

Genetic deficiency of human mast cell α -tryptase

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Summary

Background Human α - and β -tryptases are proteases secreted by mast cells. Beta (but not α) tryptases are implicated in asthma. Genes encoding both types of tryptases cluster on chromosome 16p13.3.

Objective This study examines the hypothesis, generated from mapping data, that α -alleles compete with some β -alleles at one locus and that an adjacent locus contains β -alleles exclusively. This hypothesis predicts that β -alleles outnumber α and that some genomes lack α genes altogether.

Methods To test this hypothesis, we developed PCR-based techniques to distinguish α from β genes. We then genotyped genomic DNA from individuals and tryptase-expressing cell lines.

Results In support of our hypothesis, we find that α -tryptase deficiency affects 80/274 (29%) of individuals surveyed. The genotype of the α -deficient individuals is $\beta\beta\beta\beta$, due to inheritance of four β genes. The percentage of the population with the mixed genotypes $\alpha\alpha\beta\beta$ and $\alpha\beta\beta\beta$ is 21% and 50%, respectively. Accounting for all α - and β -alleles at the tandem loci on 16p13.3, overall α -allele frequency is only 0.23, with β -alleles considerably outnumbering α as hypothesized. In samples of defined ethnicity, α deficiency affects 45% of Caucasians, but a much lower percentage of other backgrounds, including African-Americans and Asians. Examination of cell lines reveals that HMC-1 and U-937 lack α -genes; thus, lack of α transcripts in these cells is due to absence of α -genes rather than β -selective transcription. By contrast, α -transcribing Mono Mac 6 and KU812 cells contain α - and β -genes.

Conclusions Genetic α -tryptase deficiency is common and varies strikingly between ethnic groups. Because β -tryptases are implicated in allergic disorders, inherited differences in α/β -genotype may affect disease susceptibility, severity and response to tryptase inhibitor therapy.

Keywords HMC-1, KU812, mast cell, mono Mac 6, tryptase, U-937

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Introduction

Tryptases are the most abundant proteins expressed and secreted by human mast cells and are postulated to contribute to the pathology of asthma and other allergic diseases [1, 2]. α -Tryptase was the first human tryptase characterized by cDNA cloning [3]. Later it became clear that the enzymes isolated from tissue extracts and purified mast cells belong to a second group of tryptases, termed β [4, 5]. α -Tryptases circulate in the blood of most normal individuals at baseline and can reach very high levels in the setting of systemic mastocytosis [6], while β -tryptase is the principal type identified in the circulation following systemic anaphylaxis [7]. β -Tryptases are 98 to 99% identical to each other by amino acid sequence [4, 8–10], while α -tryptases are *c.* 93% identical to β -tryptases in amino acid sequence. Notwithstanding the predicted differences in amino acid sequence, α and β genes are remarkably similar in

nucleotide sequence. For example, genomic clones AF099144 and AF098328 encompassing the α II and β I genes and flanking regions are 98% identical in nucleotide sequence over more than 5 kilobases of aligned sequence [9].

Despite their similarity to β -tryptases, α -tryptases possess unique features that call into question their biological importance. They lack a basic amino acid in the propeptide thought to be crucial for activation from the zymogen form in which they are first synthesized [11]. Although expression of catalytically active α -tryptase can be achieved in insect cells by provisioning the proenzyme with an engineered, cleavable propeptide [12], no active forms of α -tryptase have been identified in human tissues or cell lines. Thus, α -tryptases may be released in a form devoid of catalytic activity. If enzymatic activity is as important for function of α -tryptases as it is for most proteases, α -tryptases may have limited biological roles and less inflammatory potential than β -tryptases. Although most preparations of tissue mast cells contain ample mRNA encoding both α - and β -types of tryptases [13], protein is only found from β -tryptase, and α -tryptase in plasma is hypothesized to be made up of the inactive proenzyme form secreted from mast cells [6, 14]. Basophils, which may express α mRNA preferentially [13], also

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potentially contribute to plasma levels, especially in allergic conditions [15]. However, in normal individuals, levels of tryptase mRNA and protein expression in basophils is orders of magnitude lower than in mast cells.

This laboratory's efforts to identify tryptase polymorphisms and to uncover previously undetected members of the tryptase gene family led to the discovery and mapping of a collection of closely associated tryptase genes on human chromosome 16p13.3 [9, 10]. This cluster accommodates genes encoding α , β and γ /transmembrane tryptases, as well as pseudogenes encoding homologues of mouse mast cell protease (mMCP)-7, canine mastin, and additional serine protease genes. Evidence suggesting that α and β genes are arrayed tandemly in two loci *c.* 12 kb apart, includes: (i) sequencing of genes; (ii) characterization and alignment of overlapping segments of 16p13.3 contained in bacterial artificial chromosomes (BACs); (iii) identification and characterization of restriction fragment length polymorphisms (RFLPs); (iv) PCR analysis of tryptase isoforms; and (v) comparisons with sequences contributed by the Human Genome Project [9, 10]. This laboratory's prior analysis suggests that the locus accommodating α -tryptase also accommodates β I-tryptase [10]; however, only β -tryptases II and III are found at the adjacent locus. There is no evidence that β -alleles at the α locus can also serve as alleles at the adjacent β -only locus. Thus, at one of two loci, α - and some β -tryptases appear to have an allelic relationship. The γ -tryptase, mMCP-7-like/ δ -tryptase and mastin-like genes reside nearby in separate loci. The unusual finding that α - and some β -alleles appear to compete at one locus led us to hypothesize that some individuals may have an 'all- β ' genotype, with no α -genes. The current work tests this prediction by assaying genomic DNA from individuals and established tryptase-expressing cell lines. We find that genetic α -tryptase deficiency is surprisingly common in human populations and that it also affects cell lines commonly used to study mast cell and tryptase function. Because the biological behaviour of the two types of tryptases differs, the identified variations in inherited tryptase genotype may be pathophysiologically important.

Materials and methods

Prediction of tryptase polymorphisms and allele assignment to specific loci

To assist in the design of PCR strategies with which to distinguish α and β tryptases, we examined cDNA and gene sequences from the following sources: (i) our published sequences of β and γ cDNAs (β I-III, γ I, γ II) [4, 10] and α , β , γ and mMCP-7-like genes (α II, β I-III, γ I, γ II, mMCP-7-like I and II) [4, 9, 10]; (ii) α and β cDNAs from human tissues, mast cell preparations, and cell lines sequenced and published by other investigators [3, 5, 16–18]; and (iii) partial cDNA and genomic tryptase sequence deposited in GenBank expressed sequence tag (EST) and high throughput genome sequence (HTGS) databases. To assign tryptase alleles to specific loci in the multigene cluster, we correlated our sequence analysis with our recently published maps of the chromosome 16p13.3 tryptase locus derived from extensive sequencing, restriction mapping and alignments of BACs containing large, overlapping portions of the cluster [9, 10].

Detection of an EcoRV polymorphism in exon 4

We developed a semiquantitative PCR-based assay to amplify a 1028-bp fragment from all known α - and β -tryptase genes and to bracket exon 4, which contains an *EcoRV* RFLP. Amplimers from α (but not β) genes are cleaved by *EcoRV* at a single site to yield fragments of 350 and 678 bp. The primers used in the amplification are as follows: 5'-GCCCTGCCCT GTGA GGCCC-3' and 5'-CCGTGTAGGC GCCAAGGTG-3'. These primers do not recognize γ -tryptase genes, mMCP-7-like or mastin pseudogenes in the cluster. Cycling was conducted as follows: 95 °C for 10 min (one cycle); 95 °C for 30 s, 63 °C for 60 s and 72 °C for 60 s (35 cycles). The resulting amplimers were digested with *EcoRV*, electrophoresed in 1% agarose gels and visualized with ethidium bromide. As a positive control for amplification of α genes we used subclone λ 324F of BAC 324 as template. As reported by this laboratory [9], this subclone contains a complete α gene but no β genes. As a further positive control for the detection of α in the presence of β genes, we used all of BAC 324 as a template. This BAC contains one α and one β gene [9]. Similarly, as a negative control for α -tryptase we used the λ 48B subclone of BAC 48 as template. This subclone contains a β I gene but no α gene [9]. In this assay, samples with equal numbers of α and β genes (e.g. those of genotype $\alpha\alpha\beta\beta$) produce a 678-bp band that is only about one-third less intense than the 1028-bp fragment after digestion with *EcoRV*. However, samples with a 3 : 1 excess of β genes (e.g. those of genotype $\alpha\beta\beta\beta$) generate a much weaker α band.

Detection of a deletion in α -tryptase intron 4

Comparison of α and β tryptase gene sequence reveals extensive homology in introns as well as exons [9]. However, a conspicuous difference between the α gene sequence and all known β sequences is a 10- or 11-bp deletion in intron 4 [9]. To exploit this deletion as a means of detecting α -tryptase genes in genomic DNA, we designed PCR primers (5'-GCGATGTGGA CAATGATGGT GGGTCTGG-3' and 5'-GAAGAGGGTG CAGCCTGAGG AGG-3') to bracket the deletion site. These primers do not recognize γ -tryptase, mMCP-7-like or mastin genes due to extensive sequence deviation in intron 4 compared with α/β tryptases. Cycling was conducted as follows: 95 °C for 1 min (one cycle); 95 °C for 30 s, 57 °C for 45 s and 72 °C for 45 s (35 cycles). The resulting amplimers were electrophoresed in 10% polyacrylamide or 4% agarose gels and visualized with ethidium bromide.

Screening of cell lines for genomic α -tryptase deficiency

Among several lines reported to transcribe α and/or β tryptase genes, there is diversity of tryptase phenotype, with lines expressing one or another type exclusively, or both simultaneously. To correlate genotype with phenotype in such cells, we subjected genomic DNA isolated from HMC-1, KU812, U-937 and Mono Mac 6 cells (all reported to transcribe tryptases) to the PCR assays described above. HMC-1 cells derive from a mast cell leukaemia, express β - and γ -tryptase protein [10, 19] and transcribe two variants of β I tryptase mRNA [18, 20]. These cells were obtained from Dr Joseph Butterfield at the Mayo Clinic. KU812, a line with basophilic features transcribing β II tryptase [17] and, to a lesser extent, α [21], was obtained from Dr Edward Goetzl at the University of California at San Francisco. U-937, a myelomonocytic line transcribing β I

trypsinase [16], was obtained from the University of California at San Francisco Cell Culture Facility. Mono Mac 6 cells, also myelomonocytic and reported to transcribe α -trypsinase exclusively [16], were obtained from Dr William Mobley at the University of California at San Francisco. All cell lines were cultured at 37 °C/5% CO₂ in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, L-glutamine, penicillin and streptomycin.

Screening for α -trypsinase deficiency in human populations

To detect α -trypsinase deficiency in genetically diverse individuals, we used the above PCR assays to screen genomic DNA from the DNA Polymorphism Discovery Resource [22] developed by the National Human Genome Research Institute. The samples were obtained from the Coriell Institute for Medical Research via the Nelson Freimer Laboratory at the University of California at San Francisco. The individuals in the samples we tested are of African, Asian, Caucasian, Mexican and Native American descent and resident in the United States of America (USA). Additionally, we examined 31 samples of individually unspecified ethnicity from a group of samples with ethnic ratios of African, Asian, Caucasian, Mexican and Native Americans of 4:4:4:2:1. We used the *EcoRV* RFLP assay to determine the percentage of the sampled populations with each of the three genotypes, $\alpha\alpha\beta\beta$, $\alpha\beta\beta\beta$ and $\beta\beta\beta\beta$. We then compared the observed distribution of genotype frequencies with those expected based on α alleles being in equilibrium with β I alleles at the first locus. To estimate α trypsinase allele frequency α_f we assume that β trypsinases reside at two sites in the genome, that α -genes compete with β at the first site, and that only β genes reside at the second site. Individuals with no detectable α -genes by our PCR screening approaches are assumed to be β -homozygous (i.e. to have two β alleles at each of two sites).

To calculate frequency of α -null individuals in the population, we started with the observed β homozygote frequency at the first site, which is the square of the β allele frequency β_f at the first site. Therefore, β_f is the square root of the measured α -null ($\beta\beta\beta\beta$) frequency of the population. Given that $\alpha_f + \beta_f = 1$ at the first site, $\alpha_f = 1 - \beta_f$. The frequency of α homozygosity (i.e. genotype $\alpha\alpha\beta\beta$) at the first site is α_f^2 . The frequency of $\alpha\beta$ heterozygotes (genotype $\alpha\beta\beta\beta$) at the first site is $2\alpha_f\beta_f$, assuming that the alleles are in equilibrium in the population under study. Given that only β alleles can occupy the second gene site, the overall α allele frequency α'_f is $\alpha_f/2$. To confirm the predicted absence of α genes in human genomic samples lacking PCR evidence of the *EcoRV* RFLP and the intron 4 deletion, a sample of the *EcoRV*-resistant 1028-bp amplicons was sequenced directly.

Results

Identification of α -trypsinase gene-specific features

Examination of cDNA and genomic exon sequence encoding exon 4 of known trypsinases revealed an *EcoRV* site featured in all published α -trypsinase cDNAs, ESTs and genes. As shown in the expanded map of the multigene trypsinase locus in Fig. 1, the α -specific *EcoRV* cleavage site gives rise to an RFLP in *EcoRV*-digested genomic DNA. This polymorphism arises from a single, silent nucleotide change (GAC to GAT; see Fig. 2) in the codon for Asp102 (standard chymotrypsinogen

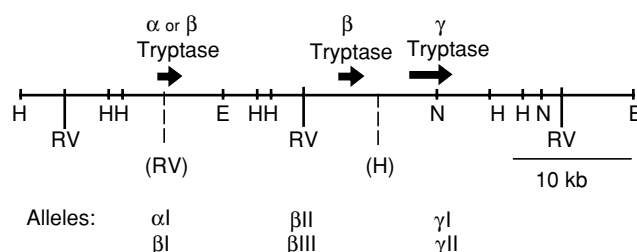


Fig. 1. Map of chromosome 16p13.3 multigene human trypsinase locus with restriction polymorphisms. Positions of known, actively transcribed trypsinase genes are shown. Arrows indicate size of genes and direction of transcription. Positions of all *HindIII* (H), *EcoRV* (RV), *EcoRI* (E) and *NotI* (N) restriction endonuclease sites in this interval are shown. Sites of genetic variation giving rise to restriction length polymorphisms, including the *EcoRV* polymorphism distinguishing α from β trypsinases, are indicated in parentheses. Known alleles accepted at each position are listed beneath the appropriate site in the map. The left locus accepts α and β trypsinase alleles. The middle locus accepts β II and β III but not α alleles. There is no evidence of interchange of alleles between these two loci. The right locus accepts only γ (transmembrane) trypsinase alleles. The α , β I-III and γ alleles correspond to previously described trypsinase genes and cDNAs.

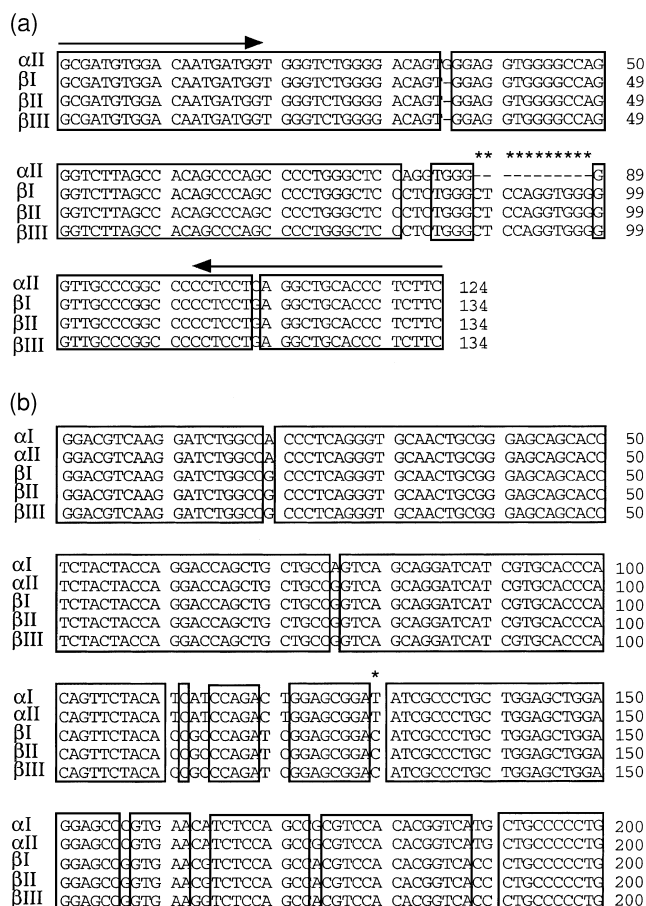


Fig. 2. Comparison of α and β trypsinase nucleotide sequences. Panel (a) shows partial α I and β II gene sequences (GenBank accession numbers AF098328, AF099144, AF099146, AF099143) in the vicinity of the 11-nucleotide deletion in intron 4 of α genes. Arrows indicate the position of PCR primers used to amplify fragments bracketing the deletion site (marked by asterisks; see Fig. 3). Panel (b) shows portions of α I, α II and β I-II exon 4 cDNA sequences (GenBank accession numbers M30038, AF098328, M33491, M33492 and M33493) surrounding the single nucleotide change (*) responsible for an *EcoRV* restriction fragment polymorphism, distinguishing α from β genes (see Figs 3 and 4).

numbering), which is one of the 'catalytic triad' residues essential for the function of every serine protease. This change is absent from any published β cDNA or gene (including unfinished β -like gene sequences in GenBank's HTGS databank), as well as from γ -tryptase genes and mMCP-7-like (δ tryptase) and mastin pseudogenes. In additional searches, an 11-bp deletion noted previously by us to reside in intron 4 of the α II gene compared with the homologous region of the β I gene [9] was not detected in any other sequenced β , γ , mMCP-7-like or mastin gene. Thus, comparisons with available tryptase sequences suggest that both the Asp102 *EcoRV* mutation and the 11-bp deletion are consistent, exclusive features of α -tryptase genes.

Genotyping of tryptase-expressing cell lines

Results of screens of tryptase-transcribing cell lines for the α -tryptase *EcoRV* polymorphism in exon 4 and the 11-bp deletion in intron 4 are shown in Fig. 3. Note that HMC-1 and U-937 cells show single bands in both tests, indicating the presence of β -like genes but absence of α . By contrast, KU812 and Mono Mac 6 cells yield three bands after *EcoRV* restriction and two bands after amplification across the intron 4 deletion site, indicating a mixture of α and β genes. In each case, the genotype appears to be $\alpha\alpha\beta\beta$ based on the relative intensity of 1028- and 678-bp bands in the *EcoRV* assay. Amplification across the 11-bp site generates a 10- (rather than 11-) nucleotide difference because α genes contain a single nucleotide insertion elsewhere in the amplified segment (see Fig. 2). Thus, two of the four known tryptase-transcribing human cell lines are genetically deficient in α -tryptase. Table 1 summarizes the findings from this and prior work concerning tryptase allele expression by human cell lines. Both of the cell lines expressing only β mRNA (i.e. HMC-1 and U-937) lack α genes. However, both cell lines that express α mRNA contain both α and β genes, consistent with the gene map and allele assignments in Fig. 1.

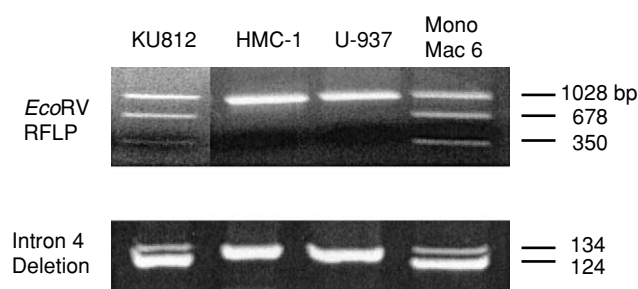


Fig. 3. Genotyping of tryptase-expressing human cell lines. Genomic DNA from KU812 cells (a line with basophilic features), HMC-1 cells (a line derived from mast cell leukaemic cells), and the myelomonocytic cell lines U-937 and Mono Mac 6 was tested for the presence of α vs. β tryptase genes by two independent PCR-based assays. Both panels show bands of amplified DNA after agarose electrophoresis and ethidium bromide staining. The top panel shows results of amplification across the polymorphic *EcoRV* restriction site that we identify in exon 4 of all known α cDNAs and genes but not in β tryptases. The PCR primers amplify a 1028-bp fragment of all α and β genes. However, only α -derived amplimers yield fragments upon digestion with *EcoRV*. The bottom panel shows results of amplification across an 11-bp deletion found in intron 4 of α -compared with β -tryptase genes. The β amplimer is 134 bp, whereas the α amplimer is only 124 bp. The results generated by both methods are mutually consistent and suggest that KU812 and Mono Mac 6 cells express a mixture of α - and β -tryptases, whereas HMC-1 and U-937 express only β -tryptases. Thus, HMC-1 and U-937 genotype is 'all- β '.

Genotyping of individuals

Based on screening of individual samples of genomic DNA using the PCR-based *EcoRV* RFLP and intron 4 deletion assays (see Fig. 4), we estimate α_f (frequency of the α allele at the single site that accepts α genes) to be 0.46. Direct sequencing of *EcoRV*-resistant amplimers from the two individuals predicted to lack α genes in Fig. 4 (lanes 1 and 4) reveals only β transcripts, as predicted. Overall α allele frequency α'_f , accounting for the two additional β alleles occupying the second genomic locus that does not appear to accept α genes, is 0.23. Therefore, overall β allele frequency β'_f , accounting for both loci, is 0.77. In this population sample, the frequency of $\alpha\alpha\beta\beta$, $\alpha\beta\beta\beta$ and $\beta\beta\beta\beta$ genotypes is 0.21, 0.50 and 0.29, respectively (Table 2). Using the observed frequency of individuals with the $\beta\beta\beta\beta$ genotype as the starting point for predicting the frequency of $\alpha\alpha\beta\beta$ and $\alpha\beta\beta\beta$ genotypes, one obtains nearly exactly the same frequencies (0.21 and 0.5) by applying the Hardy-Weinberg formula. This is evidence that α and β alleles are in equilibrium at the α/β site. The 274 individuals analysed are

Table 1. Tryptase gene and allele expression in cell lines

Cell line	Genes	mRNA	Citation
KU812	$\alpha + \beta$	$\alpha + \beta$ II	[17]
JHMC-1	β	β Ia + β Ib + γ	[10, 20, 21]
U-937	β	β Ia	[16]
Mono Mac 6	$\alpha + \beta$	α	[16]

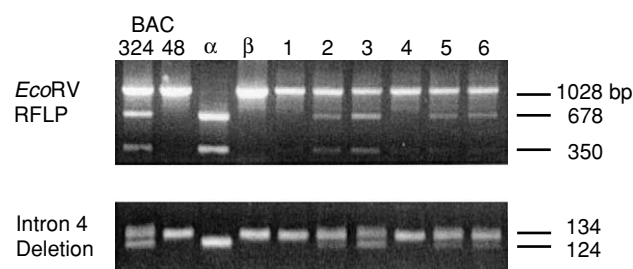


Fig. 4. Genotyping of DNA from genetically diverse individuals. The figure shows representative results of PCR-based screening for α - and β -tryptase genes based on the presence or absence of the exon 4 *EcoRV* RFLP and a 11-bp deletion in intron 4. See Fig. 3 legend for explanation of bands. The first 4 lanes are positive and negative controls for the detection of α -tryptase genes. The 'BAC 324' lane contains DNA amplified from a chromosome 16p13.3 BAC clone containing most of the multigene human tryptase locus, including α and β II genes. The 'BAC 48' lane contains DNA amplified from a 16p13.3 BAC clone containing β I and β III (but not α) genes. The ' α ' lane contains amplimers derived from λ 324F, which is a BAC 324 subclone containing an α -tryptase (but not β) gene. The ' β ' lane contains amplimers derived from I48B, which is a subclone of BAC 48 containing the β I tryptase (but no α) gene. Each of the remaining lanes (labelled 1 to 6) contains amplimers derived from genomic DNA of a different individual. Every individual yields a β band (1028 and 134 bp) but individuals '1' and '4' lack α bands (678, 350 and 124 bp). Note the concurrence (in controls as well as individuals) between the results of the two independent PCR approaches, i.e. lanes lacking α bands by one approach also lack α bands by the other approach. Individuals '1' and '4' are assumed to possess the $\beta\beta\beta\beta$ genotype. Note also the differences in relative intensity between α and β bands in different lanes. The α bands in lanes '2', '5' and '6' are weak relative to lane '3' α bands, which resemble those of BAC 48, which has equal numbers of α and β genes. Thus, individual '3' may have two α and two β genes ($\alpha\alpha\beta\beta$ genotype), whereas individuals '2', '5' and '6' may have just one α and three β ($\alpha\beta\beta\beta$) genotype.

Table 2. Tryptase genotype in human populations

Background	n*	Genotype(%)†		
		$\alpha\alpha\beta\beta$	$\alpha\beta\beta\beta$	$\beta\beta\beta\beta$
African American	96	12 (13%)	59 (61%)	25 (26%)
Caucasian	93	14 (15%)	37 (40%)	42 (45%)
Chinese American	10	5 (50%)	4 (40%)	1 (10%)
Japanese American	10	2 (20%)	7 (70%)	1 (10%)
South-east Asians	10	3 (30%)	5 (50%)	2 (20%)
Mexican	10	4 (40%)	5 (50%)	1 (10%)
Pacific Islander	9	2 (22%)	5 (56%)	2 (22%)
Native American	5	3 (60%)	1 (20%)	1 (20%)
Not Identified	31	12 (39%)	14 (45%)	5 (16%)
Overall	274	57 (21%)	137 (50%)	80 (29%)

*Number of samples genotyped. †Number and percentage of total with this genotype in a given population.

mainly from two groups: African-American and Caucasian. The remainder are of other groups or are not identified by ethnicity. The frequency of α -deficiency is 0.45 in Caucasians, 0.26 in African-Americans, and 0.15 in individuals of other identified backgrounds. In the entire surveyed sample, the frequency is 0.29. Thus, the α -null ($\beta\beta\beta\beta$) genotype is fairly common in the population overall.

Discussion

The findings reported here support our hypothesis, based on fine mapping of the multigene human tryptase locus, that α -tryptase alleles compete with β -alleles at one of the two sites available to β alleles and that genetic α -tryptase deficiency is common. Our findings suggest an explanation for the failure to detect α -tryptase mRNA or protein in certain samples in prior investigations. For example, many patients with systemic mastocytosis have high levels of immunoreactive α -tryptase, but some do not [6]. Also, when mast cells are purified from lung or skin of individual donors, some samples contain approximately equal levels of α and β mRNA, whereas others contain β but no detectable α mRNA [21]. Similar variations in mRNA expression are seen in cell lines (Table 1). For example, α mRNA is absent in HMC-1 and U-937 cells but present in KU812 cells, whereas β mRNA is abundant in all three cell types [16, 17, 21]. Mono Mac 6 cells, on the other hand, transcribe mainly α mRNA [16].

The basis of such variation has been presumed to be due to differences in independent expression of α and β genes from separate loci. While there may in fact be independent regulation of tryptases from the two loci (as suggested by preferential expression of α in Mono Mac 6 cells), our data indicate that the failure of some individuals or cell lines to express α has a simpler explanation: the lack of any α -gene. In the case of the HMC-1 cells, two β -alleles (Ia and Ib) are transcribed in approximately equal amounts. This finding supports our prediction that β I is the β type that shares a single locus with α -alleles. According to this prediction, expression of two β I alleles precludes the possibility of α expression. Similarly, in α -null U-937 cells, only β Ia mRNA has been found [16]. Presumably, these cells are β Ia-homozygous and thus lack α -alleles. They also fail

to express β II/ β III alleles from the expected β -only locus. This suggests that tryptase genes can be transcribed independently from the two loci. KU812 cells provide further evidence in this regard. Although simultaneous expression of β II and α alleles in KU812 cells suggests that genes are actively transcribed from alleles associated with both of the two loci, mRNA quantification suggests large inequality of expression at the two loci because β far exceeds α expression in these cells [21].

Our data also suggest a basis for this laboratory's previous finding of three different β cDNAs (β Ia, II and III) in a library prepared from a single donor [4]. This finding is inconsistent with a genetic model consisting of two tryptase loci, one exclusively α and the other β . Our new model, shown in Fig. 1, allows for as many as four different β alleles in an individual. The genotype of the donor in the prior study is presumably $\beta\beta\beta\beta$. Our new model predicts that an individual inherits at most two α genes due to unavailability of the second site to α alleles. Due to gene dosage effects, the number of inherited α and β genes may influence the relative amounts of each type produced by mast cells of a given genotype. Since α -tryptase is not stored, its levels are said to reflect total body burden of mast cells, whereas levels of β -tryptase, which rise dramatically after anaphylaxis but are often below limits of detection at baseline, reflect mast cell activation [14]. Our data predict that in persons with no α -tryptase genes ($\beta\beta\beta\beta$ genotype), total serum tryptase levels may underestimate mast cell burden due to absence of a constitutively secreted form of tryptase. Our findings also predict that laboratory tests relying on measurement of levels of α relative to β -tryptase to diagnose systemic mastocytosis [14] will not detect patients with the $\beta\beta\beta\beta$ genotype. In contrast, persons with two inherited α -tryptase genes ($\alpha\alpha\beta\beta$ genotype) may have higher serum levels of α -tryptase than those with just one gene ($\alpha\beta\beta\beta$ genotype), despite equivalent mast cell burdens. Serum tryptase levels may overestimate mast cell burden in these patients. The high frequency with which serum α -tryptase levels are elevated in patients with systemic mastocytosis suggests the possibility that inheritance of α -tryptase genes increases susceptibility to development of the disorder.

The results reported in this paper clarify findings in connection with an *EcoRV* RFLP reported by Schwartz and colleagues [23], who detected 13- and 20-kb bands on blots of *EcoRV*-digested human genomic DNA hybridized with β II tryptase cDNA. Some individuals exhibited just the 20-kb band, others both the 20 and 13-kb bands. None had the 13-kb band alone. The constancy of the 20-kb band can now be understood by examining the restriction map of the extended locus shown in Fig. 1. In an individual with no inherited α gene, three *EcoRV* sites bracket the two loci occupied by β -tryptases. The three *EcoRV* sites are separated by 20-kb intervals, so that a restriction digest generates superimposed 20-kb bands upon electrophoresis, blotting and hybridization with tryptase probe. When an α -tryptase gene occupies one of the loci, it introduces a new *EcoRV* site interrupting one of the 20-kb fragments. Because β -tryptase cross-hybridizes with α -tryptase, a new 13-kb hybridizing band is generated in the blot of the *EcoRV* digest. In a survey of 28 unrelated Caucasians, the frequency of the α -derived 13-kb 'allele' was 0.32, although this may have underestimated true allele frequency in the population because the method did not distinguish α single allele genotypes from double allele genotypes ($\alpha\beta\beta\beta$ vs. $\alpha\alpha\beta\beta$, respectively). Nonetheless, this estimate of allele frequency is similar to our estimate of

0.23 for overall α -allele frequency. In retrospect, the blots of genomic DNA with only 20-kb bands were early sightings of α -tryptase deficiency.

The two PCR-based tests introduced here for distinguishing α - from β -tryptases improve upon our previously reported use of *SacII* polymorphisms [9], which are less specific because one of the restriction sites is shared by the mMCP-7-like pseudogene. Techniques that use blotting approaches to distinguish α and β sequences are neither as convenient nor as well adapted to the use of small biological samples. A *BseAI* restriction site polymorphism was used previously to distinguish between α and β tryptases [6]. However, this approach does not distinguish α and β III genes, neither of which is cleaved by *BseAI*, and therefore overestimates α allele frequency.

Our findings are consistent with results of a survey of tryptase single nucleotide polymorphisms (SNPs) reported recently by Guida et al. [24], although we reach different conclusions concerning the prevalence and nature of α -tryptase gene deficiency. β genes are not as polymorphic as the SNP study initially suggested, because, as we show in Fig. 1 as well as in other recent work [9, 10], β genes are distributed among two loci rather than merely one. The Guida study did not identify SNPs specifically associated with the β III allele, even though this allele is likely to be common based on the identification of a cDNA, full-length gene and ESTs from several individuals [4, 9, 10]. This failure to detect the β III allele may be due to a PCR strategy involving β primers mismatched in the region of β III exon 3. Their PCR approach, which is designed to detect single nucleotide variations rather than larger insertions or deletions, also misses the 11-bp α intron 4 deletion that we use in the current study to distinguish α and β genes.

Interestingly, Guida et al. were unable to amplify α gene fragments from c. 20% of screened samples of genomic DNA from individual donors. They hypothesized that a sizeable fraction of the surveyed population possesses large genomic deletions of the α locus. Our own data suggest that these are not persons with genomic deletions but with allelic substitution of β for α at the $\alpha + \beta$ locus. Nonetheless, the observed overall prevalence of α -deficiency is similar to the one we report here. One SNP reported by Guida et al. is a single base insertion found in 20% of Caucasians and causing a frameshift that would lead to formation of an inactive product. Assuming the hypothesis that α -tryptases are non-functional is correct, we predict that the rare individual inheriting two α genes and two insertionally mutated β genes will have no functional mast cell tryptases.

Additional support for our gene alignments and allelic assignments in the tryptase cluster stems from the recently published results of an international effort to fully sequence and annotate chromosome 16p13.3 [25]. These investigators detect a β I gene at the predicted α/β site, and a β III gene at the β -only site, followed by a γ I-gene, consistent with our scheme [10] (see Fig. 1). Interestingly, they also provide evidence that the chromosome in the immediate vicinity of the tryptase cluster manifests a number of unusual features, including high rates of genetic recombination and chromosomal breakage, delayed synthesis or separation of chromatids during chromosomal replication, and resistance to cloning and sequencing. In addition, three of the tryptase loci are part of tandem and inverted repeats that are the largest such repeats at any site in 16p13.3's ~2 megabases of DNA. The present condition of the human

tryptase locus may have resulted from fairly recent gene duplications and conversions in a particularly unstable region of the genome [9, 10].

In summary, genetic α -tryptase deficiency is widespread and fairly common, although variable, in human populations. It remains to be seen whether ethnic variations in α -deficiency are linked to variations in susceptibility to allergic diseases and systemic mastocytosis. However, it is tempting to speculate that α -genes are partially protective in allergy because they occupy a site that would otherwise contain β -genes producing the stored, active forms of tryptases released during immediate hypersensitivity reactions.

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