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Humans are protected from mast cell tryptase deficiency despite frequent inheritance of loss-of-function mutations

Neil N. Trivedi, M.D.[1,2,6], **Bani Tamraz, Pharm. D.**[1,4], **Catherine Chu, B.S.**[3,4], **Pui-Yan Kwok, M.D., Ph.D.**[1,3,4], and **George H. Caughey, M.D.**[1,2,5,6]

[1] Cardiovascular Research Institute of the University of California at San Francisco, California

[2] Department of Medicine of the University of California at San Francisco, California

[3] Department of Dermatology of the University of California at San Francisco, California

[4] Institute for Human Genetics of the University of California at San Francisco, California

[5] Veterans Health Research Institute, San Francisco, California

[6] Veterans Affairs Medical Center, San Francisco, California

Abstract

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—To compare functional and nonfunctional tryptase allele frequencies and establish haplotypes in human populations.

Methods—Tryptase allele frequencies were determined by 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 (βIII^{FS}) carried by 23% and 19% of individuals of European and African ancestry, but 0% of Asians. Homology models predict that βIII^{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 two major and five minor tryptase haplotypes.

Conclusions—Tryptase deficiency alleles (α and newly discovered βIII^{FS}) are common, causing the number of inherited active genes to range from a minimum of two to a maximum of four, with major differences between populations in proportion of individuals inheriting two versus four active alleles. African and Asian populations are especially enriched in genes encoding functional and non-functional tryptases, respectively. Strong linkage of *TPSAB1* and *TPSB2*, and pairing of deficiency alleles with functional alleles in observed haplotypes, protect humans from "knockout" genomes, and indeed from inheritance of fewer than two active alleles.

Address correspondence to: Dr. George H. Caughey, Veterans Affairs Medical Center 111D, 4150 Clement Street, San Francisco, CA 94121, George.Caughey@ucsf.edu, Tel: 415-221-4810 x2385, Fax: 415-387-3568.

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

Keywords

tryptase; mast cells; human genetics

INTRODUCTION

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

Human soluble tryptases are produced by three closely spaced genes: *TPSD1*, *TPSAB1* and *TPSB2* (Fig 1). *TPSD1* (δ-tryptase) genes developed inactivating truncation mutations in the great ape lineage.[21] Thus, enzymatically active soluble tryptases in humans are products of *TPSAB1* and *TPSAB2*, which are polymorphic. These two loci produce four major tryptase isoforms: α, βI, βII, and βIII. Work from our laboratory suggests that α and βI are restricted to *TPSAB1* and that βII and βIII are restricted to *TPSB2*.[22, 23] α-Tryptases have minimal to no catalytic activity and are genetically absent in many humans.[24–28] In contrast, βI tryptase is an active peptidase that may recruit neutrophils.[29] βII tryptase substrate specificity and heparin-dependent activation are equivalent to those of βI.[30, 31] Less is known of βIII tryptases, although they are predicted to be active.[32] To explore tryptase gene prevalence and variation, the present study evaluates ethnically diverse backgrounds. The results reveal greater 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 cells and HMC-1 cells were obtained from Dr. Dean and Dr. Joseph Butterfield, respectively. Sequences of LAD2-derived α and βII tryptase and of HMC-1 βI and βIII were determined *de novo*. Briefly, LAD2 genomic DNA was extracted using GenElute Miniprep kits (Sigma, St Louis, MO). HMC-1 mRNA was extracted using RNeasy kits (Qiagen, Germantown, MD) and converted to cDNA by Superscript III (Invitrogen, Carlsbad, CA). Tryptase genes and transcripts were PCR-amplified using the Advantage 2 system (Clontech, Mountain View, CA). 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 βIII (βIII^{FS}) tetramers were generated with Swiss-Model (http://swissmodel.expasy.org) by threading deduced amino acid sequence onto each monomer of crystal-based βII tetramer 1AOL.10. Images were created from resulting files using Chimera (www.cgl.ucsf.edu/chimera). HMC-1 Glu^{121}Lys βI and Thr^{141}Ala βIII tryptase were analyzed similarly.

Population samples

Tryptase alleles were analyzed in 270 samples from the HapMap project (www.hapmap.org) representing individuals from four 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 individuals in Tokyo (JPT), and 45 unrelated Han Chinese individuals in Beijing (CHB), 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 SNPs in coding regions (see appendix E1 in the Online Repository). Sequencing was performed with Big Dye Terminator kits and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). Primers were designed using Primer3.[33]

Statistical analysis

Allele frequencies were compared using two-tailed Fisher's Exact Tests. Hardy–Weinberg equilibrium, linkage disequilibrium, and haplotype frequencies were estimated using Helixtree software. Haplotype frequencies were compared using two-tailed Fisher's exact tests.

RESULTS

Identification of α and β tryptase genes in human mast cell lines

LAD2 cells, which are monozygous for chromosome 16, possess one α and one βII gene.[34] Compared to α tryptase cDNA sequence M30038.1, LAD2 α gene (accession #FJ931116) exons contain synonymous SNPs c.309A>G and c.333A>C and nonsynonymous SNPs c.44–46GCG>CGG, c.253A>G, and c.508C>T, which lead to Arg^{15}Pro, Thr^{85}Ala, and Pro^{170}Ser, respectively.[35] LAD-2 βII gene (accession #FJ9311117) exons are identical to M33492.[32] HMC-1 cells lack both tryptases identified in LAD2 cells, instead having two βI and two βIII genes. One HMC-1 βI cDNA is identical to M33494, whereas the other (accession #FJ9311118) contains SNP c.382G>A, which results in an amino acid change (Glu^{121}Lys).[32] Of two HMC-1 βIII cDNAs identified, one matches AF099143 except for a one-base insertion (c.980_981insC, accession# FJ9311119).[22] The other (accession #FJ9311120) contains synonymous SNP c.420C>T and non-synonymous SNP c.421A>G (Thr^{141}Ala and c.719G>A (Gly^{240}Ala).

Discovery and characterization of a frame-shifted βIII tryptase allele

The c.980_981insC mutation identified in a HMC-1 βIII cDNA results in a frame-shifted tryptase (βIII^{FS}) with truncation of 109 amino acids (Fig 2) and creates a *Bsl*I restriction fragment length polymorphism, which we confirmed using PCR of genomic DNA followed by *Bsl*I digestion (data not shown). Databank mining revealed a small intestine partial cDNA (DC387615) that is identical to βIII^{FS} in regions of overlap. To establish whether βIII^{FS} genes are common, we determined carrier frequency in several populations using PCR-based genotyping, which reveals that βIII^{FS} 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.

Modeling of βIII and frame-shifted βIII tryptases

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

Tracking additional β-tryptase polymorphisms

The HMC-1 $Glu^{121}Lys$ variant of βI-tryptase is detected solely in the Europeans, where it is present in 7% of genomes. Indeed, every individual with this βI SNP also possesses the βIII^{FS} mutation, comprising a haplotype perhaps limited to individuals of European ancestry. Only 22% of genomes with a βIII^{FS} mutation also possess the βI $Glu^{121}Lys$ mutation, suggesting that the βIII^{FS} mutation, which is also present in Africans, occurred earlier in tryptase evolution. Homology modeling of this βI variant does not predict a functional change (data not shown). By contrast, the c.421A>G ($Thr^{141}Ala$) βIII SNP is present in all four populations, and, indeed, is the dominant form, representing 90%, 91% and 70% of African, Asian and European βIII genes, respectively. Modeling of $Thr^{141}Ala$ βIII tryptase does not predict altered protease function. Based on the likely functional significance of its truncation, we prioritized βIII^{FS} for genotyping in population screens.

α/β Tryptase genotyping assay

We developed an assay that simultaneously identifies α and β tryptase alleles, including α, βI, βII, βIII and βIII^{FS} in individual samples. Since there are two relevant loci (*TPSAB1* and *TPSB2*), each individual inherits four alleles. All known α-tryptases uniquely feature a SNP (c.733G>C), which results in a $Gly^{215}Asp$ mutation that distorts the substrate binding site and limits peptidase activity. [25, 26] βII tryptases are defined by another SNP (c.396C>G), which results in an $Asn^{102}Lys$ mutation that eliminates a highly conserved glycosylation site.[37] βI and βIII genes are detected by allele-specific SNPs c.158C and c.158G, respectively. This assay accurately genotypes two BACs (324 and 48, of established tryptase content) and two cell lines (LAD2 and HMC-1), whose tryptase gene content is determined here by sequencing individual cDNAs and genes.[23]

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

Using this assay, we identified alleles in 270 individuals with a range of ancestries. Overall, as revealed in Fig 4 and Table E1 in the Online Repository, βII is the most common allele (frequency 0.33). The least abundant allele type is βIII (including βIII^{FS}), being half as common (frequency 0.17) as βII ($P < 0.0001$). Nonfunctional α frequency (0.26) does not differ substantially from that of functional βI (0.23). Further analysis by subgroups reveals striking population-specific differences. For example, α tryptase is nearly twice as common in Asians than Africans (JPT/CHB = 0.35/0.34 vs. YRI = 0.18; $P < 0.0001$) and is slightly more common in Asians than in Europeans (JPT/CHB = 0.35/0.34 vs. CEU = 0.26; $P = 0.01$). Similarly, βII is over twice as prevalent in Asians as in Africans or Europeans (JPT/CHB = 0.55/0.49, YRI = 0.18, CEU = 0.26; $P < 0.0001$). Conversely, βI and βIII are strikingly enriched in Africans and Europeans (0.36 and 0.17 in YRI, 0.27 and 0.18 in CEU) compared to Asians (JPT/CHB = 0.06/0.09 and 0.04/0.07; all $P < 0.0001$). Africans and Europeans differ in frequency of α (0.18 vs. 0.26, respectively; $P = 0.01$) and βI (0.36 and 0.27, respectively; $P = 0.02$). Japanese and Chinese do not differ significantly with respect to any allele. Individual populations are in

Hardy-Weinberg equilibrium; however, pooled populations depart from equilibrium (P <0.001 and <0.04 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 two measures of linkage disequilibrium (r^2 and D'), which is strongly positive when populations are pooled (r^2 = 0.83, D' = 0.85, P <0.0001) or considered individually (r^2/D' is 0.94/0.99, 0.63/0.96, 0.80/0.99, 0.90/0.91 in CEU, YRI, CHB, and JPT, respectively; all P <0.0001).

Haplotype predictions

Tryptase haplotypes and their corresponding frequencies in each population are shown in Fig 5 and Table E2 in the Online Repository. Given five alleles and two loci, 15 haplotypes are possible but only seven are encountered. The two major haplotypes (Fig 5), defined as frequency ≥15%, are α–βII and βI–βIII, which together represent 77% of all chromosomes. Indeed, α-βII and βI-βIII are the only haplotypes present in all populations. Consistent with observed allele frequencies, α-βII is much more common in Asians than in Africans and Europeans (JPT/CHB = 0.68/0.71, YRI = 0.36, CEU = 0.44; P <0.0001), whereas βI-βIII exhibits the opposite trend (JPT/CHB = 0.09/0.14, YRI = 0.34, CEU = 0.36; P <0.0001). Africans and Europeans do not differ in frequency of the two major haplotypes, as is also true of Japanese vs. Chinese populations.

In addition to the two major haplotypes, we found five minor haplotypes (Fig 5) with frequencies <15%, some of which are enriched in specific populations. For example, βII-βII is much more common in Asians than in others. Indeed, in the Japanese, βII–βII is more prevalent than βI–βIII (P = 0.04), 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 α–βI is "European" whereas βI–βII and βI–βI are "African". The βI-βIII^{FS} haplotype is found solely in the Africans and Europeans, and does not differ between them.

The haplotype data also indicate that α and βIII (including βIII^{FS}) are restricted to *TPSAB1* and *TPSB2*, respectively, which is consistent with maps of tryptase loci cloned into BACs.[22, 23] Thus, no α–α or βIII-βIII chromosomes are detected. Furthermore, the α-tryptase gene is almost always co-inherited with βII at the neighboring locus (linkage frequency = 0.94; 95% CI 0.91–0.98). More strikingly, βIII and βIII^{FS} are 100% linked to βI at the neighboring locus. On most chromosomes, βI and βII are restricted to *TPSAB1* and *TPSB2*, respectively, but in 16% of chromosomes βII and βI apparently occupy the adjacent locus and both can form haplotypes with themselves (i.e., βII-βII and βI-βI).

Testing of haplotype predictions in family trios

Modes of inheritance of alleles based on the seven 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 the Online Repository).

Population-selective differences in active tryptase gene dosage

Strikingly, not a single Asian (JPT and CHB) sample possesses the βIII^{FS} deficiency allele (Fig 4). However, Asians are enriched for the other known deficiency allele, α. Consequently, overall frequency of dysfunctional alleles (α + βIII^{FS}) in Europeans, Chinese, and Japanese is not statistically different, and the Africans have the lowest frequency (Fig 4; P = 0.007 YRI vs. CEU; P <0.0001 YRI vs. JPT or CHB). Analysis of individuals with respect to number of inherited active alleles reveals dramatic skewing between populations (Fig 6). Specifically,

30% of Africans inherit four active alleles compared to just 2% of Japanese (P <0.0001). Four active alleles are found in 13% and 9%, respectively, of Europeans and Chinese (P = 0.002 for both YRI vs. CEU and YRI vs. CHB). At the other extreme, 21% of Africans inherit just two active tryptase alleles, compared to 41%, 47%, and 40% of Europeans, Chinese, and Japanese, respectively (P = 0.03 YRI vs. JPT; P = 0.006 YRI vs. CEU; P = 0.003 YRI vs. CHB). Europeans, Chinese, and Japanese do not differ significantly with respect to number of inherited active tryptases.

Protection against total tryptase deficiency

The results suggest that dysfunctional tryptase alleles are always co-inherited with functional alleles. Despite overall high prevalence of dysfunctional alleles, and the existence of dysfunctional alleles at *TPSAB1* and *TPSB2*, we find no individual chromosomes with two dysfunctional alleles (i.e., theoretical haplotypes α–α, α–βIII^{FS}, and βIII^{FS}-βIII^{FS}). Thus, individuals with fewer than two functional alleles are notably absent. The observed strong linkage of deficiency and functional alleles protects from inheritance of less than two functional alleles.

DISCUSSION

Tryptases are implicated in asthma and other allergic and non-allergic conditions, as well as in normal host defense.[1] Work from our laboratory and others demonstrated that the human multi-gene tryptase cluster is polymorphic and that nonfunctional α competes with functional β at one of the loci.[22, 32, 38] Nevertheless, the scope of human tryptase genetic variation was unexplored and underappreciated. The present work expands understanding of tryptase diversity and reveals marked population-specific differences in allele distribution. An intriguing finding is that humans may be protected from inheriting four 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).[22] Previously, we published evidence that duplication in the tryptase cluster occurred during primate evolution, and that tryptases “hyper-evolved” during mammalian speciation.[37] As a manifestation of this instability, tryptases are susceptible to loss-of-function mutations, as in the case of δ-tryptases, which are dominant in macaques but likely nonfunctional in great apes, including humans.[21] α-Tryptases provide additional examples of mutational loss of function. However, unlike δ, α genes compete with functional β genes at the same locus, and are absent in many humans, including members of each of the populations surveyed here.[27] The present study also identifies a new deficiency allele, βIII^{FS}, which is frequent in Europeans and Africans, but not Asians. Molecular modeling predicts that βIII^{FS} lacks crucial components of active site and catalytic machinery. Because the βIII^{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 five 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 may 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 two major haplotypes being α-βII and βI-βIII. 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 α and βIII (and βIII^{FS}) appear to be restricted to one locus in the cluster, βI and βII appear able to occupy either locus, and even can form haplotypes with themselves. Because βI is the only allele observed to pair with all other alleles, it may be the α/β tryptase with the oldest lineage.

Our results are consistent with general observations about allele distribution arising from the HapMap project (www.hapmap.org) in that major haplotypes occur in all populations studied, yet some are more prevalent in certain populations than others.[39] Also, some minor tryptase haplotypes appear to be limited to single populations. The Africans contain all seven haplotypes and, therefore, exhibit the most diversity as well as the greatest haplotype heterozygosity (74%). In contrast, the Asians collectively contain five 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 humans migrating from Africa ~100,000 years ago reduced heterogeneity in Eastern Asian descendents.[40, 41] Indeed, violation of Hardy-Weinberg equilibrium in our pooled populations likely reflects African and Asian allele frequency differences, which may result from mutation, migration, inbreeding, and natural selection.

The absence of chromosomes with two nonfunctional alleles combined with the observed lack of humans inheriting fewer than two functional alleles hint at the potential importance of active tryptases to human survival. α-Tryptases are clearly not essential for life since many living adult humans completely lack α genes. However, our data suggest the possibility that inheritance of fewer than two β tryptases is deleterious. Because mouse studies with deleted tryptase genes suggest roles for tryptases in anti-microbial defense, haplotypes containing active tryptases may be positively selected.[17, 18] Indeed, mouse studies suggest that contributions may be gene dose-related, because mice inheriting one functional allele exhibit mortality between that of tryptase-null and wild type mice with *Klebsiella* peritonitis.[17] In some humans, there also may be adverse consequences (e.g., excessive inflammation) of inheriting active tryptases, such that although one may be not enough, four may be too many. If a penalty is paid for inheriting four active tryptases, then there may be an "optimum" gene dosage, most likely two or three genes, given that most individuals in each population fall into these categories. If "more is better", it is improbable that one would see skewing towards fewer active tryptases, which is most marked in the Japanese population, in which only 2% of individuals inherit four active genes.

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Abbreviations used

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

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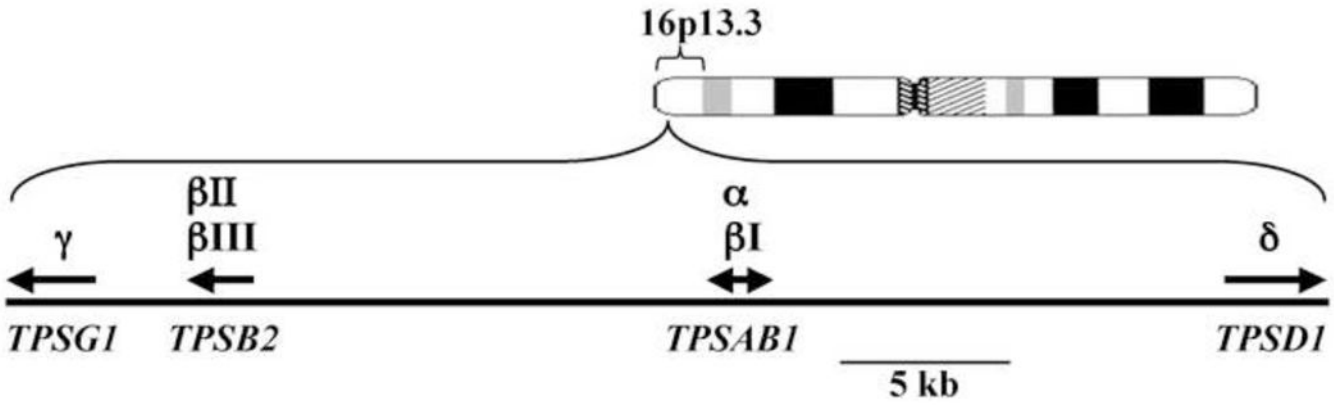

FIG 1.

The tryptase gene cluster on chromosome 16p13.3. *TPSG1*, which encodes transmembrane tryptase γ, anchors the telomeric end. At adjacent *TPSB2*, βII and βIII tryptase genes compete as alleles. Similarly, α and βI genes compete as alleles at adjacent locus *TPSAB1*. *TPSD1* is the δ locus, which encodes a truncated, largely inactive peptidase. Arrows depict transcriptional orientation.

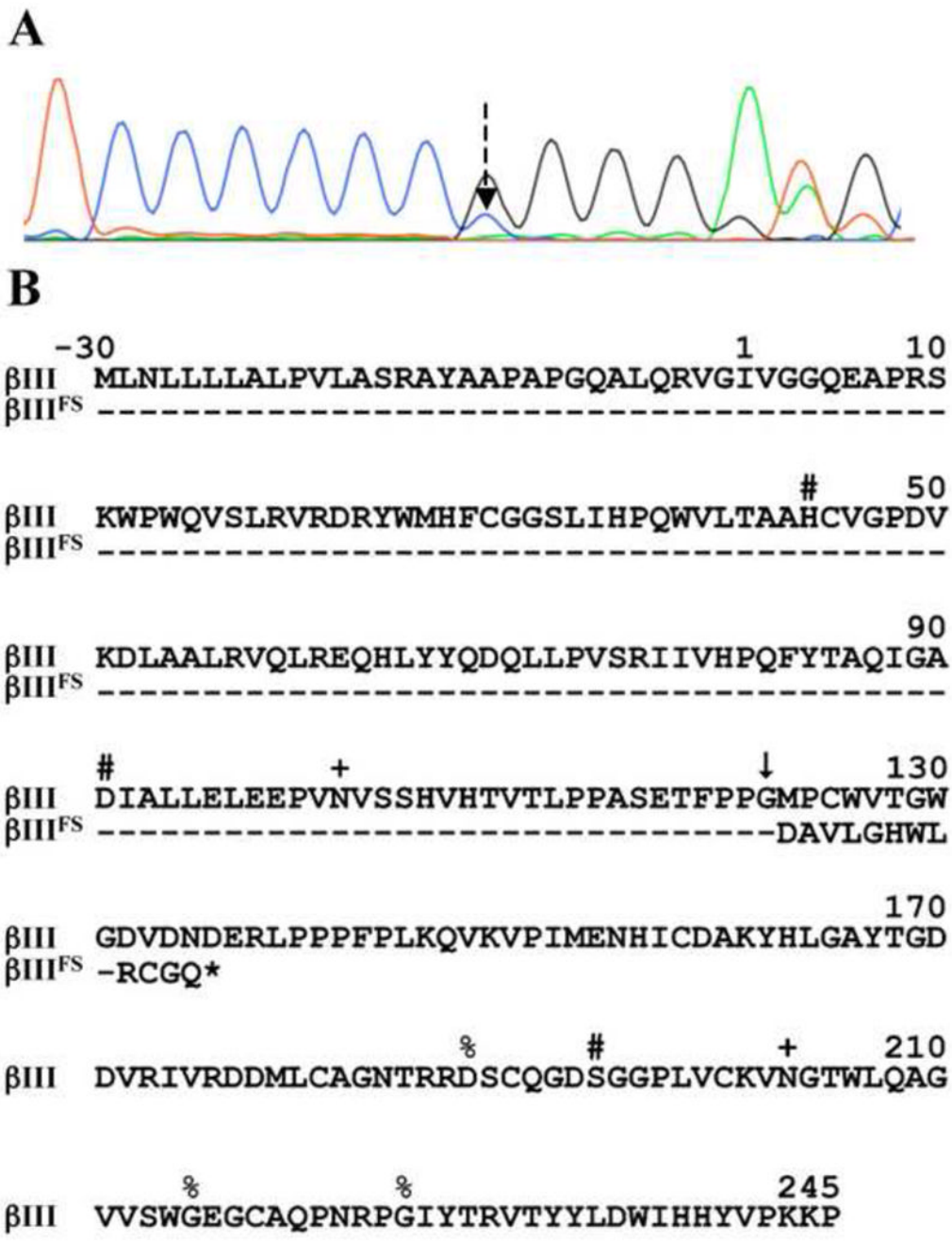

FIG 2.

Alignment of frame-shifted and full-length βIII tryptases. The Panel A chromatogram identifies the cytosine insertion (arrow) in βIII^{FS}. Red, blue, black, and green signify thymine, cytosine, guanine, and adenine, respectively. Panel B compares amino acid sequence of βIII^{FS} and full-length βIII: −, identical residue; #, conserved "catalytic triad" residue; %, conserved "specificity triad" residue; +, N-glycosylation site. The arrow identifies the residue whose codon contains the frame-shifting insertion. Mature enzyme begins with residue 1.

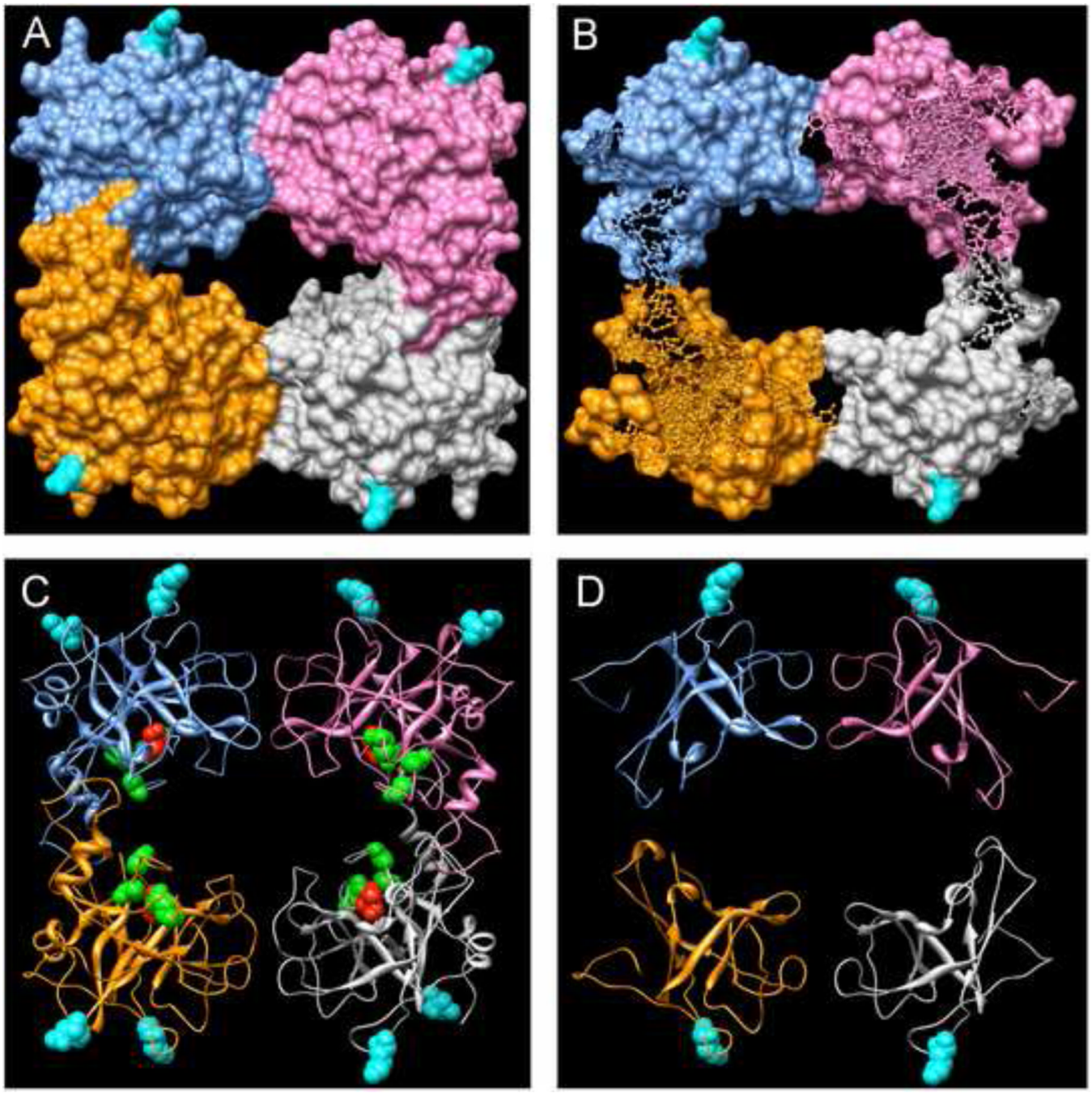

FIG 3.

Models of full-length and frame-shifted βIII tryptases. Full-length (A and C) and frame-shifted (B and D) models are shown with "specificity triad" Asp^{188}, Gly^{215}, and Gly^{225} in green, catalytic Ser^{195} in red, and *N*-glycosylation sites Asn^{102} and Asn^{203} in cyan. Residues truncated in $βIII^{FS}$ are ball and stick models in panel B. Blue, pink, grey, and orange residues, respectively, identify monomers 1–4 of the tryptase tetramer.

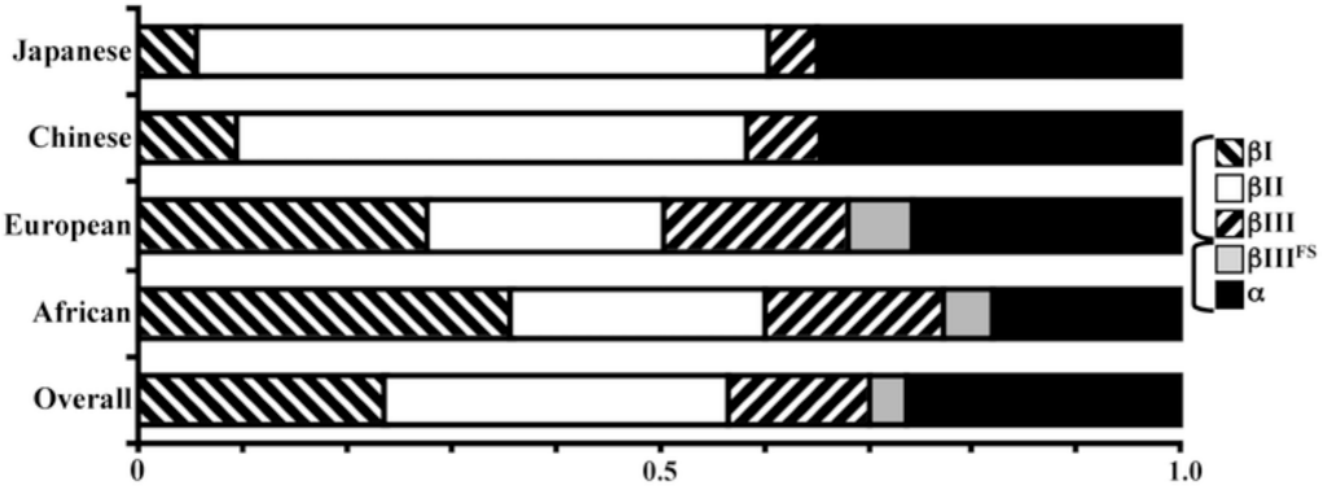

FIG 4.

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.

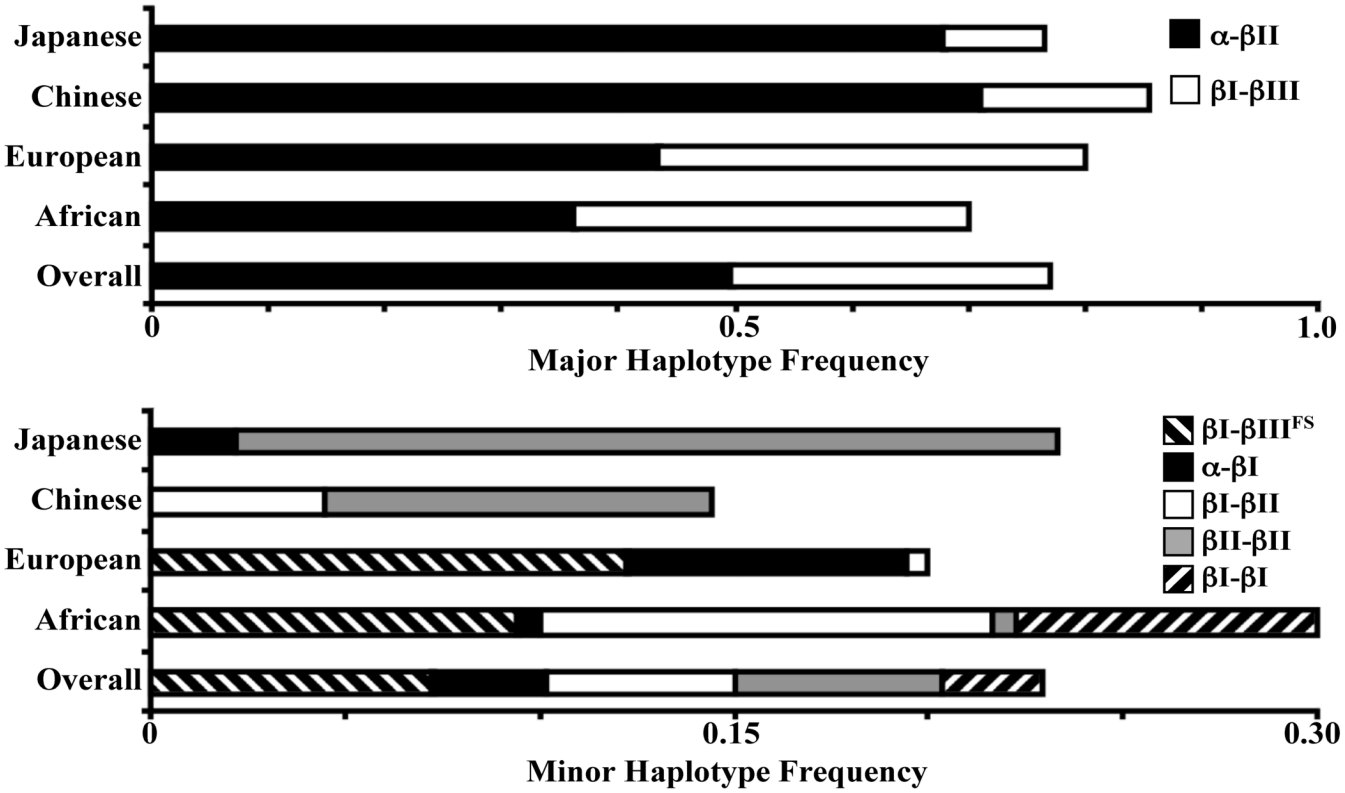

FIG 5.

Tryptase haplotype frequencies in four populations. Haplotypes with overall frequency ≥ 0.15 and < 0.15 are defined as major and minor, respectively.

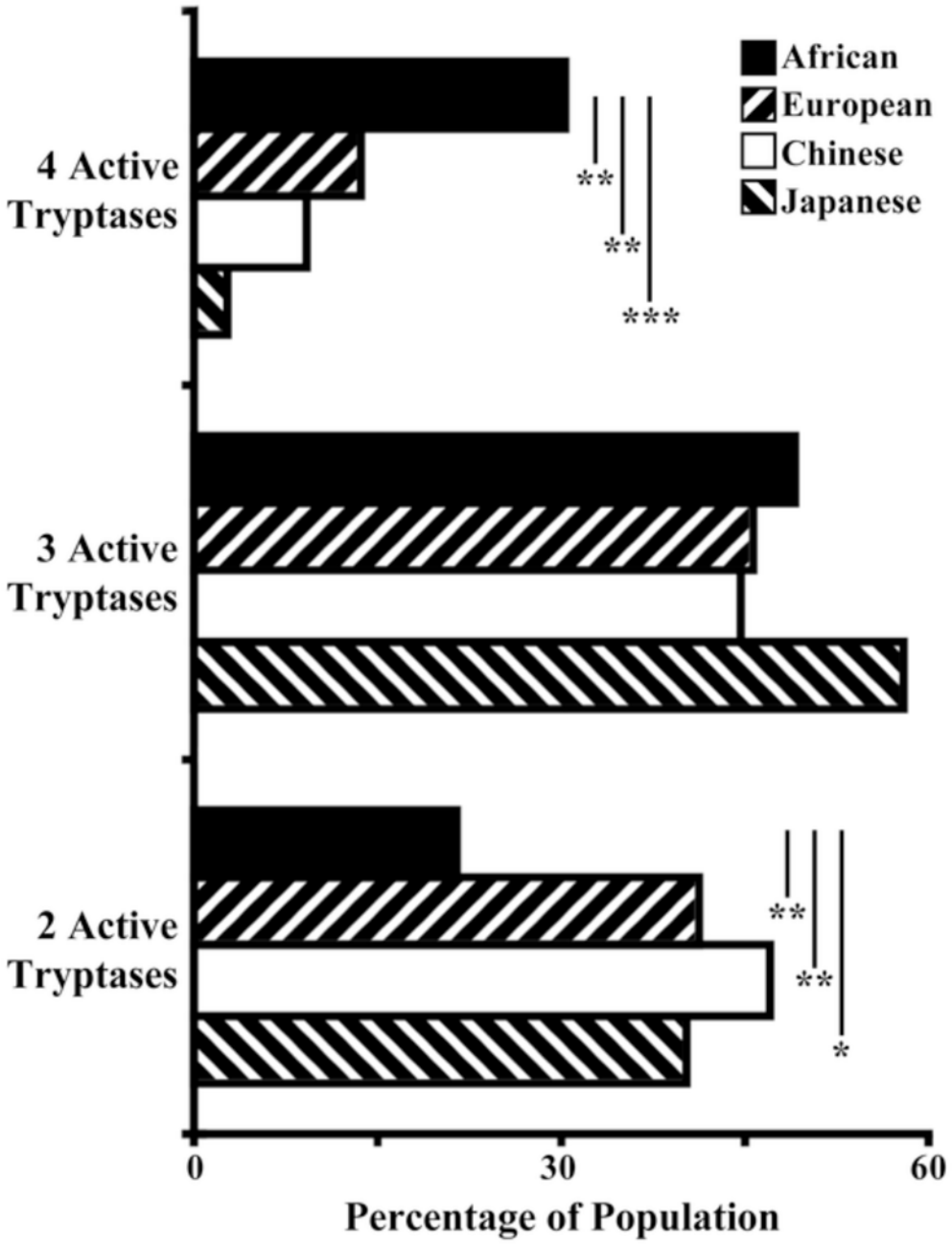

FIG 6.

Active tryptase gene dosage in four populations. *, **, *** represent P values < 0.05, < 0.001, and < 0.0001, respectively.