Heritability and Shared Genetic Effects of Asthma and Hay Fever: An Italian Study of Young Twins

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A number of studies have provided evidence of a significant familial aggregation for both asthma and hay fever, and have reported a substantial comorbidity between the two conditions. However, far fewer, especially in Italy, have aimed at clarifying the origins of such comorbidity. The main aims of the present study were (a) to estimate heritability of asthma and hay fever, (b) to measure the association between asthma and hay fever at the individual level, and (c) to assess the extent to which genetic and environmental factors, shared by the two conditions, mediate this association. The twin method was used. The study sample was derived from the Italian Twin Registry, and included 392 twin pairs aged 8 to 17 years. Data collection was performed through parent self-administered questionnaire. Bivariate structural equation twin modeling was applied to asthma and hay fever. Genetic factors accounted for 92% and 78% of the variance in liability to asthma and hay fever, respectively, with the remaining contributions due to unique environmental influences. The within-individual association between asthma and hay fever was substantial. The genetic correlation between the two conditions was .58, whereas no evidence of overlapping unique environmental effects was found. In conclusion, this study showed a high heritability of asthma and hay fever in the Italian child and adolescent population. It also indicated that asthma and hay fever share, to a large extent, a common genetic background, and environmental factors are not relevant to explain the comorbidity.

Allergic diseases such as asthma (AS) and hay fever (HF) are among the most common chronic diseases worldwide. It is known that immunoglobulin E (IgE)-mediated mechanisms play an important role in these conditions.

Although the patterns of AS and HF vary throughout the world, considerable increases in the prevalence of these diseases have occurred globally over recent years (Aberg, 1989; Burney et al., 1990; Burr et al., 2006; Fleming & Crombie, 1987; Galassi et al., 2006; Isolauri et al., 2004). Because the rise has been far too rapid to implicate any genetic basis for change, various environmental and lifestyle factors have been proposed, and most recently the 'hygiene hypothesis' has also been explored as a possible explanation (Bach, 2002). According to this hypothesis, allergic diseases have increased because infectious diseases have diminished. In Italy, lifetime prevalence rates of AS and HF have been reported to be, respectively, 9.3% and 8.9% in children (6–7 years old), and, respectively, 10.3% and 16.6% in adolescents (13–14 years old; SIDRIA-2 Collaborative Group, 2005).

Among the factors that have been associated with AS, HF, and other respiratory symptoms in Italian children and adolescents are environmental and infectious exposures (parental smoking, air pollution, early respiratory infections), socioeconomic status (parental education and occupation), as well as perinatal factors (birthweight, gestational age; SIDRIA-2 Collaborative Group, 2005).

Studies on families have shown a substantial familial aggregation for both AS and HF (Burke et al., 2003; Lee et al., 2004), but have failed to clarify whether the within-family resemblance in the disease pattern has a genetic or an environmental basis. Also, there is ample evidence of a close relationship between AS and HF at the individual level (Bugiani et al., 2005; Linneberg et al., 2002), but the contribution of genetic and environmental factors, shared by the two allergic conditions, in determining the individual clustering is much less known and characterized. Previous family data have supported the hypothesis of the alliance of genes and environment in comorbidity (Annesi-Maesano et al., 2001).

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Twins provide a unique setting to establish the role of genes and environment both in the development of a disease and in the comorbidity of multiple disorders. The potential of the twin design has enormously increased after the implementation of population-based registries in several countries (Busjahn & Hur, 2006), including Italy (Fagnani et al., 2006; Stazi et al., 2002a); such registries are of extreme value to genetic epidemiological research, due to the lack of ascertainment bias (Boomsma et al., 2002). By comparing the concordance for a disease in genetically identical monozygotic (MZ) twins with that in dizygotic (DZ) twins who represent full sibs, and assuming that relevant environmental exposures are shared by MZ and DZ twins to the same extent ('equal environments assumption'), it is possible to estimate the relative weight of genetic and environmental factors in the occurrence of the disease. Furthermore, cross-twin/cross-trait similarity (between one trait in a twin and another trait in the co-twin) in MZ and DZ twin pairs gives information on whether genetic or environmental influences common to the two traits are implicated (Boomsma et al., 2002; Spector et al., 2000).

In twin studies, the estimated genetic effects for AS and HF have varied greatly, depending on the population and study design; the heritability estimates have ranged from 35% to 91% for AS (Duffy et al., 1990; Hallstrand et al., 2005; Harris et al., 1997; Koeppen-Schomerus et al., 2001; Laitinen et al., 1998; Nieminen et al., 1991; Skadhauge et al., 1999; van Beijsterveldt & Boomsma, 2007), and from 33% to 95% for HF (Lichtenstein & Svartengren, 1997; Rasanen et al., 1998; Thomsen et al., 2006a; van Beijsterveldt & Boomsma, 2007).

Only a few twin studies have quantified the role of overlapping genetic factors in AS-HF comorbidity. The genetic correlation between the two traits has been estimated to be .52 from male and .65 from female Australian twins (Duffy et al., 1990), .90 and .47 from Swedish (Lichtenstein & Svartengren, 1997) and Dutch twins (van Beijsterveldt & Boomsma, 2007), respectively, and .57 from a Danish twin sample (Thomsen et al., 2006b). Unraveling the genetic and environmental basis of the comorbidity between AS and HF might give insights into the pathogenetic processes underlying respiratory allergy. The estimation of the degree of genetic relatedness of AS and HF might also be relevant for the search for pleiotropic genes affecting both traits. Furthermore, evaluating the environmental correlation between AS and HF might provide information on the existence of environmental risk factors shared by the two diseases; these factors, if identified, could be targeted by intervention programs to prevent both conditions simultaneously.

In this study, data on parent-reported AS and HF in a sample of young twins from the Italian Twin Registry (ITR; Stazi et al., 2002a; Fagnani et al., 2006) were analyzed to (a) confirm known factors associated to each trait, (b) estimate prevalence, concordance, and heritability of each trait, (c) measure the association between the two diseases at the individual level (comorbidity), and (d) assess the magnitude of shared genetic and environmental cause that could explain the comorbidity.

Materials and Methods

Study Design

The study sample was derived from the ITR. The procedure that led to the establishment of the ITR is described in detail elsewhere (Stazi et al., 2002a). Briefly, a nationwide database of all 'possible twins' in the Italian population was set up in 1996 by means of the 'codice fiscale' (fiscal code), an alphanumeric code that uniquely identifies every Italian citizen, based on first and last name, and place and date of birth. Subjects born before 31 December 1995, for whom the fiscal code indicated the same last name and the same place and date of birth were identified as 'possible twins'.

This study is part of an ongoing project aimed at investigating health-related characteristics and behaviors in Italy. In 2003, 682 twin pairs from the ITR, aged 8–17 years and resident in the North Italian Provinces of Milano and Lecco, were contacted by mail and their parents were invited to take part in a survey concerning atopic diseases in twins. Of the 682 families, 404 (59.2%) agreed to participate, and 392 twin pairs with data on both AS and HF entered the study. The mean age of twins slightly, but significantly, differed between the 404 families who agreed to participate versus the 278 remaining families (respectively, 12.93 ± 2.63 vs. 14.01 ± 2.91, $p = 9.48*10^{-7}$).

Zygosity was assessed by means of the parent-rated Goldsmith questionnaire (Goldsmith, 1991), that includes items about the similarity of appearance and the frequency of confusion of the twins by family members and strangers. This instrument has recently been reported to have an accuracy of over 94% (van Beijsterveldt et al., 2004). According to the Goldsmith questionnaire, there were 141 MZ and 251 DZ twin pairs in our sample, subdivided into 74 MZ male, 67 MZ female, 53 DZ male, 79 DZ female, and 119 opposite-sex pairs. The zygosity distribution of the 404 pairs involved in the survey was not different from that of the 278 nonparticipating pairs; furthermore, the MZ / DZ same sex/DZ opposite-sex ratio was 1.1/1.0/0.9, which did not deviate substantially from the expected 1/1/1 population ratio.

Data collection was performed through an eight-page parent-administered questionnaire that encompassed sections regarding the health status of twins, with a particular focus on allergic diseases and respiratory symptoms. For these diseases and symptoms, the standardized structure of the questionnaire previously adopted in the Italian SIDRIA study (SIDRIA Collaborative Group, 1997) within the ISAAC project (Asher et al., 1995) was followed.

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Twins were classified as having AS or HF in the case of a positive response to questions on whether they had ever suffered from these diseases, and, for AS only, a doctor diagnosis was also required. Sections concerning several markers of environmental (lorry traffic in the living area, parental smoking etc.) and infectious exposure (which included respiratory infections during the first 2 years of life), perinatal factors (premature birth, type of feeding in infancy etc.), family history of atopic syndromes in first-degree relatives, and socioeconomic status were included as well.

Statistical Analysis

Prevalence Rates

AS and HF prevalence rates were estimated in twins as individuals, and Pearson's χ^2 test for association was used to examine possible differences between sex, zygosity and age groups. Age comparisons involved two groups, 8 to 12 years and 13 to 17 years, defined on the basis of the sample median age, and approximately corresponding to puberty and adolescence.

Risk-Factor Analysis

For both AS and HF, the association with known environmental, infectious and perinatal risk factors, adjusted for potential confounders (age, sex, and socioeconomic status), was investigated by means of a generalized estimating equations (GEE) model, considering twins as individuals, and correcting standard errors for clustering on pairs. This analysis was performed using the software Stata (Stata Statistical Software, Release 8, 2003).

Concordance Rates

For both AS and HF, pairwise and probandwise concordance rates were estimated for MZ and DZ pairs separately.

Pairwise concordance is expressed as $P_P = C/(C+D)$ and probandwise concordance is given by $P_C = 2C/(2C+D)$, where C and D are the numbers of concordant affected and discordant pairs, respectively (Witte et al., 1999).

Odds Ratios

Probability and odds ratio (a) of a twin having a trait given the presence or absence of the other trait, and (b) of a twin having a trait given that his/her co-twin had or had not either the same or the other trait were calculated for MZ and DZ pairs separately.

Correlations

Tetrachoric correlations (a) between twin 1 and twin 2 for the same trait (cross-twin/within-trait), (b) between trait 1 and trait 2 in the same twin (within-twin/cross-trait), and (c) between one trait in a twin and the other trait in the co-twin (cross-twin/cross-trait) were estimated for MZ and DZ pairs separately.

Tetrachoric correlation is defined under a 'liability-threshold' model: it is assumed that the influence of many genes and environmental factors results in an underlying, normally distributed liability (i.e., susceptibility) to the disease, with a threshold that divides the population into affected and unaffected subjects, and that is inferred from the disease prevalence in the sample; tetrachoric correlation is the correlation between twins for the underlying liability (Sham, 1998).

To estimate tetrachoric correlations, a saturated model was fitted with the software Mx (Neale et al., 2006). This model was specified constraining the threshold of each trait and the within-twin/cross-trait correlation to be the same for twin 1 and twin 2, MZ and DZ pairs, and also constraining cross-twin/cross-trait correlations (of AS in twin 1 with HF in twin 2 and vice-versa) to be equal within each zygosity group; this implements the natural assumption that there is symmetry between twin and co-twin within pairs, and that twins (as individuals) are from the same reference population.

Genetic Analysis

Standard structural equation twin modeling was performed. A bivariate Cholesky decomposition, under a liability-threshold model, was fitted to AS and HF in MZ and DZ twins with the software Mx, considering raw data as input.

The assumptions and features of such a decomposition are described in detail elsewhere (Neale & Cardon, 1992). Briefly, the goal is to estimate the effect of additive genetic influences (A), either common environmental factors (C, shared by twins within pairs) or dominant genetic factors (D), and unique environmental influences (E, not shared by twins within pairs) on the variation and co-variation in liabilities to AS and HF. Consider, for example, an AE decomposition (Figure 1). For each causal source (A, E), two independent latent factors are specified: the first one (A_1, E_1) loads on both AS and HF (a_{11}, e_{11} on AS; a_{21}, e_{21} on HF), while the second one (A_2, E_2) loads on HF only (a_{22}, e_{22}); this formalizes the assumption on the existence of genetic and environmental influences common to the two diseases, supplemented by effects specific to at least one of the traits. Additive and dominant genetic factors affecting the two twins in a pair correlate 100% between MZ twins (all genes are shared), while they correlate, respectively, 50% and 25% between DZ twins [DZ twins share half of the additive genetic and one quarter of the dominant genetic variance on average (Neale & Cardon, 1992)]; common environmental factors correlate 100% between both MZ and DZ twins [equal environments assumption (Spector et al., 2000)].

Relevant statistics that can be derived from the model include: (a) heritability (h^2) of AS and HF $\{h^2(\text{AS}) = a_{11}^2/(a_{11}^2 + e_{11}^2)$; $h^2(\text{HF}) = (a_{21}^2 + a_{22}^2)/[(a_{21}^2 + a_{22}^2) + (e_{21}^2 + e_{22}^2)]\}$; (b) proportions of total covariance between AS and HF due to genetic and environmental factors $[a_{11}a_{21}/(a_{11}a_{21} + e_{11}e_{21})$, $e_{11}e_{21}/(a_{11}a_{21} + e_{11}e_{21})]$; and (c) genetic and environmental correlations between AS and HF $\{a_{11}a_{21}/[a_{11}^2(a_{21}^2 + a_{22}^2)]^{1/2}$, $e_{11}e_{21}/[e_{11}^2(e_{21}^2 + e_{22}^2)]^{1/2}\}$. The quantities (b) and (c) provide information

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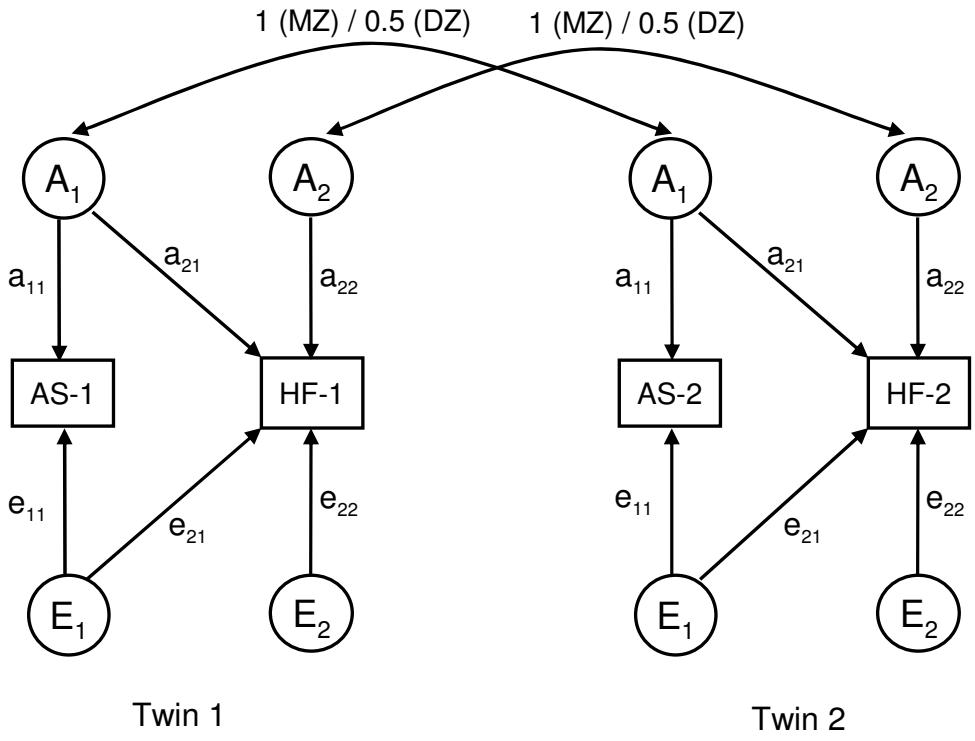

Figure 1

Bivariate (AE) Cholesky model with asthma (AS) entered as the first and hay fever (HF) as the second variable.

Note: Observed phenotypes are shown in rectangles. Latent factors are shown in circles. A_1 and E_1 represent additive genetic and unique environmental influences common to AS and HF. A_2 and E_2 represent additive genetic and unique environmental influences specific to HF. Reported beside the arrows are factor loadings of observed variables on latent factors: a_{11} = additive genetic influence on AS; a_{21} = additive genetic influence common to AS and HF; a_{22} = additive genetic influence specific to HF; e_{11} = unique environmental influence on AS; e_{21} = unique environmental influence common to AS and HF; e_{22} = unique environmental influence specific to HF. Latent additive genetic factors correlate 1 in MZ twins and .5 in DZ twins. Models including C (ACE) and D (ADE) were also tested, but C and D are not shown in the figure for reasons of clarity.

on the role of genes and environment in AS-HF comorbidity. In particular, (c) can be regarded as a measure of the extent to which genetic or environmental influences on the two diseases overlap; for example, a genetic correlation of 0 would suggest that the two traits are affected by completely distinct sets of genes, while a genetic correlation of 1 would indicate that the same genes influence both syndromes.

Model fitting started with full ACE and ADE decompositions, and then proceeded with a series of submodels to test the significance of the various parameters by likelihood-ratio χ^2 tests: the difference between twice the negative log-likelihood for the reduced and the full model has a χ^2 distribution, with the difference of the degrees of freedom of the two models; if the χ^2 test is not significant, the sub-model is preferred. Parameter estimates were reported under the most parsimonious solution (best model).

Results

Prevalence Rates

Table 1 summarizes the characteristics of the study sample and the prevalence rates of AS and HF according to sex, zygosity, and age group. Information on both AS and HF was available for 392 complete pairs (784 twins). The mean age of the twins was 12.9 years (range: 8.3–17.6). Of the 784 twins, 373 (47.6%) were males and 411 (52.4%) were females. The estimated prevalence rate of AS was 8.2%, and it did not significantly differ by sex (males: 8.6%; females: 7.8%; p = .69), zygosity (MZ: 6.4%; DZ: 9.2%;

Table 1

Sample Descriptives and Prevalence Rates of Asthma and Hay Fever

	Mean age	Prevalence (%)	
		Asthma	Hay fever
Sex			
Males (*N* 373)	12.8	8.6	16.6
Females (*N* 411)	13.0	7.8	18.7
	[a]p = .38	[b]p = .69	[b]p = .44
Zygosity			
MZ (*N* 282)	13.1	6.4	19.5
DZ (*N* 502)	12.8	9.2	16.7
	[a]p = .18	[b]p = .17	[b]p = .33
Age group			
8–12 years (*N* 392)		8.4	14.8
13–17 years (*N* 392)		7.9	20.7
		[b]p = 0.79	[b]p = .03
Total sample (*N* 784)	12.9	8.2	17.7

Note: *N* = number of individuals; MZ = monozygotic; DZ = dizygotic; [a] = *t* test probability; [b] = χ^2 test probability

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Table 2

Factors Related to Asthma and Hay Fever According to Multivariate GEE-Models (Twins as Individuals — Standard Errors Adjusted for Clustering on Twin Pairs)

Covariate	Asthma		Hay fever	
	OR	95% CI	OR	95% CI
Age (years)	1.01	(0.87–1.17)	1.13	(1.02–1.24)
Sex				
Males	1		1	
Females	1.29	(0.71–2.36)	0.97	(0.60–1.56)
Parental education				
Secondary school	1		1	
High school	2.16	(0.41–11.47)	1.20	(0.54–2.66)
Degree	2.89	(0.52–16.09)	1.70	(0.73–3.95)
Lorry traffic in living area				
Absent	1		1	
Low	1.87	(0.67–5.18)	0.97	(0.52–1.81)
Moderate	2.04	(0.66–6.32)	1.21	(0.64–2.29)
High	1.46	(0.37–5.77)	0.78	(0.29–2.09)
Maternal smoking				
Never	1		1	
Ex	2.42	(0.85–6.83)	2.03	(1.07–3.85)
Current	2.40	(1.01–5.68)	2.32	(1.27–4.22)
Paternal smoking				
Never	1		1	
Ex	1.04	(0.40–2.69)	1.19	(0.68–2.09)
Current	0.91	(0.33–2.45)	0.84	(0.42–1.69)
Premature birth (gestational age < 37 weeks)				
No	1		1	
Yes	1.04	(0.45–2.39)	1.11	(0.68–1.80)
Feeding in infancy				
Breast	1		1	
Artificial	0.82	(0.30–2.23)	1.10	(0.54–2.23)
Mixed	1.28	(0.46–3.55)	1.87	(0.93–3.76)
Respiratory infections during the first 2 years				
No	1		1	
Yes	12.24	(3.40–44.05)	1.42	(0.88–2.30)
Parental disease history				
No	1		1	
Yes	3.26	(1.37–7.74)	2.54	(1.51–4.28)

Note: OR = adjusted odds ratio; 95% CI = 95% confidence interval

p = .17) or age group (8–12 years: 8.4%; 13–17 years: 7.9%; p = .79). For HF, the prevalence rate was estimated at 17.7% in the total sample. While this rate was rather similar between sexes (males: 16.6%; females: 18.7%; p = .44) and zygosity groups (MZ: 19.5%; DZ: 16.7%; p = .33), it was significantly higher in older twins (8–12 years: 14.8%; 13–17 years: 20.7%; p = .03); this agreed well with a significant positive biserial correlation (.15) between age (as measured on a continuous scale) and HF (as a dichotomous trait).

Risk Factor Analysis

Table 2 shows the adjusted odds ratios of AS and HF estimated by two separate GEE-models incorporating known risk factors and potential confounders for these diseases. Risk factors considered in the models were lorry traffic in the living area, parental smoking, premature birth, type of feeding in infancy, respiratory infections during the first 2 years of life, and family history for either AS or HF; the latter variable was defined as the lifetime occurrence of the disease in at least one of the parents. Potential confounding factors included sex, age, and socioeconomic status as indicated by the highest educational attainment of either parent. The multivariate analysis showed a significant association of maternal smoking and parental disease history with each of AS and HF, and of early respiratory infections only with AS. The adjusted odds ratio of AS was 2.40 (95% CI: 1.01–5.68) for twins with currently smoking mothers compared to those whose mothers had never smoked, and the odds ratios of HF were 2.32 (95% CI: 1.27–4.22) and 2.03 (95% CI: 1.07–3.85) for children whose mothers were current and ex smokers, respectively, compared to children of never-smoking mothers. A parental disease history was associated with more than three times the risk of AS (OR = 3.26; 95% CI: 1.37–7.74) and with about two and a half times the risk of HF (OR = 2.54; 95% CI: 1.51–4.28). The odds ratio of AS was 12.24 (95% CI: 3.40–44.05) for twins who had been affected by respiratory infections during the first 2 years of life. Furthermore, the significant effect of age on HF occurrence was confirmed (OR = 1.13; 95% CI: 1.02–1.24).

Concordance Rates

In Table 3, twin concordance for AS and HF by zygosity is reported. With respect to AS, out of the 141 MZ pairs, 6 (4.3%) were concordant and another 6 (4.3%) were discordant for the condition; the numbers of concordant and discordant pairs among the 251 DZ pairs were 8 (3.2%) and 30 (12.0%), respectively. For HF, 18 MZ (12.8%) and 13 DZ pairs (5.2%) had both twins with a lifetime

Table 3

Concordance Rates of Asthma and Hay Fever

	Asthma		Hay fever	
	MZ	DZ	MZ	DZ
Affection status				
+ +	6	8	18	13
+ –	6	30	19	58
– –	129	213	104	180
Total pairs	141	251		
P_P (95% CI)	0.50 (0.22–0.78)	0.21 (0.08–0.34)	0.49 (0.33–0.65)	0.18 (0.09–0.27)
P_C (95% CI)	0.67 (0.42–0.92)	0.35 (0.17–0.52)	0.65 (0.51–0.80)	0.31 (0.18–0.44)

Note: + + = concordant affected pairs; + – = discordant pairs; – – = concordant unaffected pairs
MZ = monozygotic; DZ = dizygotic; P_P = pairwise concordance; P_C = probandwise concordance
95% CI = 95% confidence interval

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Table 4a

Within-Individual Categorical Associations Between Asthma and Hay Fever: Probabilities of an Individual Having Trait 2 Given Trait 1

Trait 1	Affection	Trait 2	
		Asthma	Hay fever
Asthma	Yes	—	0.48
	No	—	0.15
Hay fever	Yes	0.22	—
	No	0.05	—
	OR	5.32	
	(95% CI)	(3.13–9.06)	

disease occurrence, while in 19 MZ (13.5%) and in 58 DZ pairs (23.1%) the disease had occurred only in one twin. This resulted in significantly higher concordance rates in MZ than in DZ twins for both traits, which was compatible with genetic influences. The estimated probandwise concordances were 0.67 (MZ) and 0.35 (DZ) for AS, and 0.65 (MZ) and 0.31 (DZ) for HF.

Odds Ratios

The odds ratios describing the categorical association between AS and HF both within an individual and within a pair were reported in Table 4a and 4b. The probabilities of a twin having AS in the presence and absence of HF were .22 and .05, respectively; on the other way around, the proportions of twins with HF among twins with and without AS were .48 and .15, respectively. This gave a within-individual/cross-trait odds ratio of 5.32 (95% CI: 3.13–9.06), clearly indicating a substantial co-occurrence of the two conditions.

The within-pair association between AS and HF was also significant and stronger in MZ versus DZ pairs; the estimated odds ratio between AS in one twin and HF in the co-twin was 5.55 (95% CI: 1.47–20.94) in MZ pairs and 3.55 (95% CI: 1.37–9.20) in DZ pairs. This represented a first indication of the possible existence of genetic factors common to AS and HF.

Correlations

Table 5 provides the tetrachoric twin correlations for AS and HF. These correlations were estimated with the software *Mx* under a saturated model specified with a series of constraints (see the paragraph 'Correlations' in the section 'Statistical Analysis'). As indicated by a likelihood-ratio χ^2 test, the fit of this model was not significantly worse than that of the more general model without the constraints ($\chi^2_{11} = 8$, $p = .713$). The within-twin correlation between AS and HF liabilities was .49, and confirmed the substantial association of the two traits at the individual level. For each condition, the within-pair correlation was higher in MZ versus DZ twins; the estimates were .92 (MZ) and .55 (DZ) for AS, and .81 (MZ) and .32 (DZ) for HF. This pointed to large genetic effects on susceptibility to each of AS and HF, and provided no evidence for shared environmental influences on either trait; possible dominant genetic effects for HF were also suggested. Finally, the estimated cross-twin/cross-trait correlation was sizeable and higher in MZ (.49) than in DZ pairs (.38), revealing that pleiotropic genes affecting both AS and HF are likely to come into play.

Genetic Analysis

Table 6 shows the goodness-of-fit statistics of the full ACE (Model 1) and ADE (Model 2) Cholesky

Table 5

Tetrachoric Twin Correlations for Asthma and Hay Fever

	Estimate (95% CI)	
	MZ	DZ
Cross-twin/Within-trait		
Asthma	.92 (.73–.99)	.55 (.29–.76)
Hay fever	.81 (.63–.92)	.32 (.06–.54)
Cross-twin/Cross-trait		
Asthma — Hay fever	.49 (.29–.61)	.38 (.10–.56)
Within-twin/Cross-trait		
Asthma — Hay fever	.49 (.34–.63)	

Note: MZ = monozygotic; DZ = dizygotic; 95% CI = 95% confidence interval

Table 4b

Within-Pair Categorical Associations Between Asthma and Hay Fever: Probabilities of Co-Twin Being Affected Given Twin Affection

Trait	Twin affection	Co-twin			
		Asthma		Hay fever	
		MZ	DZ	MZ	DZ
Asthma	Yes	0.75	0.32	—	—
	No	0.03	0.06	—	—
	OR	96.75	7.71	—	—
	(95% CI)	(14.70–636.87)	(2.81–21.17)	—	—
Hay fever	Yes	0.20	0.19	0.60	0.31
	No	0.04	0.06	0.06	0.14
	OR	5.55	3.55	22.29	2.78
	(95% CI)	(1.47–20.94)	(1.37–9.20)	(7.74–64.19)	(1.30–5.97)

Note: Cross-trait probabilities and odds ratios are reported only for twin's hay fever and co-twin's asthma, because of symmetry; MZ = monozygotic; DZ = dizygotic; OR = odds ratio; 95% CI = 95% confidence interval

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Table 6

Function Values (–2lnL) and Likelihood-Ratio χ^2 tests of Submodels of the Full ACE and ADE Cholesky Decompositions for Asthma and Hay Fever

Model	–2lnL	*df*	c.t.m.	χ^2	Δdf	*p*
1. Full ACE	1055.499	1559	—	—	—	—
2. Full ADE	1054.654	1559	—	—	—	—
3. CE	1071.093	1562	1	15.595	3	0.001
4. AE	1056.482	1562	1	0.983	3	0.805
			2	1.828	3	0.609
5. AE + no genetic correlation (a_{21}=0)	1082.333	1563	4	25.851	1	0.000
6. AE + no unique environmental correlation (e_{21}=0)	**1056.678**	**1563**	**4**	**0.195**	**1**	**0.659**

Note: A = additive genetic factors; C = common environmental factors; D = dominant genetic factors; E = unique environmental factors
–2lnL = minus twice the log-likelihood; df = degrees of freedom; c.t.m. = compared to model; χ^2 = (–2lnL sub-model) – (–2lnL full model)
Δdf = (*df* sub-model) – (*df* full model); Best model is printed in **bold** type

decompositions along with those of the nested models. As a first test, a CE model (Model 3) was considered by dropping all 3 parameters describing the additive genetic effects on AS and HF; this produced a significant deterioration of the goodness of fit, and indicated the importance of additive genetic influences on the two traits. By fitting an AE model (Model 4), environmental influences shared by twins were confirmed not to be relevant in explaining the observed variance-covariance structure of AS and HF liabilities, and dominant genetic effects were also found not to be significant. Possible genetic pleiotropic effects for AS and HF were specifically tested in Model 5 by setting the genetic correlation between the two diseases to zero; this resulted in a significant change in the log-likelihood, pointing to genetic factors simultaneously affecting the two conditions. On the contrary, Model 6 suggested that unique (individual-specific) environmental influences were unlikely to explain the co-occurrence of AS and HF. Therefore, the best model was one in which shared environmental influences on AS and HF, and environmental factors not shared by twins but common to the two traits were ruled out.

Genetic and environmental proportions of variance and covariance for AS and HF, as well as path coefficients as estimated under the best model (Model 6), were reported in Table 7a and 7b. Additive genetic factors accounted for 92% and 78% of variance in liability to AS and HF, respectively, while the remaining 8% and 22% were explained by unique environmental effects.

These estimates closely matched those obtained in the univariate analyses (not shown): for both AS and HF, univariate AE models gave the best fit. However, for HF, a nonsignificant estimate of 0.29 for dominant genetic effects under a full ADE model should be carefully interpreted due to the low power of this study.

Given that the effect of environmental factors common to AS and HF was set to zero in the best model, genetic factors explained the entire phenotypic correlation between the two conditions (i.e., the genetic proportion of total covariance between AS and HF liabilities was 100%). The genetic correlation was estimated at .58, suggesting that AS and HF share many of the same genes.

Table 7a

Standardized Genetic and Environmental Components of Variance and Covariance of Asthma and Hay Fever as Estimated From the Best Structural Equation Model

	A	E
Asthma	0.92 (0.76–0.99)	0.08 (0.01–0.24)
Hay fever	0.78 (0.60–0.90)	0.22 (0.10–0.40)
Asthma – Hay fever	[a]1.00 (1.00–1.00)	—
	[b]0.58 (0.40–0.75)	—

Note: A = additive genetic factors; E = unique environmental factors; [a] = additive genetic proportion of covariance between asthma and hay fever; [b] = additive genetic correlation between asthma and hay fever; — = fixed to zero in the best model; numbers in parentheses are 95% confidence limits

Table 7b

Standardized Genetic and Environmental Components of Variance and Covariance of Asthma and Hay Fever and Path Coefficients as Estimated From the Best Structural Equation Model

	A		E	
	Factor 1	Factor 2	Factor 1	Factor 2
Asthma	0.96 (0.87–0.99)		0.27 (0.11–0.49)	
Hay fever	0.52 (0.35–0.66)	0.72 (0.55–0.84)	—	0.46 (0.31–0.63)

Note: A = additive genetic factors; E = unique environmental factors; — = fixed to zero in the best model; numbers in parentheses are 95% confidence limits

Discussion

In the present study, the heritability of parentally reported asthma (AS) and hay fever (HF), and the magnitude of shared genetic and environmental effects that could explain the comorbidity between the two conditions have, for the first time, been explored in a

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sample of young twins from the Italian Twin Registry, using a structural equation modeling approach.

The prevalence rates of AS and HF were slightly, but not significantly, different between sex, zygosity, and age groups (8–12 and 13–17), with the exception of HF that was observed to be more frequent in older twins. The estimated rates were essentially in line with those reported for a large sample of Italian children (6–7 years) and adolescents (13–14 years) involved in the international ISAAC project [SIDRIA study, (SIDRIA-2 Collaborative Group, 2005)], which may represent an indication against major effects of twinning in terms of AS or HF risk, at least for the age range considered.

The multivariate analysis confirmed the association of known factors with AS or HF risk. More precisely, maternal smoking and parental disease history were found to be associated with each of AS and HF, and respiratory infections (bronchitis, bronchiolitis, or pneumonia) occurring within the first 2 years of life were detected to affect the risk of AS. The role of passive smoking, and, in general, of indoor pollution in the development or worsening of respiratory symptoms and diseases is well recognized, and so is the impact of early respiratory infections in terms of enhanced airway sensitization and increased risk of AS later in life (Stazi et al., 2002b). With respect to parental history, its relevance as an individual etiological factor predisposing to respiratory disorders and allergies is also very well known; furthermore, it has been estimated that about 27% of the population risk in Italian children and adolescents could be attributed to this factor (SIDRIA-2 Collaborative Group, 2005).

Strong genetic effects on each of AS and HF were detected. The bivariate genetic analysis provided a heritability estimate of 92% for AS and 78% for HF, with the remaining 8% and 22% of the variance in liabilities due to unique environmental influences. Although there is a great deal of variation in the estimated genetic effects for AS and HF in twins, depending on the population and study design, the heritabilities obtained here appear somewhat higher compared to previous results, especially for AS. Only two twin studies show similar estimates for parentally reported AS: van Beijsterveldt & Boomsma (2007) find a heritability of 91% in 5-year-old Dutch twins, and Laitinen et al. (1998) report a heritability of 87% in 16-year-old Finnish twins, but from families where one of the parents is asthmatic. Since this is the first time that the heritability of AS and HF has been estimated in Italy, future studies on larger twin samples in the same age range may help to clarify whether the present estimates reflect the genetic factors truly affecting AS and HF liabilities in the Italian child and adolescent population. The estimates of genetic influence may, for example, be inflated if a violation of the assumption of equal environment for MZ and DZ twins occurs; however, the lack of zygosity differences in AS and HF prevalence rates in these data indicated a minor effect of being a MZ twin compared to a DZ twin with regard to environmental exposures associated with an increased risk of the examined conditions.

In agreement with other twin studies of AS and HF, no shared environmental influences were detected; in other words, factors related to home and family environment do not seem to contribute to the variance in AS and HF liabilities. Very little information was gained on this issue from the multivariate analysis; in fact, just two factors related to family environment were considered in this analysis, namely maternal and paternal smoking, with only the former being significant. A more precise definition of parental smoking behavior, as well as the inclusion of additional variables such as diet, indoor pollution, and management of the diseases would have been necessary to better characterize and evaluate the role of family environment in the risk of AS and HF. However, the result that shared environment has a minor impact seems to contrast with studies suggesting not only parental smoking, but also indoor pollution, domestic pets, and number of siblings as important risk factors especially for AS. Gene–environment interactions, not easily detectable within the classical twin design, could explain the lack of evidence for shared environmental effects in twin studies of AS and HF (see van Beijsterveldt & Boomsma, this issue). It is possible that some factors in the family environment have an effect on AS or HF only in subjects with a higher genetic predisposition to allergic diseases, or equivalently, that some genes affect AS or HF risk only in the presence of determined environmental conditions. For example, Colilla et al. (2003) found evidence for linkage between certain chromosomal regions and AS after stratifying subjects on the basis of cigarette smoke exposure during infancy. The small environmental impact emerging from this study should not be seen as incompatible with the widely recognized increasing secular trend of AS and HF, which is difficult to explain on a genetic basis only. Indeed, this trend may well be ascribed, at least partially, to gene–environment interaction effects, not modeled here.

The main purpose of the present study was to investigate the association between AS and HF at the individual level, and to establish the role of shared genetic and environmental factors in explaining this association. The degree of comorbidity between AS and HF was substantial, and cross-twin/cross-trait odds ratios and tetrachoric correlations in MZ versus DZ pairs suggested that genetic effects common to both diseases could explain the comorbidity. The estimated genetic correlation between AS and HF under the best fitting Cholesky model was .58. This value is similar to those reported in male (.52) and female (.65) Australian twins (Duffy et al., 1990), and is practically equal to that (.57) shown by a Danish twin study (Thomsen et al., 2006b). A slightly weaker genetic correlation (.47) was obtained in Dutch twins (van Beijsterveldt & Boomsma, 2007), whereas a

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much higher estimate (.90) was found in a Swedish twin sample (Lichtenstein & Svartengren, 1997). The significant genetic correlation obtained in the various twin studies, including the present one, is well in agreement with the results of a recent genome scan conducted in French families (Dizier et al., 2007), showing evidence for specific linkage of a locus in the 1p31 region with the comorbidity of AS and allergic rhinitis. The fact that the estimated genetic correlation was less than unity is itself noteworthy, and can be interpreted as an indication of the existence of additional genetic influences specific to AS or HF. In the present study, no evidence of overlapping environmental influences for AS and HF was detected, implying that the phenotypic correlation between the two conditions was entirely genetically mediated. This is not in agreement with the above-mentioned twin reports (Duffy et al., 1990; Thomsen et al., 2006b; van Beijsterveldt & Boomsma, 2007) that show significant environmental correlations ranging from .33 to .73, and is also probably not consistent with the importance of aeroallergens for both AS and HF; on the contrary, environmental effects specific to AS or HF, and unique to an individual may, for instance, represent the role of respiratory infections in the etiology of AS, as shown in the multivariate analysis.

The biggest limitation of this study is the small sample size, which seriously reduces the statistical power in a context where dichotomous traits are implicated. This may have affected the model selection process and, more importantly, did not make it feasible to address crucial issues such as sex or age differences in the estimated parameters; future studies on larger samples would allow testing of whether heritability of AS and HF, and their genetic and environmental correlation, are different between males and females, or between children and adolescents. A second caveat that must be kept in mind when interpreting these results is that parental report of AS and HF is completely relied upon, and objective verification of disease status is lacking in this study. Other limitations pertain to the assumptions of the twin model itself (equal environments assumption, no interaction or correlation between genes and the environment) that may be violated in the case of allergic diseases, leading to biased estimates of the genetic and environmental influences. In particular, the assumption that shared environmental exposures contribute equally to MZ and DZ within-pair similarity is crucial to these diseases. Genetic effects will be overestimated if environments predisposing to AS or HF are shared to a greater extent among MZ compared to DZ co-twins. A direct test of possible violations of the assumption could not be performed in this study, and therefore the impact of these violations in terms of excess concordance of MZ twins could not be precisely evaluated. Especially for AS, given the nature of relevant exposures (parental smoking, air pollution, mites, domestic pets), it is unlikely that MZ and DZ twins were differentially correlated with regard to these exposures when they were infants, while exposure similarity may have increased in MZ twins at older ages, if they spent more time together than did DZ twins.

Another issue that is important to be aware of is the broad phenotype definition adopted. Phenotypic heterogeneity represents a major challenge for the genetic dissection of several complex traits. In particular, AS includes both allergic and nonallergic subtypes that might have different genetic as well as environmental architectures in the population. The distinction of these subtypes was not possible in the present study, and this may have affected the results in the case of a significant heterogeneity.

In conclusion, this study showed that AS and HF are highly heritable in the Italian child and adolescent population. It also indicated that, to a large extent, AS and HF share a common genetic basis, with disease-specific genes also playing an important role, and environmental factors are not relevant to explain the comorbidity of the two conditions. These results can be of use in the counseling of families of allergic children and adolescents, and may encourage the search for pleiotropic genes affecting the individual predisposition to suffer from both AS and HF.

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