Genetics of parentally reported asthma, eczema and rhinitis in 5-yr-old twins

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ABSTRACT: The aim of the present study was to examine the genetic and environmental contributions to the individual differences in susceptibility to asthma, eczema and rhinitis in childhood and their role in the association among these conditions.

Information on asthma, eczema and rhinitis was obtained by parental report. Parents were asked whether a physician had ever diagnosed the condition. Complete data were available for 8,633 5-yr-old twin pairs born between 1986 and 1998.

The frequency of parentally reported asthma, eczema and rhinitis was 8.7, 16.8 and 4.4%, respectively, and was higher in males than females. Genetic factors accounted for ~90% of the variance in the susceptibility to asthma, eczema and rhinitis. The magnitude of genetic factors did not differ between males and females. The remaining part of the variance was explained by environmental factors not shared by family members. The phenotypic correlations between parentally reported asthma, eczema and rhinitis were moderate and mainly mediated by the same genetic factors.

The high heritability and the limited influence of shared environmental factors may point to gene x environment interactions. Future research should focus on this type of interaction processes.

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Over recent decades the prevalence of asthma and allergic diseases has increased substantially [1]. These diseases often start in childhood, have a chronic character and can have a considerable impact on the quality of life and well-being of children [2]. Although many twin studies showed that genetic factors play an important role in the development of asthma [3], several issues remain worthy of study. First, most studies reported on the genetics of asthma, but the genetics of eczema and rhinitis are less well studied. Secondly, most twin studies were carried out in adolescents and adults. Thirdly, the aetiology of the association between asthma, eczema and rhinitis in very young children has not previously been studied.

To derive the genetic and environmental contribution to complex diseases, the twin design is powerful [4]. The general result from twin studies is that asthma is a highly heritable trait. Approximately 70% of the variance in liability to asthma is explained by genetic factors [5–11]. If there were sex differences in genetic factors, then the influence of genetic factors was usually lower for females than males. Another consistent finding from twin studies is that the environment shared by family members does not contribute to the variation in susceptibility to asthma. Only environmental influences not shared by family members play a role. In the light of the increasing rate of asthma during recent decades, this minor role of environmental factors seems remarkable, as the time period of two decades is simply too short for changes to occur in allele frequencies in genes that could account for the increasing rate of asthma.

In contrast to asthma, the heritability of hay fever and eczema has not often been studied. In a sample of Norwegian twins aged 18–35 yrs, genetic factors accounted for ~72 and 68% of the variance in liability to eczema and hay fever, respectively [10]. In a Finnish sample of adolescent twins, genetic factors accounted for 74–82% of the variance in liability to hay fever [12]. In children aged 7–9 yrs, the heritability for hay fever was 0.33 for males and 0.70 for females, and the heritability for eczema was ~70% for both boys and girls [9].

Several studies provided evidence that asthma and related diseases tend to cluster within families and that overlapping genes may play a major role in this clustering of diseases [5, 9, 13, 14]. However, only a few studies have used structural equation modelling to estimate the extent to which the same genes contribute to association between asthma and related diseases [5, 9].

In the present study, susceptibility to asthma, eczema and rhinitis was assessed by parental report in a large sample of 5-yr-old Dutch twin pairs. The first aim was to examine the genetic and environmental contributions to variance in susceptibility to parentally reported asthma, eczema and rhinitis. The second aim was to estimate the extent that shared genes and/or environments contribute to the comorbidity of asthma, eczema and rhinitis.

METHOD

Participants and measures

The data presented in the current paper come from a longitudinal study, which examines the genetic and environmental influences on the development of behavioural and emotional problems in twins from birth onwards. The twin families are volunteer members of the Netherlands Twin Register (NTR), established by the Dept of Biological Psychology at the Free University in Amsterdam (Amsterdam, The Netherlands) [15]. From 1987 onwards, the NTR has recruited families with twins a few weeks or months after birth. Currently, 40–50% of all multiple births are registered by the NTR. For the present study, data were included that were obtained by questionnaires mailed to parents of 5-yr-old twin pairs born between 1986 and 1997.

Parents were asked to report (yes/no) whether a physician had ever diagnosed the following conditions: asthma, eczema, hay fever or rhinitis. A child was defined as affected by one of the specific conditions when the item was positively answered. Children with hay fever or allergic rhinitis were considered as cases for "rhinitis".

For 1,218 same-sex twin pairs, zygosity was based on blood (n=407) or DNA (n=811) group polymorphisms. For the remaining twins, zygosity was determined by questionnaire items about physical similarity and frequency of confusion of the twins by family and strangers [16]. If zygosity was missing, the zygosity status was determined by items from surveys sent on other ages. This left 16 twin pairs without information on zygosity. Complete data were available for 1,349 monozygotic (MZ) male twin pairs, 1,461 dizygotic (DZ) male twin pairs, 1,592 MZ female twin pairs, 1,387 DZ female twin pairs and 2,844 opposite-sex (OS) twin pairs.

Data analysis

Prevalence

Prevalence rates were estimated using Mx [17], a structural equation modelling package, in order to obtain unbiased estimates for data assessed in family members. Likelihood-ratio Chi-squared tests were used to examine the significance of sex and zygosity on differences in prevalence.

Genetic analysis

The relative contribution of genetic and environmental factors to variance in susceptibility to complex diseases, such as asthma, can be inferred with data from genetically related subjects, such as MZ and DZ twins. The twin method compares the resemblance for a certain trait in MZ twin pairs, who are genetically identical, with the resemblance in DZ pairs, who share on average 50% of their segregating genes. The twin method assumes that MZ and DZ twin pairs share their family environment to the same extent. If the MZ

resemblance, often expressed in correlations, is twice as large as the DZ resemblance, the trait is influenced by genetic factors since the only difference between the two zygosity groups is in genetic relatedness. If the DZ correlation is the same as or larger than half the MZ correlation, then a trait is influenced by shared environmental factors. Shared environmental factors include experiences due to growing up in the same family (e.g. exposure to the same smoking behaviour of parents) but also due to in utero experiences. Any true differences, free from measurement error, between MZ twins are attributable to their non-shared environment. Non-shared environmental influences denote experiences that affect only one of the twins.

For dichotomous variables, tetrachoric correlations can be used to index twin similarity. This is the correlation between twins for an underlying, continuously distributed trait that is often called the "liability" or "susceptibility" to disease. It is assumed that many genes and environmental influences affect susceptibility, resulting in its normal distribution. A threshold on the susceptibility distribution divides the population into affected and unaffected subjects [17, 18]. The threshold, expressed as a z-value of the normal distribution, is inferred from the prevalence of the disease in the sample.

With structural equation modelling, the influence of additive genetic effects (A), dominant genetic factors (D), shared or common environment effects (C) and non-shared environmental (E) factors [4] on variation in susceptibility can be estimated. The modelling procedure starts with a model with A, C and E factors (ACE full model) or A, D and E factors (ADE full model). The significance of A, D, C or E can be tested by omitting them from the model. A significant decrease in goodness-of-fit implies that the omission is not allowed and the factor contributed significantly to the variance of the trait. Sex differences in genetic architecture were tested by constraining the parameters that represent the influence of A, D, C or E to be equal across sex. Again, if the goodness-of-fit deteriorates significantly then the constraint is not allowed and there are significant sex differences in genetic and/or environmental influences. Goodness-of-fit statistics obtained for the different models were compared with likelihood-ratio Chi-squared tests. Mx jobs for the genetic analyses were obtained from the Mx library [19].

To estimate the genetic and environmental influences on the association of asthma, eczema and rhinitis, cross-twin, cross-trait correlations (e.g. asthma in one twin and allergy in the other twin) in MZ and DZ twin pairs were used. If there is an overlap of genes for asthma and eczema, it is expected that the cross-trait, cross-twin MZ correlation will be higher than the cross-trait, cross-twin DZ correlation. Shared environmental influences to the co-occurrence of diseases are suggested if the DZ correlation is larger than half the MZ correlation. To estimate the genetic and environment influences on the association between asthma, eczema and rhinitis, a bivariate genetic model was applied to the data [20]. The model provides estimates of the extent to which shared genetic factors and/or environmental factors contribute to the phenotypic correlation. If the genetic correlation ($r_{(g)}$) is 1, then the same genes influence both diseases. A correlation of 0 indicates that each disease is affected by a different set of genes. To estimate what proportion of the phenotypic correlation

between asthma, eczema and rhinitis is due to genetic factors, the genetic correlation was weighted by the square roots of the heritabilities of the traits and divided by the phenotypic correlation.

RESULTS

Prevalence

Table 1 provides a summary of the characteristics of the sample according to sex and birth cohort. The prevalence of asthma, eczema, and rhinitis was higher for males than females (asthma: ΔChi-squared=49.62, Δ degrees of freedom (d.f.)=1, p<0.001; eczema: ΔChi-squared=12.97, Δd.f.=1, p<0.001; allergy: ΔChi-squared=32.90, Δd.f.=1, p<0.001). Within each sex, the frequency of asthma, eczema and rhinitis was not different in MZ and DZ twins (asthma: ΔChi-squared=1.90, Δd.f.=2, p=0.39; eczema: ΔChi-squared=3.79, Δd.f.=2, p=0.15; allergy: ΔChi-squared=0.07, Δd.f.=2, p=0.96).

Genetic analysis

The present authors first explored whether the twin correlations for asthma, eczema and rhinitis were different among full-term born twins ($\geqslant$37 weeks), pre-term born twins ($\geqslant$32 and <37 weeks) and very pre-term born twins (<32 weeks). As prevalence differences were observed for asthma between very pre-term twins (15.5%) and full-term twins (7.2%), it is possible that the aetiology explaining the variance in susceptibility to asthma differs across these groups. A different pattern of MZ and DZ correlations may point to a different underlying aetiology. As shown in table 2, the difference between MZ and DZ twin correlations of asthma and rhinitis in the very pre-term group was smaller than the difference between the MZ and DZ correlations in the pre-term and full-term twins. Constraining the DZ correlation to be equal in the very pre-term to that of the twins born after 32 weeks resulted in a significant deterioration of the fit for asthma (ΔChi-squared=3.94; Δd.f.=1; p=0.047) but not for rhinitis (ΔChi-squared=1.93; Δd.f.=1; p=0.165). These results could indicate that for the very pre-term twins, both C and A factors contribute to variance in susceptibility to asthma, while for twins born after 32 weeks only genetic influences are suggested. However, structural equating modelling did not demonstrate the significance of C in the very pre-term group. The detection of C in the classical twin design requires large

samples and it is likely that the number of twins was too small to draw any firm conclusion about C. Since the present study is ongoing, it is hoped that its sample size can be extended in the future, which may lead to a decisive answer to the question whether the genetic and environmental contribution to asthma and rhinitis is different in very pre-term children. For the present analyses, the very pre-term twins were excluded (n=363) from further genetic analyses.

Table 3 gives the phenotypic correlations between asthma, eczema and rhinitis, the twin correlations, and the cross-twin, cross-trait correlations. For all three conditions, the MZ correlations were higher than the DZ correlations, suggesting genetic influences. The results of the univariate genetic analyses are presented in table 4. The analyses revealed no sex differences in genetic influences for asthma, eczema and rhinitis. Additive genetic factors accounted for the most part of the variance in liability to asthma (h^2=95%) and to rhinitis (h^2=91%). Heritability (h^2) refers to the percentage of variance explained by genetic differences between individuals. The remaining part of the variance was explained by non-shared environmental factors. For eczema, an ADE model was the best fitting model; this indicated that a large part of the variance in susceptibility of eczema could be explained by genetic factors (84%), but that both additive (35%) and dominant (49%) genetic factors were important.

Based on the univariate results, which suggested no sex differences in heritability and no evidence for shared environmental effects, bivariate analyses were performed without sex differences in estimates for A and E; shared environmental factors were not included in the analyses. For eczema, a genetic dominance factor was included in the model. The phenotypic correlation of the first born twin (with the correlation for the second born twin in parentheses) between asthma and eczema was 0.348 (0.357); between asthma and rhinitis, 0.517 (0.475); and between eczema and rhinitis, 0.375 (0.391). The structural equation modelling revealed that both genetic and environmental factors contributed to the phenotypic correlations of asthma, eczema and rhinitis. The extent to which the same genes contribute to phenotypic correlation was estimated at 0.55 for asthma and eczema, at 0.47 for asthma and rhinitis,

TABLE 1	Distribution of asthma, eczema and rhinitis across males and females and across birth cohort					
Cohort	**Asthma**		**Eczema**		**Rhinitis**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Subjects n	8807	9135	8781	9152	8665	9035
1986–1989	6.5	4.0	16.4	14.8	6.6	4.5
1990–1992	8.9	7.3	17.1	16.7	5.3	3.4
1993–1995	12.9	8.0	18.6	15.4	5.9	3.6
1996–1998	11.7	9.0	18.5	16.6	4.5	3.7
Total	10.2	7.2	17.7	15.9	5.6	3.8

Data are presented as %, unless otherwise stated.

TABLE 2	Twin correlations separately for very pre-term, pre-term and full-term pregnancies	
	MZ	**DZ**
Very pre-term (<32 weeks) n	146	217
Asthma	0.92	0.65
Eczema	0.88	0.36
Rhinitis	0.97	0.74
Pre-term ($\geqslant$32 weeks and <37 weeks) n	1089	1806
Asthma	0.91	0.36
Eczema	0.86	0.21
Rhinitis	0.97	0.52
Full-term ($\geqslant$37 weeks) n	1706	3669
Asthma	0.91	0.43
Eczema	0.83	0.33
Rhinitis	0.93	0.53

TABLE 3	Twin correlations, cross-trait correlations and cross-trait, cross-twin correlations for asthma, eczema and rhinitis across zygosity and sex groups					
	MZM	**DZM**	**MZF**	**DZF**	**OS-mf**	**OS-fm**
Twin pairs n	1286	1394	1509	1343	1392	1346
Twin correlations						
Asthma	0.89	0.37	0.92	0.47	0.40	0.44
Eczema	0.83	0.27	0.85	0.32	0.24	0.36
Rhinitis	0.93	0.55	0.97	0.37	0.61	0.54
Within-twin, cross-trait correlations						
Asthma–eczema	0.45	0.34	0.37	0.33	0.35	0.28
Asthma–rhinitis	0.48	0.48	0.49	0.52	0.44	0.52
Eczema–rhinitis	0.38	0.43	0.37	0.35	0.32	0.41
Cross-trait, cross-twin correlations						
Asthma–eczema	0.43	0.05	0.34	0.18	0.09	0.06
Asthma–rhinitis	0.38	0.21	0.43	0.28	0.19	0.18
Eczema–rhinitis	0.35	0.17	0.35	0.15	0.25	0.15

MZM: monozygotic male twin pairs; DZM: dizygotic male twin pairs; MZF: monozygotic female twin pairs; DZF: dizygotic female twin pairs; OS-mf: opposite sex twin pairs, with male as the first born and female as the second born; OS-fm: opposite sex twin pairs, with female as the first born and male as the second born.

and at 0.62 for eczema and rhinitis. These correlations also emphasised the importance of unique genetic factors for each condition. The extent to which the same environmental factors overlap was estimated at 0.18 for asthma and eczema, 0.73 for asthma and rhinitis, and 0.39 for eczema and rhinitis. The last two entries of table 4 show the proportion of the phenotypic correlation that is due to same genetic and same environmental factors. It shows that the phenotypic correlation between asthma–eczema, asthma–rhinitis and eczema–rhinitis was mediated mainly by genetic factors.

DISCUSSION
In the present study, the genetic effects of parentally reported asthma, eczema and rhinitis have been explored in a large group of 5-yr-old children covering birth cohorts from 1986–1998. The prevalence of asthma, eczema and rhinitis was 10.2, 17.7 and 5.6%, respectively, for males and 7.2, 15.9 and 3.8%, respectively, for females. Genetic factors accounted for a large part of the variance in susceptibility to asthma, eczema and rhinitis, and the influence of these factors were of the same magnitude for males and females. The influence of environmental factors was small (6–14%) and factors were non-shared between twins. The phenotypic correlations among the three diseases were moderate and the overlap between the diseases was mainly due to overlapping genes. Before the findings of the present study are further interpreted, it should be mentioned that the parental report of asthma and related conditions, and not a clinical assessment, is relied

upon. The present findings should be seen in the light of this limitation.

The parentally reported frequency of asthma (10.2% for males and 7.2% for females) was higher than the prevalence of asthma obtained by registration of the general practitioners in the Netherlands (6% for males and 4.2% for females) [21]. In the light of this findings, it could be questioned whether the twins are representative for the general population. However, several explanations are possible to account for these prevalence differences. In the present study, the frequency of asthma, eczema and rhinitis was based on parental report, in which the question asked was: ''Had the child ever had doctor-diagnosed asthma since birth?'' Thus, the question involved a larger time span than the 1 single year in which the registration by the physician is based. In addition, it is a consistent finding that the prevalence of asthma and related conditions obtained by self-report is higher than the prevalence reported by the physicians [22]. The prevalence of asthma and eczema in the present study are comparable to other studies based on parental reports of asthma in 4–6-yr-old Dutch children [23], with a prevalence aged 17.7% for skin rash ever in life reported for Dutch children aged 7–12 yrs [24]. Nevertheless, that twins may form a special population cannot be excluded. The percentage of pre-term births is higher in twin than in singleton births. In addition, it is known that babies who are born prematurely may have an increased risk for lung damage, which in turn may increase the sensitivity of developing asthma [25]. Conversely, there also is evidence that the prevalence rate of asthma may simply be lower in twins than in singletons since growing up with a co-twin increases the chance of exposure to infections in early life, which in turn protects against the development of allergic diseases [26, 27].

In the present study, the authors obtained a heritability of ~90% for parentally reported asthma, eczema and rhinitis. This high heritability corresponds to the general finding of large genetic influences for asthma, but the heritability is somewhat higher than the general finding of 70%. The fact that most research of asthma is carried out in adolescents and adults may explain this difference. Adolescents and adults may be exposed to a wider range of environments than young twins and, as a result, the relative environmental influences may be larger in adolescents. A high heritability could also be the result of violation of the assumption of equal environment for MZ and DZ twins. If MZ twins experience more equal environments than DZ twins, then the influence of genetic factors could be overestimated. It seems unlikely that the exposition to risk factors is more similar for MZ than for DZ twins in 5-yr-old twin pairs who grow up in the same home. The influence of genetic factors may be overestimated as a result of the parents' expectations regarding the MZ twin's resemblance. If this expectancy effect plays a role, then it would be expected that the twin correlations will be different between twins who were correctly classified as MZ twins and who where misclassified. The present findings do not suggest any parental bias. In a group of 214 MZ twin pairs misclassified by their parents, the twin correlations for asthma, eczema and rhinitis were also very high (0.86, 0.77 and 0.92, respectively, for asthma, eczema and rhinitis).

TABLE 4	Model fitting results of genetic analyses[#]		
	Asthma	**Eczema**	**Rhinitis**
Full model	ACE	ADE	ADE
Single trait analyses			
ΔChi-squared value			
Sex differences[¶]	1.386	0.408	0.905
Drop C (D)[+,§]	0	18.627[ƒ]	0
Parameter estimates % (95% CI)			
A^2	91 (82–93)	35 (13–57)	95 (78–97)
D^2		49 (26–72)	
E^2	9 (6–13)	16 (13–20)	5 (3–8)
Bivariate trait analyses			
Asthma and eczema			
r_g	0.55		
r_e	0.18		
Asthma and rhinitis			
r_g		0.47	
r_e		0.73	
Eczema and rhinitis			
r_g			0.62
r_e			0.38
Phenotypic correlation explained by genetic and environmental factors %			
Genetic	82	88	96
Environmental	18	12	4

[#]: the genetic model includes additive genetic factors (A), common environment (C), genetic dominance (D) and unique environmental (E) influences on liability to asthma, eczema and rhinitis. CI: confidence interval; a^2, d^2 and e^2: estimates giving the percentages of variance explained by additive genetic, dominant genetic and unique environmental influences; r_g and r_e: parameter estimates giving the genetic and environmental correlation between two traits. [¶]: Δ degrees of freedom (d.f.)=2; [+]: Δd.f.=1; [+]: the AE model omits C or D from the model (if the increase in Chi-squared value is significant, this indicates the significance of this parameter); [ƒ]: significant increase in Chi-squared value.

In agreement with other twin studies of asthma, the present results do not provide evidence for the influence of shared environmental factors. Thus, the fact that twins grow up in the same house and experience the same environmental risk factors does not seem to contribute to the variance in susceptibility to asthma. These findings seem to be in conflict with studies that suggested environmental risk factors, such as parental smoking, number of siblings and air pollution, for the increase of asthma during recent decades.

The lack of evidence for shared environmental factors could be explained by gene–environment interactions. It is likely that environmental risk factors trigger asthma only in persons with a larger genetic susceptibility for allergic and asthmatic diseases [28]. In a twin design, it is not possible to distinguish an interaction between genotype and shared environmental factors from genetic effects. There is some evidence for the possibility of gene–environment interactions for asthma and related diseases [29, 30]. These studies reported that the linkage results depended on the kind of environment to which the patient was exposed as a child. Evidence for linkage between certain chromosomes and asthma were only found for persons who where exposed to cigarette smoke in early childhood. Thus, certain genes may be expressed only when the person is exposed to certain risk factors. These results

clearly show the need for inclusion of the environmental information in genetic studies of asthma and related diseases.

An important question posed in the present study was to what extent the same genes play a role in asthma, eczema and rhinitis. The genetic correlations ranged from 0.47 (asthma–rhinitis) to 0.62 (rhinitis–eczema), meaning that some common genes play a role in more than one disease. It is noteworthy that the genetic correlations were <1; thus, there were also genetic influences unique to each disease. These results of the present study agree with those of LICHTENSTEIN and SVAERTENGREN [9], who examined asthma, eczema and hay fever in 7–9-yr-old twins. In their study, the genetic correlation between asthma and hay fever was 0.90; between asthma and eczema, 0.35; and between hay fever and eczema, 0.73. Although the sizes of the genetic correlations differ from the present study's correlations, in both studies the phenotypic correlations were found to be mainly genetically mediated.

In conclusion, the present study has shown that additive genetic factors contributed to the variance of susceptibility to asthma, eczema and allergy in 5-yr-old twins. The lack of shared environmental influences on the variability of asthma and allergic diseases may point to the importance of gene–environment interactions. Therefore, future studies should

focus on the importance of the interplay between genetic and environmental factors.

REFERENCES

1 ISAAC steering committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitus, and atopic eczema: ISAAC. *Lancet* 1998; 351: 1225–1232.

2 Crane J, Wickens K, Beasley R, Fitzharris P. Asthma and allergy: a worldwide problem of meanings and management? *Allergy* 2002; 57: 663–672.

3 Los H, Postmus P, Boomsma D. Asthma genetics and intermediate phenotypes: a review from twin studies. *Twin Res* 2001; 4: 81–93.

4 Boomsma D, Busjahn A, Peltonen L. The classical twin study and beyond. *Nat Rev Genet* 2002; 3: 872–882.

5 Duffy D, Martin N, Battistutta D, Hopper J, Mathews J. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* 1990; 142: 1351–1358.

6 Harris J, Magnus P, Samuelsen S, Tambs K. No evidence for effects of family environment on asthma. A retrospective study of Norwegian twins. *Am J Respir Crit Care Med* 1997; 156: 43–49.

7 Koeppen-Schomerus G, Stevenson J, Plomin R. Genes and environment in asthma: a study of 4 year old twins. *Arch Dis Child* 2001; 85: 398–400.

8 Laitinen T, Rasanen M, Kaprio J, Koskenvuo M, Laitinen L. Importance of genetic factors in adolescent asthma: a population-based twin-family study. *Am J Respir Crit Care Med* 1998; 157: 1073–1078.

9 Lichtenstein P, Svaertengren M. Genes, environments, and sex: factors of importance in atopic diseases in 7–9-year-old Swedish twins. *Allergy* 1997; 52: 1079–1086.

10 Nystad W, Røysamb E, Magnus P, Tambs K, Harris R. A comparison of genetic and environmental variance structures for asthma, hay fever and eczema with symptoms of the same diseases: a study of Norwegian twins. *Int J Epidemiol* 2005; 34: 1–8.

11 Skadhauge L, Christensen K, Kyvik K, Sigsgaard T. Genetic and environmental influence on asthma: a population-based study of 11,688 Danish twin pairs. *Eur Respir J* 1999; 13: 8–14.

12 Rasanen M, Laitinen T, Kaprio J, Koskenvuo M, Laitinen L. Hay fever – a Finnish nationwide study of adolescent twins and their parents. *Allergy* 1998; 53: 885–890.

13 Clarke J, Jenkins M, Hopper J, *et al.* Evidence for genetic associations between asthma, atopy, and bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2000; 162: 2188–2193.

14 Hopper J, Murray C, Macaskill G, Mathews J. Twin concordance for a binary trait: III. A bivariate analysis of hay fever and asthma. *Genet Epidemiol* 1990; 7: 277–289.

15 Boomsma D, Vink J, Van Beijsterveldt C, *et al.* Netherlands Twin Register: A focus on longitudinal research. *Twin Res* 2002; 5: 1–6.

16 Rietveld M, Van der Valk J, Bongers I, Stroet T, Slagboom P, Boomsma D. Zygosity diagnosis in young twins by parental report. *Twin Res* 2000; 3: 134–141.

17 Neale M, Boker S, Xie G, Maes H. Mx: Statistical Modeling. 5th Edn. Richmond, Virginia Commonwealth University, 1999.

18 Falconer DS, Mackay TFC. Introduction to Quantitative Genetics. 4th Edn. London, Longman, 1996.

19 Posthuma D, Boomsma D. Mx Scripts Library: Structural equation modeling scripts for twin and family data. *Behav Genet* 2005; 35: 499–505.

20 Neale MC, Cardon LR. Methodology for Genetic Studies of Twins and Families. Dordrecht, Kluwer Academic Publishers BV, 1992.

21 Poos MJR, Gijsen RR. Prevalentie, incidentie en sterfte naar leeftijd en geslacht. Volksgezondheid Toekomst Verkenning NKVBR. [Prevalence, incidence and mortality according to age and sex]. www.rivm.nl/vtv/object_class/kom_cara.html and www.rivm.nl/vtv/object_class/kom_copd.html. Date last updated: December 14, 2006 . Date last accessed: January 9, 2007.

22 Mohangoo A, van der Linden M, Schellevis F, Raat H. Prevalence estimates of asthma or COPD from a health interview survey and from general practitioner registration: what's the difference? *Eur J Public Health* 2006; 16: 101–105.

23 Hirasing R, Aardoom H, van den Heuvel E, Sanavro F, Leeuwenburg J. Prevalentie van door ouders gerapporteerde chronische aandoeningen bij schoolkinderen. [Prevalence of parental-reported chronic disorders in schoolchildren.]. *Nederlands Tijdschrift Geneeskunde* 1995; 139: 934–938.

24 Meerwaldt R, Rodink R, Landaeta R, *et al.* A lower prevalence of atopy symptoms in children with type 1 diabetes mellitus. *Clin Exp Allergy* 2002; 32: 254–255.

25 Halvorsen T, Skadberg B, Eide G, Roksund O, Carlsen K, Bakke P. Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study. *Acta Paediatr* 2004; 93: 1294–1300.

26 McKeever T, Lewis S, Smith C, *et al.* Siblings, multiple births, and the incidence of allergic disease: a birth cohort study using the West Midlands general practice research database. *Thorax* 2001; 56: 758–762.

27 Strachan D, Moran S, McInneny K, Smalls M. Reduced risk of hospital admission for childhood asthma among Scottish twins: record linkage study. *BMJ* 2000; 321: 732–733.

28 Boomsma D, Martin N. Gene–environment interactions. *In*: D'haenen H, Boer JAd, Willner P, eds. Biological Psychiatry. Chichester, John Wiley & Sons, 2002; pp. 181–187.

29 Colilla S, Nicolae D, Pluzhnikov A, *et al.* Collaborative Study for the Genetics of Asthma. Evidence for gene–environment interactions in a linkage study of asthma and smoking exposure. *J Allergy Clin Immun* 2003; 111: 840–846.

30 Meyers DA, Postma D, Stine OC, *et al.* Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. *J Allergy Clin Immun* 2005; 115: 1169–1175.