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Genetic factors account for most of the variation in serum tryptase—a twin study

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ABSTRACT

Background: Mast cells are involved in a number of diseases, including inflammatory diseases such as asthma. Tryptase is a known marker of mast cell burden and activity. However, little is known about the genetic influence on serum tryptase variation. Also, only few and conflicting data exist on serum tryptase in asthma.
Objective: To estimate the overall contribution of genetic and environmental factors to the variation in serum tryptase and to examine the correlation between serum tryptase and asthma, rhinitis, markers of allergy, airway inflammation, and airway hyperresponsiveness (AHR) in a sample of Danish twins.
Methods: A total of 575 twins underwent a skin prick test and had lung function, AHR to methacholine, exhaled nitric oxide and serum tryptase measured. Multiple regression and variance components models (using the statistical package SOLAR) were computed.
Results: Serum tryptase values were available in 569 subjects. Intraclass correlations of serum tryptase in monozygotic and dizygotic twin pairs were 0.84 and 0.42 ($P < .001$). Variance decomposition showed that genetic factors accounted for 82% (95% confidence interval 74–90, $P < .001$) of the variation in serum tryptase. Body mass index and sex, but not asthma, rhinitis, or AHR, were correlated to serum tryptase.
Conclusion: As much as 82% of the variation in serum tryptase is due to genetic factors. Body mass index and sex, but not asthma or AHR to methacholine, correlate to serum tryptase. A genetic overlap may exist between serum tryptase and body mass index.
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Introduction

Mast cells (MCs) are involved in different diseases, ranging from primary MC disorders (mastocytosis) to anaphylaxis and inflammatory disorders such as asthma.[1] Serum tryptase is a marker of MC burden and activity[2] and is the most abundant mediator of MCs, constituting up to 25% of all protein in the cell. Aside from MCs, basophils are the only other cells that normally express tryptase, but in quantities less than 1% of that in MCs.[3]

Tryptase exists in 2 major forms, α-tryptase and β-tryptase. Tryptase exits the endoplasmic reticulum as a catalytically inactive pro-tryptase or a catalytically active mature tryptase. Pro-tryptase is constitutively secreted from the cell[4] and therefore is a useful marker of total MC burden. It consists of pro–β-tryptase alone or pro–β-tryptase in combination with pro–α-tryptase, depending on the genetic constitution of the subject. Mature tryptase is stored in secretory granules ready to be released whenever the cell is activated[4] and is a marker of acute activation. All known genes that code for human MC tryptase are on the short arm of chromosome 16,[5] and 3 genotypes exist, $\beta\beta$:$\beta\beta$, $\beta\beta$:$\beta\alpha$, and $\beta\alpha$:$\beta\alpha$, accounting for 29%, 50%, and 21% of the population, respectively.[6]

Elevated total serum tryptase is used to confirm cases of anaphylaxis[7] and an elevated basal serum tryptase level higher than 20 μg/L is a minor diagnostic criterion for mastocytosis.[1] Factors that have been reported to influence basal levels of serum tryptase include age, sex, obesity, severe chronic renal failure, haplotype ($\beta\alpha$), and severe alcohol consumption,[8–11] whereas food intake, pregnancy, or physical exercise do not seem to affect the level of serum tryptase.[8,9,12,13] For most factors associated with serum tryptase, however, correlations are rather weak and only partly explain variations in serum tryptase levels.

No study has ever used twin data to estimate the percentage of variation in serum tryptase that is associated with genetic factors. Furthermore, MCs are key effector cells in asthma and airway

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hyperresponsiveness (AHR),[14,15] but only 2 studies have been conducted on the association between serum tryptase and asthma and AHR, showing conflicting results.[12,13] Also, body mass index (BMI) has been shown to be associated to the level of serum tryptase in adults, and recent findings have suggested that MCs directly and indirectly interact and activate adipose tissue.[16] However, no study has estimated the overlap in genetic and environmental effects between serum tryptase and BMI.

Therefore, using data from a large sample of Danish twins, the authors estimated the overall contribution of genetic and environmental factors to variation in serum tryptase levels and studied the correlation between serum tryptase and variables related to atopic disease (asthma, rhinitis, and dermatitis).

Methods

Study Population

A total of 21,133 twins (born from 1953 through 1982 and registered in the Danish Twin Registry) answered a mailed questionnaire concerning health and lifestyle, with a response rate of 75%. Of these, pairs with at least 1 member with self-reported asthma (based on an affirmative response to the question, "Do you have or have you ever had asthma?") and who lived in the eastern part of Denmark were invited to take part in a clinical examination. The invited group of twins consisted of 438 pairs, of whom 575 subjects agreed to undergo examination (89 monozygotic [MZ] pairs, 167 dizygotic [DZ] pairs, and 63 single twins).[17] Twin zygosity was determined using 4 questions of similarity and mistaken identity, which assign zygosity correctly in more than 95% of cases.[18] The scientific ethics committee approved the study (S-VF-20010202). Written informed consent was obtained from all subjects before entering the study.

Clinical Examination

At the clinical examination, subjects were interviewed about symptoms of asthma and time of last symptomatic episode. Clinical asthma was defined as recurrent episodes of wheezing, shortness of breath, chest tightness, and/or cough at rest, after exertion, or subsequent to allergenic or unspecific stimuli in the absence of a respiratory tract infection. For this study, subjects were classified with asthma only if symptoms had been present within 4 weeks before examination. Rhinitis was defined as recurrent episodes of runny or stuffy nose and/or sneezing within the past year in the absence of a common cold.

Spirometric measurements of forced expiratory volume in 1 second (FEV_1) and forced vital capacity were performed using a dry wedge spirometer (Vitalograph, Maids Moreton, Buckingham, United Kingdom). The ratio of FEV_1 to forced vital capacity was used to calculate the degree of airway obstruction.

Airway responsiveness to inhaled methacholine was measured according to the method of Yan et al.[19] Each aerosol was inhaled, starting with saline and followed by increasing doses of methacholine until a cumulative dose of 7.8 μmol had been reached. The test was terminated when the maximum concentration had been reached or when a 20% decrease in FEV_1 had occurred before the end of the dosing regimen. For all subjects with at least a 20% decrease in FEV_1, the concentration causing a 20% decrease in FEV_1 (PD_{20}) was calculated. AHR was defined as a PD_{20} below 3.9 μmol.

Skin prick tests were performed using standard dilutions of 10 common aeroallergens, a negative control with normal saline, and a positive control with histamine 10 mg/mL. The allergens used were birch, grass, mugwort, horse, dog, cat, house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and mold (*Alternaria iridis* and *Cladosporium herbarium*; Soluprick SQ System, ALK Abelló, Hørsholm, Denmark). Reactions were read after 15 minutes. A positive result was defined as a positive reaction to at least 1 of the allergens, and a reaction was considered positive if the mean wheal diameter was at least 3 mm.

Fractional exhaled nitric oxide levels were measured online (rate 0.05 L/s) with a nitric oxide analyzer (NIOX, Aerocrine AB, Solna, Sweden) and according to American Thoracic Society guidelines.[20]

Serum Tryptase

Blood was collected in tubes with no additives and stored at 4°C after sampling for a maximum of 2 hours. After centrifuging at 3,000 rpm for 15 minutes, serum was separated and stored at −80°C. Total serum tryptase was measured using a fluoro enzyme immunoassay (ImmunoCap, Phadia, Sweden) that detects the sum of pro−α-tryptase, pro−β-tryptase, and mature α- and β-tryptase (detection range 1−200 μg/L).

Statistical Analysis

Univariate analyses were performed using the Student t test comparing serum tryptase levels based on sex, age, atopy, BMI, smoking, AHR to methacholine, asthma, rhinitis, atopic dermatitis, use of inhaled corticosteroids, and level of fractional exhaled nitric oxide. All variables with a P value less than or equal to .3 in the univariate analysis were entered into a linear regression, with serum tryptase as the dependent variable. To meet the assumption of normality, serum tryptase was logarithmically transformed ($\log_{10}$) before analyses. The correlation of serum tryptase levels between MZ and DZ twin pairs was assessed using Pearson correlation analysis. To test whether the correlation in MZ and DZ twin pairs differed, an interaction term between zygosity and serum tryptase was added to the linear regression model, with serum tryptase (twin 1) as the dependent variable and serum tryptase (twin 2), zygosity, and the interaction term as independent variables.

A variance components model was used to partition the variation in serum tryptase into genetic and environmental sources, namely additive genetic variance (A), shared environmental variance (C), and nonshared environmental variance (E).[21] Although the C component reflects environmental effects that render members of the same family more alike, the E component reflects those environmental factors that create dissimilarity among members of the same family. The E component also includes variance owing to measurement error. The expected covariance for MZ twins is $A + C$, whereas that for DZ twins is $0.5 \times A + C$. Variables that were significantly correlated to serum tryptase in the multiple regression model were entered as covariates in the means model of the variance components model. Overlap in genetic and environmental effects between serum tryptase and BMI was assessed in a model that included the variance components A and E. Using the SOLAR statistical package (SOLAR Software, Westerville, Ohio), the parameter estimates in the model were adjusted for ascertainment, ie, for the selection of the population through a proband with asthma.

Results

Of the 438 twin pairs invited, 575 subjects (ie, 66%; 89 MZ pairs, 167 DZ pairs [112 same-sex pairs], and 63 single twins) participated in the clinical examination. Mean age was 36 years (range 21−51) and 335 (58%) were women. Baseline characteristics are presented in Table 1. A total of 299 subjects (52%) had a history of asthma, 211 of whom reported symptoms within the past month.

Serum tryptase values were available in 569 of the 575 subjects examined. Median serum tryptase level was 3.69 μg/L (interquartile range 2.41 μg/L, minimum <1 μg/L, maximum 18.70 μg/L).

Table 1
Baseline characteristic and multiple regression model[a]

	Baseline characteristics of 569 subjects with available serum tryptase values			Multiple regression		
	n	Serum tryptase (μg/mL), median (IQR)	*P* value	Unstandardized B (SE)	Standardized B	Adjusted *P* value
Sex				0.047 (0.020)	0.099	.02
Male	239	4.00 (2.41)				
Female	330	3.51 (2.29)	.01			
Age (y)				0.002 (0.001)	0.077	.07
20–29 (reference)	155	3.44 (2.25)				
30–39	192	3.75 (2.30)	.16			
40–49	203	3.82 (2.52)	.04			
50–59	19	5.34 (2.82)	.17			
Skin prick test result				—	—	—
Negative	254	3.63 (2.77)				
Positive	315	3.74 (2.22)	.08			
BMI (kg/m^2)				0.008 (0.002)	0.156	<.001
≤25 (reference)	310	3.45 (2.39)				
25–30	187	3.91 (2.15)	<.01			
>30	72	4.41 (3.03)	<.01			
Smoking				0.016 (0.024)	0.030	.49
No	427	3.68 (2.31)				
Yes	142	3.81 (2.78)	.13			
AHR to methacholine				—	—	—
No	376	3.68 (2.40)				
Yes	149	3.88 (2.25)	.51			
Current asthma				—	—	—
No	359	3.69 (2.40)				
Yes	210	3.64 (2.41)	.65			
Current rhinitis				−0.032 (0.021)	−0.067	.12
No	236	3.82 (2.69)				
Yes	333	3.62 (2.34)	.03			
Atopic dermatitis				—	—	—
No	509	3.69 (2.42)				
Current	60	3.58 (2.36)	.93			
Current ICS				−0.033 (0.023)	−0.060	.16
No	446	3.75 (2.40)				
Yes	123	3.61 (2.30)	.12			
FeNO (ppb)				0.0003 (0.0003)	−0.035	.42
≤25	387	3.82 (2.46)				
>25	176	3.60 (2.37)	.27			

Abbreviations: AHR, airway hyperresponsiveness; BMI, body mass index; FeNO, fractional exhaled nitric oxide; ICS, inhaled corticosteroids; IQR, interquartile range; SE, standard error.
[a]In the regression model, $\log_{10}$ tryptase was the dependent variable. Age and BMI were entered as continuous variables.

Intraclass correlations for serum tryptase were 0.84 ($P < .001$) within MZ twin pairs and 0.42 ($P < .001$) within same-sex DZ twin pairs (Fig 1). The difference between MZ and DZ twins was statistically significant ($P < .001$), whereas the difference between male and female DZ twin pairs was not ($P = .11$). Variance decomposition showed that genetic factors accounted for 82% (95% confidence interval [CI] 74–90, $P < .001$) of the variation in serum tryptase, whereas nonshared environment accounted for the remaining 18%. The heritability of BMI was 72% (95% CI 62–82). The genetic correlation between BMI and serum tryptase was 0.27 (95% CI 0.08–0.46, $P = .008$), indicating that the 2 traits are in part influenced by similar genetic factors; the overlap in unique environmental effects on serum tryptase and BMI was insignificant (−0.03, 95% CI −0.26 to 0.20, $P = .81$).

In a univariate analysis, asthma ($P = .65$) and AHR ($P = .51$) were not correlated to serum tryptase, whereas sex, age, BMI, skin prick test, smoking, rhinitis, use of inhaled corticosteroids, and fractional exhaled nitric oxide showed *P* values below .3 and subsequently were included in the multiple regression analysis. Because symptoms of rhinitis and the result of a skin prick test are closely correlated, only the variable that was most significantly correlated in the regression model, namely rhinitis, was included. Only BMI and sex remained statistically significantly related to serum tryptase after adjusting for other covariates (Table 1). The overall fit of the model with these 7 predictors was $R^2 = 0.06$, meaning that the multiple regression model explained 6% of the variation in serum tryptase. The unstandardized B of BMI (0.008) indicated that increasing BMI by 1 unit increases serum tryptase by 0.8%, whereas the standardized B of 0.16 indicated that increasing BMI 1 standard deviation increases serum tryptase 0.16 standard deviation. Male sex increases serum tryptase by 4.7% (unstandardized B), corresponding to a standardized B of 0.10, ie, an increase of 0.10 standard deviation.

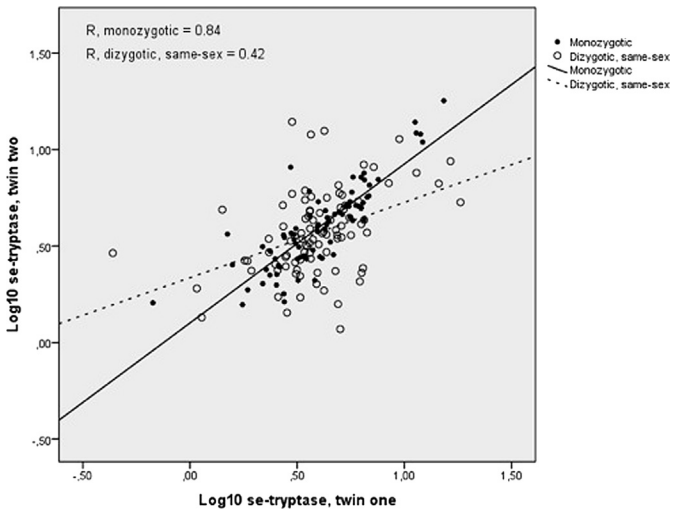

Figure 1. Distribution of serum tryptase in 88 monozygotic twin pairs and 112 same-sex dizygotic twin pairs.

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Discussion

In the first part of this study, the authors examined the extent to which genetic factors affect variation of serum tryptase levels. The intraclass correlation in MZ twins was high and significantly higher than in DZ twins, indicating that genes influence variation in serum tryptase levels: as much as 82% of the variation in serum tryptase was due to additive genetic effects. This means that of the individual differences observed in serum tryptase levels, 82% was due to individual differences on a genetic level. This provides information about the expected correlation of serum tryptase levels among relatives and can be used to enhance gene mapping efforts and monitor patients and families with diseases that cause elevated serum tryptase, such as systemic mastocytosis and other neoplastic MC diseases.

In the second part of the study, the authors examined the relation between serum tryptase and MC-related diseases in 575 Danish twins. Asthma, rhinitis, and AHR to methacholine showed no influence on the level of serum tryptase. In a multiple variable linear regression model, only BMI and sex remained statistically significant predictors of serum tryptase levels, with serum tryptase levels being higher for men and for subjects with higher BMI. The model including sex and BMI, however, explained only 6% of the variance in serum tryptase, which suggests that most of the variation in serum tryptase levels is explained by other factors.

Higher BMI and male sex have been reported to be related to elevated levels of serum tryptase in adults, and it has been suggested that the relation of serum tryptase to high BMI is due to systemic inflammation in obese subjects.[9] Also, new results have suggested that MCs directly and indirectly interact and activate adipose tissue.[16] The overlap in genetic effects between serum tryptase and BMI in this study indicates that some of the genetic factors responsible for the variation in serum tryptase may contribute to the variation in BMI.

Age has been identified as a significant predictor for serum tryptase levels,[9,12] whereas the relation with age was not statistically significant in the present study ($P = .07$). This might be explained by a narrower age range and the limited number of 50- to 59-year-old subjects in the present study compared with previous publications.

Only 2 other studies have investigated serum tryptase as a marker of asthma, with conflicting results.[12,13] Asthma is a heterogeneous disease, and the lack of consistency in serum tryptase in subjects with and without asthma might be due to the existence of different phenotypes of asthma with different degrees of MC involvement. It also has been suggested that asthma (and rhinitis) with disease processes limited to the airways does not induce MC degranulation and subsequent "spillover" into the blood stream large enough to significantly influence serum tryptase levels.[22,23] However, given the consistency of the present study's result with those reported by Fenger et al[12] in a study with a large sample (n = 1,195), the authors find it reasonable to assume that serum tryptase does not constitute a marker of asthma and that the quantitative and qualitative significance of MCs in atopic diseases needs to be established through investigations other than serum tryptase in subjects with atopic disease.

A possible limitation is that the group of participating twins may not be fully representative of the general population because of the used inclusion criterion of self-reported asthma in at least 1 member of each twin pair. However, at present, there is no reason to believe that the relative genetic and environmental influences on serum tryptase are different in subjects with asthma compared with healthy subjects. Therefore, the authors presume that their results are representative of the population as a whole, but future research in a large population-based twin study is recommended.

In conclusion, as much as 82% of the variation in serum tryptase is due to genetics factors, and genetic effects overlap serum tryptase and BMI. This information may be useful not only in future gene mapping but also in monitoring patients and families with diseases that cause elevated serum tryptase, such as systemic mastocytosis and other neoplastic MC diseases.

No significant correlation between serum tryptase and MC-associated diseases such as asthma was found. However, BMI and sex did influence the variation in serum tryptase. The authors suggest that the quantitative and qualitative significance of MCs in atopic disease needs to be established through other investigations than through serum tryptase.

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