Published in final edited form as:
Clin Immunol. 2007 June ; 123(3): 268–271.

Tryptase haplotype in mastocytosis: Relationship to disease variant and diagnostic utility of total tryptase levels

Cem Akin[1,5], **Darya Soto**[2], **Erica Brittain**[3], **Adhuna Chhabra**[1], **Lawrence B. Schwartz**[4], **George H. Caughey**[2], and **Dean D. Metcalfe**[1]

1*Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases; NIH, Bethesda, MD 20892*

2*Department of Medicine and Cardiovascular Research Institute, The University of California at San Francisco, San Francisco, CA 94143*

3*Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases; NIH, Bethesda, MD 20892*

4*Division of Rheumatology, Allergy and Immunology, Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA 23298*

Abstract

Serum mast cell tryptase levels are used as a diagnostic criterion and surrogate marker of disease severity in mastocytosis. Approximately 29% of the healthy population lacks α tryptase genes; however, it is not known whether lack of α tryptase genes leads to variability in tryptase levels or impacts on disease severity in mastocytosis. We have thus analyzed tryptase haplotype in patients with mastocytosis, computing correlations between haplotype and plasma total and mature tryptase levels; and disease category. We found: (1) the distribution of tryptase haplotype in patients with mastocytosis appeared consistent with Harvey-Weinberg equilibrium and the distribution in the general population;(2) the disease severity and plasma tryptase levels were not affected by the number of α or β tryptase alleles in this study; and (3) information about the tryptase haplotype did not provide any prognostic value about the severity of disease. Total and mature tryptase levels positively correlated with disease severity, as well as prothrombin time and partial thromboplastin time, and negatively correlated with the hemoglobin concentration.

Keywords

Mastocytosis; tryptase; haplotype; genotype; allele; prothrombin time; partial thromboplastin time

Introduction

An elevated baseline total tryptase level is a minor diagnostic criterion for systemic mastocytosis according to World Health Organization criteria[1]. Tryptases are serine proteases produced by mast cells [2;3]. Among members of the tryptase family in humans, including α, β, δ [4], ε [5] and γ (transmembrane) [6;7], the two that are most abundantly

Corresponding author: Cem Akin, M.D., Ph.D., University of Michigan, 5520-B, MSRB-1, Box 0600, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-0638, Phone: 734-647-6234, Fax: 734-763-4151, E-mail: cemakin@umich.edu
[5]Currently at the Division of Allergy and Immunology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109

expressed in and secreted by mast cells are α and β tryptases. Tryptases ε and γ are ≤50% identical to α and β tryptases, while δ-tryptase, though more closely related to α/β-tryptases, is truncated 40 amino acids shy of the C-terminus of α/β tryptases and appears to be less abundantly expressed [8;9]. Human mature β tryptase is stored in mast cell granules and released upon activation while α tryptase is apparently processed only to the proenzyme stage and is constitutively secreted from mast cells along with β protryptase [10]. Therefore, serum or plasma levels of mature β tryptase are found transiently elevated after a mast cell degranulation event such as anaphylaxis, while the levels of the precursors of α/β tryptase are reported to reflect the total body mast cell burden; and to be elevated in patients with mastocytosis when compared to the general population [11;12;13].

The genes encoding α and β tryptases are located in 2 loci in close proximity to each other on chromosome 16p13.3 [4]. Mapping data suggests that α alleles compete with β alleles at one locus, while an adjacent locus contains β alleles exclusively (αβ versus ββ haplotype) [7]. Accordingly, it has been shown that 20-29% of the normal population lacks α tryptase and has a ββ:ββ genotype [10;14;15].

The αβ tryptase haploytpe and female gender are associated with a small but statistically significant elevation of circulating tryptase levels in healthy individuals [16]. It is, however, not known whether patients with mastocytosis have the same allelic distribution of α and β tryptase genes as the general population and whether those lacking the α tryptase gene have a corresponding decrease in circulating tryptase protein levels. The World Health Organization's (WHO) diagnostic criteria for systemic mastocytosis do not take the α/β tryptase haplotype into account. Because of the potential impact of this haplotype on these criteria, we have examined the α/β tryptase genes in a cohort of patients with mastocytosis and evaluated the correlations of the α/β tryptase genotype with category of disease, serum tryptase levels and hematologic laboratory values.

Patients and Methods

Thirty one patients (13 males) with mastocytosis were examined after signing informed consent. All patients were diagnosed and classified into a disease category according to the World Health Organization's criteria. The numbers of patients in each disease category in the order of increasing disease severity were as follows: 4 patients with pediatric-onset cutaneous mastocytosis (CM), 24 with indolent systemic mastocytosis (ISM) (9 with the subvariant smouldering systemic mastocytosis [SSM]), 2 with systemic mastocytosis with associated clonal hematologic non-mast-cell lineage disease (SM-AHNMD) and 1 with aggressive systemic mastocytosis (ASM).

Plasma was collected in EDTA tubes and separated with centrifugation for 10 minutes at 750g. Tryptase levels were measured by immunoassay, using G5 and G4 monoclonal anti-tryptase antibodies to capture mature (mainly β) and total tryptase (combination of α, and β protryptases and mature [primarily β] tryptases) respectively, as described [12]. Direct ELISA measurement of α protryptase is not currently possible as there is no antibody that distinguishes α from β pro or mature tryptases. Determination of α and β tryptase haplotypes in the genomic DNA from peripheral blood leukocytes was performed by PCR using primers across the site of the intron 4 deletion in the α tryptase gene as described [15].

Statistical analyses were carried out by using non-parametric Spearman's correlation test. For the purpose of correlating disease severity with other parameters, each disease category described above was assigned a numerical severity value from 1-5 according to extent of disease and prognosis, with cutaneous mastocytosis as 1 and aggressive systemic mastocytosis as 5. Results with p-values less than 0.05 were considered statistically significant.

Results

Seven of 31 (23%) patients with mastocytosis lacked the α tryptase gene while 8 (26%) patients had 2 α tryptase alleles. This distribution is similar to that of the general population and is consistent with a population in Hardy-Weinberg equilibrium. These results suggest that patients with mastocytosis do not have a skewed distribution of α tryptase alleles.

There was a strong correlation between the severity of mastocytosis category and plasma total, and mature tryptase levels (Spearman's r 0.81, $p<0.001$; and 0.55, $p<0.01$ respectively). There was, however, no correlation between the category of mastocytosis and tryptase genotype (Table 1).

Total and mature plasma tryptase levels were not significantly associated with the number of β alleles in tryptase genotypes (Figure 1). Although there appeared to be a trend of increasing mature tryptase levels with the number of β alleles, this was not statistically significant ($p= 0.218$).

Analysis of tryptase haplotype with respect to routine hematologic parameters also failed to show any significant correlation with WBC or platelet counts. Total and mature tryptase levels inversely correlated with hemoglobin ($r= -0.36$ and -0.37 respectively, $p<0.05$), and positively correlated with the prothrombin time (PT) ($r=0.65$, $p<0.001$ and $r=0.56$, $p<0.01$, respectively) and the partial thromboplastin time (PTT) ($r=0.46$ and 0.37; $p<0.05$ for both). These parameters did not correlate with the number of β alleles and the correlations of both PT and PTT with these tryptase levels were essentially unchanged after adjustment was made for the α/β tryptase genotype.

Discussion

Baseline total tryptase levels in mastocytosis are thought to reflect enzymatically inactive α and β protryptases that are constitutively secreted, even though a portion of β-protryptase undergoes intracellular processing to yield mature tryptase and is then stored in secretory granules [2]. Circulating protryptase levels thus appear to reflect the spontaneous secretion of both α and β protryptases, and possibly other tryptase precursors. Our results support the conclusion that while some patients with mastocytosis lack the α tryptase gene (ββ:ββ tryptase genotype), the deficiency of α tryptase does not appear to impact disease severity or total tryptase levels.

Min et al. have examined the effect of tryptase haplotype and gender on total tryptase levels in 106 healthy subjects, and found that the presence of an αβ haplotype was associated with a slight but statistically significant increase (0.5 ng/ml) above the mean in the serum level of total tryptase [16]. As mature tryptase levels are normally undetectable in healthy subjects at baseline, this study could not correlate the number of beta alleles with the serum level of mature tryptase.

Analysis of our data also revealed a previously unreported correlation between tryptase levels (both total and mature) and PT and PTT, although neither parameter correlated with tryptase genotype. The exact mechanism of this correlation is beyond the scope of this paper, however, one possible explanation may be the partial fibrinogenolytic activity of tryptase [17]. While the vast majority of circulating tryptase is enzymatically inactive protryptase, small quantities of mature tryptase released over time may increase fibrinogen degradation.

In conclusion, our data highlight the contribution of non-α protryptases (such as β protryptase) to baseline tryptase levels in patients with mastocytosis, and suggest that routine genotyping of tryptase alleles does not have any place in routine diagnostic workup of the patients with

mastocytosis. The recent discovery of α tryptase deficiency thus should not lead to reconsideration of the WHO diagnostic criteria for systemic mastocytosis.

Acknowledgements

This work is supported by NIH grants HL024136 (G.H.C. and D.S.) and AI20487 (LBS), and the Division of Intramural Research, NIAID.

References

1. Valent P, Horny H, Escribano L, Longley BJ, Li CY, Schwartz LB, Marone G, Nunez R, Akin C, Sotlar K, Sperr WR, Wolff K, Brunning RD, Parwaresch RM, Austen KF, Lennert K, Metcalfe DD, Vardiman JW, Bennett JM. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res 2001;25:603–25. [PubMed: 11377686]
2. Schwartz LB, Irani AM. Serum tryptase and the laboratory diagnosis of systemic mastocytosis. Hematol Oncol Clin North Am 2000;14:641–57. [PubMed: 10909044]
3. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin North Am 2006;26:451–63. [PubMed: 16931288]
4. 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. [PubMed: 9920877]
5. Wong GW, Yasuda S, Madhusudhan MS, Li L, Yang Y, Krilis SA, Sali A, Stevens RL. Human tryptase epsilon (PRSS22), a new member of the chromosome 16p13.3 family of human serine proteases expressed in airway epithelial cells. J Biol Chem 2001;276:49169–82. [PubMed: 11602603]Epub 2001 Oct 15
6. Wong GW, Foster PS, Yasuda S, Qi JC, Mahalingam S, Mellor EA, Katsoulotos G, Li L, Boyce JA, Krilis SA, Stevens RL. Biochemical and functional characterization of human transmembrane tryptase (TMT)/tryptase gamma. TMT is an exocytosed mast cell protease that induces airway hyperresponsiveness in vivo via an interleukin-13/interleukin-4 receptor alpha/signal transducer and activator of transcription (STAT) 6-dependent pathway. J Biol Chem 2002;277:41906–15. [PubMed: 12194977]
7. Caughey GH, Raymond WW, Blount JL, Hau LW, Pallaoro M, Wolters PJ, Verghese GM. Characterization of human gamma-tryptases, novel members of the chromosome 16p mast cell tryptase and prostasin gene families. J Immunol 2000;164:6566–75. [PubMed: 10843716]
8. Min HK, Kambe N, Schwartz LB. Human mouse mast cell protease 7-like tryptase genes are pseudogenes. J Allergy Clin Immunol 2001;107:315–21. [PubMed: 11174199]
9. Wang HW, McNeil HP, Husain A, Liu K, Tedla N, Thomas PS, Raftery M, King GC, Cai ZY, Hunt JE. Delta tryptase is expressed in multiple human tissues, and a recombinant form has proteolytic activity. J Immunol 2002;169:5145–52. [PubMed: 12391231]
10. Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, Moxley G, Fukuoka Y. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. J Immunol 2003;170:5667–73. [PubMed: 12759448]
11. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med 1987;316:1622–6. [PubMed: 3295549]
12. Schwartz LB, Sakai K, Bradford TR, Ren S, Zweiman B, Worobec AS, Metcalfe DD. The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. J Clin Invest 1995;96:2702–10. [PubMed: 8675637]
13. Schwartz LB. Clinical utility of tryptase levels in systemic mastocytosis and associated hematologic disorders. Leuk Res 2001;25:553–62. [PubMed: 11377680]
14. Guida M, Riedy M, Lee D, Hall J. Characterization of two highly polymorphic human tryptase loci and comparison with a newly discovered monkey tryptase ortholog. Pharmacogenetics 2000;10:389–96. [PubMed: 10898108]

15. 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. [PubMed: 12100045]

16. 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. [PubMed: 15241343]

17. Schwartz LB, Bradford TR, Littman BH, Wintroub BU. The fibrinogenolytic activity of purified tryptase from human lung mast cells. J Immunol 1985;135:2762–7. [PubMed: 3161948]

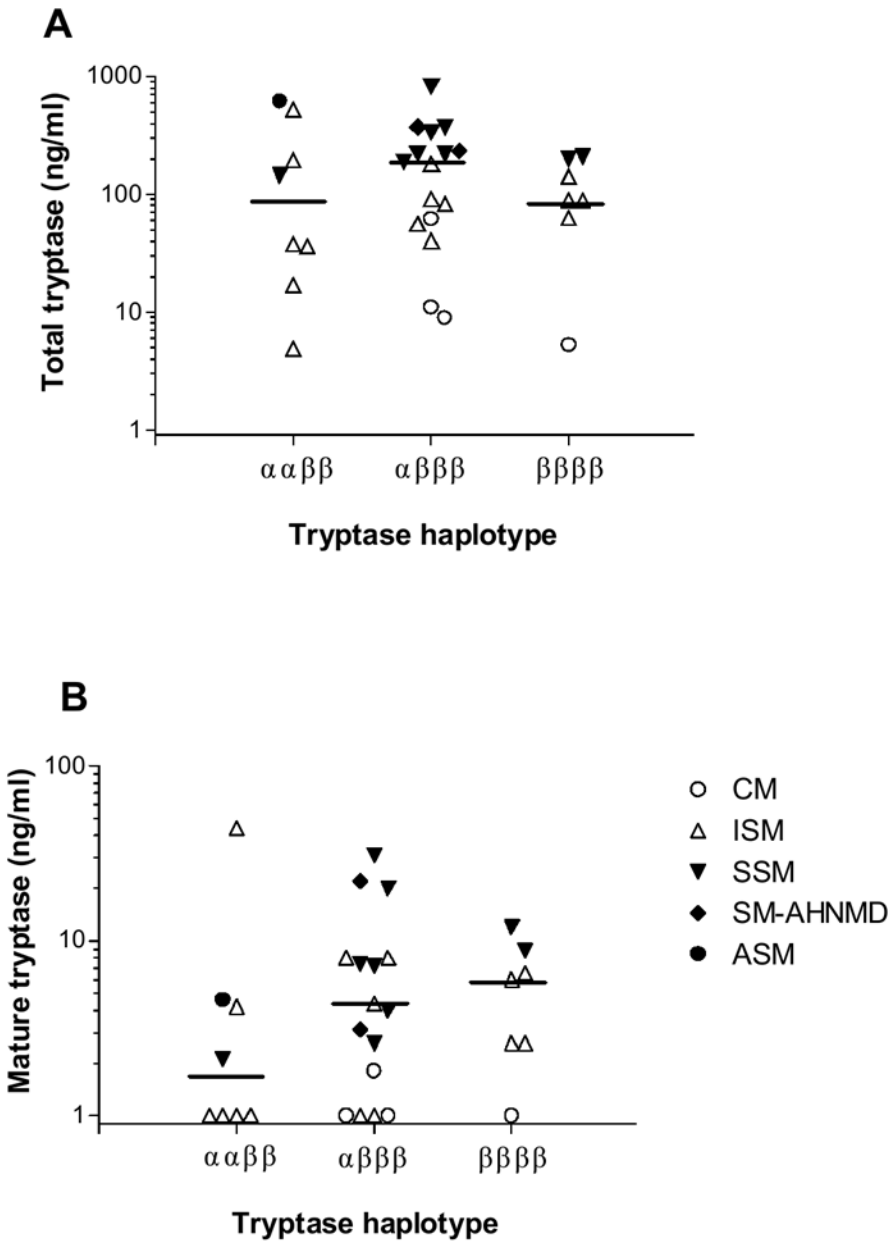

Figure 1.

Total (A) and mature (B) tryptase levels in patients with mastocytosis according to tryptase haplotype. Lines indicate median values for each category. CM, cutaneous mastocytosis; ISM, indolent systemic mastocytosis; SSM, smoldering systemic mastocytosis; SM-AHNMD, systemic mastocytosis with an associated hematologic clonal non-mast cell lineage disease; ASM, aggressive systemic mastocytosis.

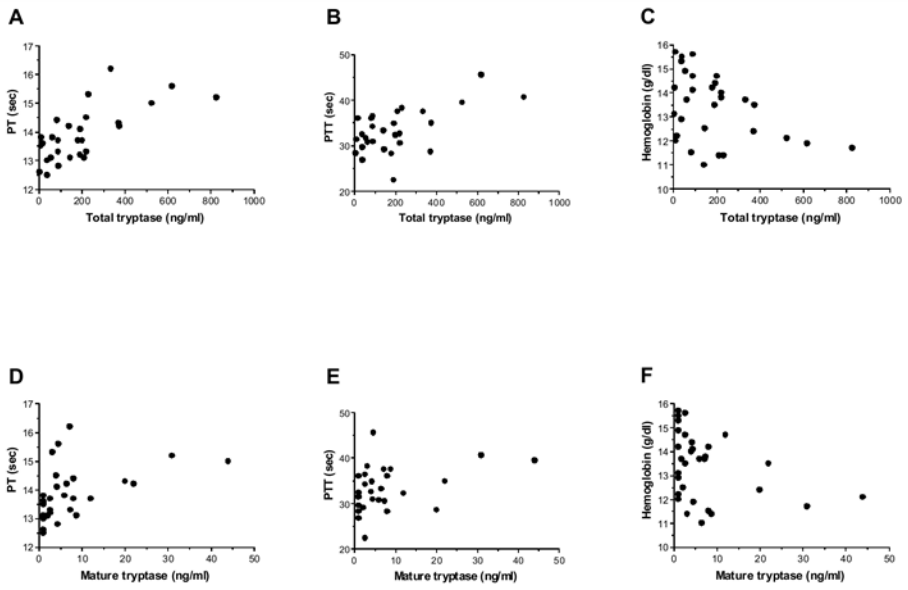

Figure 2.

Correlations of total (A-C) and mature (D-F) tryptase levels with prothrombin time (PT), partial thromboplastin time (PTT) and hemoglobin levels. Prothrombin time and partial thromboplastin time values were not available for all patients.

Table 1

Tryptase haplotype in patients with mastocytosis according to World Health Organization category of disease. CM, cutaneous mastocytosis; ISM, indolent systemic mastocytosis; SSM, systemic smoldering mastocytosis (a subcategory of ISM with extensive mast cell burden); SM-AHNMD, systemic mastocytosis with associated hematological clonal non-mast-cell lineage disease; ASM, aggressive systemic mastocytosis.

		Tryptase haplotype		
Category	**n**	**ααββ**	**αβββ**	**ββββ**
CM	4	0	3	1
ISM	15	6	5	4
SSM	9	1	6	2
SM-AHNMD	2	0	2	0
ASM	1	1	0	0
Total	31	8 (26%)	16 (52%)	7 (23%)