Testing for Hereditary α-Tryptasemia:
TPSAB1 Copy Number Analysis by Droplet Digital Polymerase Chain Reaction

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ABSTRACT

Hereditary α-tryptasemia (HαT) is the most common genetic cause of elevated basal serum tryptase (BST) levels. HαT is a risk factor for severe anaphylaxis, particularly in individuals with venom allergy, and an established modifier of mast cell activation syndromes and mastocytosis. In individuals with systemic mastocytosis (SM), the World Health Organization (WHO) classification[1] recommends a BST adjustment in the presence of HαT. Detection of an increased α-tryptase-encoding *TPSAB1* gene copy number defines HαT and aids in the interpretation, prognosis, and clinical management of patients with elevated serum tryptase.

Genetic Basis of Hereditary α-Tryptasemia

The multigene tryptase locus on chromosome 16 includes *TPSAB1* and *TPSB2,* which are inherited as a haplotype and primarily expressed by mast cells. The *TPSB2* gene encodes only β-tryptase, whereas the *TPSAB1* gene encodes either α- or β-tryptase (**Figure 1**). HαT is defined by increased copies of the *TPSAB1* gene encoding α-tryptase (**Figure 2**).[2]

Figure 1. Human tryptase gene locus

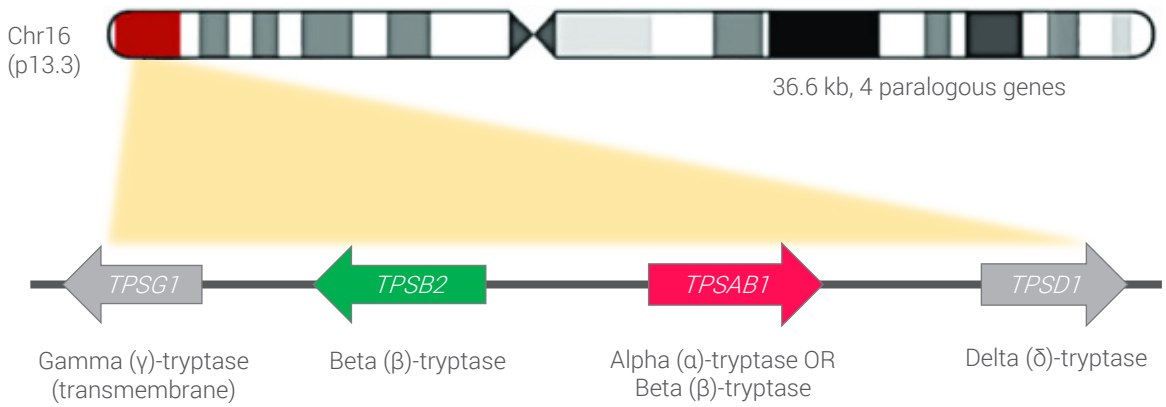
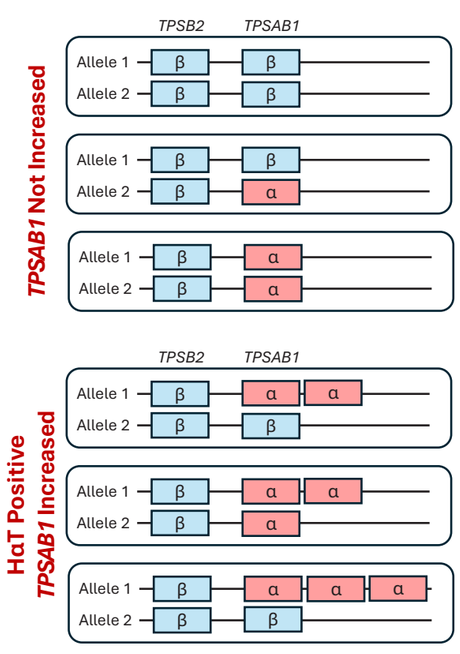


Image credit: Malia Deshotel, PhD

Figure 2. Common *TPSB2*/*TPSAB1* genotypes



α-tryptase	β-tryptase	Total α+β
0	4	**4**
1	3	**4**
2	2	**4**

α-tryptase	β-tryptase	Total α+β
2	3	**5**
3	2	**5**
3	3	**6**

Image credit: Malia Deshotel, PhD

Pathophysiology

Elevated serum tryptase levels correlate with the number of additional α-tryptase-encoding *TPSAB1* copies, demonstrating a gene-dosage effect.[3] Although the underlying mechanism is still under investigation, current research suggests that HαT increases formation of α/β-tryptase heterotetramers. These heterotetramers are mature, enzymatically active forms of tryptase that are released upon mast cell activation and function in immune response.[4]

Clinical Significance

HαT is estimated to affect approximately 4–6% of individuals based on study populations from the United States, United Kingdom, and European Union.[3] This prevalence is significantly higher (approximately 15%) among individuals diagnosed with clonal mast cell disease,[3] severe Hymenoptera venom-induced anaphylaxis, and idiopathic anaphylaxis (IA).[3,4] Although HαT can be asymptomatic,[4] elevated serum tryptase levels have been associated with cutaneous flushing, pruritus, dysautonomia, functional gastrointestinal symptoms, chronic pain, and connective tissue anomalies.[2]

Testing Utility in Hematology/Oncology

Testing for HαT in the context of hematology/oncology:

- Supports diagnostic evaluation of systemic mastocytosis
  - The WHO 5th classification[1] recommends adjustment of the serum tryptase in the presence of HαT. A total serum tryptase level of >20 ng/mL is considered a minor diagnostic criterion for SM. However, in individuals with HαT, this threshold is adjusted based on their *TPSAB1* copy number status. There are multiple suggested methods for this adjustment; refer to the WHO 5th classification[1] for more information.
- Supports diagnosis of mast cell activation syndrome
  - Identification of HαT may reduce the need for bone marrow biopsy.
- Informs anaphylaxis risk assessment in patients diagnosed with both HαT and SM, as this combination increases the risk of severe anaphylaxis and mediator-related symptoms[3,4,5]

Testing Utility in Immunology/Allergy

Testing for HαT in the context of immunology/allergy:

- Identifies individuals at risk for severe mast cell-mediated reactions
- Provides a possible explanation for elevated BST in patients with multisystem symptoms including skin flushing, pruritus, gastrointestinal complaints (diarrhea, irritable bowel syndrome), autonomic dysfunction, and connective tissue anomalies (Ehlers-Danlos syndrome)
- Informs risk assessment for severe Hymenoptera venom-induced anaphylaxis, as HαT is associated with increased reaction severity, particularly when occurring in conjunction with clonal mast cell disease
- Informs the evaluation of idiopathic anaphylaxis[3]

Testing for Hereditary α-Tryptasemia With ARUP Laboratories

Quantitative testing determines germline copy numbers of the α- and β-tryptase isoforms encoded by *TPSAB1* and *TPSB2*. Genotypes with increased *TPSAB1* copies include either three or more α-encoding *TPSAB1* copies or a total of five or more combined copies of *TPSAB1*/*TPSB2*, with at least two α-encoding *TPSAB1* copies. ARUP is among the few laboratories offering this specialized testing, and results are typically available within 10–14 days.

TPSAB1 Copy Number Analysis by ddPCR (ARUP Test 3017399)

Methodology: *TPSAB1*, *TPSB2*, and a control two-copy gene, *AP3B1*, are amplified in oil-immersed droplets using a droplet digital PCR (ddPCR) instrument. Following amplification, the detection component of a droplet reader platform scores absolute numbers and fluorescence intensities of each reaction droplet. Detection is based on three allele-specific fluorescent hydrolysis probes: FAM and HEX probes are targeted to α- or β-tryptase isoforms with three different nucleotide bases to ensure specificity (**Figure 3**); the ROX probe detects the *AP3B1* gene. Enzymatic digestion with the restriction enzyme BamH1 during polymerase chain reaction (PCR) ensures independent assortment of tandem gene copies into individual droplets for accuracy in quantification of copy numbers.

Figure 3. Allele-specific probe-binding regions of *TPSAB1* and *TPSB2*

Image credit: Sabine Hellwig, PhD

Sensitivity and Specificity

Clinical and Analytic Sensitivity and Specificity of *TPSAB1* Copy Number Analysis by ddPCR		
	Sensitivity	**Specificity**
Clinical	100%[2]	90% in individuals with elevated BST[2]
Analytic	>99%	>99%

Reporting

Results are reported as integer copy numbers. Copy number calculations are based on the allelic ratio of *TPSAB1* to *AP3B1*, and *TPSB2* to *AP3B1*.

For more information on specimen requirements and examples of test reports, scan the following QR code:

aruplab.com/TPSAB1

For additional technical information, scan the following QR code:

aruplab.com/TPSAB1-TFS

References

1. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia.* 2022;36(7):1703-1719.
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3. Shin H, Lyons JJ. Alpha-tryptase as a risk-modifying factor for mast cell-mediated reactions. *Curr Allergy Asthma Rep.* 2024;24(4):199-209.
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5. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: systemic mastocytosis. Version 1.2025. Published Feb 2025; accessed Jul 2025.