



SERIES: GENETICS IN ALLERGY (I)

## Tryptase: Genetic and functional considerations

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**Abstract** Tryptase is one of the main proteases located in the secretory granules of the mast cells, and is released through degranulation. It is therefore assumed to play an important role in inflammatory and allergic processes. Four genes are known to encode for these enzymes, with different alleles that give rise to different types of tryptases. The term "tryptase" generally refers to  $\beta$ -tryptase, which in vivo is a heterotetramer, possessing a structure of vital importance for enabling drug and substrate access to the active site of the molecule. Tryptase has been reported to possess antagonistic functions, since it plays an important role both in inflammatory phenomena and as a protector against infection. In allergic processes it is associated to bronchial hyperresponsiveness in asthmatic patients, where PAR-2 is of great importance as an airway receptor. Lastly, the genes that encode for tryptase are highly polymorphic and complex. As a result, it is important to establish a relationship between genotype and phenotype in disorders such as asthma, and to identify mutations that are presumably of pharmacological relevance. © 2012 SEICAP. Published by Elsevier España, S.L. All rights reserved.

### Introduction

Tryptase is a serine protease related to trypsin, from which it differs in form, activity and expression patterns. The genes encoding for tryptase, and its protein structure, were characterised in the 1980s. Tryptase is the most abundant of the endopeptidases stored in the secretory granules of basophils

and mast cells. In turn, mast cells have been divided into two main subtypes according to the endopeptidases contained in their granules: MC<sub>T</sub> (mast cells containing  $\beta$ -tryptase and which are located mainly in the lungs) and MC<sub>TC</sub> (mast cells that also contain chymase and which predominate in the skin and intestinal submucosa).

The functions of tryptase have not been fully clarified to date. It has been reported to participate in inflammatory and anti-inflammatory phenomena, and even in the control of infection through the recruitment of neutrophils. Due to its presence in mast cells and its participation in inflam-

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matory and immediate hypersensitivity reactions, tryptase is considered to play an important role in allergic processes. This protease reportedly worsens inflammation in asthma of allergic origin; facilitates the appearance and persistence of local oedema; causes bronchoconstriction; and increases airway smooth muscle mass. Recent studies also suggest the existence of asthma susceptibility determinants in chromosome 16, where the tryptase gene is located.<sup>1</sup> The study of this protease is therefore important, since it constitutes a promising target for future therapeutic interventions.

## Types of tryptases

The term tryptase comprises four different proteases:  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ , which show minor differences in terms of their enzymatic properties.<sup>2</sup> The tryptase best characterised to date is  $\beta$ -tryptase, and the term "tryptase" is often used as a synonym in reference to this molecule. Three  $\beta$ -tryptase subtypes have been described ( $\beta$ I,  $\beta$ II and  $\beta$ III), with a similarity among each other of up to 99%. Tryptases  $\beta$ I and  $\beta$ III differ from  $\beta$ II in position 142, while  $\beta$ I and  $\beta$ II differ from  $\beta$ III in positions 60–63. The so-called "specific triad" (Asp<sup>188</sup>Gly<sup>215</sup>Gly<sup>225</sup>), located in the catalytic domain, determines substrate affinity, and is highly preserved in the different mammalian species.<sup>3</sup>

Tryptase is stored in its active form, although it is maintained with little or no activity within the secretory granules thanks to the low pH value and stabilisation mediated by other proteins. It is expressed as a pre-protein, and is processed via the elimination of a signalling peptide through autocatalysis of a peptide sequence – thereby giving rise to a tryptase pro-form. Posteriorly, after the elimination of two amino acids, the tryptase monomer is produced. In the absence of the factors needed for stabilisation, the structure dissociates into monomers that were initially considered to be inactive, although recent studies have demonstrated the existence of active  $\beta$ -tryptase monomers.<sup>4</sup>

The next protein in order of importance is  $\alpha$ -tryptase, which is unable to auto-process itself to the mature molecular form. The activity of the  $\alpha$ -tryptase tetramer has been shown to be clearly inferior to that of  $\beta$ -tryptase. The residue in position 216 confers important differences in terms of activation, secretion and activity. On the other hand,  $\alpha$ -tryptase is not stored in the secretory granules; instead, constitutive secretion has been described, determining low levels in the bloodstream that are not affected by the sudden release of the mast cell mediators contained in the granules. As a result,  $\alpha$ -tryptase is not used as a marker of the amount of granules or of their load or acute activation. It is therefore considered less likely for  $\alpha$ -tryptase to play a role in the physiopathology of anaphylactic reactions. It has been seen that the precursor forms of both  $\alpha$ - and  $\beta$ -tryptases are secreted constitutively, representing an important fraction of the total circulating tryptase.

$\gamma$ -Tryptase is less related to the rest of the tryptases, and exhibits only 48% similarity with  $\alpha/\beta$ -tryptase. It has not been established whether  $\gamma$ -tryptase contributes together with the rest of the tryptases to increase the circulating molecular levels after an anaphylactic episode, although it is known that this type of tryptase does not acquire

the tetrameric conformation. Nevertheless, it has been reported that this enzyme is able to induce the production of IL-13, thereby promoting bronchial hyperresponsiveness.

The structural similarity between  $\delta$ -tryptase and  $\alpha$ -tryptase is about 80%. This molecule is secreted constitutively, and since it has a premature stop codon, its sequence is shorter – a fact that significantly affects its substrate specificity, though the catalytic triad is not affected.

Lastly, while  $\varepsilon$ -tryptase is a protein directly related to the tryptases, it is a product of the *PRSS22 (Protease serine 51 family member 22)* gene. Its similarity to  $\alpha$ - and  $\beta$  tryptases is 40%, although in contrast to these molecules,  $\varepsilon$ -tryptase does not adopt a tetrameric conformation.

## Structure, processing and activation

Tryptase has a molecular weight of 140–142 kDa, and is composed of identical 30–36 kDa subunits. The structure is tetrameric, and the monomers are distributed at the corners of a flat rectangular structure, with the active sites oriented towards the centre. Stabilisation of this molecular conformation requires the presence of heparin proteoglycans or other polymers with a large negative charge; as a result, tryptase is included among the so-called *self-compartmentalising proteases*. It has been shown that peptides with a certain alpha-helix diameter (12 Å) can interact with the active sites; this occurs with the calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and peptidic hormones such as fibrinogen and complement system proteins, among others. The best defined substrate is protease activated receptor 2 (PAR-2), a transmembrane receptor expressed by the bronchial mucosa and exhibiting differential expression in asthmatic patients. Also important is LDTI (leech-derived tryptase inhibitor), a tryptase inhibitor, because of its potency and capacity to directly access the active sites.<sup>5</sup>

Assembly of the tetramer and posterior enzyme activation only takes place at pH values under 6.5, and is notoriously dependent upon heparin. It has been shown that compounds less sulphated than heparin are comparatively poorer tryptase activators. Selwood et al.<sup>6</sup> have found that  $\alpha$ -tryptase, in contrast to  $\beta$ -tryptase, appears to be independent of heparin – a fact that may be of great importance in acid pH environments such as the airways of asthmatic patients, or in poorly vascularised zones.

The tryptase content of the basophils is about 0.05 pg/cell, versus 11–35 pg/cell in the case of the mast cells. In turn, the different types of mast cells show different responses to drugs such as disodium cromoglycate or theophylline, which inhibits degranulation of a specific mast cell subtype (MC<sub>TC</sub>). This could offer a target for pharmaceutical development.

## Function

Since tryptase is a prevalent element of the secretory granules within the mast cells, it seems logical to assume that it participates in those processes in which cell degranulation takes place, such as anaphylaxis or mastocytosis. One of the most widely studied effects refers to the implication of tryptase in inflammatory allergic reactions. In this context,

it is presently assumed that the molecule may exert a pro- or anti-inflammatory effect, depending on the context.

Studies in the lung suggest that allergic reactions are characterised by bronchoconstriction secondary to histamine release. In this sense, tryptase may represent a mechanism for amplifying mast cell response on inducing degranulation. In turn, local oedema may develop by inhibiting fibrin formation, causing the accumulation of eosinophils and neutrophils. Tryptase stimulates the release of IL-8, ICAM (intercellular adhesion molecule) and IL-1 $\beta$ , inducing leukocyte infiltration, which in turn results in the amplification and maintenance of mast cell response. These inflammatory molecules stimulate cell proliferation and migration in the context of a positive feedback mechanism that results in further infiltration of the vascular wall and further mediator release. Tryptase increases the expression of other mediators such as TNF $\alpha$  and IL-6 through the activation of PAR-2. It has, however, been shown that not all tryptases have the same effect.

Studies in mast cell-deficient mice in which pneumonia was induced with *Mycoplasma pneumoniae* have shown mast cells to play an antibacterial and anti-inflammatory role through the intervention of their mediators. Other studies in which human tryptase  $\beta$ I was injected into the airways of mice revealed a protective effect against infection by *Klebsiella pneumoniae*, due to the fact that tryptase is able to recruit neutrophils in those locations where mast cells degranulate. The in vivo anti-inflammatory capacity of the mast cell peptidases has not been fully clarified. Caughey summarised these apparently antagonistic effects suggesting that the mast cell tryptases include dichotomic benefit versus damage, inflammation control versus spread, and tissue defence versus destruction effects.

In the airways, tryptase is able to cause inflammation and remodelling, with an important effect in relation to bronchoconstriction. In 1997, the accumulation of tryptase resulting from the degranulation of mast cells in the airways was found to produce bronchial hyperresponsiveness associated to asthma. In turn, tryptase has been shown to favour contraction promoted by histamine, since this effect can be blocked by antihistamines, in bronchi previously sensitised to common antigens, but not in bronchi not previously sensitised.

Tryptase acts as an airway muscle cell mitogen, and moreover can attract further mast cells through mediators such as TGF- $\beta$ 1, increasing the smooth muscle cells and number of mast cells in the airways – this being a characteristic inherent to asthma. In this disease, tissue remodelling occurs as a result of an increase in smooth muscle mass accompanied by angiogenesis – this represents an important contribution to wall thickening and diminished airflow that tends to become irreversible. The infiltration of mast cells in the smooth muscle of the airways is important from the functional perspective, since the number of mast cells in this tissue layer in asthmatic patients has been correlated to the degree of hyperresponsiveness. Recently, it has been shown that tryptase is able to stimulate neuropeptide release, modifying bronchial tone. Previous studies in dogs suggested that tryptase could be implicated in asthma through the inhibition of vasoactive intestinal peptide (VIP) activity.

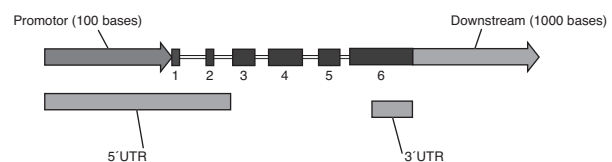
On the other hand, the PAR-2 signalling transmission pathway has been implicated in the mediation of "emergency conditions". The location of the PAR-2 receptor in the airways of asthmatic patients has been confirmed from tissue biopsies. Over-expression of the receptor induces exacerbation of airway response; in contrast, the suppression of such expression gives rise to a decrease in inflammation, with a reduction in the infiltration of eosinophils in the tissue and in bronchial hyperresponsiveness. It has been described that the inhalation of human tryptase in aerosol form in allergic sheep causes bronchoconstriction and airway hyperresponsiveness. Pre-treatment with human tryptase inhibitors before sensitisation to the allergen occurs reduces the early and delayed response and the development of airway hyperresponsiveness and eosinophilic inflammation. Mice lacking the PAR-2 receptor show less airway inflammation following antigen stimulation.<sup>7</sup>

According to Sommerhoff,<sup>8</sup> the polymorphisms described by Guida in the year 2000<sup>9</sup> for the tryptase gene may result in bronchial tone regulation mechanisms and responses very similar to those observed as a consequence of allergen inhalation in mice through modifications in the splicing sites. The first clinical trials with tryptase inhibitors (APC-366 and BABIM) in individuals with mild asthma have shown a decrease in response after inhalation of the allergen.

## Tryptase genes

At least four genes grouped in position 16p13.3 have been described. The first is the *TPSG1* gene, which encodes for  $\gamma$ -tryptase, and is closely related to the gene encoding for human prostatin. It in turn is followed by the *TPSB2* gene, which encodes for tryptases  $\beta$ II/ $\beta$ III, and the *TPSAB1* gene (90% similar to the previous gene) (Fig. 1), which encodes for  $\alpha$ -tryptase and  $\beta$ -tryptase. Lastly, the *TPSD1* gene encodes for  $\delta$ -tryptase. Although the genes encoding for tryptases  $\beta$ I and  $\beta$ III have been previously localised within the mentioned chromosome, there is some controversy regarding their precise position. CpG islands have been identified in this region – a fact that complicates the cloning and sequencing process.<sup>10</sup>

Estimations have been made of the frequency of the  $\alpha$  allele and of the  $\beta$  allele, assuming that both are in equilibrium in the *TPSAB1* gene. The gene encoding for  $\alpha$ -tryptase presents a deletion of 10–11 base pairs in intron 4, and exon 4 moreover exhibits a sequence recognised by the *EcoRV* restriction enzyme, identified as RFLP



**Figure 1** Schematic representation of the *TPSAB1* gene. The exons are represented with boxes and are numbered from 1 to 6; the lines between them represent the introns. At both extremities, arrows indicate the promoter region and 1000 bp in 3' sense. The lower boxes in turn represent the 5' UTR and 3' UTR regions.

(restriction fragment length polymorphism). Some individuals lack the  $\alpha$  allele, and their genotype is therefore referred to as "all  $\beta$ ". The  $\alpha$  and  $\beta$  alleles share the  $\alpha/\beta$  site,<sup>11</sup> but the  $\beta$  allele moreover resides in locus *TPSB2*. The genotype lacking the  $\alpha$  allele ( $\beta\beta\beta\beta$ ) is observed in 29% of all individuals (37% in our population, unpublished results).

The genes of this complex present a markedly preserved 3'UTR region (untranslated region) and a 5'UTR region with repetitions of 19bp rich in guanine that are essential for regulating the high expression levels of mast cell specific protease. It has been postulated that there are at least five gene duplication phenomena from an ancestral gene. It is also possible that alternative adjustment processes (splicing) are implicated.<sup>12</sup>

Transcriptional and post-transcriptional modulation mechanisms have been described, capable of modifying the individual levels of expression. Such regulation is positively controlled by the *mi* transcription factor (MITF), initially described for mmcp-6 (murine mast cell protease used for the study of human tryptase). At the same time, other proteins are reported to present synergic activity with the activation of the mentioned murine tryptase, such as PEB2 (polyomavirus enhancer binding protein 2), MAZR (myc-associated zinc-finger protein related factor) and c-jun. This trans-activation occurs through the binding of these factors to regions of the promoter that respond to the CANNTG sequence. As an example, it has been shown that Transforming Growth Factor  $\beta$  (TGF- $\beta$ ) induces an increase in regulation through smad3.<sup>13</sup>

It is necessary to take the complexity of these genes into account, in view of the similarity among the different alleles and the existence of polymorphic regions in the gene sequences. In general, it is known that single nucleotide polymorphisms (SNPs) located in coding regions modify the protein structure. They may be silent, acting through mechanisms that are still not fully clear, or may be located in the promoter – modifying the expression of the protein. It has been reported that gene variants can contribute to complex diseases such as atopy or asthma. In this context, our group has identified certain polymorphisms associated to allergic disease.<sup>14,15</sup> In the case of tryptase, it is important to establish a relationship between genotype and phenotype in disorders such as asthma or osteoarthritis, where tryptase has been shown to be implicated in animal models and to define the mutations which affect the response to anti-peptidases – a condition assumed to be of great pharmacological importance.

The most common mutations of the tryptase gene lead to loss of membrane anchoring, defective zymogen activation or loss of catalytic function – thereby giving rise to changes in specificity. In the year 2000, Guida et al.,<sup>9</sup> described 21 SNPs in what they refer to as the *TPS1* gene, and 17 SNPs in a chromosomal region referred to as *TPS2*. The *TPS1* SNPs are distributed along the entire gene, while in the case of the *TPS2* gene, 59% of the polymorphisms are concentrated in 250 base pairs located between exon 5 and intron 5.<sup>9</sup> Our group has analysed the *TPSAB1* gene, analysing the previously described polymorphisms and reporting new specific sites in highly polymorphic regions of the gene, their importance, and repercussions upon the encoded protein is being

determined by in silico analysis. On the other hand, the study of the mutations in the promoter region of the gene may establish their importance in relation to protein expression and their possible influence upon the tryptase levels in the serum of patients with allergic diseases.

Another factor recently defined as a cause of variations in genetic expression refers to epigenetics, i.e., modifications in DNA or chromatin that do not induce variations in genotype and which play a key role in genetic regulation. One of the epigenetic phenomena giving rise to variations in expression is DNA methylation, involving the addition of methyl groups to cytosine nucleotides in CpG regions. These sequences have been described in the region where the tryptase genes are located. It would therefore be interesting to carry out studies to explore the existence of epigenetic regulatory phenomena capable of producing variations in tryptase expression.

Knowledge of the different tryptase gene polymorphisms and of their importance may contribute to clarify the role of this protein as a possible marker in identifying the factors that predispose to certain diseases, or for clarifying the correlations to serum tryptase levels or mast cell load.

## Conflict of interest

The authors have no conflict of interest to declare.

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