ONLINE REPOSITORY

Methods

Description of the Tryptase Locus and Protein Isoforms of TPSAB1 and TPSB2

The tryptase locus on chromosome 16 is composed of four tightly linked tryptase genes *TPSG1*, *TPSB2*, *TPSAB1*, and *TPSD1* that are inherited as a haplotype. Each person has two haplotypes corresponding to their two chromosomes. Clinical testing for serum tryptase measures only the secreted isoforms encoded by *TPSAB1* and *TPSB2* genes. The *TPSB2* gene encodes only beta tryptase protein isoforms. By contrast, *TPSAB1* can encode either beta or alpha tryptase protein isoforms. Hereditary-alpha tryptasemia (H□T) occurs when there are tandem increased *TPSAB1* copy number. For example, a tryptase genotype of 3 alpha 2 beta corresponds to one tryptase haplotype of 1 alpha tryptase-encoding *TPSAB1* gene linked to 1 *TPSB2* gene and a second haplotype of 2 tandem alpha-tryptase encoding *TPSAB1* linked to 1 *TPSB2*. See reference (1) for a comprehensive review.

Genetic Testing Methodology for Diagnosis of H□T

All tryptase genotypes were collected from clinical genetic reports issued by Gene by Gene (Houston, TX) found in the medical records of subjects. Digital droplet polymerase chain reaction is the technique used by the company for clinical tryptase genotype testing as described in (1).

Figure E1

Schematic of study eligibility determination.

Figure E2

Distribution of diagnoses associated with high-normal BST with maximum basal tryptase ≥ 7.5 and < 11.5 ng/mL. Mastocytosis includes 2 subjects with ISM and 3 with CM. Other myeloid neoplasms include 2

subjects with MDS-EB-1 and 1 with a *JAK2*-associated neoplasm. Five subjects had two associated diagnoses.

Figure E3

Subjects with tryptase variability that exceeded the 20% plus 2 threshold had basal tryptase values graphed by time. Horizontal dashed lines represent the threshold of the formula. Shaded regions corresponded to concurrent use of imatinib, prednisone, or hydroxyurea. (a) H□T (b) no associated diagnosis (c) SM-PV (d) mastocytosis (e) multiple diagnoses (f) CKD.

Abbreviations

CKD – Chronic kidney disease

CM – Cutaneous mastocytosis

EoE – Eosinophilic esophagitis

H□T – Hereditary-alpha tryptasemia

HES – Hypereosinophilic syndrome

ISM – Indolent systemic mastocytosis

MDS – Myelodysplastic syndrome

MMAS – Monoclonal mast cell activation syndrome

PV – Polycythemia vera

RA – Rheumatoid arthritis

SM – Systemic mastocytosis

Bibliography

1. Lyons JJ. Hereditary alpha tryptasemia: genotyping and associated clinical features. Immunol Allergy Clin North Am. 2018 Aug;38(3):483–95.

TABLE E1 Testing in subjects with elevated BST ≥ 11.5 ng/mL with 1 or 2 associated diagnoses

Disorder	Test	Total Tested	Positive
H□T (33*)			
	KIT p.D816V blood	30	0
	N-MH	27	0
	Bone marrow biopsy	3	0
Mastocytosis (8**)			
	H□T test	6	0
	KIT p.D816V blood	5	3
	N-MH	4	3
	Bone marrow biopsy	6	3 ISM, 1 ASM, 1 MCL, 1 MMAS
HES (6)			
	FIP1L1-PDGFRα FISH***	4	1
	H□T test	3	2
	KIT p.D816V blood/marrow	2	0
	N-MH	2	0
	Bone marrow biopsy	2	1 CEL
	CKD (GFR < 60)	6	1 hemodialysis
CKD (6)			
	H□T test	6	2
	KIT p.D816V blood	6	1
	N-MH	5	0
	Bone marrow biopsy	4	1 ISM, 1 MDS
Other myeloid neoplasm (1)			
	H□T test	0	0
	KIT p.D816V marrow	1	1
	JAK2 p.V617F	1	1
	N-MH	0	0

Bone marrow biopsy	1	1 SM-PV
No associated diagnosis (4)		
H□T test	4	0
KIT p.D816V blood	4	0
N-MH	4	0
Bone marrow biopsy	3	0

CEL, chronic eosinophilic leukemia; FISH, fluorescence in situ hybridization

Note: Each subject is represented under only one disorder. All subjects had absolute eosinophil counts less than 1500 x 10^9/L and normal GFRs unless otherwise noted.

* One subject with H□T also had rheumatoid arthritis; the other 32/33 subjects had no other associated conditions

** Two subjects were diagnosed with CM and one subject with ISM by bone marrow also had been previously diagnosed with CM

*** No subjects had FIP1L1-PDGFRA testing by RT-PCR

TABLE E2 Testing in subjects with BST between 7.5 – 11.4 ng/mL with 1 or 2 associated diagnoses

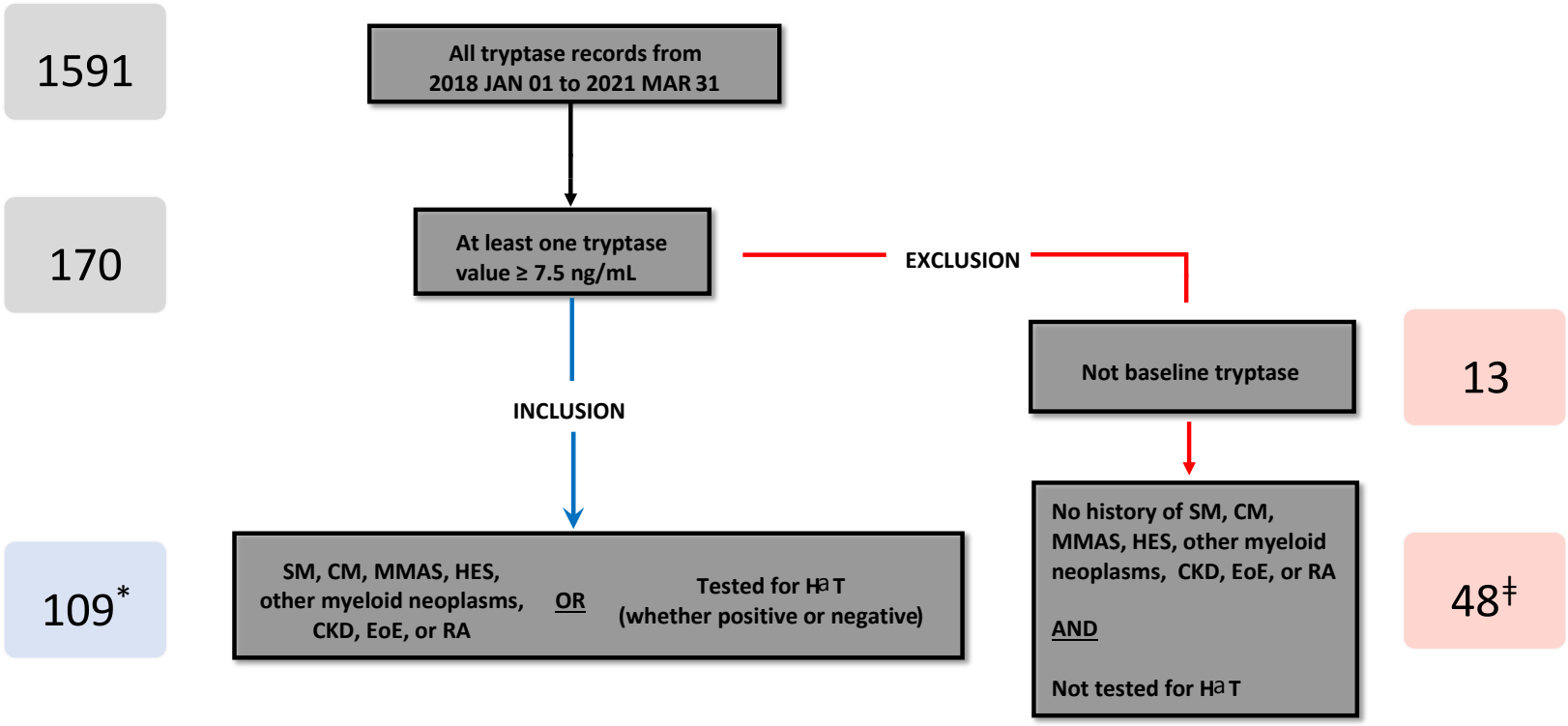
Disorder	Test	Total Tested	Positive
H□T (6)			
	KIT p.D816V blood	4	0
	N-MH	3	0
	Bone marrow biopsy	1	0
Mastocytosis (5*)			
	H□T test	5	0
	KIT p.D816V blood	5	1
	N-MH	4	1
	Bone marrow biopsy	3	2 ISM
HES (11)			
	FIP1L1-PDGFRα	10	0
	H□T test	7	0
	KIT p.D816V blood/marrow	8	0
	N-MH	0	0
	Bone marrow biopsy	5	0
	CKD (GFR < 60)	11	1 HD, 1 CKD Stage 3b
EoE (5)			
	H□T test	5	0
	KIT p.D816V blood	5	0
	N-MH	5	1
	Bone marrow biopsy	1	0
	Absolute Eosinophil Count	5	2 HES-EoE overlap
CKD (7)			
	H□T test	2	0
	KIT p.D816V blood	0	0
	N-MH	1	0

	Bone marrow biopsy	1	1 MDS-EB-1
Other myeloid neoplasm (2)			
	H□T test	0	0
	KIT p.D816V blood	1	0
	JAK2 p.V617F blood	1	1
	N-MH	1	0
	Bone marrow biopsy	1	1 MDS-EB-1
RA (1)			
	H□T test	1	0
	KIT p.D816V blood	1	0
	N-MH	1	0
	Bone marrow biopsy	0	0
No associated diagnosis (14)			
	H□T test	14	0
	KIT p.D816V blood	11	0
	N-MH	9	0
	Bone marrow biopsy	2	0

HD, hemodialysis

Note: Each subject is represented under only one disorder. All subjects had absolute eosinophil counts less than 1500 x 10^9/L and normal GFRs unless otherwise noted.

* 3 subjects with mastocytosis were diagnosed with cutaneous mastocytosis



* **Breakdown of subjects by tryptase values**
58 subjects with at least one BST ≥ 11.5 ng/mL
51 subjects with all BST values < 11.5 ng/mL

‡ **Breakdown of subjects by tryptase values**
8 subjects with at least one BST ≥ 11.5 ng/mL
40 subjects with all BST values < 11.5 ng/mL

Figure E1

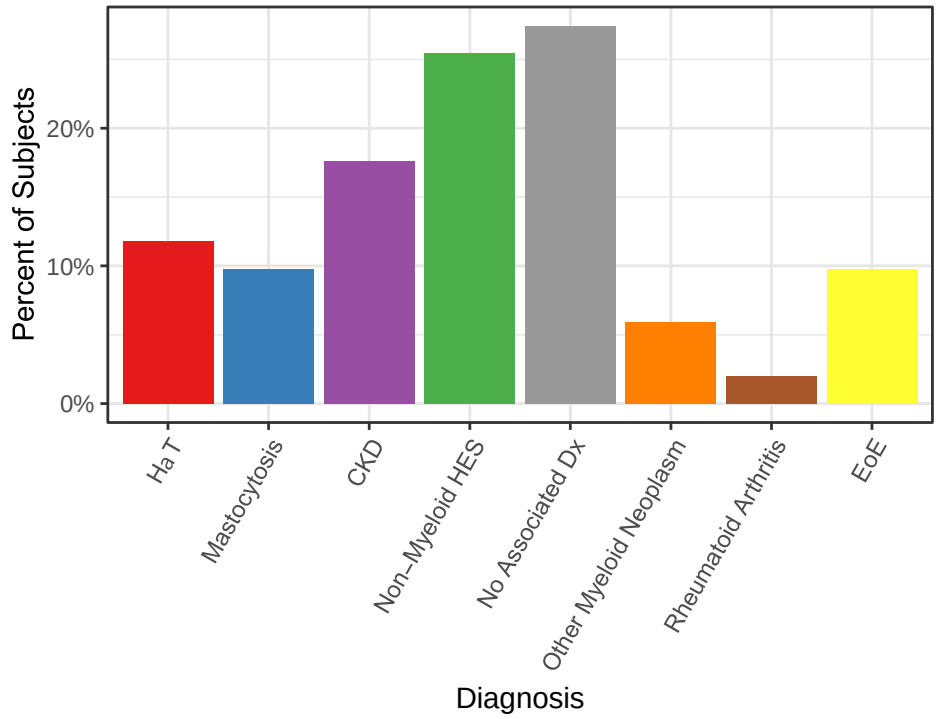

Percent of Subjects
20%
10%
0%
Ha T
Mastocytosis
CKD
Non–Myeloid HES
No Associated Dx
Other Myeloid Neoplasm
Rheumatoid Arthritis
EoE
Diagnosis

Figure E2

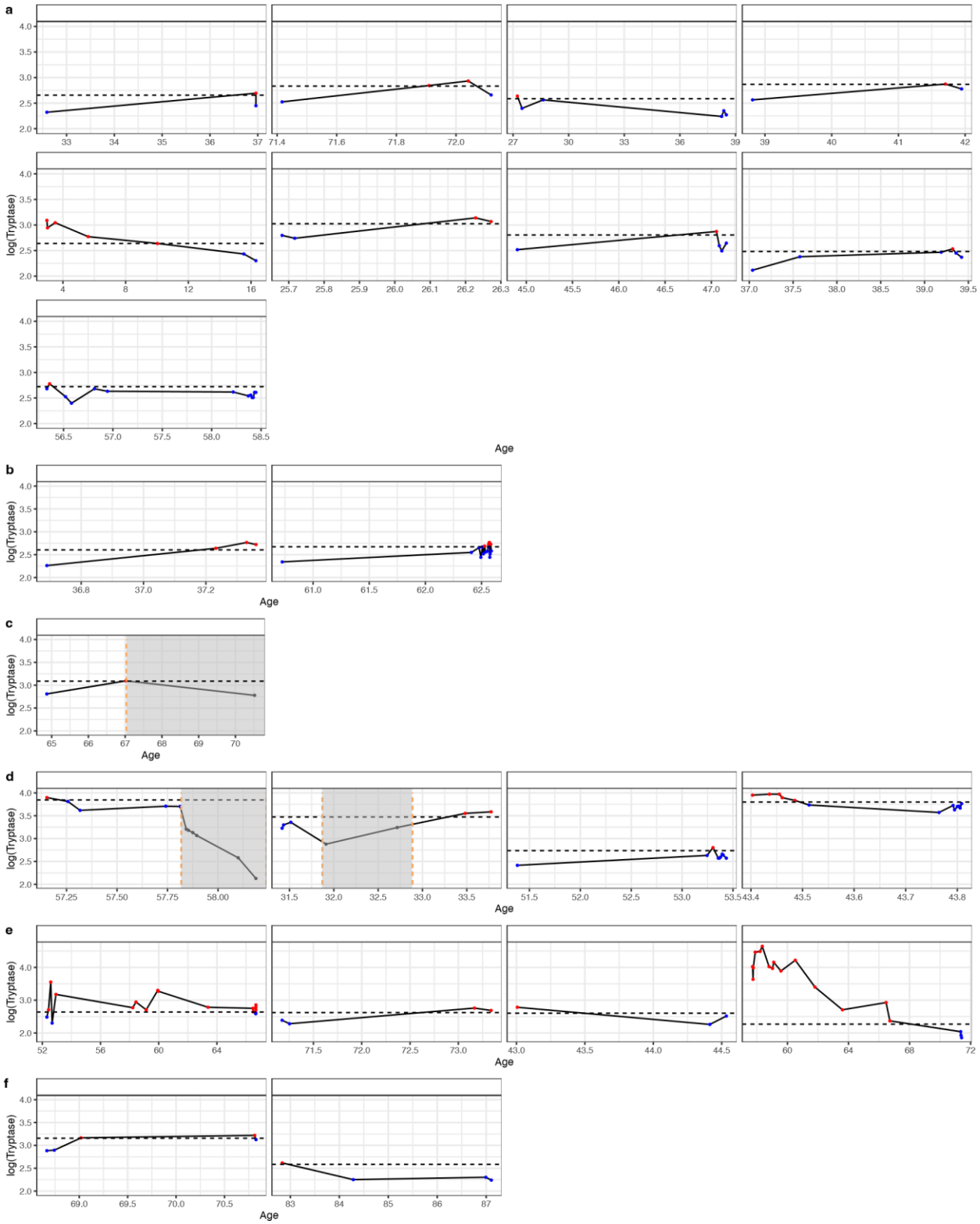


Figure E3 a-f