

Original article

A quantitative genetic analysis of intermediate asthma phenotypes

Aim: To study the relative contribution of genetic and environmental factors to the correlation between exhaled nitric oxide (FeNO), airway responsiveness, airway obstruction, and serum total immunoglobulin E (IgE).

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. Traits were measured using standard techniques. Latent factor models were fitted to the observed data using maximum likelihood methods.

Results: Additive genetic factors explained 67% of the variation in FeNO, 43% in airway responsiveness, 22% in airway obstruction, and 81% in serum total IgE. In general, traits had genetically and environmentally distinct variance structures. The most substantial genetic similarity was observed between FeNO and serum total IgE, genetic correlation (ρ_A) = 0.37, whereas the strongest environmental resemblance was observed between airway responsiveness and airway obstruction, specific environmental correlation (ρ_E) = -0.46, and between FeNO and airway responsiveness, ρ_E = 0.34.

Conclusions: Asthma is a complex disease characterized by a set of etiologically heterogeneous biomarkers, which likely constitute diverse targets of intervention.

S. F. Thomsen¹, M. A. R. Ferreira^{2,3},
K. O. Kyvik⁴, M. Fenger⁵, V. Backer¹

¹Department of Respiratory Medicine, Bispebjerg Hospital, Copenhagen, Denmark; ²Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; ³Queensland Institute of Medical Research, Brisbane, Qld, Australia; ⁴The Danish Twin Registry, University of Southern Denmark, Odense, Denmark; ⁵Department of Clinical Biochemistry, Hvidovre Hospital, Copenhagen, Denmark

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S. F. Thomsen
Department of Respiratory Medicine
Bispebjerg Hospital
DK-2400 Copenhagen NV
Denmark

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Asthma is a heterogeneous inflammatory disorder of the airways characterized by wheezing, shortness of breath, chest-tightness, and cough. Clinical manifestations range from mild periodical discomfort during exercise or in the course of exposure to certain types of allergen, to severe debilitating disease with frequent exacerbations and hospitalizations. In a considerable proportion of cases, asthma is complicated by rhinitis and atopic eczema. Respiratory symptoms are often poor indicators of the underlying pathological process of the asthmatic airway and clinical symptoms correlate weakly with objectively measurable biomarkers of disease (1). Several intermediate (or endophenotypes) like airway responsiveness, eosinophil count, serum immunoglobulin E (IgE), exhaled nitric oxide (FeNO) and lung function, underlie the clinical expression of asthma. These intermediate phenotypes are less subjective and tend to be more informative and statistically powerful to genetic analyses than binary clinical definitions of disease (2). These advantages have been exploited by both linkage and association studies of atopic traits leading to the discovery of several candidate genes (3).

Understanding the interplay between different pathways underlying asthma is central to the discovery of targeted treatments and interventional measures.

However, our present knowledge of the relationship between intermediate asthma phenotypes is limited. Twin studies can provide important clues, since they can be used to quantify the genetic and environmental sources of variation in human traits (4). Furthermore, information from multivariate datasets enables us to examine the degree of genetic and environmental overlap between different traits. In the present study, we aimed to evaluate whether there are common genetic and environmental factors influencing intermediate phenotypes underlying asthma. In particular, we examined the association between levels of FeNO, airway responsiveness to inhaled methacholine, degree of airway obstruction, and levels of serum total IgE.

Methods

Sample. The studied sample was comprised of twins born between 1953 and 1982 who were enrolled in the Danish Twin Registry (5). Twin zygosity was determined using four questions of similarity and mistaken identity, which have been shown to assign zygosity correctly in more than 96% of the cases (6). In an initial screening phase, 21 162 twin individuals, 20–49 years of age, answered a mailed questionnaire concerning health and lifestyle. The response rate was 73%. Of these, 7317 twin pairs were intact and 6528

subjects were single responders. A total of 6791 pairs had known zygosity [33% monozygotic (MZ) twins, and 67% dizygotic (DZ) twins] and complete data on asthma. Among these, pairs with at least one proband with self-reported asthma according to an affirmative response to a 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 ever had asthma?' (7). In the clinical examination, airway responsiveness to inhaled methacholine, FeNO, serum total IgE, and airway obstruction were measured. A total of 575 subjects (256 intact pairs and 63 single twins) underwent clinical examination (individual response rate 67%; pair-wise response rate 60%).

Lung function and airway responsiveness. Spirometric measurements of forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were performed. The ratio of the preprovocation values of FEV₁ and FVC was used to calculate degree of airway obstruction. The method of Yan et al. was used to measure airway responsiveness to inhaled methacholine (8). 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 methacholine until a cumulative dose of 7.8 µM had been reached. The response was measured by determining 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. The dose response slope (DRS) was calculated as the decline in FEV₁ divided by the highest dose of methacholine given.

Exhaled nitric oxide (FeNO). 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 (9). All measurements were performed in triplicate and the mean concentration (in parts per billion [ppb]) was registered.

Serum total IgE. Levels of serum total IgE were measured with an enzyme linked radio immunosorbent assay (ELISA) (Immunit 2500, DPC, New York, USA). Results were expressed in KIU/l.

Statistical analysis. Data were analyzed with latent factor models. These are standard twin analyses and assume that the variance of each trait can be partitioned into additive genetic influences (loci contributing additively to trait variance, A), shared environmental influences (environmental factors that increase the resemblance

between members of the same family, C), and nonshared environmental influences (influences unique to an individual, E). The E-component also includes error variance (4). From biometrical genetic theory, the expected covariance for MZ twins is $A + C$, while for DZ twins it is $0.5 \times A + C$. The significance of the contribution of the individual parameters to the trait variance was determined by a likelihood ratio test for the difference between the full ACE-model and subsequently fitted nested models (AE and CE models).

Genetic and environmental sharing between the different traits was assessed under the AE models and was expressed as additive genetic (polygenic) 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 (10). All models were fitted with SOLAR with correction for ascertainment. DZ twin pairs of the same and the opposite sex were pooled in the analysis; age, sex, use of inhaled corticosteroids, smoking, and current airway infection were screened as covariates. The Scientific Ethics Committee of Copenhagen approved the protocol.

Results

Characteristics of the studied subjects are given in Table 1. The mean age was 36 years (age range 21–51 years) and 58% of the subjects were females. Variances were homogeneous between the two sexes and the zygosity groups for the different traits. Sex was a significant predictor for FeNO ($P = 0.003$) and FEV₁/FVC ($P < 0.001$), smoking for FeNO ($P < 0.001$), DRS ($P = 0.01$), and serum total IgE ($P = 0.02$), and current airway infection for FeNO ($P < 0.001$).

Results from univariate variance components analysis are shown in Table 2. Additive genetic factors explained 67% of the variation in FeNO, 43% in DRS, 22% in FEV₁/FVC, and 81% in serum total IgE.

Table 3 shows the results from bivariate variance components analysis. In general, traits had genetically and environmentally distinct variance structures. The most substantial genetic similarity was observed between FeNO and serum total IgE ($\rho_A = 0.37$), followed by

Table 1. Distribution of asthma-related traits in a sample of 575 twins, 21–51 years of age

	<i>n</i> †	Age‡	Asthma§	Clinically measured traits*			
				FeNO	DRS	FEV ₁ /FVC	Total IgE
Males	240 (29)	35.6 (7.9)	129 (54)	21.8 (19.7–24.2)	2.9 (2.3–3.5)	0.81 (0.80–0.82)	63.6 (52.2–77.6)
Females	335 (34)	36.1 (8.4)	208 (62)	16.6 (15.2–18.2)	2.8 (2.4–3.3)	0.86 (0.85–0.87)	50.5 (42.5–60.0)
MZ twins	191 (13)	34.9 (7.4)	126 (66)	19.6 (17.4–22.1)	3.0 (2.4–3.8)	0.85 (0.83–0.86)	59.5 (47.7–74.4)
DZ twins	384 (50)	36.5 (8.5)	211 (55)	18.2 (16.7–19.7)	2.7 (2.3–3.2)	0.84 (0.83–0.85)	53.7 (45.7–63.1)
Total	575 (63)	35.9 (8.2)	337 (59)	18.6 (17.4–19.8)	2.8 (2.5–3.2)	0.84 (0.83–0.85)	54.4 (48.8–63.3)

*Geometric mean values (95% CI). NO is ppb. Total IgE is KIU/l.

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

‡Mean age in years (standard deviation).

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

Table 2. Variance components analysis of asthma-related traits in a sample of 575 twins, 21–51 years of age

	Variance components*			Fit statistics		
	A	C	E	$\Delta-2 \log L$	Δdf	P-value
FeNO						
ACE	0.67 (0.56–0.78)	0.00	(0.00–0.11)	0.33 (0.22–0.44)		
AE	0.67 (0.56–0.78)	–	0.33 (0.22–0.44)	0.00	1	1.00
CE	–	0.36 (0.25–0.47)	0.64 (0.53–0.75)	11.30	1	<0.001
DRS						
ACE	0.43 (0.25–0.61)	0.00	(0.00–0.18)	0.57 (0.39–0.75)		
AE	0.43 (0.25–0.61)	–	0.57 (0.39–0.75)	0.00	1	1.00
CE	–	0.21 (0.08–0.34)	0.79 (0.66–0.92)	3.73	1	0.05
FEV ₁ /FVC						
ACE	0.22 (0.00–0.49)	0.44 (0.22–0.66)	0.34	(0.25–0.43)		
AE	0.69 (0.61–0.77)	–	0.31 (0.23–0.39)	5.40	1	0.02
CE	–	0.60 (0.52–0.68)	0.40 (0.32–0.48)	1.30	1	0.25
Total IgE						
ACE	0.81 (0.75–0.87)	0.00 (0.00–0.06)	0.19 (0.13–0.25)			
AE	0.81 (0.75–0.87)	–	0.19 (0.13–0.25)	0.00	1	1.00
CE	–	0.53 (0.44–0.62)	0.47 (0.38–0.56)	17.95	1	<0.001

*Standardized parameter estimates of additive genetic factors (A), shared environmental factors (C), and nonshared environmental factors (E).

$\Delta-2 \log L$, difference in model fit (log-likelihood) between saturated model and nested model.

Δdf , difference in degrees of freedom between saturated model and nested model.

Table 3. Correlations (95% CI) between asthma-related traits in a sample of 575 twins, 21–51 years of age

	Correlation‡	ρ_A §	ρ_E ¶	Proportion of phenotypic correlation due to†	
				Genetic factors	Environmental factors
FeNO–DRS	0.25	0.20 (–0.02;0.42)	0.34 (0.15;0.53)**	0.42	0.58
FeNO–FEV ₁ /FVC	–0.05	–0.02 (–0.15;0.13)	–0.11 (–0.30;0.08)	0.29	0.71
FeNO–Total IgE	0.29	0.37 (0.24;0.50)***	0.08 (–0.13;0.29)	0.93	0.07
DRS–FEV ₁ /FVC	–0.33	–0.26 (–0.47;–0.05)*	–0.46 (–0.64;–0.28)***	0.42	0.58
DRS–Total IgE	0.22	0.24 (0.05;0.43)*	0.24 (0.03;0.45)*	0.64	0.36
FEV ₁ /FVC–Total IgE	0.02	0.00 (–0.13;0.13)	0.07 (–0.11;0.25)	0.15	0.85

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Numbers in bold print represent significant P values after adjustment for multiple testing.

Negative genetic and environmental correlations indicate that genetic and environmental factors induce a negative phenotypic correlation between traits.

†Proportion of the phenotypic correlation that is attributable to genetic and environmental factors, shared between the traits.

‡Phenotypic (within subject) correlation.

§Additive genetic correlation.

¶Environmental correlation.

DRS and FEV₁/FVC ($\rho_A = -0.26$), and by DRS and serum total IgE ($\rho_A = 0.24$). On the contrary, the same environmental risk factors influenced, to a large extent, DRS and FEV₁/FVC ($\rho_E = -0.46$), and FeNO and DRS ($\rho_E = 0.34$), whereas DRS and serum total IgE shared environmental determinants to a smaller extent ($\rho_E = 0.24$). FEV₁/FVC and FeNO, and FEV₁/FVC and serum total IgE seemed to be regulated by distinct genetic and environmental factors.

Discussion

We used the classical twin design to investigate the overlap between genetic and environmental determinants

of key intermediate asthma phenotypes. Our analyses suggest that

- (1) genetic factors account for a large proportion of the variation in FeNO, airway obstruction, airway responsiveness, and serum total IgE; however, as expected, environmental influences also contribute considerably to the variation in these traits;
- (2) the correlation between FeNO and serum total IgE is mainly explained by genetic factors, whereas environmental factors mainly explain the correlation between FeNO and airway responsiveness, and between airway responsiveness and airway obstruction; and

- (3) the genetic and environmental correlations between these traits are low to moderate, indicating that trait variation arises to a large extent from distinct determinants.

Little is known about the genetic architecture of intermediate asthma phenotypes. In particular, few studies have investigated the heritability of FeNO and its correlation with other intermediate phenotypes. A recent Norwegian study found that genetic influences accounted for 60% of the variation in FeNO, which is comparable to our estimate of 67% (11). However, in contrast to our results the Norwegian study found that genes primarily explained the association between FeNO and airway responsiveness. This may, however, allude to an adjustment for atopic status in the Norwegian sample. Atopy is a strong predictor of elevated FeNO levels whereas subjects without atopy usually have normal levels of FeNO (12). We found some evidence of pleiotropic effects on the association between FeNO and serum total IgE, which could have confounded the relationship between FeNO and airway responsiveness. Particularly, the degree of airway responsiveness may reflect severity of airway inflammation only in atopic individuals (13). We found a low genetic correlation between airway responsiveness

and serum total IgE, which has also been reported by others (14, 15). It is therefore possible that airway inflammation in asthmatics is associated with airway hyper-responsiveness independently of a shared genetic basis with atopy.

We found that airway obstruction also correlated well with airway responsiveness and that environmental factors primarily mediated this relationship. Our analysis was adjusted for smoking, current airway infection, and use of inhaled corticosteroids, which are known predictors of airway responsiveness and airway obstruction in asthmatics. This signals that other factors may be of importance for this association, particularly allergens, like house dust mite could be contributory factors (15).

In conclusion, this study showed that FeNO, airway obstruction, airway responsiveness and serum total IgE have moderate to high heritabilities. There were similarities in the genetic and environmental architecture of some of these traits. However, a large proportion of the variance of each trait was attributable to factors unshared between traits, indicating that each trait's variability to a large extent arises from unique sources. These results underline that asthma is a complex disease characterized by a set of etiologically heterogeneous biomarkers, which therefore likely constitute diverse targets of intervention.

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