A study of asthma severity in adult twins

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Abstract

Introduction: The tendency to develop asthma runs in families, but whether the severity of asthma symptoms is inherited is not known.

Objectives: The aim of this study was to examine whether genetic factors influence the variation in the severity of asthma.

Methods: Of a sample of 21 133 adult twins from the Danish Twin Registry, a total of 575 subjects (256 intact pairs and 63 single twins) who themselves and/or their co-twins reported a history of asthma at a questionnaire survey were clinically examined. The severity of asthma symptoms was graded according to a clinical interview, and markers of airway impairment and allergy were measured.

Results: After adjusting for confounders, genetic factors explained 24% (10%–37%), $P = 0.0004$, of the variation in overall asthma symptom severity, whereas non-shared environment accounted for the remaining 76% of the variation. A significant genetic component was also found for the severity of specific asthma symptoms; wheezing 12% (3%–22%), $P = 0.007$ and shortness of breath 17% (7%–27%), $P = 0.0006$, but not for chest tightness and cough. Asthma symptom severity correlated weakly with rhinitis severity as well as with objective markers of lung function, airway inflammation, airway responsiveness and allergic sensitization.

Conclusion: The individual variation in asthma symptom severity is to some degree influenced by genetic factors, but environmental factors explain the main part of the variation. The genetic architectures underlying the severity of asthma symptoms and objectively measured asthma-related traits, respectively, seem to differ.

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Simon Francis Thomsen designed the study, collected and analyzed the data and wrote the paper. Sophie van der Sluis analyzed the data and wrote the paper. Kirsten Ohm Kyvik and Vibeke Backer designed the study and wrote the paper.

Ethics
The Committee of Biomedical Research Ethics of Copenhagen approved the study.

Conflicts of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Introduction

The clinical expression of asthma is highly variable; some individuals are affected only to a limited extent, whereas others suffer from severe disease with frequent exacerbations and objective signs of airflow limitation (1). In a considerable number of cases, the disease is further complicated by rhinoconjunctivitis and sensitization to aeroallergens. Most cases of asthma require daily treatment with inhaled corticosteroids, whereas mild intermittent asthma probably is controlled sufficiently with short acting bronchodilators as needed. Several factors are known to influence the severity of asthma. Particularly, smoking (2, 3), adherence to prescribed medications (2, 4) and probably also hereditary factors (5) have been implicated in disease expression.

There is a paucity of studies investigating the genetic variation underlying the clinical expression of asthma (6). Notably, much investigation into the genetics of asthma has concerned the susceptibility to the disease and, to a much smaller extent, more elaborated aspects of its clinical expression such as, for example, its

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symptomatology. A first line of evidence in which genetics influences the variation in the clinical manifestation of the disease would be to show that the severity of asthma symptoms aggregates within families. This would implicate that some families with asthma are more likely to cluster at the one mild end of the disease spectrum, whereas other families are more likely to cluster at the other severe end of the disease spectrum. To test this hypothesis, we studied a population of adult twins and examined whether genetic factors contribute to the variation in the severity of asthma symptoms. Particularly, we estimated the heritability of asthma severity as judged by overall symptom frequency as well as the severity of specific asthma symptoms. As supportive secondary analysis, we examined the severity of asthma by a medication use index as well as by objective measures of airway impairment, such as lung function, airway responsiveness, airway inflammation and atopic sensitization. Although some of these analyses have been reported previously for this population of twins, particularly in regard to the genetic relationships between airway hyperresponsiveness (AHR), serum total immunoglobulin E (IgE), skin prick test reactivity, exhaled nitric oxide levels and degree of airway obstruction, we present them herein for completeness (7, 8). Furthermore, we studied to what extent possible underlying genetic effects for clinical asthma severity were shared with genetic effects for objectively measured asthma-related traits.

Materials and methods

Study population

The studied sample consisted of twins born between 1953 and 1982 who were registered in the Danish Twin Registry (9). In an initial screening phase, 21 133 twin individuals answered a mailed questionnaire concerning health and lifestyle. The response rate was 75%. Of these, a total of 6731 individuals were single twins, whereas 7201 were intact twin pairs with complete data on asthma [2351 monozygotic (MZ) twin pairs, 4671 dizygotic (DZ) twin pairs and 179 pairs with unknown zygosity]. Among these, pairs with at least one 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 geographical region surrounding the study site were invited to take part in a clinical examination. This invited group of twins consisted of 438 pairs. Twin zygosity was determined using four questions of similarity and mistaken identity, which assign zygosity correctly in more than 96% of the cases (10). The Scientific Ethics Committee of Copenhagen approved 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 considerable recurrent episodes of wheezing, shortness of breath, chest tightness, and/or cough at rest following exertion or subsequent to allergenic or to unspecific stimuli when not having a respiratory tract infection. The severity of asthma symptoms was graded according to the Global Initiative for Asthma guidelines into intermittent, mild persistent, moderate persistent and severe persistent based on symptoms experienced within the past month (1). Intermittent asthma was defined as the presence of symptoms less than one day per week and/or symptoms less than two nights per month; mild persistent asthma was defined as symptoms one or more days per week but less than every day, and/or symptoms three nights or more per month but less than one night per week. Moderate persistent asthma was defined as symptoms once daily, and/or symptoms one night or more per week but less than every night, whereas severe persistent asthma was defined as symptoms more than once daily, and/or symptoms every or almost every night. The adherence to and the type of asthma medication used were also recorded at the clinical interview. To qualify as *adherent*, an individual should not have skipped any prescribed dose of inhaled medication during the last month.

According to Ungar and colleagues, a medication severity index of asthma was also constructed based on current amount and type of inhaled and other medications for asthma comprising severity classes: no intake of medication (non-asthmatic), mild, moderate and severe asthma (11). This index defines mild asthma as a condition that requires bronchodilator monotherapy that does not exceed 4 puffs/day, and moderate asthma as a condition that requires bronchodilator monotherapy that does exceed 4 puffs/day, inhaled anti-inflammatory monotherapy that does not exceed the equivalent of beclomethasone 800 μg/day or treatment with two types of medication; neither of which is an oral corticosteroid. If one or both medications are inhaled anti-inflammatory agents, the combined dose does not exceed the equivalent of beclomethasone 800 μg/day; and severe asthma as a condition that requires treatment with one or two types of medication, where one is an inhaled anti-inflammatory drug with a dosage greater than the

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equivalent of beclomethasone 800 μg/day OR treatment with three or more types of medication where one is an inhaled anti-inflammatory OR treatment with oral corticosteroids.

Rhinitis was defined as considerable recurrent episodes of runny or stuffy nose, and/or sneezing when not having a cold. The severity of rhinitis was graded according to the Allergic Rhinitis and its Impact on Asthma guidelines into mild intermittent, severe intermittent, mild persistent and severe persistent based on symptoms experienced within the past year (12).

Skin prick tests were performed using standard dilutions of 10 common aeroallergens. The allergens used were birch, grass, mugwort, horse, dog, cat, house dust mite (HDM) (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and mould (*Alternaria iridis* and *Cladosporium herbarium*) (Soluprick SQ system; ALK Albelló, Denmark). Reactions were read after 15 min. A positive result was defined as a positive reaction to at least one of the allergens, and a reaction was considered positive if the mean wheal diameter was at least 3 mm. HDM sensitivity was defined as a positive test result to one or two of the HDM allergens.

Serum total IgE was measured with an enzyme-linked immunosorbent assay on Immulite 2500 (DPC, New York, USA).

Spirometric measurements of forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were performed using a dry wedge spirometer (Vitalograph, Buckingham, United Kingdom). The ratio of the preprovocation values of FEV_1 and FVC was used to calculate the degree of airway obstruction. Airway responsiveness to inhaled methacholine was measured according to the method by Yan et al. (13). Each aerosol was inhaled starting with saline 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% decline in FEV_1 had occurred before the end of the dosing regimen. For all subjects experiencing at least a 20% decline in FEV_1, the concentration causing a 20% fall in FEV_1 (provocative dose, PD_{20}) was calculated. AHR was defined as a PD_{20} below 3.9 μmol.

Fractional exhaled nitric oxide (FeNO) was measured using a chemiluminescence analyser (NIOX, Aerocrine AB; Solna, Sweden). Measurements were taken for 10 s maintaining a constant flow rate of 50 mL/s at each exhalation procedure. All measurements were performed in triplicate, and the mean concentration (in parts per billion) was registered (14).

Statistical analysis

Variance components models were used to examine the relative importance of genetic and environmental effects on asthma severity. These models assume that the variation in the severity of asthma symptoms can be partitioned into genetic and environmental sources, i.e. additive genetic variance (A), shared environmental variance (C) and non-shared environmental variance (E). The E-component also includes variance because of measurement error (15). The expected covariance for MZ twins is A + C, while for DZ twins, it is 0.5*A + C. In this analysis, the severity of asthma symptoms was regarded a quantitative phenotype; subjects with any respiratory symptom (wheeze, shortness of breath, chest tightness and/or cough) within the preceding month were categorized into four severity groups (score 1–4), whereas subjects who did not meet this criterion were classified as asymptomatic (score 0). A similar analysis was employed for the genetics of rhinitis severity assigning subjects to severity categories 0 through 4 and for the asthma medication severity index (categories 0 through 3). The degree of overlap between genetic and environmental effects, respectively, for asthma symptom severity and associated traits was expressed as the additive genetic correlation (ρ_A) and the non-shared environmental correlation (ρ_E), respectively. These were estimated under a model that included the variance components A and E (16). All models were fitted in SOLAR with correction for ascertainment using the *probnd* option. DZ twin pairs of the same and the opposite sex were considered together in the analysis; age, sex, smoking, current airway infection and body mass index (BMI) were incorporated as covariates. Descriptive analyses were carried out in SPSS 16.0 (SPSS Inc., Chicago, IL, USA), whereas analyses within concordant twins were performed in Mx (17).

Results

Descriptive analysis

A total of 575 subjects (256 intact pairs; 89 MZ pairs, 167 DZ pairs and 63 single twins) underwent the clinical examination. This corresponded to an individual response rate of 67% and a pair-wise response rate of 60%. The prevalence of self-reported asthma at the screening questionnaire in single twins and in intact twin pairs was the same (60 vs 58%), $P = 0.770$. The mean age of the population was 36 years (age range 21–51 years); 58% of the subjects were females, and 25% were smokers (Table 1). Of 337 subjects who

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Table 1. Characteristics of 575 Danish adult twins according to the severity of asthma symptoms experienced during the preceding month

	Asymptomatic (n = 314)	Intermittent symptoms (n = 81)	Mild symptoms (n = 61)	Moderate symptoms (n = 32)	Severe symptoms (n = 87)	Total (n = 575)
Age	35.1 (8.4)	36.4 (8.5)	35.1 (7.3)	39.8 (8.1)	37.7 (7.3)	35.9 (8.2)
Males	138 (43.9)	33 (40.7)	23 (37.7)	12 (37.5)	32 (39.1)	240 (41.7)
BMI	25.0 (4.2)	24.0 (3.2)	25.4 (3.8)	28.7 (8.0)	25.9 (5.0)	25.2 (4.6)
MZ twins	96 (30.6)	29 (35.8)	23 (37.7)	11 (34.4)	32 (36.8)	191 (33.2)
Current smoking	67 (21.3)	15 (18.5)	15 (24.6)	12 (37.5)	35 (40.2)	144 (25.0)
Ever smoking	124 (39.5)	37 (45.7)	34 (55.7)	18 (56.2)	49 (56.3)	262 (45.6)
Self-reported ever asthma	123 (39.2)	72 (88.9)	48 (78.7)	26 (81.2)	68 (78.2)	337 (58.6)
Clinical ever asthma						
Certain asthma	100 (31.8)	68 (84.0)	50 (82.0)	20 (62.5)	61 (70.1)	299 (52.0)
Probable asthma	10 (3.2)	5 (6.2)	4 (6.6)	4 (12.5)	6 (6.9)	29 (5.0)
Doubtful asthma	15 (4.8)	2 (2.5)	0 (0)	1 (3.1)	9 (10.3)	27 (4.7)
Daytime symptoms						
Wheezing	0 (0)	56 (69.1)	41 (67.2)	20 (62.5)	53 (60.9)	170 (29.6)
Shortness of breath	0 (0)	34 (42.0)	35 (57.4)	15 (46.9)	46 (52.9)	130 (22.6)
Chest tightness	0 (0)	27 (33.3)	19 (31.1)	7 (21.9)	19 (21.8)	72 (12.5)
Cough	0 (0)	41 (50.6)	41 (67.2)	24 (75.0)	78 (89.7)	184 (32.0)
Any symptom	0 (0)	81 (100)	61 (100)	32 (100)	86 (98.9)	260 (45.2)
Nighttime symptoms						
Wheezing	0 (0)	3 (3.7)	6 (9.8)	6 (18.8)	18 (20.7)	33 (5.7)
Shortness of breath	0 (0)	0 (0)	4 (6.6)	4 (12.5)	15 (17.2)	23 (4.0)
Chest tightness	0 (0)	0 (0)	2 (3.3)	0 (0)	8 (9.2)	10 (1.7)
Cough	0 (0)	3 (3.7)	10 (16.4)	3 (9.4)	29 (33.3)	45 (7.8)
Any symptom	0 (0)	5 (6.2)	14 (23.0)	7 (21.9)	31 (35.6)	57 (9.9)
Current SABA	36 (11.5)	43 (53.1)	33 (54.1)	17 (53.1)	39 (44.8)	168 (29.2)
Current LABA	6 (1.9)	8 (9.9)	14 (23.0)	4 (12.5)	16 (18.4)	48 (8.3)
Current ICS	19 (6.1)	30 (37.0)	29 (47.5)	13 (40.6)	32 (36.8)	123 (21.4)
Any inhaled medication	38 (12.1)	48 (59.3)	38 (62.3)	20 (62.5)	46 (52.9)	190 (33.0)
Adherence (among treated)	34 (89.5)	36 (75.0)	21 (55.3)	13 (65.0)	28 (60.9)	132 (69.5)
Medication severity index	0.20 (0.03)	1.07 (0.12)	1.39 (0.16)	1.16 (1.19)	1.08 (0.13)	0.64 (0.04)
Rhinitis (in the past year)	153 (48.7)	59 (72.8)	50 (82.0)	19 (59.4)	55 (63.2)	336 (58.4)
AHR	52 (17.6)	28 (35.9)	25 (44.6)	15 (50.0)	29 (40.8)	149 (28.1)
$DRS_{methacholine}$	2.0 (1.1)	3.6 (1.2)	4.4 (1.3)	6.2 (1.3)	4.3 (1.2)	2.8 (1.1)
FeNO	17 (1.9)	24 (2.2)	24 (2.6)	20 (2.3)	16 (2.5)	19 (1.4)
FEV_1% predicted	93.9 (11.3)	91.3 (13.4)	89.8 (15.8)	89.1 (14.0)	83.3 (15.7)	91.2 (13.5)
FVC% predicted	96.1 (13.6)	94.0 (14.0)	95.7 (15.4)	92.6 (15.6)	86.6 (15.8)	94.1 (14.6)
FEV_1/FVC (%)	84.5 (7.3)	83.8 (8.2)	81.0 (10.3)	82.9 (7.3)	83.2 (11.5)	83.7 (8.6)
Positive SPT	154 (49.0)	56 (69.1)	46 (75.4)	18 (56.2)	44 (50.6)	318 (55.3)
HDM sensitization	71 (22.6)	34 (42.0)	23 (37.7)	5 (15.6)	22 (25.3)	155 (27.0)
Serum total IgE	47 (4.3)	64 (4.0)	91 (4.1)	44 (6.1)	59 (4.2)	54 (4.3)
Current airway infection	13 (4.1)	6 (7.4)	4 (6.6)	4 (12.5)	5 (5.7)	32 (5.6)

All figures are numbers (%). Age, BMI, FEV_1% predicted, FVC% predicted, FEV_1/FVC (%) and medication severity index are mean values (standard deviation). $DRS_{methacholine}$, FeNO and IgE are geometric means.

SABA, short-acting beta-2 agonist; LABA, long-acting beta-2 agonist; ICS, inhaled corticosteroids; AHR, airway hyperresponsiveness; IgE, immunoglobulin E; BMI, body mass index; MZ, monozygotic twins; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; SPT, skin prick test; DRS, dose-response slope; HDM, house dust mite.

reported a history of asthma at the screening questionnaire, a total of 38 (11.3%) were subsequently diagnosed not to have (or not to have had) asthma based on the findings at the clinical examination. Conversely, of 238 subjects who denied a history of asthma at the screening questionnaire, a total of 29 (12.2%) were diagnosed with asthma at the clinical examination giving a misclassification rate of 11.7% (sensitivity of

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the asthma screening question, 87.8%; specificity, 88.7%). In total, 261 subjects had had asthma symptoms within the preceding month (Table 1).

Characteristics of asthma severity in the whole sample

Among individuals with any current asthma symptom, a clinical diagnosis of asthma was made more often in the intermittent and mild severity categories (over 80%), whereas fewer were given a clinical asthma diagnosis in the more severe categories, particularly in individuals with moderate symptoms. Specific asthma symptoms experienced during daytime were distributed evenly, albeit not equally frequently, between severity categories except for cough that increased in prevalence for every increase in severity score, whereas symptoms during nighttime were more common among the severe. The prevalence of rhinitis and positive skin prick test was substantial in subjects with asthma symptoms (around 70%) but tended to be less frequent in more severe asthma cases. Also, the prevalence of AHR increased with increasing severity score with a small decrease in prevalence among subjects with severe persistent asthma symptoms. Around 40% of the subjects with asthma symptoms used inhaled corticosteroids, whereas around 50% used inhaled bronchodilators. The adherence rate for inhaled medications was around 60% in symptomatic subjects but tended to decrease with increasing severity of the symptoms. The asthma medication severity index tended to decrease among the most severe asthmatics. The rate and amount of smoking increased, with increasing asthma severity. Subjects who reported asthma at the screening questionnaire were quite evenly distributed among the clinical severity categories, indicating that initially reporting asthma at the questionnaire was independent of the clinically assigned severity of the disease at the examination.

Analysis of asthma severity within the individual

The severity of asthma symptoms was significantly correlated within the individual (Table 2). Particularly, cough and overall severity were highly correlated, r = 0.89, as were wheezing and shortness of breath, r = 0.72, whereas chest tightness correlated least with the other symptoms. The severity of all the specific asthma symptoms correlated significantly with the medication severity index. Severity scores for wheezing and shortness of breath, and to some extent also for overall severity correlated significantly, but only moderately, with most of the objectively measured traits;

Table 2. Within-subject correlations between asthma severity indicators in a sample of 575 Danish adult twins

	Over	Whee	Short	Chest	Cough	Medi	Rhin	FEV_1	FVC	FE/FV	DRS	FeNO	IgE	SPT	HDM
Overall symptom severity															
Wheezing	0.62***														
Shortness of breath	0.55***	0.72***													
Chest tightness	0.34***	0.38***	0.45***												
Cough	0.89***	0.41***	0.38***	0.25***											
Medication severity index	0.38***	0.43***	0.36***	0.21***	0.27***										
Rhinitis severity	0.13**	0.18***	0.13**	0.14***	0.09*	0.18***									
FEV_1	−0.24***	−0.25***	−0.25***	−0.15**	−0.21**	−0.21***	−0.02								
FVC	−0.19***	−0.16***	−0.15***	−0.12**	−0.17***	−0.13**	−0.04	0.92***							
FEV_1/FVC	−0.08*	−0.20***	−0.21***	−0.04	−0.05	−0.18***	0.05	−0.01	0.39***						
$DRS_{methacholine}$	0.24***	0.37***	0.37***	0.12**	0.16***	0.28***	0.19***	0.23***	−0.08	0.31***					
FeNO	0.01	0.15***	0.12**	0.01	−0.02	0.13**	0.28***	0.15***	0.15***	−0.05	0.24***				
Serum total IgE	0.07	0.12**	0.10*	0.05	0.05	0.20***	0.29***	0.00	−0.01	0.01	0.22***	0.28***			
Positive SPT	0.04	0.10*	0.09*	0.06	0.02	0.28***	0.45***	0.11**	0.11**	−0.04	0.32***	0.37***	0.54***		
HDM sensitization	0.07	0.14***	0.10*	0.00	0.05	0.18***	0.30***	0.14**	0.16**	−0.08	0.23***	0.36***	0.38***	0.70***	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; SPT, skin prick test; DRS, dose-response slope; HDM, house dust mite.

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Table 3. Resemblance between asthma concordant twin pairs for severity related traits

	Correlation within concordant twin pairs (95% CI)		
Trait	MZ twin pairs ($n = 38$)	DZ twin pairs ($n = 21$)	*P* value*
Overall symptom severity	0.37 (0.07 to 0.61)	0.11 (–0.31 to 0.50)	0.301
Wheezing	0.39 (0.09 to 0.62)	0.07 (–0.35 to 0.47)	0.211
Shortness of breath	0.37 (0.07 to 0.61)	–0.20 (–0.56 to 0.23)	0.031
Chest tightness	–0.01 (–0.32 to 0.30)	0.04 (–0.37 to 0.44)	0.855
Cough	0.30 (–0.01 to 0.56)	0.01 (–0.40 to 0.42)	0.273
Medication severity index	0.13 (–0.10 to 0.42)	0.27 (–0.16 to 0.61)	0.593
Rhinitis severity	0.18 (–0.14 to 0.47)	0.43 (0.02 to 0.71)	0.315
FEV_1	0.84 (0.72 to 0.91)	0.36 (–0.05 to 0.67)	0.002
FVC	0.86 (0.76 to 0.93)	0.42 (0.01 to 0.71)	0.002
FEV_1/FVC	0.79 (0.63 to 0.88)	0.63 (0.29 to 0.82)	0.235
$DRS_{methacholine}$	0.36 (0.01 to 0.63)	0.54 (0.13 to 0.78)	0.455
FeNO	0.67 (0.45 to 0.81)	0.03 (–0.38 to 0.44)	0.005
Serum total IgE	0.86 (0.75 to 0.92)	0.52 (0.14 to 0.77)	0.010
Positive SPT	0.73 (0.54 to 0.85)	0.14 (–0.29 to 0.52)	0.005
HDM sensitization	0.48 (0.20 to 0.69)	0.58 (0.22 to 0.80)	0.618

*Test for equality of correlations in MZ and DZ twin pairs.

MZ, monozygotic twins; DZ, dizygotic twins; IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; SPT, skin prick test; DRS, dose-response slope; HDM, house dust mite.

particularly, having a high severity score on these symptoms predicted a low lung function, an increased degree of airway obstruction and an increased level of airway responsiveness. On the contrary, cough and chest tightness were poor indicators of variation in the objective traits. In general, the severity of asthma symptoms correlated weakly with the objective markers of allergic sensitization, i.e. positive skin prick test, serum total IgE and HDM sensitization.

Analysis of asthma severity within concordant twin pairs

The study identified a total of 38 MZ and 21 DZ twin pairs concordant for clinical asthma (Table 3). Among these, the correlation between asthma severity characteristics was consistently higher among MZ than among DZ twin pairs for the overall symptom severity score as well as for the specific asthma symptoms except for chest tightness. The same pattern was observed for all other traits except the asthma medication severity index, rhinitis severity, airway responsiveness and HDM sensitization. Statistically significant differences between MZ and DZ twin pairs were found for shortness of breath, FEV_1, FVC, FeNO, serum total IgE and positive skin prick test.

Variance components analysis of asthma severity

Genetic factors explained 26% (12%–39%), $P = 0.0002$ of the variation in overall asthma symptom severity, whereas the remaining 74% of the variation was explained by non-shared environment. After adjusting for age, sex, current airway infection, smoking and BMI, genetic factors explained 24% (10%–37%), $P = 0.0004$, of the variation in overall asthma symptom severity, whereas non-shared environment accounted for the remaining 76% (Table 4). When current use of inhaled corticosteroids was also incorporated as covariate, the heritability of overall asthma symptom severity was 21%, $P = 0.004$, whereas adjusting instead also for current use of any medication for asthma, the heritability of overall asthma symptom severity was 18%, $P = 0.011$. A significant heritable component was also observed for the severity of specific asthma symptoms, wheezing 12% (3%–22%), $P = 0.007$, and shortness of breath 17% (7%–27%), $P = 0.0006$, but not for the severity of chest tightness and cough. Also, the genetic contribution to the asthma medication severity index was small and statistically insignificant.

Genetic determinants of overall asthma symptom severity correlated generally highly with genetic determinants of the severity of the specific asthma symptoms, whereas the specific symptoms that had the greatest genetic similarity were wheezing and shortness of breath. These two traits also had a high environmental similarity (Table 5). Asthma symptom severity and rhinitis severity correlated weakly in regard to underlying genetic and environmental influences. The objective traits that had the most marked genetic and environmental correlations with asthma symptom severity were airway responsiveness (DRS) and lung

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Table 4. Variance components analysis of asthma severity in Danish adult twin pairs

	Variance components†		
	A	C	E
Overall symptom severity	0.24***	–	0.76
Wheezing	0.12**	–	0.90
Shortness of breath	0.17***	–	0.83
Chest tightness	0.00	0.01	0.99
Cough	0.10	0.05	0.85
Medication severity index	0.02	–	0.98
Rhinitis severity	0.23**	–	0.77
FEV_1	0.68***	–	0.32
FVC	0.58***	0.27*	0.15
FEV_1/FVC	0.22	0.44**	0.34
$DRS_{methacholine}$	0.43***	–	0.57
FeNO	0.67***	–	0.33
Serum total IgE	0.81***	–	0.19
Positive SPT	0.54***	–	0.46
HDM sensitization	0.06	0.26*	0.68

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

†Proportion of variance in asthma severity scores and related traits because of additive genetic effects (A), shared environmental effects (C) and non-shared environmental effects (E) adjusted for age, sex, smoking, current airway infection and body mass index; only significant covariates were included in final model. Variance components sum to 1.00 (100%).

IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; SPT, skin prick test; DRS, dose-response slope; HDM, house dust mite.

function (FEV_1 and FVC). Genetic and environmental effects for airway inflammation (FeNO) and markers of atopic sensitization (IgE and skin test reactivity) did not significantly overlap with the severity of any of the specific asthma symptoms or overall symptom severity.

Discussion

This study showed that the individual variation in asthma symptom severity, as judged by overall symptom frequency, is influenced by genetic factors. Notably, around one fourth of the variation in asthma symptom severity was explained by individual differences in genotypes, whereas the rest of the variation could be attributed to non-genetic factors. An influence of genetic factors could furthermore be demonstrated for variation in the severity of specific asthma symptoms, particularly wheezing and shortness of breath. Conversely, the severity of asthma, as judged by an asthma medication severity index, did not prove to have a heritable component. The severity of wheezing and shortness of breath was found to share underlying genetic effects to a high degree. On the contrary, asthma symptom severity correlated weakly both on the phenotypic level and in respect to underlying genetic and environmental effects, with rhinitis severity, and with the objectively measured characteristics of asthma severity and markers of allergic sensitization.

Previously, we and others have shown that relatives tend to exhibit the same degree of impairment in several intermediate phenotypes underlying asthma, such as lung function, airway responsiveness, airway obstruction and airway inflammation, with a moderate-to-high heritability of these parameters (7, 8, 18). Furthermore, the variation in these phenotypes has been extensively associated with several genetic variants, signifying that the pathogenic mechanisms underlying asthma severity are under genetic control. However, it is not completely clear whether this also applies to the clinical severity of asthma, i.e. whether a particular degree of symptomatology in a family member predicts the same pattern of disease expression in a relative. A study by Sarafino and Goldfedder examined the overall asthma symptom severity in concordant MZ and DZ twin children based on parent report of the frequency and intensity of asthma symptoms in the child (19). They found a correlation of 0.63 for overall asthma symptom severity in 23 MZ twin pairs, which significantly exceeded the correlation of –0.12 in 13 DZ twin pairs. Although the study was performed in a smaller and selected sample of twins, the results suggested a familial aggregation of asthma symptom severity that is consistent with our findings among concordant twins. The French Epidemiological study on Genetics and Environment of Asthma, bronchial hyperresponsivensess and Atopy (EGEA) found a familial tendency for the severity of asthma symptoms (20). Particularly, the correlation between first-degree relatives for a clinical asthma severity score, which was a composite measure of asthma attack frequency in the past year, persisting symptoms between attacks, and hospitalization for asthma within the past year, was 0.23. However, the same study did not find an increased risk of asthma in relatives according to asthma severity in the index case, indicating that the risk of asthma in a relative is independent of the severity of asthma in his or her family members.

A genetic propensity to the clinical severity of asthma is also supported by several molecular genetic studies, although the collective evidence is sparse. The GABRIEL (A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community) Consortium analyzed data from several populations from Canada, Australia and United Kingdom, and even though genome wide association significance was not reached for the studied

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Table 5. Genetic and environmental correlations between asthma severity indicators in a sample of 575 Danish adult twins

	Over	Whee	Short	Chest	Cough	Medi	Rhin	FEV_1	FVC	FE/FV	DRS	FeNO	IgE	SPT	HDM
Overall symptom severity		**0.50*****	**0.47*****	**0.26*****	**0.86*****	**0.30*****	0.20*	–0.22*	–0.20*	–0.18*	**0.32*****	0.09	0.08	0.22*	0.15
Wheezing	0.68**		**0.64*****	0.17	0.24**	**0.34*****	0.17*	–0.10	–0.09	–0.07	**0.34*****	0.16*	0.11	0.18*	0.19*
Shortness of breath	0.72**	**1.00*****		0.17**	0.20*	0.11	0.14	**–0.26*****	–0.13	–0.19*	0.44**	0.12	–0.05	0.15*	0.11
Chest tightness	0.61	–0.15**	0.27		**0.27*****	–0.04	–0.06	–0.02	–0.04	–0.03	0.11*	–0.03	0.00	0.01	–0.01
Cough	1.00***	0.52	0.58*	0.68*		**0.25*****	0.12	–0.10	–0.01	–0.23**	0.21*	0.12	0.05	0.22**	0.05
Medication severity index	1.00*	**1.00*****	1.00*	1.00	0.34		–0.01	–0.07	–0.03	–0.10	0.08	0.09	0.12*	0.12*	0.08
Rhinitis severity	0.02	0.63*	0.37	–0.43	0.00	1.00**		0.03	–0.04	0.06	0.27**	0.22*	0.12	**0.31*****	0.12
FEV_1	–0.27	–0.40	–0.45**	0.07	–0.04	–0.52	0.02		**0.75*****	**0.53*****	0.35**	–0.02	–0.02	0.00	0.01
FVC	–0.01	–0.27	–0.44**	–0.59	–0.07	–0.40	–0.01	**0.90*****		0.13	–0.01	0.04	–0.06	–0.02	–0.02
FEV_1/FVC	0.09	0.02	0.14	–0.37	0.22	0.05	0.16	–0.30**	**0.67*****		**0.46*****	–0.11	0.07	0.01	0.05
$DRS_{methacholine}$	0.25	0.62**	0.43*	–0.34	0.22	1.00**	0.09	0.39**	–0.37**	0.26*		0.34**	0.24*	0.27*	0.12
FeNO	0.15	0.13	0.09	–0.50	–0.03	0.82*	0.31	0.29*	0.19	–0.02	0.20		0.08	0.29**	0.26**
Serum total IgE	–0.03	0.32	0.11	0.90	–0.03	0.47	0.40*	0.13	–0.03	0.00	0.24*	**0.37*****		**0.35*****	0.28**
Positive SPT	–0.12	0.24	0.10	–0.07	–0.23	0.33	**0.90*****	0.21	0.08	0.14	0.26*	**0.41*****	0.34**		**0.46*****
HDM sensitization	–0.17	0.21	0.11	–0.63	–0.22	0.65	**0.54*****	0.18	0.04	0.17	0.31*	**0.44*****	0.25*	**0.73*****	

Additive genetic correlation below diagonal, non-shared environmental correlation above diagonal; adjusted for age, sex, smoking, current airway infection and BMI; only significant covariates were included in final model.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (highlighted in bold print).

IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; SPT, skin prick test; DRS, dose-response slope; HDM, house dust mite.

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alleles, a suggestive association was found for a single-nucleotide polymorphism on chromosome 5, situated within the thymic stromal lymphopoietin gene, among patients with severe asthma (21). Moreover, several other studies have associated genetic variants with asthma severity. For example, polymorphisms within the beta2-adrenergic receptor gene (22, 23), the interleukin 4 gene (24–26), and the transforming growth factor-β1 and cluster of differentiation 14 genes (27) have been shown to play a role in asthma severity.

Although we found that clinical asthma severity has a heritable component, it is evident from our study that most of the variation in this phenotype is explained by non-genetic factors. One strong predictor of asthma severity is non-compliance with inhaled corticosteroids, which has been shown to contribute to airway remodeling and refractory asthma in the long run (28). Whether airway remodeling is a precursor for severe symptomatic asthma or severe symptomatic asthma precedes airway remodeling is not completely clear, and the interaction between symptoms, for example in the form of exacerbations, and concomitant airway remodeling is a dynamic process that is also influenced by host factors. For example, the ADAM (a disintegrin and metalloprotease domain) 33 gene has been shown to contribute to airway remodeling and progression, and to severity of asthma and also chronic obstructive pulmonary disease (29). These mechanisms are likely not encompassed by our definition of symptomatic asthma severity. Particularly, the symptom score used herein to grade asthma severity did not have a uniform relationship with the different objectively measured traits. For example, low lung function, high degree of airway obstruction and increased airway inflammation were more pronounced in the intermediate asthma severity categories, which is somewhat counterintuitive, particular because for example, compliance with prescribed medications as well as the asthma medication severity index were also highest in these categories. Moreover, the frequency of smoking, which could also be speculated to influence these traits, was highest in the most severe asthma categories. At the same time, cough, which is a rather unspecific symptom of asthma, notably in adult smokers more than any other symptom defined overall asthma symptom, including probably too many subjects in the most severe symptom category. This could explain why the symptom severity scale used herein did not reflect objectively defined asthma severity in terms of low lung function, airway inflammation and AHR, but instead could reflect the presence of unspecific symptoms, for example those elicited by smoking. On the other hand, the more specific asthma symptoms such as wheezing and shortness of breath also correlated weakly with the objective severity traits. As such, it is apparent from this study that the severity of asthma, as judged only by the frequency of respiratory symptoms, reflects rather poorly the underlying pathophysiological process of the asthmatic airway. An additional uncertainty alludes to whether the severity of asthma symptoms in an individual can be assumed to be stable over time, i.e. whether a cross-sectional diagnosis of severity can be assumed to apply to that person in general or just reflects present disease activity. In this instance, it would have increased the reliability of our findings if we had had longitudinal data on symptomatic asthma severity and objectively measured traits, for example invasive measures of airway remodeling. Furthermore, we cannot be sure that our conclusions are specific to the twin population or whether they apply to the population as a whole. However, we have no reason to believe that the genetics and pathophysiology of asthma differs between twins and singletons.

We conclude that the individual variation in asthma symptom severity to some extent is influenced by genetic factors. However, the clinical expression of asthma appears to be multidimensional and is influenced, besides genetic factors, also to a large degree by external factors. The severity of asthma judged only by symptoms correlates weekly with the pathophysiological impairments in asthma, such as airway obstruction, airway responsiveness and airway inflammation. This may partially be due to the fact that the genetic architecture underlying the severity of asthma symptoms seems different from the genetic architecture underlying these objectively measured asthma-related traits.

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