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Published in:
Journal of Allergy and Clinical Immunology

DOI:
[10.1016/j.jaci.2020.11.017](https://doi.org/10.1016/j.jaci.2020.11.017)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Giannetti, M. P., Akin, C., Hufdhi, R., Hamilton, M. J., Weller, E., Anrooij, B. V., Lyons, J. J., Hornick, J. L., Pinkus, G., Castells, M., & Pozdnyakova, O. (2021). Patients with mast cell activation symptoms and elevated baseline serum tryptase level have unique bone marrow morphology. *Journal of Allergy and Clinical Immunology*, 147(4), 1497-1501.e1. <https://doi.org/10.1016/j.jaci.2020.11.017>

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Patients with mast cell activation symptoms and elevated baseline serum tryptase level have unique bone marrow morphology

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Background: Patients with mast cell (MC) activation symptoms and elevated baseline serum tryptase level (MCAS-T) may not necessarily have a clonal MC disorder. Many are diagnosed with hereditary α -tryptasemia (H α T), a genetic trait characterized by autosomal dominant inheritance of multiple copies of *TPSAB1* encoding α -tryptase and increased risk for severe anaphylaxis. **Objective:** The aim of our study was to identify and characterize bone marrow MC histopathologic features specific for MCAS-T. **Methods:** A total of 43 patients with MCAS-T underwent evaluation, including bone marrow biopsy, for a MC disorder. The results of the work-up for clonal MC disorders such as systemic mastocytosis and monoclonal MC activation syndrome were negative. Bone marrow MC histopathology was reviewed to identify characteristic features of MCAS-T. A subgroup of patients was available for tryptase genotyping. **Results:** Patients with MCAS-T showed unique morphologic and histologic features when compared with controls. MCs were larger ($P < .01$), hypogranular ($P < .01$), frequently detected in paratrabecular ($P < .05$) and perivascular ($P < .01$) locations, and associated with bone marrow eosinophilia ($P < .01$). A total of 10 patients who were available for tryptase genotyping were

all confirmed to have H α T. This subgroup was representative of the larger MCAS-T cohort.

Conclusion: We report unique bone marrow MC phenotypic and histopathologic changes in patients with MCAS-T. These morphologic changes are associated with an elevated tryptase level that has been confirmed to be caused by H α T in all patients available for testing. (J Allergy Clin Immunol 2021;147:1497-501.)

Key words: Mastocytosis, MC activation syndrome, hereditary α -tryptasemia, tryptase, mast cell morphology, bone marrow biopsy

INTRODUCTION

Mast cells (MCs) are tissue-resident cells that perform a variety of effector functions of the immune system.^{1,2} Clinical disorders of MCs have traditionally been classified into clonal and nonclonal entities on the basis of their molecular and histologic characterization.^{3,4} Regardless of their classification, all MC disorders are manifested with characteristic and paroxysmal symptoms involving multiple organ systems.^{5,6}

Systemic mastocytosis is the prototypical clonal MC disorder. It is histopathologically well characterized. Characteristic bone marrow findings include large (>15 MCs), typically paratrabecular or perivascular aggregates of MCs with aberrant coexpression of CD25 and/or CD2 and an atypical spindle-shaped MC morphology.⁷ The disease is almost invariably associated with activating mutations in the *KIT* gene.⁸ In contrast, there is little published literature regarding the pathologic characterization of nonclonal MC disorders.⁹ Idiopathic MC activation syndrome is a nonclonal MC disorder defined by clinical and laboratory criteria.^{10,11} As the diagnosis of idiopathic MC activation syndrome is based on clinical and laboratory data, patients do not routinely undergo bone marrow biopsy specimens and there are few studies characterizing bone marrow histopathologic features in nonclonal MC disorders aside from the absence of evidence for clonal disease.¹²

It has also been established that many patients with an elevated baseline serum tryptase level have symptoms of MC activation but do not meet the criteria for a clonal MC disorder. These patients may have hereditary α -tryptasemia (H α T), a genetic trait described by Lyons et al in 2016 and the most common cause of elevated baseline serum tryptase level in the general population.¹³ H α T is characterized by an elevated baseline serum tryptase level and multiple copies of *TPSAB1* gene encoding α -tryptase. This trait is present in approximately 5% of Western populations and

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Supported in part by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Disclosure of potential conflict of interest: M. Giannetti, M. Castells, and C. Akin receive salary support from Blueprint Medicines. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 18, 2020; revised November 13, 2020; accepted for publication November 18, 2020.

Available online November 25, 2020.

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0091-6749/\$36.00

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<https://doi.org/10.1016/j.jaci.2020.11.017>

Abbreviations used

HαT:	Hereditary α-tryptasemia
ISM:	Indolent systemic mastocytosis
MC:	Mast cell
MCAS-T:	Mast cell activation symptoms and elevated baseline serum tryptase level

is inherited in an autosomal dominant manner.^{14,15} HαT has been associated with a variety of symptoms, including anaphylaxis, flushing, irritable bowel syndrome, arthralgias, and small fiber neuropathy.¹⁶ Diagnosis is confirmed by identifying extra allelic copies of *TPSAB1* encoding α-tryptase. In 5 patients, bone marrow biopsy specimen histopathology was notable for increased MC numbers, lack of MC aggregates, and absence of CD2 and CD25 expression.¹²

Here we have characterized the bone marrow MC morphologic findings in a cohort of patients with elevated baseline serum tryptase level and symptoms of MC activation. Our data identify atypical MC bone marrow features associated with mast cell activation symptoms and elevated baseline serum tryptase level (MCAS-T) that may predispose to clinical symptoms.

RESULTS AND DISCUSSION

A total of 43 patients were included in this study. All patients had an elevated baseline serum tryptase level ranging from 11.3 to 43.0 ng/mL (mean 19.5 ng/mL). Patients' age ranged from 28 to 84 years and the majority were female (29 of 43 [67%]). Physician-diagnosed anaphylaxis was reported in 19 of 43 patients (44%). Other allergic conditions such as allergic rhinitis, food allergy, and atopic dermatitis were reported in 21 of 43 patients (49%). The skin was the most commonly affected organ system (27 of 43 [63%]), followed by gastrointestinal (19 of 43 [44%]), pulmonary (13 of 43 [30%]), psychiatric (11 of 43 [26%]), and neurologic (7 of 43 [16%]) symptoms. The majority of patients were treated with medications to block MC mediators. Clinical symptoms and treatments are detailed in Table I.

We selected 12 histologic parameters for evaluation of biopsy specimens on the basis of observations from patients with idiopathic SM (ISM). Table II details the bone marrow MC morphology and topography in all groups. As expected, the biopsy specimens from patients with ISM showed a much higher MC burden than in patients with MCAS-T (12% vs 0.8% [$P < .001$]). The percentage of MCs in the MCAS-T group was similar to that in the normal control group (0.8% vs 0.6% [$P = .28$]). The majority of patients with MCAS-T had MC infiltration less than 1% of the overall marrow cellularity (27 of 43 [63%]). MCs in the MCAS-T group demonstrated atypical cell morphology that resembled the morphology found in ISM. In comparison with the MCs of the normal control group, the MCs of patients with MCAS-T and ISM were larger; however, the MCs in patients with ISM were significantly larger than those in the MCAS-T group (1.8 vs 1.3 [$P < .01$]); all MC size measurements were relative to a resting lymphocyte. Patients with MCAS-T had hypogranular MCs in 56% of biopsy specimens (24 of 43 patients), which was similar to the percentage in the biopsy specimens of patients with ISM (69% [9 of 13 patients]) ($P = .31$) but significantly higher than in the controls ($P < .01$). The MCAS-T group demonstrated MC clusters in 9% of cases (4 of 43 patients);

unlike in patients with ISM, these clusters were small and contained only 2 MCs. No such clusters were present in the normal control group (Fig 1).

Patients in the MCAS-T group demonstrated abnormal MC perivascular localization (28 of 43 patients [65%]). This percentage was similar to that in the ISM group but increased compared with the percentage in the control group ($P < .01$). Similarly, MC paratrabecular localization was frequently noted in the group with MCAS-T (24 of 43 [56%]), which was significantly more than in the normal control group ($P < .05$). Eosinophilia was seen in 44% of MCAS-T biopsy specimens (from 19 patients), a percentage similar to that in the ISM group but significantly increased compared with the percentage in the normal control group ($P < .001$). Fig 2, A graphically represents the bone marrow phenotype in the MCAS-T cohort.

A total of 10 patients (10 of 43 [23%]) within the MCAS-T group were available for confirmatory genetic testing for the diagnosis of HαT; all were found to have HαT. Complete morphologic features seen in these 10 patients are represented in Table II and Fig 2, B. The MC morphologic patterns in the HαT group were similar to those in the entire MCAS-T group, reproducing an increased MC burden of more than 0.5% in 8 of 10 patients (80%), larger MCs, hypogranulation, paratrabecular and/or perivascular localization, and associated eosinophilia. There were no statistically significant differences in any category between patients with a confirmed genetic diagnosis of HαT ($n = 10$) and the remainder of the MCAS-T cohort ($n = 33$).

We report a unique bone marrow phenotype of patients with elevated baseline tryptase level without evidence of clonal MC disorder, likely representing histologic changes associated with HαT. HαT is a genetic trait associated with extra allelic copies of *TPSAB1* on chromosome 16 encoding α-tryptase and resulting in a basal tryptase level higher than 8.0 ng/mL.^{13,14} HαT is associated with complex symptomatology and associated MC activation, including anaphylaxis. This study describes patients with symptoms of MCAS-T who underwent a bone marrow biopsy and were not found to have a clonal MC disorder based on lack of MC aggregates of more than 15 cells, *KIT* p.D816V, and absence of aberrant MC CD2 or CD25 expression.

Patients presented with clinical symptoms consistent with MC activation and an elevated baseline serum tryptase level. The mean tryptase level in the MCAS-T cohort was 19.4 ng/mL, which is similar to the level in the initial description of HαT by Lyons et al.¹³ Symptoms involved multiple organ systems, with the skin and GI tract being the most prominent. It is also notable that almost half of the patients (19 of 43) reported physician-diagnosed anaphylaxis, which is consistent with the findings of prior studies.¹⁵ Patients with anaphylaxis had increased total IgE levels compared with those in patients without anaphylaxis ($P < .05$), which is consistent with prior observations. Serum IgE level did not correlate with MC hypogranularity. Anaphylaxis was not more common in patients with a higher MC burden. Of note, 1 patient presented exclusively with psychiatric symptoms. Psychiatric symptoms such as mood disorder and cognitive abnormalities occur in many chronic conditions and may be associated with HαT but are not diagnostic of a MC disorder.

We utilized 12 morphologic parameters to assess similarities and differences between clonal and nonclonal MC disorders (Table II). Many patients with MCAS-T meet 1 or 2 minor criteria for SM. For example, 19 patients (44%) demonstrated the presence of abnormal MC morphology with a median of 25.8%

TABLE I. Clinical and demographic characteristics of 43 patients with MC activation symptoms and elevated baseline serum tryptase level

Patient	Age	Sex	Baseline tryptase (ng/mL)	Symptoms	Anaphylaxis	Total IgE level (IU/mL)	Medication	Tryptase copy number
1	55	F	17.3	A	N	455	S	
2	50	M	19.1	A	Y		H1	
3	46	M	14.0	G	Y		H1, H2, CR	
4	84	M	13.1	S	Y	48	H2	
5	49	F	21.6	S	N	18	H1	
6	74	F	14.5	P, MSK	N		H1, ASA	
7	65	M	27.3	P	N		ASA	
8	35	F	17.0	CV, NR	N	8	H1, H2, CR	
9	61	M	13.3	CV, R	Y	51	5LO, CR	
10	61	M	25.0	G, MSK	N	25		
11	63	F	33.7	P, R	N	66	H1, H2, SC, CR	3α, 2β
12	69	M	16.4	R, A	Y	890	H1, H2, S, omalizumab	
13	53	F	14.5	S, A	N	33	H1, H2, CR	2α, 3β
14	52	F	24.0	S, A	N	378	H1, H2, ASA	
15	59	F	18.5	S, A	Y	135	H1, LR, ASA, CR	3α, 3β
16	76	F	16.9	S, G	N	166	H1, H2, LT, ASA	
17	49	F	20.0	S, G	Y		H1, H2	
18	46	F	11.3	S, G	Y	28	H1, H2	
19	57	M	15.0	S, NR	N	9	ASA	
20	32	F	17.0	G, A, MSK	N		LT	
21	61	F	14.5	G, P, A	N		ASA, SC	
22	68	F	18.5	P, R, A	Y	50	H1, S, LT	
23	76	M	29.0	S, G, C	Y		H1 H2	
24	64	M	14.0	S, G, MSK	N	11	H1, H2, CR, SC	
25	81	F	22.9	S, G, P	N		ASA	
26	79	F	19.5	S, G, R	N	13	H1, H2	
27	63	F	33.5	S, G, R	N		H1, CR	
28	44	F	18.0	S, P, CV	N			2α, 3β
29	60	F	24.4	S, R, A	Y	857	H1 H2, CR, omalizumab	2α, 3β
30	63	F	20.0	S, R, A	Y	165	H1, CR	
31	56	M	18.7	S, R, A	Y	184	H1	
32	28	F	43.0	CV, N, A, MSK	N		H1, H2, LT, CR	
33	54	F	19.0	G, P, R, A	N		H1 H2	2α, 3β
34	63	F	19.0	S, R, N, MSK	Y	8	H1, H2, CR, LT, SC	2α, 3β
35	49	M	28.0	S, P, C, A	Y	1030	ASA	
36	69	F	17.0	S, P, CV, A	N	11	H1, H2, CR	
37	52	F	17.3	S, G, CV, A	Y	83	H1 H2	
38	54	F	12.0	S, G, P, R, A	N		H1, LT	2α, 3β
39	62	F	21.3	S, G, R, A	Y	1280	H1, SC	4α, 2β
40	59	F	12.5	S, G, CV, NR, MSK	N	8	H1, H2, CR, LR	3α, 2β
41	47	M	19.9	S, G, CV, NR, A	Y		H1, LT	
42	48	F	11.3	S, G, CV, NR, A	Y		H1, H2, ASA, CR	
43	44	M	15.0		N			

A, Allergic/immunologic; ASA, aspirin; β₂, albuterol; CR, oral cromolyn; CV, cardiovascular; GI, gastrointestinal; F, female; H1, H1 antagonist; H2, H2 antagonist; LT, cysteinyl leukotriene antagonist; M, male; MSK, musculoskeletal; NR, neurologic; N, no; P, psychiatric; R, respiratory; S, skin/cutaneous; SC, systemic corticosteroid; Y, yes. All ages are reported at the time of data analysis. In patients with confirmed HαT, α and β copy numbers are listed.

TABLE II. Bone marrow MC morphology and topography

Variable	MC infiltrate, mean %, (range)	Biopsy specimens with spindled MCs, n (%)	MC size, median	MC hypogranulation, %	Biopsy specimens with MC clusters, %	MC cluster size, cell (range)	Perivascular	Paratrabecular	Increased eosinophil count
MCAS-T (n = 43)	0.8*† (0.1-2)	19 (44%)*†	1.3*†	24 (56%)*†	4 (9%)*	2 (2)*†	28 (65%)*	24 (56%)*†	19 (44%)*
Confirmed HαT (n = 10)	0.9 (0.5-2)	4 (40%)	1.3	8 (80%)	2 (20%)	2	4 (40%)	5 (50%)	6 (60%)
ISM (n = 13)	12 (0.5-50)	13 (100%)	1.8	9 (69%)	9 (69%)	18.3 (1-50)	10 (77%)	13 (100%)	4 (31%)
Control (n = 10)	0.6 (0.5-1)	6 (60%)	1	0	0	n/a	2 (20%)	2 (20%)	0

Bone marrow morphology, topography, and associated features are shown for patients with MCAS-T and the 2 control groups (the group with ISM and the normal control group). Patients with confirmed HαT are a subset of those with MCAS-T. No statistically significant differences were seen between the HαT group and the remainder of group with MCAS-T (data not shown).

*Denotes statistical significance ($P < .05$) compared with the control group.

†Denotes statistical significance ($P < .05$) compared with the group of those with ISM.

FIG 1. Bone marrow morphologic findings in patients with ISM and H&T. **A**, Bone marrow biopsy specimen from a patient with ISM shows clusters of MCs that are positive for MC tryptase and CD117/KIT and show aberrant coexpression of CD25. Many MCs in a paratrabecular and perivascular location are present (*blue arrows*) (immunoperoxidase stain; original magnification, $\times 400$). **B** and **C**, Bone marrow morphologic findings in a patient with H&T. **B**, Scattered MCs are highlighted by MC tryptase stain (1%-2% of marrow cellularity) and are negative for CD25 (immunoperoxidase stain; original magnification, $\times 400$ [Olympus BX41 microscope]). **C**, (*Clockwise starting with the top panel*) The composite image highlights the perivascular location of MCs, a 2-cell MC cluster, paratrabecular locations, spindled MCs, and the range of MC sizes (small, intermediate, and large) (MC tryptase immunoperoxidase stain; original magnification, $\times 1000$ [Olympus BX41 microscope, Olympus Life Science, Tokyo, Japan]). The images were taken with an Olympus DP27 camera and acquired by using cellSense Entry Microscope Imaging Software (Olympus Life Science).

A																				2	1	0.5	0.1
MC infiltrate, %																							
MC size, in relation to lymphocyte																							
MC spindled shape																							
MC hypogranulation																							
MC clusters																							
MC perivascular																							
MC paratrabecular																							
Increased eosinophils																							

B																				2	1	0.5	0.1
MC infiltrate, %																							
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MC clusters																							
MC perivascular																							
MC paratrabecular																							
Increased eosinophils																							

FIG 2. **A**, Distribution of the most significant morphologic bone marrow findings in the group with MCAS-T (n = 43). Percentage of MC infiltrate was recorded as 2% (*red*), 1% (*orange*), 0.5% (*yellow*), or 0.1% (*green*). MC size was noted in relation to lymphocytes and recorded as 2 $\times$ (*red*), 1.5 $\times$ (*yellow*), or 1 $\times$ (*green*). All other variables are present if *red*, absent if blank. **B**, Distribution of the most significant morphologic bone marrow findings in patients with confirmed H&T (n = 10). MC infiltrate percentage was recorded as 2% (*red*), 1% (*orange*), 0.5% (*yellow*), or 0.1% (*green*). MC size was noted in relation to lymphocyte and recorded as 2 $\times$ (*red*), 1.5 $\times$ (*yellow*), or 1 $\times$ (*green*). All other variables are present if *red*, absent if blank.

spindled MCs (range 5%-80% of MCs) and 14 patients (33%) had a baseline serum tryptase level greater than 20 ng/mL, although the presence of H α T may now confound the latter criterion. It is important to note, however, that identification of MC clonality was an exclusion criterion; therefore, none of the bone marrow biopsy specimens from the MCAS-T cohort fulfilled the diagnostic criteria for any clonal MC disorder.

Why individuals with an elevated baseline tryptase level demonstrate abnormal morphologic MC features and unique clinical symptomatology is unclear. It is reasonable to speculate that extra allelic copies of *TPSAB1* alter normal MC function and protein expression, resulting in morphologic and topographic changes. These changes may contribute to symptomatology as H α T is more prevalent and correlates with anaphylaxis in patients with mastocytosis.¹⁷ Separately, Plum et al describe strong expression of adhesion proteins in MCs, such as CD312, sialic acid-binding Ig-like lectin 6 (SIGLEC6), SIGLEC8, and CD44.¹⁸ Upregulation of adhesion molecules may manifest as small MC cluster formation and perivascular or paratrabecular MC localization. In addition, recent data indicate that tryptase heterotetramers (2 α and 2 β) are catalytically active and can interact with PAR2 and EMR2.¹⁹ Cleavage of CD312, a receptor for dermatan sulfate, by MC-derived tryptase, may render MCs susceptible to degranulation. Overexpression of CD312 and increased levels of tryptase in H α T may contribute to hypogranular MC morphology.

There are several weaknesses of this study. First, not all individuals were available for tryptase genetic testing. Tryptase genotype was obtained in 10 of 43 patients (23%); H α T was confirmed in all 10 patients. There were no statistically significant differences between the H α T group and remainder of the MCAS-T group (Table II), which suggests that our findings are representative of H α T and might be extended to all patients with symptomatic H α T. Second, our data likely have referral bias, as all patients evaluated at the Brigham and Women's Hospital mastocytosis center presented with clinical symptoms and abnormal laboratory findings. Because of the prevalence of H α T, many patients with this trait are likely to be asymptomatic. These data will need to be confirmed in an unselected cohort of patients with H α T. Finally, histopathologic evaluation is challenging in this patient population, as the low MC burden in bone marrow biopsy specimens makes it difficult to evaluate overall MC morphology and topography. It is possible that these subtle changes represent early manifestations of a clonal MC disorder (particularly in patients without confirmed genetic H α T). At the time of biopsy, however, there was no evidence of clonal MC disease. No patient had CD2 or CD25 expression; the results of testing for *KIT* p.D816V were negative in all tested patients (37 of 43 all patients [86%]). Furthermore, none of the patients have been subsequently diagnosed with a clonal MC disorder over a follow-up period of 5 to 13 years.

In summary, we propose that patients with MC activation symptoms and an elevated baseline serum tryptase level have a distinctive bone marrow phenotype defined by both morphologic and topographic changes within the bone marrow MC compartment. Our findings suggest that the presence of increased copies of *TPASB1* encoding α -tryptase is associated with intrinsic MC abnormalities in those with symptoms of MCAS.

For detailed methods, please see the Methods section in this article's Online Repository at www.jacionline.org.

Clinical implications: H α T accounts for MCAS-T in all individuals available for testing and is associated with intrinsic bone marrow MC abnormalities and clinical features of MC activation.

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METHODS

Patient population

The study was approved by the Partners Healthcare Institutional Review Board under protocols 2012P002481 and 2018P000122. A subset of patients were active participants who had provided informed consent on National Institute of Allergy and Infectious Diseases institutional review board–approved studies to study allergic inflammation and reactions (NCT01164241 and NCT00852943). This study was a retrospective evaluation of 43 patients who presented to the Brigham and Women's Hospital Mastocytosis Center from 2007 to 2015. All patients underwent a complete medical history, physical examination, laboratory testing, and bone marrow examination as part of the evaluation. The inclusion criteria were (1) symptoms consistent with MC activation, (2) a baseline serum tryptase level greater than 11.3 ng/mL, and (3) a bone marrow biopsy at Brigham and Women's Hospital. Patients with clonal MC disorders, such as systemic mastocytosis, monoclonal MC activation syndrome, or another myeloid malignancy were excluded (however, 13 patients with ISM were used as controls [see the next section]). Clonal MC disorders were defined according to the World Health Organization criteria and included all or any 1 of the following: presence of large MC aggregates of more than 15 cells, *KIT* p.D816V variant, and CD2 or CD25 expression on MCs.

Control patients

The study included 2 control groups. The first group included 10 patients with normal bone marrow biopsy findings who underwent a bone marrow examination for staging of a lymphoma diagnosis (the control group). This group had a mean age of 67 years and a 1:1 male-to-female ratio. The second group consisted of 13 patients with ISM who underwent a bone marrow examination as a part of routine clinical evaluation (the positive control group). The mean age of the members of this group was 61 years, and their mean baseline serum tryptase level was 83.3 ng/mL. Anaphylaxis was reported in 9 of 13 patients (69%).

Bone marrow morphologic evaluation

Bone marrow biopsy specimens were fixed in Bouin solution and briefly decalcified in RapidCal-Immuno (BBC Biochemical, McKinney, Tex). Morphologic evaluation was performed on bone marrow biopsy specimens stained with hematoxylin and eosin and Giemsa stain. Antibodies against the following antigens were used on the bone marrow core samples: CD117/*KIT* (Dako, Santa Clara, Calif), MC tryptase (Dako), CD2 (Leica, Wetzlar, Germany; clone AB75), and CD25 (Lifespan Biosciences, Seattle, Wash;

clone 4C9). All morphologic parameters were assessed on the bone marrow core sample, and a minimum of 3 sections were examined for each patient. We assessed the following parameters: percentage of MCs in the total bone marrow cellularity; percentage of MCs that were spindled; MC size; MC granulation; presence of MC clusters of any size and numbers of MCs within the clusters; presence of perivascular MCs; presence of paratrabecular MCs; presence of fibrosis; presence of bone marrow eosinophilia; presence of MC, eosinophil, and lymphocyte (MEL) aggregates; and presence of CD2 and CD25 coexpression on MCs. An MC cluster was defined as at least 2 MCs that were touching each other. MC size was measured in relation to a small lymphocyte, with 1× being the same size as a small lymphocyte and 1.5× and 2× being 1.5 and 2 times larger than a small lymphocyte, respectively. Paratrabecular and/or perivascular location was considered present when no intervening cells were present between MCs and bone trabeculae or vessels. MC granularity was assessed on Giemsa-stained bone marrow biopsy specimens or May-Grunwald-Giemsa–stained bone marrow aspirates; MCs with complete absence of or only a few cytoplasmic basophilic granules were considered hypogranular. Morphologic evaluation was conducted by several hematopathologists during the clinical evaluation of bone marrow biopsy specimens. For the study, the pathology was rereviewed by 1 hematopathologist (O.P.) who was blinded to all clinical information.

Tryptase genotyping

Genetic testing to confirm the diagnosis of HαT was conducted by GenebyGene, Ltd (Houston, Tex) and on a research basis at the National Institutes of Health (J.J.L.) as described.^{E1} All results are reported as α-tryptase copy number and β-tryptase copy number.

Statistical analysis

All intergroup comparisons were conducted with the Fisher exact test or Student *t* test. Samples were considered independent (unpaired). A 2-tailed test was used for *t* test comparisons. A *P* value less than .05 was considered significant. Microsoft Excel software was used for the Student *t* test and Statsmodels software (available at [Statsmodels.org](https://statsmodels.org)) was used for the Fisher exact test.

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