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Multivariate genetic analysis of atopy phenotypes in a selected sample of twins

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Summary

Background Atopic traits often co-occur and this can potentially be caused by common aetiological relationships between traits, i.e. a common genetic or a common environmental background.

Objective To estimate to what extent the same genetic and environmental factors influence wheeze, rhinitis, airway hyper-responsiveness (AHR), and positive skin prick test (posSPT) in a sample of adult twins.

Methods Within a sampling frame of 21 162 twin subjects, 20–49 years of age, from the Danish Twin Registry, a total of 575 subjects (256 intact pairs and 63 single twins), who either themselves and/or their co-twins reported a history of asthma at a nationwide questionnaire survey, were clinically examined. Symptoms of wheeze and rhinitis were obtained by interview; airway responsiveness and skin test reactivity were measured using standard techniques. Correlations in liability between the different traits were estimated and latent factor models of genetic and environmental effects were fitted to the observed data using maximum likelihood methods.

Results The various phenotypic correlations between wheeze, rhinitis, AHR and posSPT were all significant and ranged between 0.50 and 0.86. Traits that showed highest genetic correlations were wheeze–rhinitis ($\rho_A = 0.95$), wheeze–AHR ($\rho_A = 0.85$) and rhinitis–posSPT ($\rho_A = 0.92$), whereas lower genetic correlations were observed for rhinitis–AHR ($\rho_A = 0.43$) and AHR–posSPT ($\rho_A = 0.59$). Traits with a high degree of environmental sharing were rhinitis–posSPT ($\rho_E = 0.92$) and wheeze–posSPT ($\rho_E = 0.71$), whereas a lower environmental correlation was seen for wheeze–rhinitis ($\rho_E = 0.25$). The estimates were corrected for ascertainment and adjusted for age, sex, inhaled corticosteroids and smoking.

Conclusions Different atopic conditions share, to a large extent, a common genetic background. In particular, upper and lower respiratory symptoms seem to be different phenotypic expressions of a common set of genes. These results add new insight into the origins of clinical heterogeneity within atopy and should stimulate the search for pleiotropic genes of importance for these conditions.

Keywords airway hyper-responsiveness, asthma, rhinitis, skin prick test, twin study

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Introduction

The classical twin design has been used frequently to dissect genetic from environmental causes of atopic diseases [1]. Generally, studies have found that the risk of asthma, hayfever, and eczema in a co-twin of an affected identical twin is increased 5–10 times compared

with two to four times in a co-twin of an affected fraternal twin, relative to the general population [2–5]. These findings are consistent with a genetic causation in atopic disease and stand irrespective of the definition of disease, the country of origin, and the age of the study cohorts. However, environmental causes also play a role, as exemplified by the observation that identical

twins are not always concordant for atopic disease status.

Previous twin studies of atopic traits have mainly focused on self-reported measures of disease and only sporadically have objective markers such as serum IgE levels [6, 7], skin test reactivity [7, 8] and airway responsiveness [7] been included. Furthermore, these studies have mostly used univariate analysis, whereas studies that examine the genetic and environmental basis of covariation between several traits are sparse [2, 9, 10]. The simultaneous analysis of two or more traits in twins allows one to estimate to what extent the same genetic (pleiotropy) and environmental factors influence these traits [11]. This can not only widen our understanding of atopic co-morbidity but it can also prove informative when searching for atopy susceptibility genes. In this case, disregarding information about the genetic covariation between different traits can lead to loss of power to detect genetic linkage [12].

In this study, we measured wheeze, rhinitis, airway hyper-responsiveness (AHR), and positive skin prick test (posSPT) in 575 twins from the general population who were selected through a proband with self-reported asthma. All of these traits have been shown to co-occur more often than is expected by chance and so we used multivariate analysis to estimate the magnitude of genetic and environmental sharing between traits.

Methods

Sample

The sample analysed herein was drawn from twin cohorts born between 1953 and 1982 in Denmark, who, in 2002, participated in a nationwide questionnaire survey originating from The Danish Twin Registry [13]. In these cohorts, zygosity was established in 1991 using four questions of similarity and mistaken identity, which have a frequency of misclassification of less than 4% [14]. Selection of subjects for the study is depicted in Fig. 1. In an initial screening phase, a mailed questionnaire with items aimed at identifying multiple phenotypes including asthma was answered by 21 162 twins individuals, 20–49 years of age. The response rate was 73% and 55% were females. The sample comprised of 7317 intact twin pairs, of whom 2377 were identical (monozygotic, MZ) twin pairs, 2606 were fraternal (dizygotic, DZ) same-sex twin pairs, 2154 were DZ opposite-sex twin pairs, 180 were pairs of unknown zygosity and finally 6528 were single responders. Of the 7317 intact pairs, 6791 pairs had known zygosity and complete data on asthma. Among these, pairs in which at least one twin in each pair responded affirmatively to an asthma-screening question and who lived in the geographical region surrounding the study site were invited to take part in a clinical examination. The asthma screening question was 'Do you have, or have you

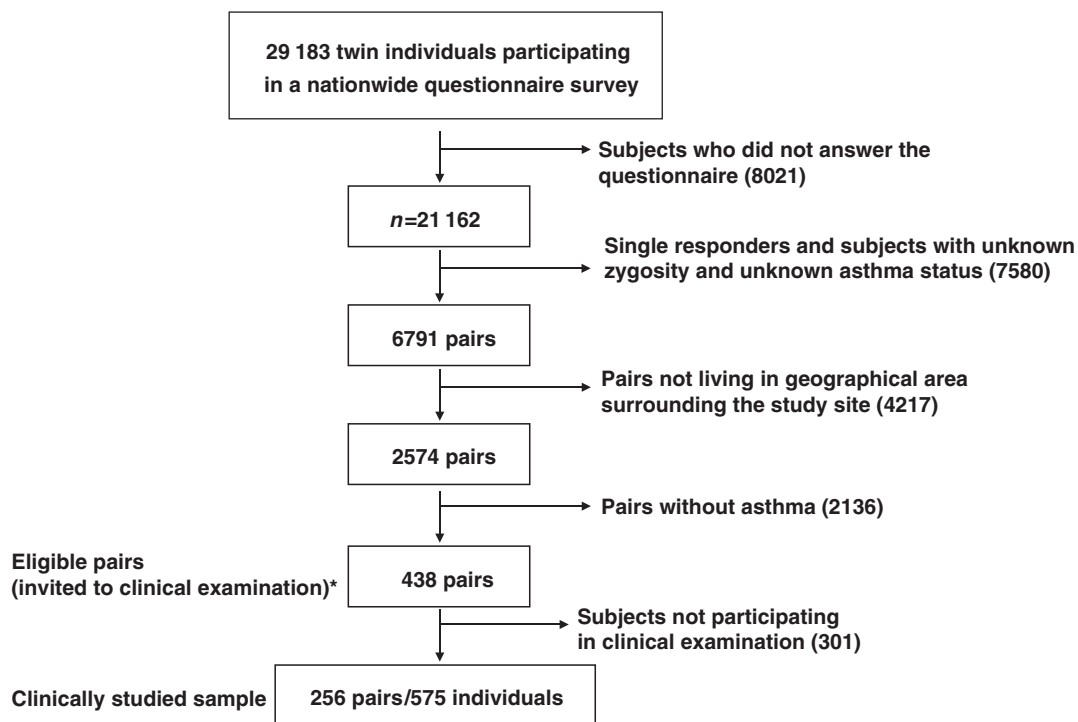


Fig. 1. Selection of 575 twin individuals who participated in a clinical examination. *Due to immigration, death in 10 twin pairs, only 428 pairs fulfilled eligibility.

ever had asthma?' This question has previously been shown to be reliable with respect to identifying subjects with asthma in population-based studies, which use questionnaire responses as the sole diagnostic criterion [15]. The clinical examination consisted of physician-based assessment of symptoms of wheeze and rhinitis, and measures of airway responsiveness and skin test reactivity. A total of 575 subjects (256 intact pairs and 63 single twins) participated in the clinical examination (individual response rate, 67%; pair-wise response rate, 60%).

Symptom assessment

Subjects were interviewed about symptoms of wheeze and rhinitis and time of last symptomatic episode. Wheeze was defined as considerable recurrent episodes of wheezing at rest, following exertion, or subsequent to allergenic or unspecific stimuli when not having a respiratory tract infection. Rhinitis was defined as considerable recurrent episodes of runny or stuffy nose, or sneezing when not having a cold. Both for wheeze and rhinitis, subjects were divided into three categories: subjects with no history of the particular symptom (never symptoms), subjects who have had symptoms no more recently than 12 months before examination (distant symptoms) and subjects who have had symptoms during the past 12 months (current symptoms).

Skin prick tests

Skin prick tests (SPTs) 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 (*Altaria iridis* and *Cladosporium herbarium*), with positive and negative references being histamine 10 mg/mL in 50% glycerol and glycerol 50%, respectively. The concentrations of allergens were 10 HEP (Soluprick SQ[®] system, ALK Abelló, Hørsholm, Denmark). Reactions were read after 15 min. A positive test result was defined as a positive reaction to at least one of the allergens. The reaction to each of the allergens was regarded as positive if the mean weal diameter $[(D1 + D2) \times 1/2]$ was at least 3 mm [16]. The participants were requested to discontinue medications that contained antihistamine at least 3 days before skin testing.

Airway responsiveness test

The method of Yan et al. [17] was used to measure airway responsiveness to inhaled methacholine. A Morgan nebulizer generated the aerosols of the test solution. Each aerosol was inhaled through the mouth starting with saline (0.9%), and followed by increasing doses of metha-

choline until a cumulative dose of 7.8 μ mol had been reached. The response was measured by determining the forced expiratory volume in 1 s (FEV₁), 2 min after each inhalation. The test was terminated when the maximum concentration had been reached or when a 20% decline in FEV₁ had occurred before the end of the dosing regimen. For all subjects experiencing at least a 20% decline in FEV₁, the concentration causing a 20% fall in FEV₁ (PD₂₀) was calculated [17]. A positive test result (AHR) was defined as a PD₂₀ below 3.9 μ mol [17].

Statistical analysis

Correlations in liability. We estimated correlations in liability between the different traits under the multifactorial threshold model [11]. This model assumes that (1) many factors (genetic and environmental) contribute to the development of the disorder, (2) each member of a twin pair is assumed to have his or her own liability, the pair of liabilities for a twin pair being assumed to be bivariate normally distributed and (3) as the number of causal factors for the disorder increases in an individual, the liability for the disorder increases. When a threshold is reached, the burden of liability becomes so great that disease results [11]. The threshold then represents the population prevalence of that disorder and is equivalent to a z-value of the (standard) normal distribution [18]. If more than one threshold is imposed on a trait, each threshold then is the distinction between the different categories assigned to that particular trait [18]. Under this model, asthma was distributed into two categories, i.e. never vs. ever having had asthma. Wheeze and rhinitis were distributed into three categories, corresponding to never, distant and current symptoms. Airway responsiveness and skin test reactivity were distributed into two categories, i.e. affected vs. unaffected. From these distributions, polychoric correlations were calculated.

Variance components analysis. In order to examine the extent to which genetic and environmental risk factors were shared between traits, we fitted latent factor models to the observed data (Fig. 2). These models assumed that the phenotypic variance of each trait was a linear function of genetic and environmental influences. Genetic influences were partitioned into additive genetic effects (loci contributing additively to disease risk, A) and non-additive genetic effects (interacting alleles, either from the same locus – genetic dominance – or from separate loci – epistasis, D). Environmental influences were partitioned into shared environmental effects (environmental factors that increase the resemblance between members of the same family, C) and non-shared – random – environmental effects (influences unique to an individual, E) [11]. For most human traits, it is reasonable to assume all four

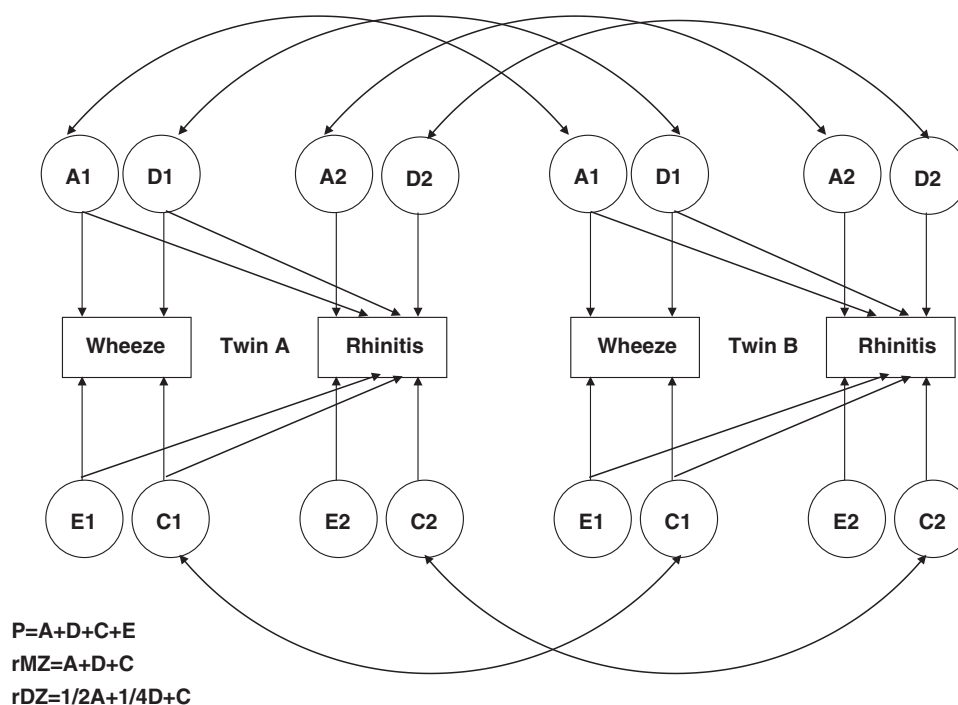


Fig. 2. Classical twin model showing latent additive genetic influences (A), non-additive genetic influences (D), shared environmental influences (C), and individual specific environmental influences (E) on two traits (wheeze and rhinitis) measured in a pair of twins. Variance components sum to total phenotypic variance (P) (see formulas). The arrows pointing from each of the latent factors to the trait directly below or above (from A1, D1, C1 and E1 on wheeze, and from A2, D2, C2, and E2 on rhinitis) account for the variation unique to that trait, whereas oblique arrows pointing to the adjacent trait (from A1, D1, C1 and E1 on rhinitis) assign genetic and environmental covariance between the two traits. The expected twin pair resemblances (the cross-twin correlations, r) for a trait in monozygotic (MZ) twins and in dizygotic (DZ) twins are given in formulas. Additive genetic factors are correlated 1.0 across MZ twins and 0.5 across DZ twins, whereas non-additive genetic factors are correlated 1.0 across MZ twins and 0.25 across DZ twins. Shared environment by definition is correlated 1.0 both across MZ and DZ twins. Non-shared environment is uncorrelated across twin pairs.

sources of variance (A, D, C and E) to act simultaneously. However, components C and D are not identified under the same model in studies that include only twins reared together [19]. Therefore, the likelihood of the data was determined under two different saturated models, which either included the components A, C and E, or A, D and E. Nested models that included the parameters A and E were subsequently fitted by fixing the appropriate parameters to zero. The difference in log likelihood between the saturated and the nested models provided an estimate of the significance of the contribution of the individual parameters to the trait variance. Estimates of genetic and environmental sharing between the different traits were assessed under the nested models and were expressed as additive genetic correlations (ρ_A) and random environmental correlations (ρ_E), respectively. These were estimated as the additive genetic or the random environmental covariance between two traits divided by the square root of the product of the additive genetic or the random environmental variances of those two traits, respectively [18].

Correction for ascertainment. Because wheeze, rhinitis, AHR, and posSPT were only measured in twin pairs with

self-reported asthma, their resulting distributions were highly skewed relative to the general population. That is, it was less likely to observe doubly unaffected pairs with these conditions in the clinically studied sample compared with the general population. This means that estimation of parameters using only data from the clinically studied sample would be biased. To circumvent this problem wheeze, rhinitis, AHR and posSPT were analysed jointly with the asthma screening question and the entire nationwide sample of twins (not just those clinically tested). This means that we had to perform either bivariate (asthma plus one clinical trait) or trivariate (asthma plus two clinical traits) analyses in order to obtain estimates of the correlations between asthma and a clinical trait and between two clinical traits, respectively. This procedure has previously been shown to be reliable with respect to correcting for ascertainment bias in populations selected through an affected twin [20].

The statistical package Mx was used for maximum likelihood estimation of the parameters [21]. DZ twin pairs of the same sex and DZ twin pairs of the opposite sex were pooled in the analysis and estimates were adjusted for influences from age, sex, inhaled corticosteroids and smoking. A P -value < 0.05 was considered to be

Table 1. Distribution of atopy phenotypes in a selected sample of twins, 21–51 years of age

	<i>N</i> [†]	Age (SD) [‡]	Asthma [§]	Clinically measured traits*			
				Wheeze	Rhinitis	AHR	PosSPT
MZ	191 (13)	34.9 (7.4)	126 (66)	133 (70)	133 (70)	52 (30)	113 (59)
Males	83 (5)	34.8 (7.2)	60 (72)	60 (72)	68 (82)	21 (29)	63 (76)
Females	108 (8)	34.9 (7.6)	66 (61)	73 (68)	65 (60)	31 (31)	50 (46)
DZ-ss	210 (22)	35.0 (8.6)	117 (56)	116 (55)	130 (62)	50 (26)	116 (55)
Males	70 (10)	33.5 (7.8)	35 (50)	36 (51)	46 (66)	18 (27)	43 (61)
Females	140 (12)	35.7 (9.0)	82 (59)	80 (57)	84 (60)	32 (25)	73 (52)
DZ-os	174 (28)	38.2 (8.1)	94 (54)	95 (55)	100 (57)	47 (28)	89 (51)
Males		38.1 (8.1)	34 (39)	39 (45)	44 (51)	22 (26)	43 (49)
Females		38.3 (8.0)	60 (69)	56 (64)	56 (64)	25 (31)	46 (53)
All DZ	384 (50)	36.5 (8.5)	211 (55)	211 (55)	230 (60)	97 (27)	205 (50)
Males	240	35.6 (7.9)	129 (54)	135 (56)	158 (66)	61 (27)	149 (62)
Females	335	36.1 (8.4)	208 (62)	209 (62)	209 (62)	88 (29)	169 (50)
Total	575 (63)	35.9 (8.2)	337 (59)	344 (60)	363 (63)	149 (28)	318 (55)

*Number of subjects from a clinically examined sample with the particular condition (percentage of examined subjects); for AHR, 530 subjects who had a test performed constitute examined subjects. Wheeze and rhinitis are cumulative (lifetime) prevalence.

[†]Total number of subjects who were clinically examined (single twins).

[‡]Mean age in years. SD, standard deviation.

[§]Number of subjects from clinically examined sample with self-reported asthma based on the screening questionnaire (percentage of examined subjects). Asthma is cumulative (lifetime) prevalence.

MZ, monozygotic; DZ-ss, dizygotic same sex; DZ-os, dizygotic opposite sex; AHR, airway hyper-responsiveness; PosSPT, positive skin prick test.

statistically significant. The protocol was evaluated and approved by the Scientific Ethics Committee.

Results

The characteristics of the studied sample are shown in Table 1. The mean age was 35.9 years (age range 21–51 years) and 58% were females. These findings were similar to what was observed in the total twin population. A total of 59% of the clinically studied subjects reported a history of asthma at the screening questionnaire, whereas 60% had a history of wheeze, 63% had a history of rhinitis, 28% had AHR and 55% had a posSPT. There was a tendency towards females having asthma (62% vs. 54%), wheeze (62% vs. 56%) and AHR (29% vs. 27%) more often than males, whereas males more often had rhinitis (66% vs. 62%) and posSPT (62% vs. 50%). According to the nationwide screening questionnaire, the lifetime prevalence of asthma was 9.2%, 8.1% and 10.0% in males and females, respectively.

Correlations in liability

For most traits, the ascertainment-uncorrected cross-twin same-trait correlations were low (Table 2), indicating that the distribution of the clinically studied sample was skewed relative to the nationwide population due to a high correlation with the selection variable – asthma. Hereafter, only estimates corrected for ascertainment are presented. The phenotypic (within subject) correlations

Table 2. Cross-twin same-trait correlations obtained from univariate analysis using only the 575 subjects from the clinical sample, uncorrected for ascertainment

	Asthma	Wheeze	Rhinitis	AHR	PosSPT
MZ	– 0.56	0.18	0.41	0.43	0.76
DZ	– 0.81	– 0.55	0.04	0.05	0.43

MZ, monozygotic; DZ, dizygotic; AHR, airway hyper-responsiveness; PosSPT, positive skin prick test.

between the different clinical traits were all significant and ranged between 0.50 and 0.86, the highest between rhinitis and posSPT, 0.86, 95% confidence interval (CI) (0.81–0.90), and the lowest between rhinitis and AHR, 0.50, 95% CI (0.35–0.55) (Table 3). The cross-twin same-trait correlations were significantly higher in MZ twins compared with DZ twins, suggesting a genetic influence on trait variability (Table 4). The cross-twin cross-trait correlations were generally higher among MZ twins compared with the DZ counterparts, suggesting that the covariance between the different traits was partially genetic (Table 4).

Variance components analysis

Table 5 shows results from variance components analysis. Trait heritabilities ranged between 0.53 and 0.85, whereas the rest of the variance of each trait was accounted for by non-shared environmental differences. The analyses suggested that genetic non-additivity influenced asthma and

Table 3. Phenotypic (within-subject) correlations between atopy phenotypes in a sample of twins, 21–51 years of age, corrected for ascertainment (95% confidence intervals)

Trait	Asthma	Wheeze	Rhinitis	AHR	PosSPT
Asthma	1				
Wheeze	0.89 (0.86–0.94)	1			
Rhinitis	0.60 (0.42–0.69)	0.64 (0.54–0.73)	1		
AHR	0.57 (0.35–0.71)	0.69 (0.53–0.73)	0.50 (0.35–0.55)	1	
PosSPT	0.64 (0.52–0.81)	0.67 (0.56–0.76)	0.86 (0.81–0.90)	0.53 (0.47–0.65)	1

AHR, airway hyper-responsiveness; PosSPT, positive skin prick test.

Table 4. Cross-twin same-trait correlations (diagonal elements) and cross-twin cross-trait correlations (off-diagonal elements) between atopy phenotypes in a sample of twins, 21–51 years of age, corrected for ascertainment (95% confidence intervals)

Trait	Monozygotic twins					Dizygotic twins				
	Asthma	Wheeze	Rhinitis	AHR	PosSPT	Asthma	Wheeze	Rhinitis	AHR	PosSPT
Asthma	0.60 (0.52–0.67)					0.18 (0.10–0.26)				
Wheeze	0.59 (0.45–0.65)	0.64 (0.47–0.82)				0.15 (0.01–0.28)	0.12 (0.00–0.31)			
Rhinitis	0.52 (0.31–0.66)	0.53 (0.35–0.68)	0.53 (0.27–0.73)			0.25 (0.05–0.38)	0.23 (0.05–0.40)	0.28 (0.04–0.40)		
AHR	0.41 (0.16–0.59)	0.55 (0.37–0.65)	0.17 (0.01–0.25)	0.58 (0.27–0.79)		0.16 (0.00–0.34)	0.21 (0.08–0.28)	0.20 (0.01–0.26)	0.28 (0.22–0.36)	
PosSPT	0.57 (0.50–0.70)	0.51 (0.38–0.55)	0.63 (0.53–0.71)	0.45 (0.37–0.62)	0.84 (0.68–0.93)	0.35 (0.22–0.49)	0.25 (0.14–0.32)	0.40 (0.38–0.53)	0.20 (0.10–0.45)	0.64 (0.45–0.77)

AHR, airway hyper-responsiveness; PosSPT, positive skin prick test.

Table 5. Variance components analysis of atopy phenotypes in a sample of twins, 21–51 years of age

Trait	Saturated models*				Nested models*		Fit statistics			
	A	C	D	E	A	E	$\Delta-2$ loglikelihood	Δdf	P-value	
Asthma	0.16 (0.00–0.47)	–	0.44 (0.09–0.67)	0.40 (0.33–0.48)	0.55 (0.48–0.62)	0.45 (0.38–0.52)	6.16	1	0.01	
	0.55 (0.46–0.57)	0.00 (0.00–0.06)	–	0.45 (0.38–0.52)	0.55 (0.48–0.62)	0.45 (0.38–0.52)	0.00	1	1.00	
Wheeze	0.31 (0.14–0.47)	–	0.34 (0.13–0.63)	0.35 (0.24–0.78)	0.64 (0.47–0.72)	0.36 (0.28–0.52)	8.42	2	0.02	
	0.64 (0.44–0.74)	0.00 (0.00–0.10)	–	0.36 (0.26–0.51)	0.64 (0.47–0.72)	0.36 (0.28–0.52)	0.00	2	1.00	
Rhinitis	0.42 (0.17–0.53)	–	0.10 (0.00–0.41)	0.48 (0.30–0.61)	0.53 (0.31–0.71)	0.47 (0.29–0.68)	1.85	2	0.40	
	0.51 (0.20–0.67)	0.02 (0.00–0.26)	–	0.47 (0.29–0.57)	0.53 (0.31–0.71)	0.47 (0.29–0.68)	0.01	2	0.99	
AHR	0.51 (0.23–0.59)	–	0.06 (0.00–0.46)	0.43 (0.37–0.55)	0.57 (0.30–0.62)	0.43 (0.22–0.54)	0.64	2	0.73	
	0.55 (0.06–0.78)	0.02 (0.00–0.40)	–	0.43 (0.22–0.73)	0.57 (0.30–0.62)	0.43 (0.22–0.54)	0.01	2	1.00	
PosSPT	0.84 (0.57–0.92)	–	0.01 (0.00–0.09)	0.15 (0.07–0.20)	0.85 (0.72–0.93)	0.15 (0.07–0.24)	0.39	2	0.82	
	0.45 (0.16–0.69)	0.38 (0.00–0.60)	–	0.17 (0.08–0.25)	0.85 (0.72–0.93)	0.15 (0.07–0.24)	4.05	2	0.13	

*Standardized parameter estimates (95% confidence intervals) of additive genetic factors (A), non-additive genetic factors (D), shared environmental factors (C), and non-shared environmental factors (E). $\Delta-2$ log-likelihood, difference in model fit between saturated model and nested model; Δdf , difference in degrees of freedom between saturated model and nested model.

AHR, airway hyper-responsiveness; PosSPT, positive skin prick test.

wheeze, whereas the remaining traits were under simple additive genetic control.

We found high correlations between genetic risk factors influencing the different traits, whereas environmental risk factors were less correlated between traits (Table 6). Traits that showed the highest genetic similarity were wheeze–rhinitis ($\rho_A = 0.95$), wheeze–AHR ($\rho_A = 0.85$) and

rhinitis–posSPT ($\rho_A = 0.92$), whereas lower genetic correlations were observed for rhinitis–AHR ($\rho_A = 0.43$) and AHR–posSPT ($\rho_A = 0.59$). Traits with a high degree of environmental sharing were rhinitis–posSPT ($\rho_E = 0.92$) and wheeze–posSPT ($\rho_E = 0.71$), whereas a lower environmental correlation was seen for wheeze–rhinitis ($\rho_E = 0.25$).

Table 6. Additive genetic correlations (below diagonal) and specific environmental correlations (above diagonal) between atopy phenotypes in a sample of twins, 21–51 years of age, corrected for ascertainment (95% confidence intervals)

Trait	Asthma	Wheeze	Rhinitis	AHR	PosSPT
Asthma	1	0.82 (0.77–0.84)	0.19 (0.11–0.26)	0.41 (0.30–0.59)	0.31 (0.11–0.47)
Wheeze	0.96 (0.89–1.00)	1	0.25 (0.23–0.29)	0.44 (0.32–0.58)	0.71 (0.59–0.83)
Rhinitis	0.94 (0.66–1.00)	0.95 (0.74–1.00)	1	0.58 (0.41–0.63)	0.92 (0.85–0.97)
AHR	0.70 (0.34–0.96)	0.85 (0.70–0.94)	0.43 (0.26–0.56)	1	0.41 (0.17–0.58)
PosSPT	0.71 (0.47–0.79)	0.64 (0.50–0.70)	0.92 (0.80–0.99)	0.59 (0.32–0.82)	1

AHR, airway hyper-responsiveness; PosSPT, positive skin prick test.

Table 7. Percentage of phenotypic correlation between atopy phenotypes that is attributable to genetic factors (below diagonal) and environmental factors (above diagonal) shared between traits

Trait	Asthma	Wheeze	Rhinitis	AHR	PosSPT
Asthma	–	37	15	32	14
Wheeze	63	–	15	25	25
Rhinitis	85	85	–	52	28
AHR	68	75	48	–	20
PosSPT	86	75	72	80	–

AHR, airway hyper-responsiveness; PosSPT, positive skin prick test.

The proportion of the phenotypic correlation between pairs of traits that was attributable to shared genetic and environmental factors is shown in Table 7. Generally, around three-quarters of the phenotypic correlation between any pair of traits was ascribable to genetic factors shared in common between those traits, whereas the rest was due to environmental factors.

Discussion

This study showed a high correlation between wheeze, rhinitis, AHR and posSPT. For any pair-wise relationship between these traits, the resulting correlation seemed to be driven primarily by genetic factors shared in common between the traits, whereas a smaller proportion of the correlation was ascribable to environmental factors. In particular, disease symptoms (wheeze and rhinitis) were, to a great extent, genetically similar but environmentally distinct. This suggests that wheeze and rhinitis are different phenotypic expressions of a common set of genes. Furthermore, wheeze and AHR were genetically very similar conditions, as opposed to rhinitis and AHR. Conversely, wheeze and posSPT seemed genetically less alike compared with rhinitis and posSPT. Finally, both rhinitis and posSPT, and wheeze and posSPT showed similar environmental architectures, suggesting that the exogenous factors, that cause atopic sensitization also cause wheeze and rhinitis.

These results expand upon previous studies that have examined the aetiological connections between atopic

traits. Hitherto, only a few twin studies have looked at the interrelationships between clinical atopy phenotypes. An Australian study considered the associations between asthma, posSPT and AHR in 381 twin pairs, 8–18 years of age. Higher cross-twin cross-trait recurrence risks in MZ twins compared with DZ twins indicated that the traits were genetically related [22]. More recently, one study examined the genetic and environmental basis for co-variation between multiple intermediate asthma phenotypes in a large sample of Australian twins and their families [10]. This study suggested a certain degree of genetic relatedness between the phenotypes considered but mostly that trait-specific genetic factors contributed to the variation of each phenotype. Especially, asthma and posSPT were genetically quite similar traits ($\rho_A = 0.46$), whereas asthma and AHR ($\rho_A = 0.24$), and AHR and posSPT ($\rho_A = 0.35$) seemed more genetically dissimilar. In contrast, the same pairs of traits showed more environmental resemblance. AHR and posSPT were most alike ($\rho_E = 0.67$), whereas the environmental correlations between asthma and AHR, and between asthma and posSPT were 0.63 and 0.44, respectively. We found considerably higher genetic correlations between traits. Particularly, we attributed the relationship between asthma/wheeze and AHR mainly to pleiotropic effects, rather than to environmental factors shared in common between the traits. In line with this, it has been shown that children with a parental history of asthma have higher levels of AHR compared with children without such a history [23]. Furthermore, it has been found that parents of asthmatic children have greater AHR compared with parents of non-asthmatic children [24]. Finally, several studies have linked AHR and asthma to the same genetic markers [25, 26]. These findings are consistent with, but not proof of, an underlying genetic vulnerability shared between asthma and AHR. Consequently, it is important to note that a strong genetic similarity does not preclude an overlap between environmental risk factors for the two traits. Factors that have been shown, at least partly, to explain this overlap are infections with respiratory syncytial virus, HDM allergens and exposure to tobacco smoke in childhood, especially in subjects predisposed to atopy [26–28].

A few studies have examined the genetic and environmental relationships between self-reported atopic diseases using variance components analysis. An Australian study of 3808 adult twin pairs found that the genetic correlation between self-reported asthma and hayfever was 0.52 for men and 0.65 for women, whereas correlations between environmental factors influencing the two traits were 0.53 and 0.33 for men and women, respectively [2]. Lichtenstein and Svartengren [9] studied 1480 Swedish twin pairs, 7–9 years of age, and estimated the genetic correlation between asthma and hayfever to be 0.90. This is largely similar to the corresponding correlation found in our study between wheeze and rhinitis and seems to concur well with the growing body of evidence linking rhinitis strongly to asthma [29, 30]. Indeed, many argue that rhinitis and asthma are different phenotypic expressions of the same underlying disease [31]. For example, the majority of patients with asthma have inflammatory changes in their noses and upper respiratory tracts. Likewise, asymptomatic inflammatory changes in the lungs often accompany rhinitis, indicating that pathophysiological aspects are common to the two disorders [32]. In agreement with the Swedish study, we found a low environmental correlation between wheeze and rhinitis. This suggests that the aetiological basis of clinical heterogeneity between these two entities is largely environmental in origin. In line with this, it has been shown that indoor allergens like HDM are strongly associated with wheeze and asthma, whereas outdoor allergens like pollen are closely related to rhinitis and hayfever [33]. Furthermore, several environmental risk factors for asthma, for example, lower respiratory tract infections and passive smoke exposure, seem to influence the risk of rhinitis to a lesser degree [28, 34]. Our study showed a strong environmental similarity both between wheeze and posSPT, and between rhinitis and posSPT, indicating that exposure and sensitization to aeroallergens are fundamental for development of both asthma and rhinitis. However, we did not distinguish between sensitization to indoor and outdoor allergens, which perhaps could have captured some additional information. Irrespective of this, the relationship between disease symptoms and pattern of sensitization is likely blurred by the fact that sensitization to indoor allergens predicts subsequent outdoor allergen sensitization and those indoor allergens in the first place not only cause wheeze but also cause rhinitis [35, 36].

The classical twin study implies that environmental influences shared between the two members of a twin pair are independent of zygosity [37]. If this is not true – for example, if MZ twins are being treated more similarly by their parents than are DZ twins, or if MZ twins seek to live in more similar environments compared with DZ twins – the genetic contribution to the traits under study can be overestimated [37]. Furthermore, the power of the classical twin study to detect an effect of shared environment is

usually low, especially when studying small sample sizes [38]. For posSPT, the influence of a shared environment, although statistically insignificant, seemed rather large. The same was the case regarding non-additive genetic effects for asthma and wheeze. Also, both for rhinitis and AHR non-additive genetic effects seemed not entirely negligible. We disregarded genetic non-additivity and shared environment when deriving genetic and environmental correlations between traits. For some traits, this may not have provided the best description of the data. Irrespective of this, estimates of variance components under the AE models compared with the saturated models suggested that the A-component, perhaps with the exception of posSPT, was an indicator of genetic effects. Furthermore, estimates of E were practically the same across all models. As a result of this, we are confident that the estimates obtained in this study are reliable.

In conclusion, different atopic conditions share, to a large extent, a common genetic background. In particular, upper and lower respiratory symptoms seem to be different phenotypic expressions of a common set of genes. These results add new insight into the origins of clinical heterogeneity within atopy and should stimulate the search for pleiotropic genes of importance for these conditions.

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