



First Identification of an Inherited *TPSAB1* Quintuplication in a Patient with Clonal Mast Cell Disease

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Abbreviations

<i>TPSAB1</i>	Tryptase, alpha/beta-1
MMAS	Monoclonal mast cell activation syndrome
BST	Basal serum tryptase
RT-PCR	Real-time polymerase chain reaction
ddPCR	Droplet digital PCR
<i>KIT</i>	<i>KIT</i> proto-oncogene receptor tyrosine kinase
H1	Histamine H1 receptor
H2	Histamine H2 receptor

To the Editor

Hereditary alpha tryptasemia is caused by increased *TPSAB1* copy number on a single allele, encoding additional copies of alpha-tryptase [1]. This genetic trait is inherited in an autosomal dominant manner, and affected individuals display elevated basal serum tryptase (BST; > 8 ng/mL). While clonal mast cell disease has not been reported among patients with this trait previously, a number of individuals present with symptoms frequently reported by patients with clonal disease including functional gastrointestinal and pain symptoms and cutaneous flushing and pruritus. Among those affected, BST and these clinical symptoms have been observed to follow a

gene-dosage relationship, with increased *TPSAB1* copy number leading to greater symptom expressivity and higher BST; however, only duplications and triplications have previously been identified [2]. Moreover, features suggestive of mast cell compartment disruption, including increased mast cell number and abnormal spindle-shaped morphology (> 25%) in bone marrow, have also been observed in patients with hereditary alpha tryptasemia [3]. Herein, we report the first identification of a *TPSAB1* quintuplication manifest in a highly symptomatic patient with clonal mast cell disease, clarifying the literature, and suggesting the potential for a link between mastocytosis and hereditary alpha tryptasemia.

A three-generation family of Belgian ancestry was recently reported with dominantly inherited BST elevations of unknown etiology, associated with clinical and biochemical evidence of mast cell mediator release; 4/7 individuals presented with recurrent episodic severe abdominal cramping and diarrhea concomitant with transient increases of serum tryptase above elevated baseline levels (> 1.2 x BST plus ≥ 2 ng/mL); family members with normal BST were asymptomatic [4]. All five adults with elevated BST underwent bone marrow and gastrointestinal biopsies, and bone marrow was screened for the presence of c.2447A>T (p.D816V) *KIT* as described in the [Online Supplement](#). No morphologic or immunophenotypic evidence of abnormal mast cells was found. However, in a significantly affected individual with elevated BST (II.3), a 22-year-old male who was also identified as having mild hepatosplenomegaly (liver 15.5 cm, spleen 12.2 cm), a somatic c.2447A>T (p.D816V) *KIT* mutation was detected (Fig. 1a; Fig. S1). Despite harboring this mutation—which is present in 90% of individuals with indolent systemic mastocytosis (ISM) and is a minor criterion for the clinical diagnosis of ISM—the patient did not meet criteria for ISM (Table S1). However, this presentation may constitute a pre-diagnostic phase of ISM [5], or represent a splenic variant of disease as has been described [6].

Affected individuals in this family have been treated with anti-H1 and anti-H2 antihistamines as well as oral sodium cromolyn and cessation of these treatments has resulted in relapses of symptoms. Using our droplet digital PCR-based

Capsule Summary

Clonal mast cell disease can be associated with hereditary alpha tryptasemia.

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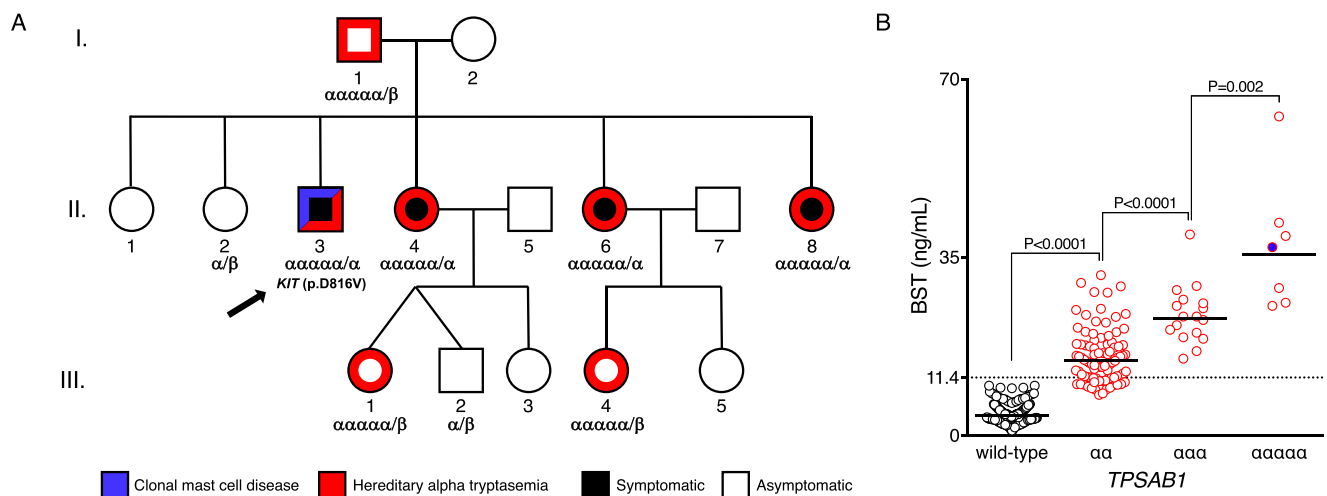


Fig. 1 Family with increased *TPSAB1* copy number and clonal mast cell disease. **a** Family pedigree. **b** Basal serum tryptase (BST) levels grouped by disease-associated *TPSAB1* genotype; wild-type includes α/α , α/β , and β/β genotypes; red circles indicate hereditary alpha tryptasemia;

blue-filled circle indicates individual with clonal mast cell disease. Mann-Whitney test; dashed-line indicates the the upper limit of normal for BST as currently defined by clinical laboratories (11.4 ng/mL)

genotyping assay [1], we found that this family carries five alpha-encoding germline *TPSAB1* copies in cis that segregate completely with elevated BST (Fig. S1). For Methods, please see the [Online Repository](#) for this manuscript. As has been observed in BST levels among individuals with duplications and triplications, a further gene-dosage effect of copy number was observed, where 5 alpha-encoding allelic *TPSAB1* copies was associated with a significantly greater BST when compared to individuals with *TPSAB1* triplications (Fig. 1b).

The prevalence of hereditary alpha tryptasemia is currently unknown, but may affect up to 6% of the general population. However, among individuals with hereditary alpha tryptasemia, two allelic *TPSAB1* copies is the most common finding. *TPSAB1* triplications are observed in less of 10% of families with hereditary alphas tryptasemia (9/95). Furthermore, triplications have not been identified through screening of patients with primary immune deficiency diseases or in healthy control cohorts [1]. Only 0.38–0.57% of the general population would be estimated to have germline *TPSAB1* triplications. The prevalence of symptomatic clonal mast cell disease that does not satisfy World Health Organization (WHO) criteria for the diagnosis of ISM remains undefined. However, prevalence estimates for mastocytosis are approximately 1:10,000 [7], suggesting that clonal mast cell populations harboring (p.D816V) *KIT* that fail to meet criteria for ISM are also likely uncommon. Identification of five allelic *TPSAB1* copies is unprecedented and is likely an exceedingly rare genotype. Based upon this, one would predict that the likelihood of an individual having both clonal mast cell disease and a *TPSAB1* quintuplication would be very low; therefore, an association may exist between the two. *TPSAB1* gene dosage may therefore have clinical relevance in patients with ISM and other forms of mastocytosis.

We have observed that individuals with *TPSAB1* triplications display more severe clinical symptoms and higher BST levels than individuals with duplications [1]. Here, we report a further gene-dosage effect, whereby 5 allelic *TPSAB1* alpha-tryptase encoding copies lead to significantly higher BST when compared to individuals with *TPSAB1* triplications (Fig. 1b). Sera containing high levels of tryptases have been shown to induce stem cell factor and granulocyte-monocyte stimulating factor from stromal cells in vitro [8], suggesting high BST may affect mast cells or other myeloid cells. Furthermore, in a small study of mastocytosis patients who commonly have significant BST elevations, individuals with ISM were reported to be twice as likely to have two alpha-tryptase containing alleles compared to the general population [9–11]. Whether increased alpha-tryptase secretion and elevated BST—in particular at modest levels seen in more commonly encountered *TPSAB1* duplications (15 ± 5 ng/mL total tryptase) or as observed in ISM patients with one or two alpha-tryptase copies—can actually modify or contribute to clonal mast cell disease in vivo however, requires additional investigation. Indeed, because of the gene-dosage effect upon both basal tryptase levels and expressivity in patients with hereditary alpha tryptasemia, should such a relationship be substantiated, we would expect a proportional effect of this genetic lesion on mast cell clonal expansion.

Elevated BST can occur with clonal expansion of mast cells and other myeloid cells, but may occur more commonly with inherited increases in *TPSAB1* copy number. Distinguishing between these causes has potential diagnostic value and clinical implications. While the potential connection between hereditary alpha tryptasemia and clonal mast cell disease requires further exploration, tryptase genotyping should be considered for inclusion as part of the work-up of patients with elevated BST.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Online Repository Material for: First identification of an inherited *TPSAB1* quintuplication in a patient with clonal mast cell disease

Methods:

Subjects

Patients and their families provided informed consent on IRB-approved at the National Institutes of Health (NCT01164241, NCT00852943) or Antwerp University Hospital (Belgium B300201525454).

Tryptase genotyping

Allele-specific alpha-/beta-tryptase genotyping of *TPSAB1* and *TPSB2* was performed by droplet digital PCR (ddPCR) as described (1).

Bone marrow and tissue biopsy and analysis

Bone marrow biopsies were performed and World Health Organization (WHO) criteria were applied to screen for the diagnosis of systemic mastocytosis (E1). Immunohistochemistry was performed on fixed paraffin embedded bone marrow core biopsies and tissue sections using anti-CD117 (Dakopatts/polyclonal rabbit) and/or anti-tryptase (Dakopatts/MCT AA1). Mast cell immunophenotyping on bone marrow aspirates by flow cytometry was performed as described (E2, E3).

***KIT* mutational analysis**

The presence of the c.2447A>T (p.D816V) *KIT* mutation in bone marrow mononuclear cells was determined as described by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) (E2) and in blood by ddPCR on a Bio-Rad QX200 droplet digital PCR system.

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Table S1. Clinical and laboratory findings among a single individual with *TPSAB1* quintuplication and clonal mast cell disease.

	A.II.3
<i>TPSAB1</i> genotype	$\alpha\alpha\alpha\alpha/\alpha$
Basal serum tryptase (ng/mL)	37
Serum IgE (kU/L)	39
Peripheral eosinophilia	+
<i>KIT</i> (p.D816V)	+
CD2 ⁺ CD25 ⁺ bone marrow mast cells	-
Clustered/spindle-shaped bone marrow mast cells	-
Bone marrow eosinophilia	+
FIP1L1/PGDFRA fusion	-
Hepatosplenomegaly	+
Anaphylaxis	-
Abdominal pain/diarrhea	+
Other symptoms	apathy, migraine

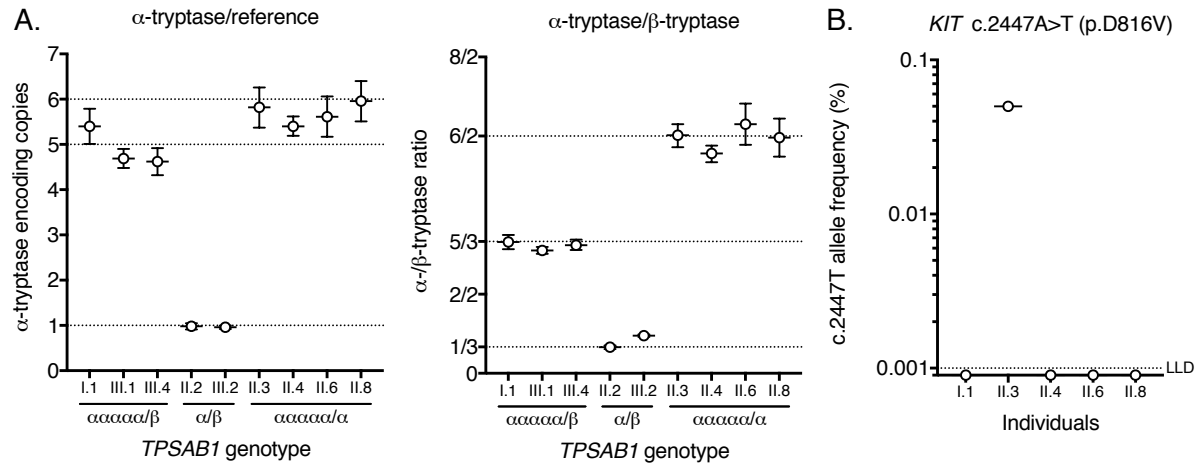


Figure S1. Primary genotyping data. **(A)** Digital droplet PCR data showing calculated α -tryptase sequence copy number from *TPSAB1* relative to conserved reference genes (left) and relative to total β -tryptase sequence copy number from *TPSAB1* and *TPSB2* (right). These data applied to the clinical pedigree confirmed the presence of an α -tryptase encoding *TPSAB1* quintuplication; two β -tryptase encoding copies at *TPSB2* are also detected by this assay and contribute to the calculated α -/ β -tryptase ratio (right). **(B)** Real-time PCR data for the 5 individuals assayed demonstrating the presence of *KIT* c.2447A>T (p.D816V) in II.3 at an estimated frequency of 0.05% transcripts present; this mutation was below (<0.001%) the lower limit of detection (LLD) in all other individuals tested.