$ ). Four active alleles are found in 13% and 9%, respectively, of European and Chinese subjects ( $P = .002$ , both YRI vs CEU and YRI vs CHB). At the other extreme, 21% of African subjects inherit just 2 active tryptase alleles compared with 41%, 47%, and 40% of European, Chinese, and Japanese subjects, respectively ( $P = .03$ , YRI vs JPT;  $P = .006$ , YRI vs CEU;  $P = .003$ , YRI vs CHB). European, Chinese, and Japanese subjects do not differ significantly with respect to the number of inherited active tryptases.

### Protection against total tryptase deficiency

The results suggest that dysfunctional tryptase alleles are always coinherited with functional alleles. Despite the overall high prevalence of dysfunctional alleles and the existence of dysfunctional alleles at *TPSAB1* and *TPSB2*, we find no individual chromosomes with 2 dysfunctional alleles (ie, theoretical haplotypes  $\alpha$ - $\alpha$ ,  $\alpha$ - $\beta$ III<sup>FS</sup>, and  $\beta$ III<sup>FS</sup>- $\beta$ III<sup>FS</sup>). Thus individuals with fewer than 2 functional alleles are notably absent. The observed strong linkage of deficiency and functional alleles protects against inheritance of less than 2 functional alleles.

### DISCUSSION

Tryptases are implicated in asthma and other allergic and nonallergic conditions, as well as in normal host defense.<sup>1</sup> Work from our laboratory and others demonstrated that the human multigene tryptase cluster is polymorphic and that nonfunctional  $\alpha$  competes with functional  $\beta$  at one of the loci.<sup>22,32,38</sup> Nevertheless, the scope of human tryptase genetic variation was unexplored and underappreciated. The present work expands our understanding of tryptase diversity and reveals marked population-specific differences in allele distribution. An intriguing finding is that human subjects might be protected against inheriting 4 dysfunctional tryptase alleles, which would create a knockout condition, as discussed in more detail below.

Enzymatically active, soluble human mast cell tryptases are produced by neighboring loci on chromosome 16p13.3 (Fig 1).<sup>22</sup> Previously, we published evidence that duplication in the tryptase cluster occurred during primate evolution and that tryptases “hyperevolved” during mammalian speciation.<sup>37</sup> As a manifestation of this instability, tryptases are susceptible to loss-of-function mutations, as in the case of  $\delta$  tryptases, which are dominant in macaques but likely nonfunctional in great apes, including human subjects.<sup>21</sup>  $\alpha$ -Tryptases provide additional examples of mutational loss of function. However, unlike  $\delta$ ,  $\alpha$  genes compete with functional  $\beta$  genes at the same locus and are absent in many human subjects, including members of each of the

populations surveyed here.<sup>27</sup> The present study also identifies a new deficiency allele,  $\beta\text{III}^{\text{FS}}$ , which is frequent in European and African subjects but not in Asian subjects. Molecular modeling predicts that  $\beta\text{III}^{\text{FS}}$  lacks crucial components of active site and catalytic machinery. Because the  $\beta\text{III}^{\text{FS}}$  gene is restricted to *TPSB2*, it is a new example of a nonfunctional tryptase gene competing with functioning alleles at the same locus.

We also report development and validation of a novel genotyping assay, which distinguishes 5 major tryptase alleles in individual samples and reveals previously unsuspected differences in allele frequency between populations of African, European, and Asian ancestry. The data indicate that the African gene pool is enriched in active tryptases, whereas Asian pools are enriched in dysfunctional tryptases. These inherited differences in active gene dosage might be important in allergic diseases and other responses in which tryptases are implicated.

This work is the first to establish tryptase haplotypes, revealing that strong linkage disequilibrium between *TPSAB1* and *TPSB2* restricts haplotypes to a fraction of those theoretically possible, with the 2 major haplotypes being  $\alpha$ - $\beta\text{II}$  and  $\beta\text{I}$ - $\beta\text{III}$ . Although the major haplotypes (constituting 77% of chromosomes) conform to the standard model of the tryptase cluster (Fig 1), several minor haplotypes are inconsistent with the model. Whereas  $\alpha$  and  $\beta\text{III}$  (and  $\beta\text{III}^{\text{FS}}$ ) appear to be restricted to 1 locus in the cluster,  $\beta\text{I}$  and  $\beta\text{II}$  appear able to occupy either locus and can even form haplotypes with themselves. Because  $\beta\text{I}$  is the only allele observed to pair with all other alleles, it might be the  $\alpha/\beta$  tryptase with the oldest lineage.

Our results are consistent with general observations about allele distribution arising from the HapMap project ([www.hapmap.org](http://www.hapmap.org)) in that major haplotypes occur in all populations studied, yet some are more prevalent in certain populations than others.<sup>39</sup> Also, some minor tryptase haplotypes appear to be limited to single populations. The African subjects contain all 7 haplotypes and therefore exhibit the most diversity, as well as the greatest haplotype heterozygosity (74%). In contrast, the Asian subjects collectively contain 5 haplotypes and exhibit the least heterozygosity (49% and 58% for CHB and JPT, respectively). These observations support the "out of Africa" hypothesis that a founder effect or bottleneck in human subjects migrating from Africa approximately 100,000 years ago reduced heterogeneity in Eastern Asian descendants.<sup>40,41</sup> Indeed, violation of Hardy-Weinberg equilibrium in our pooled populations likely reflects African and Asian allele frequency differences, which might result from mutation, migration, inbreeding, and natural selection.

The absence of chromosomes with 2 nonfunctional alleles combined with the observed lack of human subjects inheriting fewer than 2 functional alleles hints at the potential importance of active tryptases to human survival.  $\alpha$ -Tryptases are clearly not essential for life because many living adult human subjects completely lack  $\alpha$  genes. However, our data suggest the possibility that inheritance of fewer than 2  $\beta$  tryptases is deleterious. Because mouse studies with deleted tryptase genes suggest roles for tryptases in antimicrobial defense, haplotypes containing active tryptases might be positively selected.<sup>17,18</sup> Indeed, murine studies suggest that contributions might be gene dose related because mice inheriting 1 functional allele exhibit mortality between that of tryptase-null and wild-type mice with *Klebsiella* species-induced peritonitis.<sup>17</sup> In some human subjects there also might be adverse consequences (eg, excessive inflammation) of inheriting active tryptases, such that although one might be not

enough, 4 might be too many. If a penalty is paid for inheriting 4 active tryptases, then there could be an optimum gene dosage, most likely 2 or 3 genes, given that most individuals in each population fall into these categories. If more is better, it is improbable that one would see skewing toward fewer active tryptases, which is most marked in the Japanese population, in which only 2% of individuals inherit 4 active genes.

We thank Benjamin Ma for technical assistance.

**Clinical implications: Disparities in the inherited frequency in functional versus nonfunctional tryptases between populations might contribute to ethnicity-specific differences in disease phenotypes influenced by mast cell tryptase.**

## REFERENCES

1. Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* 2007;217:141-54.
2. Schwartz LB, Lewis RA, Seldin D, Austen KF. Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. *J Immunol* 1981;126:1290-4.
3. Hallgren J, Pejler G. Biology of mast cell tryptase. An inflammatory mediator. *FEBS J* 2006;273:1871-95.
4. McNeil HP, Adachi R, Stevens RL. Mast cell-restricted tryptases: structure and function in inflammation and pathogen defense. *J Biol Chem* 2007;282:20785-9.
5. Tam EK, Franconi GM, Nadel JA, Caughey GH. Protease inhibitors potentiate smooth muscle relaxation induced by vasoactive intestinal peptide in isolated human bronchi. *Am J Respir Cell Mol Biol* 1990;2:449-52.
6. Tam EK, Caughey GH. Degradation of airway neuropeptides by human lung tryptase. *Am J Respir Cell Mol Biol* 1990;3:27-32.
7. Ruoss SJ, Hartmann T, Caughey GH. Mast cell tryptase is a mitogen for cultured fibroblasts. *J Clin Invest* 1991;88:493-9.
8. Brown JK, Jones CA, Rooney LA, Caughey GH. Mast cell tryptase activates extracellular-regulated kinases (p44/p42) in airway smooth-muscle cells: importance of proteolytic events, time course, and role in mediating mitogenesis. *Am J Respir Cell Mol Biol* 2001;24:146-54.
9. Woodman L, Siddiqui S, Cruse G, Sutcliffe A, Saunders R, Kaur D, et al. Mast cells promote airway smooth muscle cell differentiation via autocrine up-regulation of TGF- $\beta$ 1. *J Immunol* 2008;181:5001-7.
10. Sekizawa K, Caughey GH, Lazarus SC, Gold WM, Nadel JA. Mast cell tryptase causes airway smooth muscle hyperresponsiveness in dogs. *J Clin Invest* 1989;83:175-9.
11. Johnson PRA, Ammit AJ, Carlin SM, Armour CL, Caughey GH, Black JL. Mast cell tryptase potentiates histamine-induced contraction in human sensitized bronchus. *Eur Respir J* 1997;10:38-43.
12. Krishna MT, Chauhan A, Little L, Sampson K, Hawksworth R, Mant T, et al. Inhibition of mast cell tryptase by inhaled APC 366 attenuates allergen-induced late-phase airway obstruction in asthma. *J Allergy Clin Immunol* 2001;107:1039-45.
13. Molinari JF, Scuri M, Moore WR, Clark J, Tanaka R, Abraham WM. Inhaled tryptase causes bronchoconstriction in sheep via histamine release. *Am J Respir Crit Care Med* 1996;154:649-53.
14. Barrios VE, Middleton SC, Kashem MA, Havill AM, Toombs CF, Wright CD. Tryptase mediates hyperresponsiveness in isolated guinea pig bronchi. *Life Sci* 1998;63:2295-303.
15. Costanzo MJ, Yabut SC, Almond HR Jr, Andrade-Gordon P, Corcoran TW, De Garavilla L, et al. Potent, small-molecule inhibitors of human mast cell tryptase. Antithiasthmatic action of a dipeptide-based transition-state analogue containing a benzothiazole ketone. *J Med Chem* 2003;46:3865-76.
16. Oh S-W, Pae CI, Lee D-K, Jones F, Chiang GKS, Kim H-O, et al. Tryptase inhibition blocks airway inflammation in a mouse asthma model. *J Immunol* 2002;168:1992-2000.
17. Thakurdas SM, Melicoff E, Sansores-Garcia L, Moreira DC, Petrova Y, Stevens RL, et al. The mast cell-restricted tryptase mMCP-6 has a critical immunoprotective role in bacterial infections. *J Biol Chem* 2007;282:20809-15.
18. Shin K, Watts GF, Oettgen HC, Friend DS, Pemberton AD, Gurish MF, et al. Mouse mast cell tryptase mMCP-6 is a critical link between adaptive and innate immunity in the chronic phase of *Trichinella spiralis* infection. *J Immunol* 2008;180:4885-91.

19. Huang C, Friend DS, Qiu WT, Wong GW, Morales G, Hunt J, et al. Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. *J Immunol* 1998;160:1910-9.
20. He S, Peng Q, Walls AF. Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. *J Immunol* 1997;159:6216-25.
21. Trivedi NN, Raymond WW, Caughey GH. Chimerism, point mutation, and truncation dramatically transformed mast cell  $\delta$ -tryptases during primate evolution. *J Allergy Clin Immunol* 2008;121:1262-8.
22. Pallaoro M, Fejzo MS, Shayesteh L, Blount JL, Caughey GH. Characterization of genes encoding known and novel human mast cell tryptases on chromosome 16p13.3. *J Biol Chem* 1999;274:3355-62.
23. Caughey GH, Raymond WW, Blount JL, Hau LW-T, Pallaoro M, Wolters PJ, et al. Characterization of human  $\gamma$ -tryptases, novel members of the chromosome 16 p mast cell tryptase and prostatic gene families. *J Immunol* 2000;164:6566-75.
24. Huang C, Li L, Krilis SA, Chanasyk K, Tang Y, Li Z, et al. Human tryptases  $\alpha$  and  $\beta$ /II are functionally distinct due, in part, to a single amino acid difference in one of the surface loops that forms the substrate-binding cleft. *J Biol Chem* 1999;274:19670-6.
25. Marquardt U, Zettl F, Huber R, Bode W, Sommerhoff C. The crystal structure of human  $\alpha$ 1-tryptase reveals a blocked substrate-binding region. *J Mol Biol* 2002;321:491-502.
26. Selwood T, Wang ZM, McCaslin DR, Schechter NM. Diverse stability and catalytic properties of human tryptase  $\alpha$  and  $\beta$  isoforms are mediated by residue differences at the S1 pocket. *Biochemistry* 2002;41:3329-40.
27. Soto D, Malmsten C, Blount JL, Muilenburg DJ, Caughey GH. Genetic deficiency of human mast cell  $\alpha$ -tryptase. *Clin Exp Allergy* 2002;32:1000-6.
28. Min HK, Moxley G, Neale MC, Schwartz LB. Effect of sex and haplotype on plasma tryptase levels in healthy adults. *J Allergy Clin Immunol* 2004;114:48-51.
29. Huang C, De Sanctis GT, O'Brien PJ, Mizgerd JP, Friend DS, Drazen JM, et al. Evaluation of the substrate specificity of human mast cell tryptase  $\beta$ I and demonstration of its importance in bacterial infections of the lung. *J Biol Chem* 2001;276:26276-84.
30. Harris JL, Niles A, Burdick K, Maffitt M, Backes BJ, Ellman JA, et al. Definition of the extended substrate specificity determinants for  $\beta$ -tryptases I and II. *J Biol Chem* 2001;276:34941-7.
31. Hallgren J, Lindahl S, Pejler G. Structural requirements and mechanism for heparin-dependent activation and tetramerization of human  $\beta$ 1- and  $\beta$ 2-tryptase. *J Mol Biol* 2005;345:129-39.
32. Vanderslice P, Ballinger SM, Tam EK, Goldstein SM, Craik CS, Caughey GH. Human mast cell tryptase: multiple cDNAs and genes reveal a multigene serine protease family. *Proc Natl Acad Sci U S A* 1990;87:3811-5.
33. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 2000;132:365-86.
34. Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA, et al. Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of Fc $\epsilon$ RI or Fc $\gamma$ RI. *Leuk Res* 2003;27:677-82.
35. Miller JS, Westin EH, Schwartz LB. Cloning and characterization of complementary DNA for human tryptase. *J Clin Invest* 1989;84:1188-95.
36. Pereira PJB, Bergner A, Macedo-Ribeiro S, Huber R, Matschiner G, Fritz H, et al. Human  $\beta$ -tryptase is a ring-like tetramer with active sites facing a central pore. *Nature* 1998;392:306-11.
37. Trivedi NN, Tong Q, Raman K, Bhagwandin VJ, Caughey GH. Mast cell  $\alpha$  and  $\beta$  tryptases changed rapidly during primate speciation and evolved from  $\gamma$ -like transmembrane peptidases in ancestral vertebrates. *J Immunol* 2007;179:6072-9.
38. Miller JS, Moxley G, Schwartz LB. Cloning and characterization of a second complementary cDNA for human tryptase. *J Clin Invest* 1990;86:864-70.
39. The International HapMap Project. *Nature* 2003;426:789-96.
40. Stoneking M, Soodyall H. Human evolution and the mitochondrial genome. *Curr Opin Genet Dev* 1996;6:731-6.
41. Swisher CC 3rd, Curtis GH, Jacob T, Getty AG, Suprijo A, Widiasmoro. Age of the earliest known hominids in Java, Indonesia. *Science* 1994;263:1118-21.

## METHODS

### Genotyping

Genomic DNA from each sample was PCR amplified with the primers GCAGGTGAGCCTGAGAGTCC and GGGACCTTCACCTGCTTCAG for amplicon 1 and the primers CTGGTGTGCAAGGTGAATGG and AGGTC CAGCACTCAGGAGGA for amplicon 2. Cycling conditions were as follows: 95°C for 5 minutes followed by 35 cycles of 1 minute at 94°C; 1 minute of annealing at 60°C and 58°C for amplicons 1 and 2, respectively; a 1-minute

extension at 72°C; and a final 10-minute extension at 72°C. After PCR cleanup (PerkinElmer Life Sciences, Inc, Boston, Mass), purified amplicons were sequenced with ABI PRISM BigDye terminator sequencing Version 3.1 on an ABI Prism 3730xl DNA analyzer (Applied Biosystems). In addition to the primers listed above, we used the primer CAGCCAGTGACCCAGCAC for sequencing with cycling conditions as follows: 96°C for 2 minutes and 25 cycles of 96°C for 15 seconds, 50°C for 1 second, and 60°C for 4 minutes. Resulting sequencing chromatograms were analyzed with SEQUENCHER 4.8 (Gene Codes Corporation, Ann Arbor, Mich).

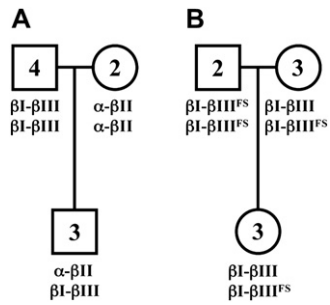


**FIG E1.** Examples of genotyped parent-child trios. The number of active tryptase genes per individual is shown within sex-specific symbols (*squares*, male; *circle*, female). **A** and **B** are families 1344 and 1350, respectively, from the Centre d'Etude du Polymorphisme Humain study collection from Utah.

**TABLE E1.** Tryptase allele frequencies

|          | $\beta$ I | $\beta$ II | $\beta$ III | $\beta$ III <sup>FS</sup> | $\alpha$ |
|----------|-----------|------------|-------------|---------------------------|----------|
| Overall  | 0.24      | 0.33       | 0.14        | 0.04                      | 0.26     |
| African  | 0.36      | 0.24       | 0.17        | 0.05                      | 0.18     |
| European | 0.28      | 0.23       | 0.18        | 0.06                      | 0.26     |
| Chinese  | 0.09      | 0.49       | 0.07        | Not detected              | 0.34     |
| Japanese | 0.06      | 0.55       | 0.04        | Not detected              | 0.35     |

**TABLE E2.** Tryptase haplotype frequencies

|          | $\alpha$ - $\beta$ II | $\beta$ I- $\beta$ III | $\beta$ I- $\beta$ III <sup>FS</sup> | $\alpha$ - $\beta$ I | $\beta$ I- $\beta$ II | $\beta$ II- $\beta$ II | $\beta$ I- $\beta$ I |
|----------|-----------------------|------------------------|--------------------------------------|----------------------|-----------------------|------------------------|----------------------|
| Overall  | 0.50                  | 0.27                   | 0.07                                 | 0.03                 | 0.05                  | 0.05                   | 0.03                 |
| African  | 0.36                  | 0.34                   | 0.09                                 | 0.01                 | 0.11                  | 0.01                   | 0.08                 |
| European | 0.43                  | 0.37                   | 0.12                                 | 0.07                 | 0.01                  | Not detected           | Not detected         |
| Chinese  | 0.71                  | 0.14                   | Not detected                         | Not detected         | 0.04                  | 0.10                   | Not detected         |
| Japanese | 0.68                  | 0.09                   | Not detected                         | 0.02                 | Not detected          | 0.21                   | Not detected         |