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Summary

Hereditary alpha-tryptasemia (HzT) is an autosomal dominant (AD) genetic trait characterized by elevated basal serum tryptase ≥8 ng/mL, caused by increased α-tryptase-encoding TPSAB1 copy number. HzT affects 5% to 7% of Western populations and has been associated with joint hypermobility. Hypermobility disorders are likewise frequently AD, but genetic etiologies are often elusive. Genotyping of individuals with hypermobility spectrum disorder (n = 132), hypermobile Ehlers-Danlos syndrome (n = 78), or axial skeletal abnormalities with hypermobility (n = 56) was performed. Clinical features of individuals with and without HzT were compared. When analyzing our combined cohorts, dysphagia (p = 0.007) and retained primary dentition (p = 0.0003) were significantly associated with HzT, while positive associations with anaphylaxis (p = 0.07) and pruritus (P = 0.5) did not reach significance likely due to limited sample size. Overall, HzT prevalence is not increased in individuals with hypermobility disorders, rather linked to a unique endotype, demonstrating how HzT may modify clinical presentations of complex patients.

Report

Hereditary alpha-tryptasemia (HzT) is an autosomal dominant genetic trait characterized by elevated basal serum tryptase ≥8 ng/mL. HzT is caused by increased α-tryptase encoding TPSAB1 copy number on a single allele and is common among Caucasians, affecting 5% to 7% of the Western populations in which this has been studied.1-5 It has been associated with symptoms suggestive of mast cell-mediator release as well as a number of multisystem complaints, notably certain congenital connective tissue abnormalities including joint hypermobility, scoliosis, retained primary teeth, and less commonly, nail/patella syndrome, ankle protonation, valgus deformity, neonatal clubbing without cardiopulmonary disease, webbed neck, torticollis, club feet, hip dysplasia, pectus excavatum, high arched palate, syndactyly, genus valgus, pes planus, tibial torsion, hyperlordosis, and alveolar mandibular hypoplasia.1-4,6-9 However, these findings have largely been reported among populations of individuals highly selected for comorbid conditions such as these, leading to potential referral or ascertainment biases.

In addition to studies of symptomatic individuals with HzT, clinical studies have also described an association between symptoms of mast cell activation and connective tissue abnormalities as well.6,10,11 While data are limited, it is estimated that two-thirds of individuals with HzT may be asymptomatic.1,3 However, two recent independent studies have demonstrated HzT to be a major modifier of clonal and non-clonal mast cell-associated disorders, including systemic mastocytosis, idiopathic anaphylaxis, and venom allergy, where individuals with HzT were 2- to 3-fold more likely to present with these disorders and to have severe mast cell-mediator symptoms including anaphylaxis.2,5 Thus, we set out to determine whether HzT was associated with connective tissue abnormalities in well-characterized cohorts of individuals recruited for connective tissue disorders and/or whether HzT modifies clinical phenotypes or presentations of these individuals, independent of recruitment or ascertainment biases linked to centers specializing in mast cell-associated disorders or syndromic presentations of allergic inflammation.

Tryptase genotyping of TPSAB1 and TPSB2 [MIM: 191801] was performed using droplet digital PCR, as described,3 in two cohorts. The first cohort was composed of individuals with hypermobility spectrum disorder (HSD) and hypermobile Ehlers-Danlos syndrome (hEDS [MIM: 130020]). These individuals were assessed by their respective rheumatologists, allergists, and clinical geneticists to determine diagnoses. The second cohort was composed of individuals with axial skeletal abnormalities, namely with pediatric-onset scoliosis or Chiari malformation, who had concomitant joint hypermobility resulting from hypermobile Ehlers-Danlos syndrome or a Beighton score of ≥6. Approximately half of the second cohort had clinical phenotypes, prompting formal medical
Among individuals with hEDS, HzT was associated with an increased prevalence of retained primary dentition requiring surgical extraction (odds ratio [OR] ≥ 4.8; p = 0.05). Among individuals with HSD, HzT was associated with an increased prevalence of retained primary dentition (OR 79.3 [6.5–1,140]; p = 0.004) and dysphagia diagnosed by barium swallow or manometry (OR 0.2 [0.01–0.6]; p = 0.02) when compared with those without HzT. No significant differences were identified based on the presence of HzT when examining our second cohort of individuals with axial skeletal abnormalities alone (Table S1), though non-significant positive associations were observed with anaphylaxis, pruritus, and dysphagia. It should be noted that of the 56 people genotyped in this second cohort, clinical manifestations were available for only 26 of them (n = 2/4 with HzT and n = 25/52 without HzT). When individuals with hEDS and HSD were combined as a single cohort, dysphagia (OR 7.3 [1.5–35.4]; p = 0.009) and retained primary dentition (OR 96 [11.6–1,240]; p = 0.0002) remained significantly associated with HzT (Table S1). After correcting for multiple comparisons, associations between HzT and retained primary dentition (adjusted p = 0.0028) in the combined hEDS/HSD cohort, remained statistically significant. When combining genotyped individuals with hEDS, HSD, and axial skeletal abnormalities (n = 236) (Table 1), the prevalence of retained primary dentition (p = 0.0003, adjusted p = 0.004) and dysphagia (p = 0.007, adjusted p = 0.098) remained significantly increased, although only the former did when accounting for multiple comparisons.

Table 1. Clinical manifestations associated with HzT among individuals with joint hypermobility disorders

| Manifestation                          | HSD and hEDS | HSD, hEDS, and axial skeletal abnormality with hypermobility |
|----------------------------------------|--------------|-----------------------------------------------------------|
|                                        | HzT (n = 9)  | no HzT (n = 201)                                          | HzT (n = 11)  | no HzT (n = 225)                                          |
|                                        | n (%)        | n (%)                                                     | p value       | n (%)                                                     | OR   | RR     | p value |
| Anaphylaxis                            | 1 (11)       | 5 (3)                                                     | 0.2           | 2 (18)                                                   | 8 (4) | 5.9    | 0.07    |
|                                        |             |                                                           |               | (1.1–26.4)                                               |      | 5.0    | (1.3–17.1) |
| Pruritus                               | 4 (57)       | 45 (42)                                                   | 0.5           | 5 (56)                                                   | 51 (39) | 1.9 | 0.5    |
|                                        |             |                                                           |               | (0.5–6.5)                                                |      | 1.4    | (0.7–2.3) |
| Inflammatory bowel disease             | 0 (0)        | 2 (1)                                                     | >0.99         | 0 (0)                                                     | 3 (1) | 0 (0.0–24.1) | >0.99 |
|                                        |             |                                                           |               | (0.0–24.1)                                               |      |       |        |
| IBS-like symptoms                      | 4 (44)       | 31 (26)                                                   | 0.3           | –                                                        | –     | –      | –       |
|                                        |             |                                                           |               | –                                                        | –     | –      | –       |
| Gastroesophageal reflux                | 7 (78)       | 134 (69)                                                  | 0.7           | –                                                        | –     | –      | –       |
|                                        |             |                                                           |               | –                                                        | –     | –      | –       |
| Retained primary dentition*            | 3 (33)       | 1 (1)                                                     | 0.0002        | 3 (27)                                                   | 1 (0) | 81 (10.2–1,048) | 59.2 |
|                                        |             |                                                           |               | (8.7–387.8)                                              |      |       | 0.0003  |
| Generalized joint hypermobility (BS ≥ 5/9) | 6 (67)       | 101 (52)                                                  | 0.5           | –                                                        | –     | –      | –       |
|                                        |             |                                                           |               | –                                                        | –     | –      | –       |
| Tilt-table test                        | 2 (100)      | 37 (47)                                                   | ≥0.5          | 2.1                                                      | 0.7–2.8 | 0.2 | –       |
|                                        |             |                                                           |               | –                                                        | –     | –      | –       |
| Headache and/or migraine               | 6 (67)       | 127 (69)                                                  | 0.9 (0.2–3.4) | 1.0 (0.5–1.3)                                           | >0.99 | 8 (73) | 146 (70) | 1.1 (0.3–4.1) | 1.1 (0.6–1.3) | 0.7 |
| Sleep disturbances                     | 7 (88)       | 106 (85)                                                  | 1.2 (0.2–14.1) | 1 (0.6–1.2)                                             | >0.99 | 9 (90) | 116 (78) | 2.5 (0.4–28.0) | 1.1 (0.8–1.3) | 0.06 |
|                                        |             |                                                           |               | –                                                        | –     | –      | –       |
| Dysphagia*                             | 7 (78)       | 56 (32)                                                   | 0.009         | 8 (73)                                                   | 61 (31) | 5.9 | 1.4–3.3 | 0.007 |
|                                        |             |                                                           |               | (1.7–21.1)                                              |      | 2.3    | (1.4–3.3) |
| Clubfeet                               | 0 (0)        | 6 (3)                                                     | >0.99         | –                                                        | –     | –      | –       |
|                                        |             |                                                           |               | –                                                        | –     | –      | –       |
| Chronic fatigue                        | 9 (100)      | 178 (91)                                                  | ≥0.2          | 1.1                                                      | 0.8–1.2 | >0.99 | 10 (91) | 193 (88) | 1.4 (0.2–15.7) | 1.0 (0.7–1.2) | >0.99 |
| Neurological bladder                   | 0 (0)        | 5 (3)                                                     | 0 (0.0–23.29) | 0 (0.0–15.9)                                            | >0.99 | 0 (0) | 5 (2) | 0 (0.7–1.0) | 0 (0.0–14.7) | >0.99 |

BS, Beighton score; HzT, hereditary alpha-tryptasemia; hEDS, hypermobile Ehlers-Danlos syndrome; HSD, hypermobility spectrum disorder; IBS, irritable bowel syndrome; OR, odds ratio; RR, relative risk; 95% CI, 95% confidence limits; –, indicates unavailable datapoints.

*Retained primary dentition and Dysphagia rows indicate statistically significant associations.
An increased prevalence of HαT was not observed in either cohort of HSD/hEDS patients or those with axial skeletal abnormalities and joint hypermobility, where genotyping could be performed. However, as has been reported in other acquired genetic conditions, we found that HαT was associated with a unique endotype, either due to independent association or modification of certain characteristics or clinical features in these individuals. Interestingly, the only connective tissue abnormality found previously to be significantly associated with HαT among unselected healthy adults was retained primary dentition.

Whether HαT may cause clinical manifestations or modify the symptomatic presentation of other clinical disorders remains a matter of scientific debate. However, the associations seen here between HαT and retained primary dentition remained statistically significant after correcting for multiple comparisons, and are remarkably consistent with prior associations reported in both selected and unselected populations. Interestingly, there was also an increased prevalence of anaphylaxis and pruritus among individuals with joint hypermobility and HαT, phenotypes also strongly linked to HαT in previous studies; however, the sample size was limited, and these did not reach statistical significance when adjusting for multiple comparisons (OR 5.9 [1.1–26.0]; p = 0.07, adjusted p = 0.98) and (OR 1.9 [-0.5–6.5]; p = 0.5, adjusted p >0.99), respectively.

While additional mechanistic work is ongoing to understand the phenotypes linked to HαT, these current data indicate that HαT is not associated with congenital joint hypermobility disorders. Additional larger studies would help confirm these findings in the future. However, given that HαT is common, it may frequently be present among individuals with such connective tissue abnormalities where it may modify and add to the diverse clinical presentations of these uniquely complex patients.

Data and code availability
The published article includes all data generated or analyzed during this study.

Supplemental information
Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2022.100094.

Acknowledgments
This research was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases, NIH and by the National Institute of Arthritis and Musculoskeletal Disease (801AR067715). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government.

Declaration of interests
The authors declare no competing interests.

Received: November 2, 2021
Accepted: February 17, 2022

Web resources
Online Mendelian Inheritance in Man, http://www.omim.org.

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Supplemental information

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Supplemental materials and methods

Subjects with hEDS and HSD

Informed consent was provided on an IRB-approved research protocol, IRB number: NP3873 Study of TPSAB1 gene copy number in a cohort of patients with hypermobile Ehlers-Danlos syndrome (hEDS) and Hypermobility Spectrum Disorders (HSD) was approved by the local Ethical Committee (Comitato Etico di Brescia, ASST degli Spedali Civili, Brescia, Italia) under Prof. Marina Colombi. Individuals were evaluated in a tertiary Italian referral center for the diagnosis and management of HDCT at the Spedali Civili University Hospital of Brescia. Personal medical histories were obtained, and complete physical examinations were performed to screen and diagnose individuals with hEDS according to the 2017 criteria (1). People with symptomatic syndromic joint hypermobility but not fulfilling the new diagnostic criteria for hEDS were classified as having HSD (2). A small subset also underwent clinically indicated Tilt-table testing for symptoms suggestive of autonomic dysfunction.

All clinical phenotypes reported in this paper - and most pertinently connective tissue phenotypes - were ascertained by clinical geneticists and/or neurologists specializing in these disorders, and their teams, prior to tryptase genotyping.

Subjects with axial skeletal abnormality and hypermobility

Informed consent was obtained on a Washington University IRB-approved research protocol (IRB ID #201102118). Subjects were recruited from cohorts of people with scoliosis or Chiari malformation. They were selected due to a concomitant clinical diagnosis of classical EDS or a Beighton score of ≥6. A subset of individuals (N=26) was available from which clinical symptoms could be queried.

Tryptase genotyping

Genotyping of TPSAB1 and TPSB2 was performed as previously described (3). In short, droplet-digital PCR was performed from extracted genomic DNA on a QX200 (Bio-Rad, Hercules, CA) using a manual
droplet generator and custom primer/probe sets for α- and β-tryptase sequences at TPSAB1 and TPSB2 employing the reference probe AP3B1 (Bio-Rad). Primary results can be provided by the author upon request.

**Statistical Analyses**

Fisher’s exact test was used where appropriate. For comparisons reaching statistical significance, a Bonferroni correction for multiple comparisons was applied to test the strength of association.
Table S1. Clinical manifestations associated with HαT among individuals with joint hypermobility disorders.

| Manifestation                        | HSD | hEDS | axial skeletal abnormality with hypermobility |
|--------------------------------------|-----|------|---------------------------------------------|
|                                       | N (%) | N (%) | OR (95%CI) | RR (95%CI) | P-value | N (%) | N (%) | OR (95%CI) | RR (95%CI) | P-value |
| anaphylaxis                          | 1 (20) | 1 (0.8) | 31 (1.3-582.9) | 24.8 (2.65-207.3) | 0.08 | - | - | - | - | - |
| pruritus                             | 3 (100) | 26 (38) | - | 2.6 (1.1-3.6) | 0.06 | - | - | - | - | - |
| inflammatory bowel disease           | 0 (0) | 2 (2) | 0.0 (0.0-60) | 0 (0.0-37.4) | >0.99 | - | - | - | - | - |
| IBS-like symptoms                    | 3 (60) | 15 (19) | 6.4 (1.2-37.4) | 3.2 (1.1-6.2) | 0.06 | - | - | - | - | - |
| gastroesophageal reflux              | 4 (80) | 74 (61) | 2.6 (0.4-32.3) | 1.3 (0.6-1.7) | 0.6 | - | - | - | - | - |
| retained primary dentition           | 2 (40) | 1 (0.8) | 79.3 (6.5-1140) | 48 (6.6-320.4) | 0.004 | - | - | - | - | - |
| generalized joint hypermobility (BS≥5/9) | 2 (40) | 30 (25) | 2.0 (0.3-10.3) | 1.6 (0.5-3.5) | 0.6 | - | - | - | - | - |
| tilt-table test                      | 1 (100) | 18 (75) | - | 1.3 (0.3-1.8) | >0.99 | - | - | - | - | - |
| headache and/or migraine             | 4 (80) | 71 (62) | 2.4 (0.4-30.3) | 1.3 (0.6-1.7) | 0.7 | - | - | - | - | - |
| sleep disturbances                   | 4 (100) | 59 (82) | - | 1.2 (0.6-1.4) | >0.99 | - | - | - | - | - |
| dysphagia                            | 4 (80) | 27 (25) | 0.2 (0.01-0.6) | 3.2 (1.4-4.9) | 0.02 | - | - | - | - | - |
| clubfeet                             | 0 (0) | 5 (4) | 0.0 (0.0-25.2) | 0.0 (0.0-13.6) | >0.99 | - | - | - | - | - |
| chronic fatigue                      | 5 (100) | 106 (86) | - | 1.2 (0.7-1.3) | >0.99 | - | - | - | - | - |
| neurological bladder                 | 0 (0) | 4 (3) | 0.0 (0.0-32) | 0.0 (0.0-19.6) | >0.99 | - | - | - | - | - |

HSD - hypermobility spectrum disorder; hEDS - hypermobile Ehlers-Danlos syndrome; HaT - hereditary alpha-tryptasemia; BS - Brighton score. OR - Odds Ratio; RR - Relative Risk; 95% CI - 95% Confidence limits; For OR or RR where the exact value could not be determined, values are indicated as the ± the lower limit of the 95% CI; bold typeface indicates statistically significant associations; grey areas indicate unavailable datapoints.