Original Article

Hereditary α-tryptasemia is Associated With Anaphylaxis to Antibiotics and Monoclonal Antibodies

Peter Korošec, PhD[a,b], Jonathan J. Lyons, MD[c,d], Manca Svetina, MSc[a,h], Monika Koudová, MD[e], Martina Bittóová, MSc[e], Mihaela Zidarn, MD, PhD[a,f], Lenka Sedláčková, MD[g], Matija Rijavec, PhD[a,h], and Peter Kopač, MD, PhD[a,f] *Golnik and Ljubljana, Slovenia; Prague, Czech Republic; and La Jolla, Calif*

What is already known about this topic? Hereditary α-tryptasemia, a genetic trait caused by increased germline α-tryptase–encoding *Tryptase α/β1* copy number, is associated with idiopathic and venom anaphylaxis.

What does this article add to our knowledge? Hereditary α-tryptasemia is more prevalent in drug-induced anaphylaxis and is associated with a greater likelihood of antibiotic-induced (approximately 20%) or mAb-induced (approximately 40%) anaphylaxis.

How does this study impact current management guidelines? Tryptase genotyping may help identify individuals with a higher risk of antibiotic- or mAb-induced anaphylaxis. Nevertheless, more data are needed to confirm this observation.

BACKGROUND: Hereditary α-tryptasemia, a genetic trait caused by increased α-tryptase copy number, is associated with idiopathic and venom anaphylaxis.
OBJECTIVE: We aimed to determine the impact of tryptase genotypes on drug-induced anaphylaxis.
METHODS: A prospective discovery cohort of 99 patients from a referral center in Slovenia with acute anaphylaxis to drugs underwent tryptase genotyping by droplet digital PCR. For validation, we included a cohort of 26 patients from the Czech Republic. Associated inciting agents and the severity of the reactions were subsequently examined.
RESULTS: Hereditary α-tryptasemia was associated with drug-induced anaphylaxis with a prevalence of 13% (n = 13 of 99) in the discovery cohort and 15% in the validation cohort (n = 4 of 26). Hereditary α-tryptasemia was identified in every individual with elevated basal serum tryptase levels (11.6-21.9 ng/mL; n = 14) within both cohorts of patients. Hereditary α-tryptasemia was more prevalent in individuals with antibiotic- or mAb-induced anaphylaxis in both the discovery and validation cohorts (n = 13 of 51; 26%) compared to those with anaphylaxis resulting from neuromuscular blocking agents, nonsteroidal anti-inflammatory drugs, contrast, chlorhexidine, or other drugs (n = 5 of 74; 7%; *P* = .02; odds ratio = 4.1; 95% CI, 1.3-11.1). Overall, we found fewer individuals with no α-tryptase than in the general population, and there was a trend for subjects with more α-tryptase copies to have more severe reactions. Thus, among subjects with three α-tryptase copies, the prevalence of severe anaphylaxis was 73%, compared with 59% with one to two α-tryptase copies and 58% for subjects without α-tryptase.
CONCLUSIONS: Risk for anaphylaxis to antibiotics and biologics is associated with inherited differences in α-tryptase–encoding copies at *Tryptase α/β1*. **(J Allergy Clin Immunol Pract 2025;13:1449-56)**

Key words: *Drug allergy; Anaphylaxis; Antibiotics; Monoclonal antibodies; α-Tryptase; Hereditary α-tryptasemia*

[a]University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia
[b]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia
[c]Division of Allergy and Immunology, Department of Medicine, University of California San Diego, La Jolla, Calif
[d]Veterans Affairs San Diego Healthcare System, La Jolla, Calif
[e]GNTlabs by GENNET, Centre of Medical Genetics and Reproductive Medicine GENNET, Prague, Czech Republic
[f]Medical Faculty, Department of Internal Medicine, University of Ljubljana, Ljubljana, Slovenia
[g]GENNET s.r.o., Department of Allergy and Clinical Immunology, Prague, Czech Republic
[h]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
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Corresponding author: Peter Korošec, PhD, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik 36, 4204 Golnik, Slovenia. E-mail: peter.korosec@klinika-golnik.si.

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Abbreviations used
BST- Basal serum tryptase
HαT- Hereditary α-tryptasemia
MC- Mast cell
NMBA- Neuromuscular blocking agent
NSAID- Nonsteroidal anti-inflammatory drug
TPSAB1- Tryptase α/β1

INTRODUCTION

Drug reactions comprise a large proportion of adult anaphylaxis, accounting for 20% to 40% of cases.[1] The incidence of drug-induced anaphylaxis increases with age, and although drug-induced anaphylaxis is still a relatively rare occurrence (1% of the population experiences at least one episode in the United States[2]), those reactions are often more severe and more frequently result in hospitalizations relative to other causes.[3,4] Indeed, drug-induced anaphylaxis is more likely to result in fatality and accounts for over half of anaphylaxis fatalities in the United States.[5] Previous studies have identified cofactors such as female sex as contributors to higher rates of drug-induced anaphylaxis and more severe reactions.[1,6] However, currently no clinical biomarker would help in identifying patients who are at higher risk for drug-induced anaphylaxis or more severe reactions.

Human tryptases are neutral serine proteases produced exclusively by mast cells (MCs) and basophils in healthy individuals.[7] Basal serum tryptase (BST) levels are dictated by the constitutive secretion of α- and β-pro-tryptases. Elevated BST levels (defined as >11.4 ng/mL)[8] are commonly associated with hereditary α-tryptasemia (HαT) resulting from increased α-tryptase—encoding *Tryptase α/β1 TPSAB1* copy number,[9] with a 6% prevalence reported for the general populations of the United Kingdom, United States, and European Union.[10] Higher relative numbers of α-tryptase—encoding gene copies increase α/β-tryptase heterotetramers, which can cleave the mechanosensing G protein-coupled receptor epidermal growth factor-like module-containing mucin-like hormone receptor-like 2 and protease-activated receptor.[2,9,11,12] Such activity has been shown to result in endothelial cell leak and MC activation *in vitro* and may potentiate anaphylaxis *in vivo*.[9,11,12] The prevalence of HαT is increased among patients with severe Hymenoptera venom-triggered anaphylaxis and idiopathic anaphylaxis.[12-14] Similarly, among patients with systemic mastocytosis, the prevalence of HαT is 12% to 18%.[12,15-18] Individuals with both systemic mastocytosis and HαT had a rate of anaphylaxis that was two- to threefold higher than in those with systemic mastocytosis alone,[12,15,16,18] but in one study, this rate was lower (1.2-fold).[17] In a study of patients with HαT and MC activation—related symptomatology, in which anaphylaxis was noted in 57% of subjects, half reported anaphylaxis to drugs.[19] Whereas it has been reported that drug-induced anaphylaxis is more common in patients with elevated BST, mainly from antibiotic use,[20] such an association has not been observed for other drugs including nonsteroidal anti-inflammatory drugs (NSAIDs).[21]

Hereditary α-tryptasemia and tryptase gene composition as a potential cofactor for drug-induced anaphylaxis has not been studied. This study aimed to assess the associations between HαT and drug-induced anaphylaxis. Furthermore, we sought to evaluate the association between tryptase gene composition and the severity of medication-induced allergy reactions. We first performed analyses in a prospective discovery cohort of patients in a referral center in Slovenia in 2021 to 2023; a smaller prospective validation cohort of patients from a referral center in Czech Republic followed the initial analyses. All subjects who chose to participate were tryptase-genotyped in a blinded fashion.

METHODS

Study cohorts

For the discovery cohort, we prospectively recruited 99 patients (55 women, aged 19-88 years; average, 55 years) with a convincing history of acute anaphylaxis to medications, who were referred to the University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia, between 2021 and 2023. All subjects had complete histories, physical examinations, verification of symptoms, and classification of the severity of acute reaction according to the Ring and Messmer grading system[22] by board-certified allergists. Skin testing and/or specific IgE and/or basophil activation test and/or graded dose challenge were performed in all subjects based on European Academy of Allergy and Clinical Immunology/European Network on Drug Allergy criteria.[23-25] Individuals with a history of immunodeficiency or known genetic disorders were excluded from the study. For the validation cohort, we recruited 26 patients (23 women, aged 22-78 years; average, 49 years) who were referred to GENNET s.r.o., Department of Allergy and Clinical Immunology, Prague, Czech Republic, with the same inclusion and exclusion criteria as for the discovery cohort. We obtained BST levels for all study subjects, and all underwent tryptase genotyping using droplet digital PCR. Ethical approval was obtained from the Slovenian National Medical Ethics Committee (150/09/13 and 0120-287/2022/3) and GENNET Institutional Ethics Committee, and all subjects provided written informed consent. Individual demographic, clinical, and laboratory details are presented in the Supplemental Methods and Tables E1 and E2 (in this article's Online Repository at www.jaci-inpractice.org).

Tryptase quantification

Total BST levels were measured by Phadia 100 or 250 fluorescence enzyme immunoassays (Thermo Fisher Scientific, Waltham, Mass). The lower limit of detection for this assay was 1 ng/mL. The normal range for BST in serum is 1 to 11.4 ng/mL.[26]

Tryptase genotyping

We implemented genotyping of *TPSAB1* and *TPSB2* by performing a multiplex droplet digital PCR assay, as described elsewhere.[27] Genomic DNA was extracted from 400 μL of EDTA-containing whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We performed droplet digital PCR on a QX200 (Bio-Rad, Hercules, Calif) using custom primer/probe sets targeting α- and β-tryptases encoding sequences at *TPSAB1* and *TPSB2* with the use of the reference probe AP3B1 or AGO1 (Bio-Rad). We used QX Manager software (Bio-Rad) for data acquisition and analysis. For details, see the Supplemental Methods.

Statistical methods

We used descriptive statistics to summarize demographic and clinical characteristics for both cohorts, with continuous variables expressed as averages or medians and ranges and categoric variables as counts and percentages. We used χ^2 test to evaluate associations between HαT or the presence of α-tryptase isoforms and drug or biologic-induced anaphylaxis. Confidence intervals for prevalence

data were determined using exact binomial calculation. Analyses were conducted in GraphPad Prism (version 10.1.1, GraphPad Software, San Diego, Calif). *P* less than .05 was considered significant.

RESULTS

Study population

A total of 125 subjects underwent tryptase genotyping between groups (99 subjects in the discovery cohort and 26 in the validation cohort). Most subjects in both groups were female, and average ages were comparable between the cohorts. Table I lists the complete demographic and clinical characteristics of the groups. Tables E1 and E2 list individual patient data.

Among the 99 subjects in the discovery cohort, approximately one third of patients had anaphylaxis to antibiotics (n = 30 of 99; 30%), one fifth to neuromuscular blocking agents (NMBAs) (n = 23 of 99; 23%), and one fifth to NSAIDs (n = 18 of 99; 18%). The others had anaphylaxis to biologics (monoclonal antibodies) (n = 8 of 99; 8%), chlorhexidine (n = 7 of 99; 7%), contrast media (n = 3 of 99; 3%), and other drugs (n = 10 of 99; 10%) (Table I). The majority had severe reactions, and more than two thirds of individuals (n = 67 of 99; 68%) had Ring and Messmer grades III or IV anaphylaxis. One third of individuals had either grade II anaphylaxis (n = 17 of 99; 17%) or grade I reactions (n = 15 of 99; 15%) (Table I).

Among the patients in the validation cohort, half of individuals had anaphylaxis to antibiotics (n = 13 of 26; 50%) and approximately one third had anaphylaxis to NMBAs (n = 7 of 26; 27%). In others the causative agent was contrast media (n = 2 of 26; 8%), chlorhexidine (n = 1 of 26; 4%), and other drugs (n = 3 of 26; 12%) (Table I). Half of patients had moderate grade II anaphylaxis (n = 14 of 26; 54%) and one third had severe grade III or IV anaphylaxis (n = 8 of 26; 31%); a smaller number had grade I reactions (n = 4 of 26; 15%). Overall, of the 125 subjects in total, most had severe reactions (60%; n = 75), and the most common inciting agents were antibiotics (34%; n = 42) and NMBA (24%; n = 30). Table E1lists detailed individual data about causative agents for the discovery cohort, and Table E2 for the validation cohort.

Tryptase genotyping

Of the 99 subjects included in the discovery cohort, 13 (13%) had HαT. In the validation cohort, four of 26 participants (15%) had HαT (Table I and Figure 1). Thus, of the 125 subjects, 14% (n = 17) had HαT. This prevalence was more than twofold higher than the reported prevalence of HαT in the general populations of the United Kingdom, United States, and European Union (6%).[10,28]

The majority of subjects in the discovery (n = 79 of 99; 80%) and validation (n = 20 of 26; 77%) cohort had at least one α-tryptase–encoding gene. Of the 125 subjects in total, only 21% (n = 26) were genotyped as β,β/β,β or β,β/β,ββ and β,β/β,0. This frequency was 35% lower than the expected prevalence of β genotypes in the general population (32%).[29] The most common tryptase genotype was α,β/β,β (n = 42 of 99; 42%), which is also the most common genotype in the general population.[30] Of the 17 subjects with HαT, 10 (59%) and six (35%) had αα,β/α,β and αα,β/β,β genotypes, respectively, which are also the most common in the individuals with HαT.[30] Table II lists complete tryptase genotypes for the two groups.

Patients with elevated BST and anaphylaxis have HαT

Elevated BST levels (>11.4 ng/mL) were found in 10 of 99 patients in the discovery cohort (10%) and four of 26 in the validation cohort (15%) (Table I and Figure 1). All subjects with elevated BST levels (14 of 14; 100%) within the discovery and validation cohort had HαT. Additionally, three subjects in the discovery cohort with normal but BST levels higher than 8 ng/mL (with values of 8.15, 9.62, and 9.7 ng/mL) also had HαT (Table E1 and Figure 1). Overall, the BST values in both cohorts were highly comparable, with median values of 5.4 ng/mL for the discovery and 5.8 ng/mL for the validation cohort. Furthermore, no individuals had markedly elevated BSTs; the highest levels of BST were 21.9 ng/mL in the discovery cohort and 20.2 ng/mL in the validation cohort (Tables E1 and E2 and Figure 1).

Prevalence of HαT is higher in patients with anaphylaxis to antibiotics and biological agents

In the discovery cohort, HαT was significantly more prevalent in individuals with antibiotic (n = 6 of 30; 20%) or mAb-induced (n = 3 of 8; 38%) anaphylaxis compared with individuals with reactions to NMBAs, NSAIDs, contrast, chlorhexidine, or other drugs (n = 4 of 61; 7%; *P* = .014; odds ratio [OR] = 4.4, 95% CI, 1.2-13.7; relative risk [RR] = 3.6, 95% CI, 1.3-10.5]) (Table E1 and Figure 2). Similarly, in the validation cohort, three of 13 subjects with antibiotics-induced anaphylaxis (23%) had HαT, whereas only one of 13 patients with reaction to other drugs (8%) had HαT (Table E2 and Figure 2). Thus, 24% of all individuals with antibiotic- or mAb-induced anaphylaxis (12 of 51) had HαT versus 7% of those with NMBA-, NSAID-, contrast-, chlorhexidine-, or other drug-induced anaphylaxis (five of 74) (Tables E1 and E2 and Figure 2) (*P* = .007; OR = 4.3, 95% CI, 1.3-11.4]; RR = 3.5, 95% CI, 1.4-9).

Overall, of nine patients with antibiotic-induced anaphylaxis and HαT, four had β-lactam (all amoxicillin), three had cephalosporins, and a single patient had sulfonamide or nitrofurantoin-induced anaphylaxis (Tables E1 and E2). Furthermore, within the three patients with mAb-induced anaphylaxis and HαT, in two individuals, the inciting biologic agents were rituximab and alemtuzumab, both resulting in severe grade 3 anaphylactic reactions at first exposure (Table E1). Cetuximab was a culprit in one patient with HαT, and after further diagnostic evaluation, IgE antibodies to α-gal were found in this patient.

Interestingly, no individuals with NMBA-induced anaphylaxis had HαT (Tables E1 and E2). In many countries, NMBAs are frequent triggers of perioperative anaphylaxis.[31] Only one individual was identified with HαT in the NSAID group (Table E1). In this subgroup, anaphylaxis was provoked by a single NSAID in all subjects, which is typical for NSAID-induced anaphylaxis.[32] Additionally, single individuals with HαT had anaphylaxis to contrast, chlorhexidine, pantoprazole, and nadroparin (classified here as other drugs) (Table E1).

Presence of α-tryptase and severity of anaphylaxis

We compared α-tryptase–encoding copy number and anaphylaxis severity grading (Ring and Messmer) in all study participants and found no significant association between α-tryptase isoform expression and severity grading. However,

TABLE I. Clinical and laboratory characteristics of entire study population with drug- or biologic-induced anaphylaxis

Characteristic	All participants (n = 125)	Discovery cohort (n = 99)	Validation cohort (n = 26)
Age, y (median [range])	54 (19-88)	55 (19-88)	46 (22-78)
Sex, n (%)			
Male	47 (38)	44 (44)	3 (12)
Female	78 (62)	55 (56)	23 (89)
Anaphylaxis severity grade, n (%)*			
I	19 (15)	15 (15)	4 (15)
II	31 (25)	17 (17)	14 (54)
III	67 (54)	61 (62)	6 (23)
IV	8 (6)	6 (6)	2 (8)
Causative drug class, n (%)			
Antibiotics	43 (34)	30 (30)	13 (50)
Biologics (mAb)	8 (6)	8 (8)	0
Neuromuscular blocking agents	30 (24)	23 (23)	7 (27)
Nonsteroidal anti-inflammatory drugs	18 (14)	18 (18)	0
Contrast	5 (4)	3 (3)	2 (8)
Chlorhexidine	8 (6)	7 (7)	1 (4)
Other drugs	13 (10)	10 (10)	3 (12)
Hereditary α-tryptasemia, n (%)			
Positive	17 (14)	13 (13)	4 (15)
Negative	108 (86)	86 (87)	22 (85)
Basal serum tryptase level, ng/mL (median [range])	5.5 (1.7-21.9)	5.4 (1.7-21.9)	5.8 (3.6-20.2)
<11.4	111 (89)	89 (90)	22 (85)
>11.4	14 (11)	10 (11)	4 (15)
>20	4 (3)	2 (2)	2 (3)
>30	0	0	0

*Grades were assigned according to Ring and Messmer.[22]

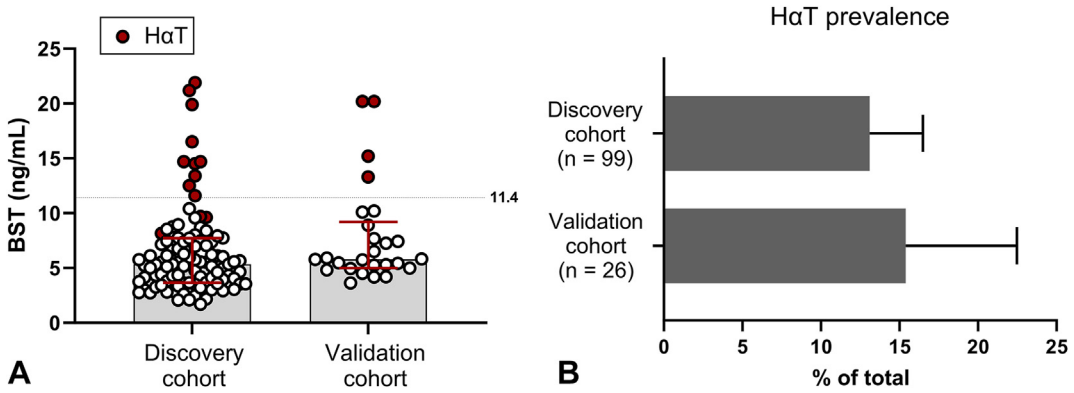

FIGURE 1. (**A**) Basal serum tryptase (BST) levels and (**B**) prevalence of hereditary α-tryptasemia (HαT) in the discovery cohort and a validation cohort of patients with medication-induced anaphylaxis. Individuals with HαT are indicated in *red*. Data are presented as (A) medians with interquartile ranges or (B) means ± SEMs.

there was a trend for subjects with more α-tryptase copies to have more severe reactions. Thus, among subjects with three α-tryptase copies, the prevalence of severe anaphylaxis (grade III/IV) was 73%, compared with 59% with one to two α-tryptase copies and 58% for subjects without α-tryptase (Figure 3) ($P = .37$; OR = 1.9, 95% CI, 0.53-6.8; RR = 1.24, 95% CI, 0.73-1.7]). Furthermore, among subjects with three α-tryptase copies, the prevalence of moderate to severe anaphylaxis (grades II to III/IV) was 91%, compared with 84% with one to two α-tryptase copies and 81% for subjects without α-tryptase (Figure 3) ($P = .51$; OR = 2, 95% CI, 0.3-22.8; RR = 1.1, 95% CI, 0.74-1.3).

DISCUSSION

Our study suggests that increased germline copies of α-tryptase–encoding sequences at *TPSAB1* are more prevalent among patients with anaphylaxis to drugs, and in both a discovery and validation cohort, the presence of HαT was associated with

TABLE II. Tryptase genotypes in discovery cohort and validation cohort

Genotype, n (%)	αα<u>α</u>,β/β,β	αα,β/α,β	αα,β/β,β	α,β/α,β	α,β/β,β	α,β/β,O	β,β/β,ββ	β,β/β,β	β ,β/β,O	Hereditary α-tryptasemia	α-Tryptase carriers	Non−α-tryptase carriers
Discovery cohort (n = 99)	0	9 (9%)	4 (4%)	22 (22%)	42 (42%)	2 (2%)	1 (1%)	18 (18%)	1 (1%)	13 (13%)	79 (80%)	20 (20%)
Validation cohort (n = 26)	1 (4%)	1 (4%)	2 (8%)	7 (27%)	9 (35%)	0	0	6 (23%)	0	4 (15%)	20 (7%)	6 (23%)

Underscored genotypes were consistent with hereditary α-tryptasemia (at least two copies of α-tryptase on the same allele).

anaphylaxis to antibiotic and/or mAb. To our knowledge, this is the first report investigating HαT and α-tryptase genotypes as a risk factor for potentially life-threatening reactions in patients with drug allergy or hypersensitivity to biological agents.

We initially explored the association of tryptase genotype in a prospective cohort from Slovenia that is served by a single referral center for all adult patients with medication-induced anaphylaxis in a country of approximately 2 million. All individuals in this cohort had a history of anaphylaxis triggered by drugs; in more than two thirds, the reactions were severe, and no patients underwent prior screening before recruitment and diagnostic allergy workup. Furthermore, no patients had BST checked before referral. We found that the number of subjects with drug-induced anaphylaxis and HαT was more than twice that of the general population,[10,28] and these findings are remarkably similar to previous data on adult patients with idiopathic anaphylaxis (17%)[12] and severe Hymenoptera venom-triggered anaphylaxis (8.6%).[12,13,33,34] To test the strength of these observations, we next recruited a group of patients from the Czech Republic with drug-induced anaphylaxis. The prevalence of HαT among subjects in the validation cohort was comparable to that in the discovery cohort and more than twice that of the general population.[10,28]

To evaluate these associations, we determined whether the prevalence of HαT might differ by causes of drug-induced anaphylaxis. Remarkably, the prevalence of HαT was higher in patients with antibiotic-induced and mAb-induced anaphylaxis; for antibiotics, the prevalence was 20% and 23% for the discovery and validation cohorts, respectively; for mAb-induced anaphylaxis, the prevalence was almost 40%. These prevalences are higher than those observed in patients with idiopathic anaphylaxis or Hymenoptera venom-triggered anaphylaxis.[12] Interestingly, an analysis of emerging causes of drug-induced anaphylaxis in the Food and Drug Administration Adverse Event Reporting System examined across multiple countries (United States, United Kingdom, Germany, France, Japan, and Canada) showed that antibiotics (15% of all) and mAbs (13% of all) are the most reported drug classes associated with anaphylaxis.[35] In particular, antibiotics were highly reported for both anaphylaxis overall and anaphylaxis followed by death. Moreover, a significant increase in anaphylaxis to mAb therapies has been observed over the past 2 decades.[35] Given the correlation between HαT and anaphylaxis to antibiotics and mAbs, tryptase genotype should be considered clinically when evaluating individuals with a history of anaphylaxis to drugs. However, further data to support this hypothesis are lacking, and additional data incorporating tryptase genotyping in larger studies are needed to determine the efficacy of this approach in risk stratification of patients with drug allergies. Additionally, further studies should also investigate the interaction between FCεRI and Mas-related G-protein coupled receptor X2 pathways of MC activation and HαT,[36] especially because NMBAs, opiates, and some antibiotics may elicit both IgE-mediated and Mas-related G-protein coupled receptor X2−mediated hypersensitivity reactions.[37]

Among the subjects in both cohorts, the prevalence of any α-tryptase−containing allele was higher, and the prevalence of only β-tryptase was lower than in the general population.[29] Furthermore, there was a trend for subjects with more α-tryptase−encoding copy numbers to have more severe drug-induced reactions. Similarly, in a recent food allergy study, the prevalence

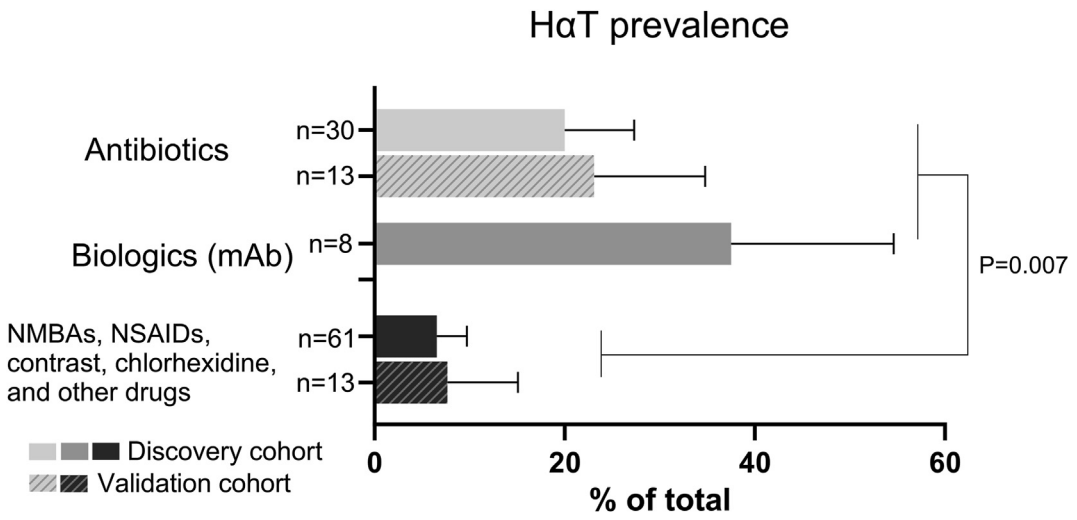

FIGURE 2. Prevalence of hereditary α-tryptasemia (HαT) in patients with antibiotic or biologic (mAb)-induced anaphylaxis compared with patients with neuromuscular blocking agents (NMBAs), nonsteroidal anti-inflammatory drugs (NSAIDs), contrast, chlorhexidine, or other drug-induced anaphylaxis. Data are represented as means ± SEMs. Chi-square test was used for calculation.

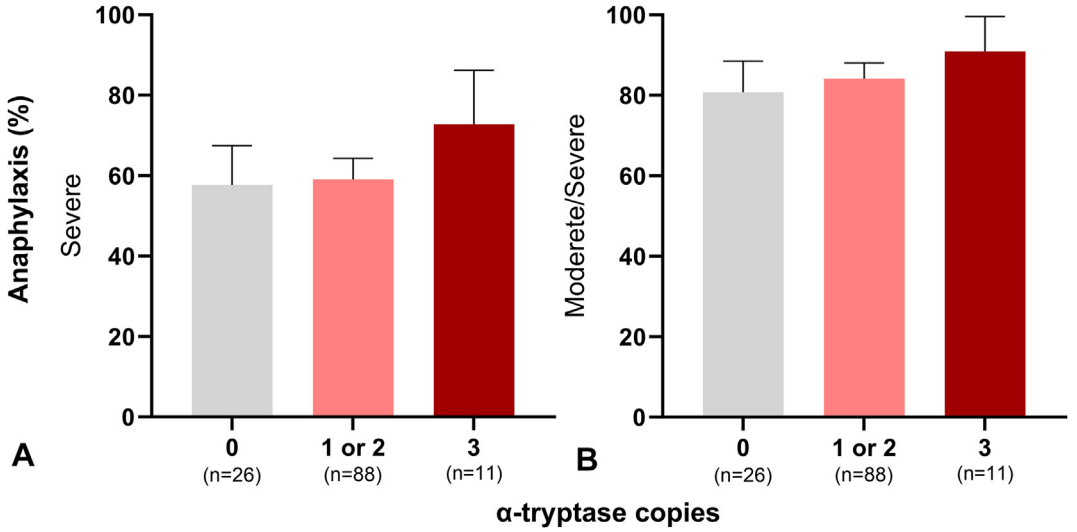

FIGURE 3. Prevalence of (**A**) severe (grade III/IV) or (**B**) moderate/severe (grades II to III/IV) anaphylaxis triggered by drug or biologic agents by relative α-tryptase–encoding copy number in all study participants. Data are represented as means ± SEMs. Grades were assigned according to Ring and Messmer.[22]

of any α-tryptase was highly comparable (74%) to that in the patients in the current study, and the presence of α-tryptase correlated with a higher prevalence of anaphylaxis or severe reaction to foods than in subjects with no α-tryptase.[29] Together, these findings support the hypothesis that increased relative amounts of α-tryptase, leading to increased heterotetrameric mature tryptases, increase the severity of allergic reactions. However, in our study, the absence of α-tryptase did not preclude the development of severe drug-induced anaphylaxis. Additional studies of the potential mechanisms underlying an association between tryptase gene composition and drug allergy severity are needed, although selective activation of protease activated receptor 2 or epidermal growth factor-like module-containing mucin-like hormone receptor-like 2 may contribute.[11]

Within the discovery and validation cohorts, all individuals with elevated BST levels had HαT. However, in most patients BST elevations were minor, and only a few individuals had levels over 20 ng/mL; none were over 30 ng/mL. In contrast, in a recent large study of patients with Hymenoptera venom-triggered anaphylaxis, the frequency of HαT among those with elevated BST was much lower (53%).[14] Interestingly, the percentages of subjects with elevated BST in the current study were highly comparable to the percentages of those with elevated BST in the Hymenoptera study (11.2% vs 11.1%[14], respectively).

Our study had several limitations. Verification of symptoms, classification, and grading was done by board-certified allergists based on the seminal and quoted Ring and Messmer[22] criteria when patients were referred to the clinic. However, we acknowledge that recent clinical definitions of anaphylaxis, such as the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network consensus clinical definition,[38] state that skin changes on their own do not constitute anaphylaxis. As such, some grade I reactions in the Ring and Messmer classification or mild reactions according to the Brown severity scale[39] would not meet criteria for anaphylaxis in these revised definitions. Overall, 15.2% of study patients (19 of 125)had grade I reactions. Furthermore, we did not consider secondary outcome measures by analyzing the data using the Sixth National Audit Project[40] or US Drug Allergy Registry[41] grading scale. We also acknowledge a lower number of subjects in the validation cohort, which may limit the generalizability and estimates of true effect size. In the validation cohort, there were also fewer patients with grade III or IV reactions (31%) than in the discovery cohort (68%). However, if we compare the percentages of moderate to severe reactions (grades II-IV), there were no differences (85% vs 92%, respectively). Despite these limitations, the results were similar in both cohorts, suggesting that HαT may be enriched in patients with anaphylaxis to certain medications. Additionally, no patients who were referred underwent prior screening; thus, they were not selected for tryptase genotyping according to BST level, severity of reaction, or causative agent for anaphylaxis.

These initial data are interesting and justify looking at tryptase genotyping in other cohorts to evaluate any potential role for genotyping in clinical evaluation. Although the understanding of HαT as a modifier of MC-mediated reactions is nascent, our evolving data suggest that in patients with antibiotics or mAb-induced anaphylaxis, HαT may be more common, similar to Hymenoptera venom or idiopathic anaphylaxis.[9,12,14] Whereas current data suggest that tryptase genotype does not appear to affect the development of allergic disease, there are no data for drug allergies. Therefore, further studies are needed to evaluate whether individuals with HαT are at higher risk of developing drug-induced anaphylaxis. Finally, we wish to highlight the importance of further studies and reproducibility in other populations, which are needed to understand how tryptase genotyping may be helpful as a biomarker.

The positive association between HαT and antibiotic- and biologic-induced anaphylaxis that we demonstrate here builds on prior links among various forms of anaphylaxis and HαT that we and others have reported. Although the overall prevalence of antibiotic-induced anaphylaxis in the general population is low, the possible life-threatening nature of these reactions necessitates a careful evaluation of risk factors. In contrast, hypersensitivity reactions to mAbs are increasingly common, and tools are needed to help assist in risk stratification of patients. These findings expand our understanding of how MC-related disorders affect hypersensitivity reactions, and additional data incorporating tryptase genotyping in larger studies of patients with drug allergies are needed to confirm these observations.

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ONLINE REPOSITORY

TRYPTASE GENOTYPING

We performed genotyping of *tryptase α/β1* and *tryptase α/β2* using a multiplex droplet digital PCR assay that precisely quantified α- and β-tryptase–encoding sequences in a single reaction.[E1] Genomic DNA was extracted from 400 μL of EDTA-containing whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Custom primer/probe sets targeting α- and β-tryptases encoding sequences were employed.[E2] The probe targeting α-tryptase was labeled with 5′-FAM, whereas the probes specific to β-tryptase and the copy number reference loci (*AP3B1* or *AGO1*) were labeled with 5′-HEX. The reaction mix was composed of 12.5 μL of 2× droplet digital PCR Supermix for probes (No dUTP) (Bio-Rad), 1.25 μL of primer and probe mix, 5 μL of BamHI-treated genomic DNA, and water to a final volume of 25 μL. A volume of 20 μL of the total mixture was used to generate droplets with the QX200 droplet generator (Bio-Rad) in an eight-channel DG8 cartridge and cartridge holder with 70 μL of Droplet Generation Oil for probes (Bio-Rad). We transferred 40 μL of the generated emulsion into a 96-well PCR plate (Bio-Rad), heat sealed with pierceable foil using PX1 PCR plate sealer (Bio-Rad), and amplified in a T100 Thermal Cycler (Bio-Rad). We performed PCR amplification with these cycling parameters: initial denaturation at 95°C for 10 minutes followed by 40 cycles (at a temperature rate of 2.5°C/s) of denaturation at 94°C for 30 seconds and a combined annealing/extension step at 60°C for 1 minute, and a final step at 98°C for 10 minute, ending at 4°C. After amplification, the plate was directly transferred to the Droplet Reader, and the QX Manager software was used for data acquisition and analysis.[E1]

TABLE E1. Individual clinical and laboratory data of discovery cohort

			Tryptase genotype					Anaphylaxis		
Age	Sex	Baseline serum tryptase, ng/mL	α	β	α/β ratio	*TPSAB1, TPSB2*	Hereditary α-tryptasemia	Severity grade	Drug class	Specific drugs/mAbs
71	F	14.7	3	2	1.5	αα,β/α,β	Yes	4	Antibiotic	Nitrofurantoin
60	F	14.5	3	2	1.5	αα,β/α,β	Yes	3	Antibiotic	Trimethoprim, sulfamethoxazole
54	F	12.5	3	2	1.5	αα,β/α,β	Yes	1	Antibiotic	Amoxicillin-clavulanic acid
36	M	9.62	3	2	1.5	αα,β/α,β	Yes	3	Antibiotic	Cefazolin
50	M	11.6	2	3	0.7	αα,β/β,β	Yes	3	Antibiotic	Cefuroxime
60	M	8.15	2	3	0.7	αα,β/β,β	Yes	2	Antibiotic	Amoxicillin
77	F	5.65	2	2	1	α,β/α,β	No	1	Antibiotic	Penicillin
59	F	5.53	2	2	1	α,β/α,β	No	3	Antibiotic	Ceftriaxone
36	M	4.76	2	2	1	α,β/α,β	No	1	Antibiotic	Amoxicillin-clavulanic acid
32	F	4.2	2	2	1	α,β/α,β	No	2	Antibiotic	Amoxicillin-clavulanic acid
34	M	3.68	2	2	1	α,β/α,β	No	1	Antibiotic	Amoxicillin
28	M	3.27	2	2	1	α,β/α,β	No	4	Antibiotic	Ceftriaxone
61	F	8.75	1	3	0.3	α,β/β,β	No	1	Antibiotic	Penicillin
59	F	8.53	1	3	0.3	α,β/β,β	No	2	Antibiotic	Moxifloxacin
88	M	7.39	1	3	0.3	α,β/β,β	No	3	Antibiotic	Amoxicillin-clavulanic acid
42	F	6.11	1	3	0.3	α,β/β,β	No	3	Antibiotic	Cefazolin
39	M	5.56	1	3	0.3	α,β/β,β	No	3	Antibiotic	Amoxicillin
53	F	5.21	1	2	0.5	α,β/0,β	No	3	Antibiotic	Amoxicillin-clavulanic acid
64	F	4.96	1	3	0.3	α,β/β,β	No	3	Antibiotic	Amoxicillin
43	F	4.33	1	3	0.3	α,β/β,β	No	3	Antibiotic	Cephalosporin
38	F	4.32	1	3	0.3	α,β/β,β	No	3	Antibiotic	Amoxicillin-clavulanic acid
42	F	2.87	1	3	0.3	α,β/β,β	No	2	Antibiotic	Phenoxymethylpenicillin
87	M	8.94	0	4	0	β,β/β,β	No	4	Antibiotic	Amoxicillin
80	M	7.74	0	4	0	β,β/β,β	No	3	Antibiotic	Amoxicillin
35	F	5.77	0	4	0	β,β/β,β	No	2	Antibiotic	Cefazoline
72	M	5.75	0	5	0	β,β/β,ββ	No	1	Antibiotic	Amoxicillin-clavulanic acid
35	M	4.42	0	4	0	β,β/β,β	No	3	Antibiotic	Amoxicillin-clavulanic acid
52	F	4.14	0	4	0	β,β/β,β	No	3	Antibiotic	Amoxicillin-clavulanic acid
60	F	3.63	0	4	0	β,β/β,β	No	2	Antibiotic	Phenoxymethylpenicillin
35	F	3.36	0	4	0	β,β/β,β	No	3	Antibiotic	Cefazoline
66	F	19.9	3	2	1.5	αα,β/α,β	Yes	3	mAb	Rituximab
64	F	9.7	3	2	1.5	αα,β/α,β	Yes	3	mAb	Alemtuzumab
76	M	16.5	2	3	0.7	αα,β/β,β	Yes	1	mAb	Cetuximab, alpha-gal
49	F	6.64	1	3	0.3	α,β/β,β	No	2	mAb	Pertuzumab
51	M	6.03	1	3	0.3	α,β/β,β	No	1	mAb	Cetuximab
77	F	3.94	1	3	0.3	α,β/β,β	No	3	mAb	Cetuximab, alpha-gal
66	F	6.22	0	4	0	β,β/β,β	No	3	mAb	Cetuximab, alpha-gal
58	M	5.5	0	4	0	β,β/β,β	No	3	mAb	Cetuximab, alpha-gal
41	F	8.7	2	2	1	α,β/α,β	No	3	NMBA	Suxamethonium
63	F	4.65	2	2	1	α,β/α,β	No	4	NMBA	Atracurium, Thiopental
60	F	4.06	2	2	1	α,β/α,β	No	3	NMBA	Rocuronium
40	F	3.81	2	2	1	α,β/α,β	No	3	NMBA	Rocuronium
39	F	3.02	2	2	1	α,β/α,β	No	3	NMBA	Rocuronium
40	F	2.81	2	2	1	α,β/α,β	No	3	NMBA	Rocuronium
35	F	2.73	2	2	1	α,β/α,β	No	3	NMBA	Suxamethonium
69	M	8.01	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
70	M	7.47	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
57	F	6.76	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
83	M	5.35	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
66	M	5.34	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
71	M	4.11	1	3	0.3	α,β/β,β	No	4	NMBA	Rocuronium
36	F	3.75	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium

(continued)

TABLE E1. *(Continued)*

			Tryptase genotype					Anaphylaxis		
Age	Sex	Baseline serum tryptase, ng/mL	α	β	α/β ratio	*TPSAB1, TPSB2*	Hereditary α-tryptasemia	Severity grade	Drug class	Specific drugs/mAbs
53	F	3.54	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
71	M	3.27	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
69	M	3.05	1	3	0.3	α,β/β,β	No	3	NMBA	Vecuronium bromide
31	F	2.77	1	3	0.3	α,β/β,β	No	3	NMBA	Rocuronium
47	F	7.15	0	3	0	β,β/β,0	No	3	NMBA	Rocuronium
63	M	5.96	0	4	0	β,β/β,β	No	1	NMBA	Rocuronium
52	F	4.28	0	4	0	β,β/β,β	No	3	NMBA	Rocuronium
61	F	2.46	0	4	0	β,β/β,β	No	3	NMBA	Vecoronium
44	F	2.17	0	4	0	β,β/β,β	No	3	NMBA	Rocuronium
48	M	21.9	3	2	1.5	αα,β/α,β	Yes	3	NSAID	Metamizole
60	M	6.97	2	2	1	α,β/α,β	No	3	NSAID	Metamizole
53	M	5.16	2	2	1	α,β/α,β	No	3	NSAID	Ketoprofen
69	F	4.37	2	2	1	α,β/α,β	No	1	NSAID	Metamizole
68	M	3.42	2	2	1	α,β/α,β	No	3	NSAID	Ketoprofen
55	F	3.02	2	2	1	α,β/α,β	No	2	NSAID	Naproxen
49	F	2.93	2	2	1	α,β/α,β	No	1	NSAID	Metamizole
56	M	10.4	1	3	0.3	α,β/β,β	No	2	NSAID	Naproxen
77	F	8.39	1	3	0.3	α,β/β,β	No	3	NSAID	Metamizole
54	F	7.77	1	3	0.3	α,β/β,β	No	3	NSAID	Etodolac
68	F	5.54	1	3	0.3	α,β/β,β	No	2	NSAID	Diclofenac
62	M	5.32	1	3	0.3	α,β/β,β	No	2	NSAID	Diclofenac
39	F	5.17	1	3	0.3	α,β/β,β	No	3	NSAID	Ketorolac
55	F	4.62	1	3	0.3	α,β/β,β	No	3	NSAID	Diclofenac
75	M	3.58	1	3	0.3	α,β/β,β	No	3	NSAID	Metamizole
35	F	3.5	1	3	0.3	α,β/β,β	No	3	NSAID	Naproxen
54	F	6.29	0	4	0	β,β/β,β	No	3	NSAID	Propyphenazone
42	M	5.02	0	4	0	β,β/β,β	No	1	NSAID	Naproxen
64	M	13.4	3	2	1.5	αα,β/α,β	Yes	2	Contrast	Contrast agents
65	F	2.61	1	3	0.3	α,β/β,β	No	3	Contrast	Gadobutrol
37	M	2.08	1	3	0.3	α,β/β,β	No	3	Contrast	Iomeprol
69	M	21.2	2	3	0.7	αα,β/β,β	Yes	2	Chlorhexidine	Chlorhexidine
42	M	7.39	1	3	0.3	α,β/β,β	No	2	Chlorhexidine	Chlorhexidine
68	M	7.05	1	3	0.3	α,β/β,β	No	4	Chlorhexidine	Chlorhexidine
63	M	5.57	1	3	0.3	α,β/β,β	No	3	Chlorhexidine	Chlorhexidine
49	M	3.66	1	2	0.5	α,β/ β,0	No	1	Chlorhexidine	Chlorhexidine
57	M	3.19	1	3	0.3	α,β/β,β	No	3	Chlorhexidine	Chlorhexidine
41	M	2.1	1	3	0.3	α,β/β,β	No	3	Chlorhexidine	Chlorhexidine
67	F	14.7	3	2	1.5	αα,β/α,β	Yes	3	Other drugs	Pantoprazole
19	F	6.14	2	2	1	α,β/α,β	No	3	Other drugs	Sevoflurane
43	M	6.11	2	2	1	α,β/α,β	No	2	Other drugs	Pantoprazole
22	F	1.7	2	2	1	α,β/α,β	No	1	Other drugs	Paracetamol
63	F	7.72	1	3	0.3	α,β/β,β	No	3	Other drugs	Unknown perioperative
71	F	7.47	1	3	0.3	α,β/β,β	No	2	Other drugs	Unknown perioperative
42	M	5.14	1	3	0.3	α,β/β,β	No	3	Other drugs	Unknown perioperative
41	M	9.57	0	4	0	β,β/β,β	No	1	Other drugs	Unknown perioperative
51	M	7.92	0	4	0	β,β/β,β	No	3	Other drugs	Unknown perioperative
58	F	3.56	0	4	0	β,β/β,β	No	1	Other drugs	Triamcinolone, Lidocaine

NMBA, neuromuscular blocking agents; *NSAID*, nonsteroidal anti-inflammatory drug.
The classification used to classify the severity of anaphylaxis was Ring and Messmer grades.[E3]

TABLE E2. Individual clinical and laboratory data of validation cohort

			Tryptase genotype					Anaphylaxis		
Age	Sex	Baseline serum tryptase, ng/mL	α	β	α/β ratio	*TPSAB1, TPSB2*	Hereditary α-tryptasemia	Severity grade	Drug class	Specific drugs
73	F	5.42	0	4	0	β,β/β,β	No	3	Antibiotic	Amoxicillin-clavulanic acid
59	M	8.91	1	3	0.3	α,β/β,β	No	2	Antibiotic	Trimethoprim, sulfamethoxazole
51	F	5.25	1	3	0.3	α,β/β,β	No	2	Antibiotic	Amoxicillin-clavulanic acid
38	F	5.46	0	4	0	β,β/β,β	No	2	Antibiotic	Amoxicillin-clavulanic acid
30	F	4.83	2	2	1	α,β/α,β	No	1	Antibiotic	Penicillin
51	F	20.2	2	3	0.7	αα,β/β,β	Yes	2	Antibiotic	Amoxicillin-clavulanic acid
47	F	7.42	0	4	0	β,β/β,β	No	2	Antibiotic	Penicillin
47	M	5.9	1	2	0.5	α,β/β,β	No	3	Antibiotic	Cefuroxim
33	F	4.95	2	2	1	α,β/α,β	No	1	Antibiotic	Penicillin
67	F	15.2	3	2	1.5	αα,β/α,β	Yes	2	Antibiotic	Cefuroxim
41	F	13.3	2	3	0.7	αα,β/β,β	Yes	3	Antibiotic	Amoxicillin-clavulanic acid
75	F	7.27	2	2	1	α,β/α,β	No	2	Antibiotic	Ampicillin
42	F	5.78	1	3	0.3	α,β/β,β	No	2	Antibiotic	Trimethoprim, sulfamethoxazole
22	F	4.99	0	4	0	β,β/β,β	No	2	NMBA	Atracurium
40	F	4.18	2	2	1	α,β/α,β	No	1	NMBA	Mivacurium
64	F	10.2	0	4	0	β,β/β,β	No	3	NMBA	Rocuronium
40	M	5.73	1	3	0.3	α,β/β,β	No	2	NMBA	Rocuronium
45	F	7.67	2	2	1	α,β/β,β	No	3	NMBA	Atracurium
65	F	4.5	1	3	0.3	α,β/β,β	No	4	NMBA	Cisatracurium
78	F	10.1	2	2	1	α,β/α,β	No	2	NMBA	Atracurium
41	F	5.83	2	2	1	α,β/α,β	No	1	Contrast	Gadobenas dimegluminum
42	F	6.5	1	3	0.3	α,β/β,β	No	2	Contrast	Iopromide
57	F	5.31	2	2	1	α,β/α,β	No	4	Chlorhexidin	Chlorhexidin
31	F	20.2	3	3	1	ααα,β/β,β	Yes	3	Other drugs	Nadroparin
40	F	4.19	0	4	0	β,β/β,β	No	2	Other drugs	Polyethylenglycol
66	F	3.64	1	3	0.3	α,β/β,β	No	2	Other drugs	Ondansetron

NMBA, neuromuscular blocking agents.
The classification used to classify the severity of anaphylaxis was Ring and Messmer grades.[E3]

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