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Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities

Jonathan J. Lyons, MD¹, Guangping Sun, MD¹, Kelly D. Stone, MD, PhD¹, Celeste Nelson, CRNP¹, Laura Wisch, MSN¹, Michelle O'Brien, RN, BSN¹, Nina Jones, RN, BSN², Andrew Lindsley, MD, PhD³, Hirsh D. Komarow, MD¹, Yun Bai, MS¹, Linda M. Scott, MS, CRNP¹, Daly Cantave, MSN⁴, Irina Maric, MD⁵, J. Pablo Abonia, MD³, Marc E. Rothenberg, MD, PhD³, Lawrence B. Schwartz, MD, PhD⁶, Joshua D. Milner, MD¹, and Todd M. Wilson, DO¹

¹Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

²Clinical Research Directorate/CMRP, Leidos Biomedical Research Inc., (formerly SAIC-Frederick, Inc.), Frederick National Laboratory for Cancer Research, Frederick, MD

³Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

⁴Clinical Center, National Institutes of Health, Bethesda, MD

⁵Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD

⁶Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA

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To the Editor

The etiology of allergic disease is generally considered to be a complex polygenic process highly influenced by environmental exposures with mast cells playing a major effector role. Genome-wide association studies and candidate gene approaches have produced an array of allelic variants which impart risk for allergic diatheses. (E1) While identification of monogenic allergic disease and inherited mast cell disorders has remained less common,

Correspondence: Joshua D. Milner, Building 10, Room 9N208C-C1, National Institutes of Health, Bethesda, MD 20892-1881, jdmilner@niaid.nih.gov, Tel: (301) 827-3662, Fax: (301) 480-8384. Todd M. Wilson, Building 10, Room 9N214-D1, National Institutes of Health, Bethesda, MD 20892-1881, twilson@mail.nih.gov, Tel: (301) 435-0768, Fax: (301) 480-3661.

Conflicts of Interest

The NIH authors declare no conflict of interest. LBS receives royalties from VCU that are collected from ThermoFisher for the tryptase UniCAP assay. MER has received consultancy fees from Immune Pharmaceuticals; has patents owned by CCHMC; may receive royalties from Teva Pharmaceuticals; and has stock/stock options in Immune Pharmaceuticals.

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several heritable mutations have been identified which help illuminate some pathophysiologic mechanisms underlying the development of atopy in the general population. These include: SPINK5 mutations in Netherton syndrome (1); dominant negative STAT3 mutations in a hyper-IgE syndrome (2); and PLCG2 mutations in a form of familial cold urticaria. (3) Within the mast cell compartment, both germ line and somatic mutations in KIT have been identified in rare familial presentations of mastocytosis. (4, 5)

While seeking to characterize patients with severe, unique, inherited allergic phenotypes among patients referred for evaluation of severe allergic skin, airway or gastrointestinal disease, or systemic mastocytosis (SM), we identified nine atopic individuals with persistent elevations in serum basal tryptase levels in the absence of evidence for a clonal mast cell disorder. Thorough clinical evaluations were undertaken, total and fractionated serum tryptase levels were obtained, *in vitro* basophil activation was assayed, and bone marrow biopsies were performed in 5 of these index patients. For a complete description see the *Methods* section in this article's Online Repository at www.jacionline.org. Elevated basal serum tryptase was found to segregate with distinct clinical features and it was noted that multiple family members of each index patient shared elements of this phenotype. Serum tryptase levels were obtained in all available family members and an autosomal dominant inheritance pattern of elevated basal total serum tryptase was revealed in 9 families. (Table I) The mean basal total serum tryptase obtained during a resting state was 21.6 ± 1.4 ng/mL among individuals with inherited tryptasemia ($n = 33$). Five family members' tryptase levels were unable to be obtained; these data were excluded from statistical analysis, but are included with unaffected family members in Table E1 of this article's Online Repository at www.jacionline.org. Tryptase fractionation in a subset of 18 affected patients from 8 of 9 families further revealed that mature tryptase in the serum was undetectable (<1 ng/mL). (see Table E2 in this article's Online Repository at www.jacionline.org)

Prominent among features segregating with basal serum tryptase elevations were symptoms consistent with chronic and episodic mast cell degranulation, with 26/33 reporting episodic urticaria, flushing, and/or cramping abdominal pain, frequently associated with urgency and/or diarrhea. These symptoms could be unprovoked, or triggered by heat, exercise, vibration, emotional stress, non-specific foods, or minor physical trauma. A history of anaphylaxis was reported in 10/33 individuals from six different families; seven occurring with exposure to foods, two due to insect stings, and another without an identified trigger. Additional triggers in three individuals with food-mediated anaphylaxis included stress/heat/exercise ($n = 2$) and anesthesia ($n = 1$). (Table I) One individual (Family 1) with normal serum tryptase (6.6 ng/mL) had a history of anaphylaxis; this reaction was due to hymenoptera envenomation. (see Table E1 in this article's Online Repository at www.jacionline.org)

Gastrointestinal manifestations, whether chronic or episodic, were another prominent feature seen in 28/33 individuals with elevated tryptase; these included eosinophilic gastrointestinal disease, gastroesophageal reflux disease (GERD), tenesmus, fecal urgency, irritable bowel syndrome (IBS), and diarrhea. Atopic symptoms were present in 31/33 tryptasemics, with environmental allergies and asthma being reported amongst 28/33 affected individuals. Connective tissue abnormalities were present in 23/33 individuals with elevated tryptase

from 8/9 families, and chronic musculoskeletal pain was present among 11/33 tryptasemic individuals from 6/9 families. Autonomic dysfunction, manifest as postural orthostatic tachycardia syndrome (POTS) was reported in 10/33 affected individuals and a neuropsychiatric diagnosis was present in 17/33 affected individuals. (Table I) Each of these five clusters of clinical characteristics (cutaneous, connective tissue, GI, atopic, neuropsychiatric) were independently and significantly associated with elevated tryptase. (Figure 1A) For additional clinical characterization of the families see the *Results* section in this article's Online Repository at www.jacionline.org.

Because some of the reported symptoms in tryptasemic individuals could have been consistent with the familial presentation of a clonal mast cell disorder, index patients in 5/9 families underwent bone marrow biopsy. A significant increase in mast cells was observed ($P<0.05$) (mean 9.6 cells/HPF, range 4–19.4) compared to normal volunteers (mean 2.5 cells/HPF, range 1–5). (see Figure E1 in this article's Online Repository at www.jacionline.org) However none of the patients met the WHO established criteria for a diagnosis of SM or Monoclonal Mast Cell Activation (MMAS). (E2) Mast cell aggregates were not present, aberrant expression of CD2/CD25 was absent, and *KIT* D816V mutation testing was negative in all 5 patients. Spindled shaped mast cells (>25%) were observed in a single patient. In the 4 families in which a bone marrow biopsy was not performed, the clinical phenotype aligned with the cohort in which SM was excluded.

In vitro basophil activation was assessed in affected individuals from 6 of 9 families ($n = 10$). While basal CD203c expression was comparable to paired healthy controls, basophils from affected individuals demonstrated reduced activation, as measured by CD203c mean fluorescence intensity (MFI) following stimulation with anti-IgE or fMLP. (Figure 1B) However, CD63 induction within the CD203c⁺ basophil population, while variable, was not significantly different between patients and paired controls. (Figure 1C)

We report a series of families with elevated basal serum pro-tryptase following a dominant inheritance pattern associated with symptoms consistent with mast cell mediator release and additional non-atopic features. Phenotypic characteristics common in this syndrome include episodic flushing, pruritus, gastrointestinal symptoms and chronic musculoskeletal pain – symptoms frequently observed in SM. Additional characteristics not classically associated with inherited or somatic clonal mast cell diseases included urticaria, EoE, and a hypermobile connective tissue phenotype. Despite bone marrow findings showing increased numbers of mast cells among those patients sampled, a diagnosis of SM or MMAS was not established in these individuals. The mechanism resulting in impaired basophil activation *in vitro* remains unknown. Suppression of CD203c by endogenous histamine, (6) or impaired activation due to a refractory state, are possible explanations. (E3) Caution must be exercised with regard to generalization of the identified associations given the small sample size and preliminary nature of this report; additional discretion must be exercised with regard to those data which were derived from a sub-population of our cohort. Furthermore, several potential biases may exist in these data with regard to referral, detection, and recall. However tryptase is a robust biomarker and the finding of families with inherited elevations is novel.

Whether elevations in serum tryptase and mast cell numbers are primary or secondary phenomena is currently under investigation, however this phenotype appears to represent a distinct familial variant in the spectrum of mast cell disorders. This cohort shares many clinical features observed in both mastocytosis and Mast Cell Activation Syndrome (MCAS) and it remains uncertain how many patients diagnosed with MCAS may have dominantly inherited tryptasemia. (7) While symptoms of mast cell mediator release among multiple first-degree relatives of patients with diagnosed mast cell disorders have been described, (E4) the presence of connective tissue disease provides further distinction of this familial phenotype. Recently other previously characterized connective tissue syndromes (Loeys-Dietz, Marfan's, and Ehlers-Danlos Syndromes) with some features common to our cohort were also found to be associated with atopic disease. (8, 9) Additional genetic study is ongoing, and as the dysfunctional pathways in these families are elucidated, we may gain additional insights into the hypermobile connective tissue phenotype, atopic disease, autonomic dysfunction, neuropsychiatric illness may be gained, and their interface with mast cell mediators.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

POTS	Positional Orthostatic Tachycardic Syndrome
EDS	Ehlers-Danlos Syndrome
LDS	Loeys-Dietz Syndrome
SPINK5	serine peptidase inhibitor, kazal type 5
PLAID	PLCG2-associated antibody deficiency and immune dysregulation
PLCG2	phospholipase C, gamma 2
STAT3	signal transducer and activator of transcription 3
GERD	gastroesophageal reflux disease

IBS	Irritable Bowel Syndrome
EoE	eosinophilic esophagitis
AD	atopic dermatitis
SM	systemic mastocytosis
WHO	World Health Organization
BSB	basophil stimulation buffer
fMLP	N-formyl-methionyl-leucyl-phenylalanine
HPF	high power field
MFI	mean fluorescence intensity
PCR/RFLP	polymerase chain reaction/restriction fragment length polymorphism
TGF-β	transforming growth factor beta
PBMCs	peripheral blood mononuclear cells

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Figure 1. Phenotypic Characteristics and *in vitro* basophil activation of patients with inherited tryptase elevation

A, Clinical traits and vibratory challenge results independently and significantly associated with inherited elevations in basal serum tryptase; Fisher's exact test. **B**, Relative difference of CD203c MFI in affected individuals normalized to paired controls, as a percent of paired control MFI, $((\text{Patient-Control})/\text{Control}) \times 100$. **C**, Difference in %CD63+ within the CD203c+ population of affected individuals, from paired controls. Data in C and D are plotted with median $\pm$ interquartile range. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.005$; Wilcoxon-signed rank test.

Table I

Pedigrees, ages, and clinical phenotypes of affected individuals

Ages	8, 10, 44, 70	11, 39, 69	6, 10, 13, 41	21, 55, 55	17, 22, 23, 48, 57, 78	24, 43, 50	2, 26, 29, 53	12, 20, 44, 68	9, 46
Symptoms No. affected									
Cutaneous	4	1	4	3	5	2	2	2	2
<i>Flushing</i>	1	-	2	3	4	1	1	1	-
<i>Urticaria</i>	4	-	3	-	5	2	2	1	1
<i>Angioedema</i>	-	-	1	-	3	-	-	1	-
<i>Pruritis</i>	4	1	2	1	5	1	1	-	2
Connective Tissue	3	3	4	3	0	2	3	3	2
<i>Hypemobility</i>	2	1	4	1	-	2	3	2	-
<i>Retained PrimaryTeeth</i>	-	3	-	1	-	-	-	1	-
<i>Arched Palate</i>	1	1	2	-	-	1	1	-	2
<i>Bone/Vascular abnormality[†]</i>	2	-	-	3	-	-	1	2	-
Atopy	4	3	4	3	6	3	2	4	2
<i>Anaphylaxis</i>	-	2	1	2(1 [†])	3	-	1	1 [†]	-
<i>Eczema</i>	3	1	3	-	3	-	1	2	2
<i>Asthma</i>	4	2	2	2	4	1	2	1	1
<i>Environmental Allergy</i>	4	2	2	2	4	3	1	3	2

<i>Food Allergy</i>	3	2	1	1	4	-	1	1	-
<i>Drug Allergy</i>	3	1	2 ⁵	1	5	2	2	1	-
Gastrointestinal	4	3	4	3	5	2	3	4	0
<i>Episodic Pain</i>	2	-	2	3	3	2	3	2	-
<i>IBS/Episodic Diarrhea</i>	1	1 ⁴	3	-	4	2	2	1	-
<i>GERD</i>	4	1	3	2	3	1	2	2	-
<i>Eosinophilic Esophagitis</i>	1	2	-	1	-	-	-	-	-
<i>Failure to Thrive in Infancy</i>	1	-	3	-	-	-	-	-	-
Neuropsychiatric	4	1	3	2	3	2	4	3	2
<i>Dysautonomia</i> ²	4	-	3	-	2	1	-	-	-
<i>Anxiety/Depression</i>	4	1	-	1	2	-	-	2	-
<i>Behavioral Disorder</i>	2	1	2	-	1	1	1	-	-
<i>Chronic Pain</i> ³	2	-	1	2	2	1	3	1	-
<i>Developmental Delay</i>	1	1	-	-	-	-	1	-	2

[†] indicates hymenoptera hypersensitivity;

¹ abnormalities include: pectus excavatum, plagio-/brachycephaly, torticollis, femoral anteversion, tibial torsion, congenital mitral valve insufficiency, tortuous/ectatic intracranial vessels, and scoliosis;

² principally manifest as Postural Orthostatic Tachycardic Syndrome (POTS);

³ arthralgia, fibromyalgia, and vulvodinia;

⁴ encopresis;

⁵ immediate hypersensitivity reaction to radiocontrast media in one patient