## Original article

# Variance components analyses of multiple asthma traits in a large sample of Australian families ascertained through a twin proband

**Background:** Intermediate phenotypes are often measured as a proxy for asthma. It is largely unclear to what extent the same set of environmental or genetic factors regulate these traits.

**Objective:** Estimate the environmental and genetic correlations between self-reported and clinical asthma traits.

**Methods:** A total of 3073 subjects from 802 families were ascertained through a twin proband. Traits measured included self-reported asthma, airway histamine responsiveness (AHR), skin prick response to common allergens including house dust mite (*Dermatophagoides pteronyssinus* [*D. pter*]), baseline lung function, total serum immunoglobulin E (IgE) and eosinophilia. Bivariate and multivariate analyses of eight traits were performed with adjustment for ascertainment and significant covariates.

**Results:** Overall 2716 participants completed an asthma questionnaire and 2087 were clinically tested, including 1289 self-reported asthmatics (92% previously diagnosed by a doctor). Asthma, AHR, markers of allergic sensitization and eosinophilia had significant environmental correlations with each other (range: 0.23–0.89). Baseline forced expiratory volume in 1 s ($FEV_1$) showed low environmental correlations with most traits. Fewer genetic correlations were significantly different from zero. Phenotypes with greatest genetic similarity were asthma and atopy (0.46), IgE and eosinophilia (0.44), AHR and *D. pter* (0.43) and AHR and airway obstruction (−0.43). Traits with greatest genetic dissimilarity were $FEV_1$ and atopy (0.05), airway obstruction and IgE (0.07) and $FEV_1$ and *D. pter* (0.11).

**Conclusion:** These results suggest that the same set of environmental factors regulates the variation of many asthma traits. In addition, although most traits are regulated to great extent by specific genetic factors, there is still some degree of genetic overlap that could be exploited by multivariate linkage approaches.

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There has been a strong interest in searching for susceptibility genes for asthma, driven by the prospect of better disease prevention, diagnosis and treatment. A common approach used for this is to measure different asthma-related traits, such as atopy, airway responsiveness and eosinophilia and subsequently test each trait individually for linkage to genetic markers genotyped throughout the genome. Regions of significant linkage are then followed up with higher resolution association studies. However, the initial stage of univariate linkage analysis has a number of caveats, including the lack of efficient methods to correct for multiple trait testing and the loss in power arising from the disregard of the additional information provided by multivariate data sets. This may result in both false-positive and false-negative linkage results. One alternative is to perform multivariate linkage analysis of related traits, a concept that has long been proposed (1–3) but that has only recently been implemented for the dissection of complex traits (4–6). Multivariate analysis not only avoids an inflated type I error as a result of multiple trait testing, as it can also provide greater statistical power to detect quantitative trait loci (QTL) than univariate analysis (7, 8).

Multivariate linkage analysis is appropriate to analyse traits that have common QTL. However, it has been demonstrated that multivariate analysis of traits that have a high genetic correlation is unlikely to be more powerful than univariate analysis (8). Thus, in order to guide the choice of traits for multivariate linkage, it is important not only to identify traits that have a common genetic aetiology but also to estimate the degree of genetic overlap.

Although a number of studies have addressed the first issue (9–12), few have provided estimates of the genetic

correlation between different asthma traits. Using a regression-based method, Palmer et al. (13) reported that the genetic overlap between asthma and baseline lung function was < 10%, whereas the overlap between airway responsiveness and total serum immunoglobulin E (IgE) was < 30% (14). Using bivariate variance components, Nystad et al. (15) estimated the genetic correlation between asthma and wheeze at 0.76.

In the present study, we describe a new sample of 802 Australian asthmatic families ascertained through a twin proband. The aim of this study was to use this large data set to estimate the genetic and environmental correlations between self-reported and clinical asthma traits through variance components methods.

## Methods

### Ascertainment of families

Two rounds of ascertainment were used to select families registered on the volunteer Australian Twin Registry (ATR) with at least one twin who had reported ever wheezing in his/her lifetime in previous studies (Fig. 1). The first round of ascertainment (1990–1992) has been described previously (11). As part of this first round, 863 twins from 443 families completed a detailed asthma questionnaire, attended clinical testing and donated blood for DNA extraction. The second round of ascertainment (1996–1998) involved two different selection approaches: first, extension of 164 round 1 families to include additional affected sibs or affected children of twins. Secondly, selection of 690 new families from five other ATR-based studies with at least one dizygotic (DZ) twin proband and any additional affected sibs, or at least one monozygotic (MZ) twin proband and additional affected sibs. As with round 1, 3824 participants from these 854 families were invited to complete a similar asthma and allergy questionnaire, donate blood or a buccal swab for DNA extraction and, if under the age of 50, to attend full clinical testing. Questionnaires were sent to 2270 participants of round 2 and 2100 (93%) from 511 families returned it completed. The outstanding 1554 participants who were not sent questionnaires were untraceable, deceased, overseas or unable to attend clinical testing. Of the 2100 participants with a completed questionnaire, 1965 (94%) also donated blood for genetic and/or haematological analyses and 1435 (68%) were clinically tested. One family consisted of one father and no other relatives and, thus, was dropped from further analysis. When the samples from both rounds were combined, the overall sample consisted of 3073 participants from 802 families: 974 participated exclusively in round 1 (292 families), 1846 exclusively in round 2 (359 families) and 253 (151 families) in both rounds. Overall, 2716 participants completed one or both asthma questionnaires, 2852 donated blood and 2087 were clinically tested. Complete data were available for 1969 participants.

### Evaluation of phenotypes

The following procedures were performed with the appropriate ethical approval of all institutions involved.

*Questionnaire administration*. The questionnaire used in round 1 was designed to validate the diagnosis of asthma and to obtain data on respiratory symptoms, environmental exposures relevant to respiratory disease and family history of asthma (11). The round 2 questionnaire included the key items of the first questionnaire plus a section for the diagnosis of chronic bronchitis (16). There were two versions of this questionnaire: one for adults (aged > 16 years) and one to be completed by parents of children (aged ≤ 16 years). A participant was considered affected for asthma if he or she answered 'Yes' or 'Yes, told to me by a doctor' to the question 'Have you ever suffered from asthma or wheezing'? and 'Yes' to the question 'Have you ever taken any medicine for asthma or wheezing'? A participant was considered affected for doctor asthma if he or she answered respectively 'Yes, told to me by a doctor' and 'Yes' to the previous two questions. Asthma and doctor asthma were highly correlated (tetrachoric correlation 0.99). For this reason, familial correlations, heritabilities and bivariate analyses are presented for asthma only.

*Lung function test*. Forced vital capacity (FVC) and forced expiratory volume in 1 s ($FEV_1$) were measured with the subject standing, using the vitalograph 5 wedge bellows spirometers (Vitalograph Ltd, Buckingham, UK) in round 1 and the Jaeger Master Scope spirometer (Viasys, Hoechberg, Germany) in round 2, both compliant with the 1995 ATS requirements (17). The measurements were repeated to a maximum of six times until a curve reproducible to within 200 ml was obtained. Both $FEV_1$ and $FEV_1/FVC$ were normalized and adjusted for significant ($P < 0.05$) covariates, which included age, gender, height ($FEV_1$) and ever smoking ($FEV_1/FVC$); the adjustment of covariates was performed prior to estimation of familial correlations or as part of variance components analyses. Residual kurtosis was 1.5 for $FEV_1$ and 0.43 for $FEV_1/FVC$.

*Airway histamine challenge test*. The test was performed in both rounds after baseline spirometry according to the protocol of Yan et al. (18) as modified by Woolcock (19). Each dose of saline or histamine was administered via a DeVilbiss No. 45 nebuliser with a hand pump (30% of round 2) or a mechanical (70% of round 2) dosimeter, calibrated at first use. The histamine provocation test was performed seated on all subjects with a $FEV_1 \geq 60\%$ (round 1) or > 70% (round 2) as predicted by the reference values of Quanjer et al. (20) and Polgar and Promadhat (21), respectively. Each consecutive dose was given at approximately 2-min intervals until either a cumulative dose of 7.8 μmol (round 1) or 2.605 μmol (round 2) of histamine was reached or a fall in $FEV_1$ of ≥ 20% of the postsaline inhalation $FEV_1$ was observed. Participants with a baseline $FEV_1$ < 60% (round 1) or ≤ 70% (round 2) of the predicted value underwent a bronchodilator test, which consisted of 400 μg of salbutamol administered over 60 s via a large spacer. An airway histamine responsiveness (AHR) test was considered positive if the participant experienced a drop in $FEV_1$ of ≥ 20% (hand-held pump) or ≥ 25% (mechanical dosimeter) of the postsaline inhalation $FEV_1$ after the last dose of histamine given. This allowed for the observed difference in histamine output between both methods. A participant with baseline airway obstruction was considered AHR-positive if the bronchodilator test induced an increase in $FEV_1$ of ≥ 15%.

*Skin prick test*. The skin prick test (SPT) was performed using a Hollister-Steier prick lancetter on the volar forearm. The longest and the perpendicular diameters of the response wheal were measured after 10 (round 1) or 15 min (round 2) to the nearest millimetre and the mean wheal diameter estimated. Eleven common allergens were tested in each round: cockroach mixture, European house dust mite *Dermatophagoides pteronyssinus* (*D. pter*), American house dust mite *D. farinae* (round 2), house dust (round 1), cat dander, dog dander, Canary grass (round 1), Timothy grass (round 1), southern grasses mixture, Rye grass, *Aspergillus* spp. mixture,
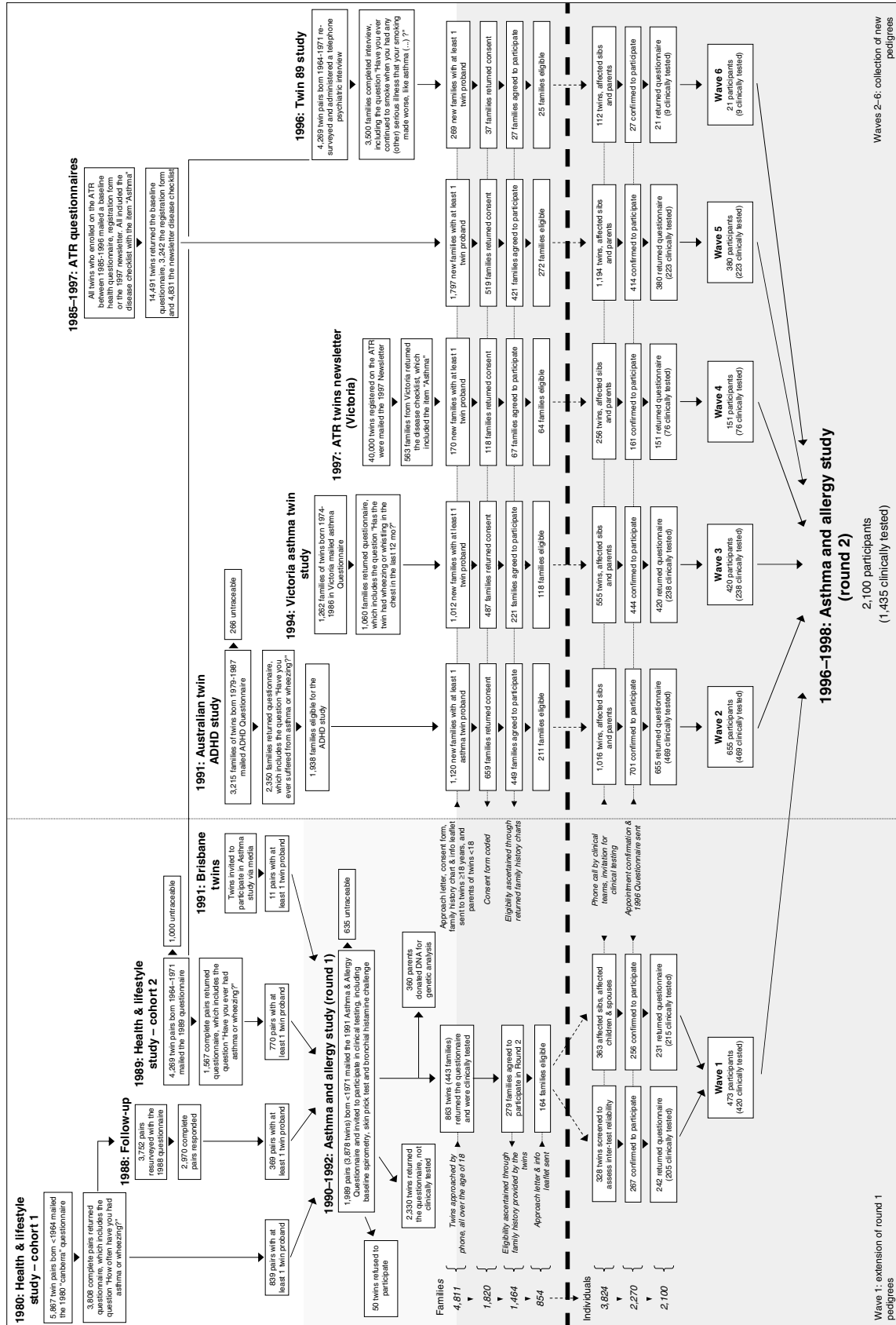
*Figure 1.* Ascertainment scheme used to select 802 families registered with the Australian Twin Registry (ATR) with at least one asthmatic twin proband. ATR families are representative of the Australian population for a number of traits, including asthma (28).

*Alternaria* spp. mixture, cows milk (round 2) and egg white (round 2). Histamine-buffered saline solution (3.125 mg/ml) was used as the positive control and glycerine as the negative control. A participant was defined as atopic if at least one of the 11 allergens tested elicited a mean wheal diameter at least 3 mm greater than the negative control wheal. Similarly, a SPT to *D. pter* was considered positive if the mean wheal diameter was 3 mm greater than the negative control wheal.

*Measurement of serum total immunoglobulin E (IgE) levels and eosinophil counts.* Total IgE was estimated in both rounds by standard enzyme immunoassay; all samples were assayed in a single batch at the conclusion of each round. Serum eosinophil counts were performed by flow cytometry for round 2 samples only. IgE was log-transformed and eosinophils normalised using an inverse normal transformulation; both were adjusted for age and gender. Residual kurtosis was −0.36 for IgE and −0.07 for eosinophils.

## Genotyping and pedigree error checking

DNA was extracted from blood or buccal swabs according to standard procedures (22). DNA from 2760 consenting participants from 735 families was genotyped at 108–440 microsatellite markers at Sequana Inc. as described by Hall and Nanthakumar (23). This genotyping data were used to verify familial relationships using GRR (24): 68 of 735 (9%) families were found to have pedigree errors, mostly due to sample duplications (4.4%), incorrect self-reported paternity (2.1%) or incorrect self-reported zygosity (1.6%). These errors were corrected prior to analyses.

## Familial correlations and heritability estimates

Pearson (continuous traits) and tetrachoric (affection traits) correlations were calculated with version 8.02 of SAS. Continuous traits were adjusted for significant covariates (defined above) prior to the estimation of familial correlations. Heritabilities for both quantitative and affection traits were estimated by maximum likelihood univariate variance components analysis using SOLAR 2.1.4 (25). Briefly, this analysis decomposed the expected phenotypic covariance between relatives into a component due to polygenic additive genetic variance ($\sigma_A^2$), dominance genetic variance ($\sigma_D^2$), environmental variance due to environmental factors shared between siblings ($\sigma_C^2$) and a component of variance because of environmental factors not shared between relatives ($\sigma_E^2$). The significance of the parameters in the model was then tested by the likelihood ratio chi-square test $2[\ln(L_{H_0}) - \ln(L_{H_1})]$ with $n$-df, where $L_{H_0}$ is the likelihood of the data under a saturated model $H_0$, $L_{H_1}$ the likelihood under a nested submodel $H_1$ where the parameters being tested were fixed to zero, and $n$ the difference in the number of parameters estimated by $H_0$ and $H_1$. Significant covariates (defined above) were retained in the means model and proband ascertainment correction implemented. A proband was defined as a twin who reported or was reported by the parents to have a history of asthma or wheeze in either the round 1 or the round 2 questionnaires.

## Bivariate analysis of asthma traits

Maximum likelihood variance components analysis was performed to estimate cross-trait correlations and to partition these into a component due to both traits sharing the same set of latent additive genetic factors and to a component due to both traits sharing the same set of latent-specific environmental factors. For this purpose, we estimated the genetic and environmental correlations for any two traits using the multivariate modelling algorithm implemented in a prerelease version of SOLAR 3.0.3 and outlined in Ref. (26). The genetic correlation ($\rho_A$) is defined here as the correlation between the latent additive genetic factors, whereas the environmental correlation ($\rho_E$) is defined as the correlation between the latent environmental factors not shared between relatives. For eight traits, there are 28 possible two-trait combinations and, therefore, 28 bivariate analyses were performed. Each bivariate analysis estimated six parameters of interest: $\sigma_A^2$ and $\sigma_E^2$ for each trait and $\rho_A$ and $\rho_E$ between the traits. In addition, the significance of $\rho_A$ and $\rho_E$ was tested as described above. To correct for multiple testing, the significance threshold for these parameters was set at $P = 0.05/28 = 0.0018$. As with the univariate approach, the effects of significant covariates and proband ascertainment correction were included in the analyses. The overall cross-trait correlation for any two traits $t_i$ and $t_j$ was then calculated as $r(t_i, t_j) = \sqrt{\sigma_A^2(t_i)} \times \rho_A(t_i, t_j) \times \sqrt{\sigma_A^2(t_j)} + \sqrt{\sigma_E^2(t_i)} \times \rho_E(t_i, t_j) \times \sqrt{\sigma_E^2(t_j)}$. In this formula, $\sqrt{\sigma_A^2(t_i)} \times \rho_A(t_i, t_j) \times \sqrt{\sigma_A^2(t_j)}$ corresponds to the component of the cross-trait correlation that results from traits $t_i$ and $t_j$ sharing the same set of latent additive genetic factors and $\sqrt{\sigma_E^2(t_i)} \times \rho_E(t_i, t_j) \times \sqrt{\sigma_E^2(t_j)}$ corresponds to the component of the correlation that results from both traits sharing the same set of latent-specific environmental factors. For most traits $\sigma_D^2$ and $\sigma_C^2$ did not significantly influence trait variance in the univariate analyses. For this reason, these parameters were fixed at zero in bivariate analyses. At present, SOLAR cannot test the significance of $\rho_A$ or $\rho_E$ when analysing more than two traits. In addition, the simultaneous analysis of continuous and affection traits restricted the number of phenotypes SOLAR could analyse and still achieve convergence to only four. Nonetheless, multivariate analyses of up to four traits were performed with parameter estimates that correlated > 0.99 with those obtained with the corresponding bivariate analyses (data not shown). For this reason, only results from the bivariate analyses are presented.

## Results

### Participants

Overall, 802 families ascertained via at least one twin proband participated in this study. These families were of arbitrary size, ranging from 1 to 10 relatives (mean 4) and from 1 to 3 generations (mean 2). Over 75% of the families consisted of two twins (21%), two twins and one or two parents (36%) or two twins, two parents and one sib (19%). There were 817 sibships available for analysis, of which 533 (65%) were of size 2, 211 (26%) of size 3, 63 (8%) of sizes 4 and 10 (1%) of size 5 or larger. The families included 490 (61%) DZ twins and 312 (39%) MZ twins. Ancestry was reported by 511 families: 72% reported being of European ancestry, 3% of native Australian or New Zealander ancestry, 1% American or Asian, 9% of mixed ancestry and 15% reported ambiguous or unknown ancestry. Data were collected from 3073 relatives from these 802 families, including 1289 self-reported asthmatics (Table 1). Of these, 35% used bronchodilators once a week or more often in the last 12 months and 30% inhaled steroids; 23% reported having previously been admitted to hospital as a result

of an asthma attack. These figures were similar for the 919 probands. Males reported a mean age at onset of asthma of 8.1 and females of 11.9 ($P < 0.001$).

Familial correlations and heritabilities

Most phenotypes had a high reliability, with a test–retest correlation $>0.8$ (Table 2). Typical familial correlations, uncorrected for ascertainment, ranged from 0.15 to 0.39 for parent–offspring pairs, 0.26 to 0.44 for sib pairs, 0.17 to 0.64 for DZ twin pairs and 0.63 to 0.90 for MZ pairs. The exception was asthma, which, as expected, showed low familial correlations as a result of the selective ascertainment procedure used. Heritabilities corrected for ascertainment ranged from 0.28 for eosinophils to 0.71 for $FEV_1$. The ascertainment correction had a negligible effect in variance components modelling for all traits other than asthma.

Bivariate analyses of asthma traits

All traits were found to be regulated by additive genetic factors but also by environmental factors not shared between relatives. We then investigated the extent to which different traits shared the same set of additive genetic factors or the same set of specific environmental factors (Table 3). First, large environmental correlations were observed between AHR and atopy (0.67), AHR and

asthma (0.63) and between AHR and eosinophils (0.60). By contrast, the two lung function measurements showed low environmental correlations with most traits, particularly with atopy, *D. pter* and IgE. Secondly, when compared with the environmental effects, fewer genetic correlations were significantly different from zero. As expected, atopy, *D. pter* and IgE showed a considerable degree of genetic overlap but the three genetic correlations were significantly different from 1 ($P < 0.0018$). This excluded complete pleiotropy between these traits. As observed with the environmental factors, the genetic correlations between $FEV_1$ and atopy, *D. pter* and IgE were close to zero. Finally, eosinophils had a significant genetic correlation with IgE (0.44) but with no other trait. Multivariate analyses of up to four traits were also performed and parameter estimates were equivalent to those obtained with the bivariate analyses (not shown). The genetic and environmental correlations were then combined with the trait heritabilities to estimate the overall cross-trait correlations (Fig. 2). As expected from the low genetic and environmental correlations, $FEV_1$ showed low cross-trait correlations with all traits other then $FEV_1/FVC$. By contrast, AHR showed high and comparable cross-trait correlations with most traits, with equivalent contributions from genetic and environmental effects shared between traits. The exception was eosinophils. The correlation observed between this trait and AHR, or between this trait and other traits other than

Table 1. Characteristics of study participants in the full sample and for asthmatics and probands only

| | Full sample | | | | | | Subsamples | |
|---|---|---|---|---|---|---|---|---|
| | Parents | Twins | Sibs | Children | Spouses | Total | Probands | Asthmatics |
| Subjects total | 1011 | 1568 | 376 | 94 | 24 | 3073 | 919 | 1289 |
| With questionnaire (%) | 65 | 100 | 100 | 100 | 100 | 88 | 100 | 100 |
| Clinically tested (%) | 8 | 98 | 98 | 99 | 54 | 68 | 98 | 87 |
| Donated blood (%) | 90 | 94 | 96 | 89 | 88 | 93 | 95 | 94 |
| Full testing (%) | 8 | 92 | 94 | 89 | 54 | 64 | 93 | 82 |
| Female sex (%) | 56 | 58 | 50 | 49 | 17 | 56 | 59 | 57 |
| Age, mean (range) | 53 (34–83) | 29 (7–76) | 24 (7–50) | 22 (7–43) | 51 (38–67) | 36 (7–83) | 28 (7–76) | 30 (7–79) |
| Ever smoker* (%) | 48 | 32 | 22 | 29 | 58 | 34 | 31 | 31 |
| Current smoker (%) | 12 | 17 | 10 | 11 | 13 | 14 | 16 | 13 |
| Asthma (%) | 28 | 47 | 77 | 83 | 26 | 47 | 80 | 100 |
| Doctor asthma (%) | 26 | 43 | 73 | 81 | 25 | 44 | 72 | 92 |
| Positive AHR test† (%) | 15 | 43 | 39 | 42 | 0 | 41 | 54 | 50 |
| Atopy (%) | 60 | 68 | 75 | 77 | 38 | 69 | 75 | 75 |
| Positive SPT to *D. pter* (%) | 39 | 54 | 63 | 66 | 8 | 56 | 62 | 63 |
| Mean $FEV_1$ [1] | 3.0 | 3.1 | 3.2 | 3.2 | 3.5 | 3.1 | 3.0 | 3.0 |
| Mean $FEV_1$/FVC | 0.80 | 0.83 | 0.83 | 0.81 | 0.79 | 0.82 | 0.82 | 0.82 |
| $FEV_1 \leq 0.7$ predicted (%) | 16 | 7 | 7 | 9 | 0 | 7 | 8 | 9 |
| IgE [IU/ml; geometric mean (*N*)] | 55 (440) | 81 (1344) | 131 (309) | 168 (69) | 52 (17) | 82 (2179) | 101 (785) | 109 (1224) |
| Eosinophil [$\times 10^9$/l; geometric mean (*N*)] | 0.19 (542) | 0.25 (831) | 0.28 (347) | 0.35 (74) | 0.18 (16) | 0.24 (1810) | 0.27 (519) | 0.28 (1042) |

*Sample size for ever smoker, current smoker, asthma and doctor asthma corresponds to the number of subjects with questionnaire.
†Sample size for AHR, atopy, *D. pter*, $FEV_1$ and $FEV_1$/FVC corresponds to the number of subjects that were clinically tested.
AHR, airway histamine responsiveness; $FEV_1$, *D. pter*, *Dermatophagoides pteronyssinus*; forced expiratory volume in 1 s; FVC, forced vital capacity; SPT, skin prick test; IgE, immunoglobulin E.

Table 2. Test–retest and familial correlations for asthma and related traits

| | Trait correlations* (N pairs) | | | | | Variance components† | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Test–retest‡ | Parent–offspring pairs | Sib pairs | DZ twins | MZ twins | $\sigma_A^2$ | $\sigma_D^2$ | $\sigma_C^2$ | $\sigma_E^2$ |
| Asthma | 0.99 (225) | −0.01 (1795) | 0.14 (1251) | −0.16 (430) | 0.34 (263) | 0.38 | – | – | 0.62 |
| AHR | 0.77 (110) | 0.39 (185) | 0.32 (675) | 0.32 (246) | 0.63 (169) | 0.58 | – | – | 0.42 |
| Atopy | 0.95 (210) | 0.25 (324) | 0.44 (1232) | 0.38 (439) | 0.78 (281) | 0.49 | – | 0.16 | 0.35 |
| *D. pter* | 0.93 (210) | 0.05 (312) | 0.33 (1225) | 0.20 (436) | 0.76 (280) | 0.30 | 0.32 | – | 0.38 |
| $FEV_1$ | 0.87 (209) | 0.15 (303) | 0.37 (1199) | 0.64 (435) | 0.90 (278) | 0.71 | – | – | 0.29 |
| $FEV_1/FVC$ | 0.67 (208) | 0.25 (305) | 0.28 (1196) | 0.37 (434) | 0.65 (278) | 0.51 | – | – | 0.49 |
| IgE | 0.87 (179) | 0.24 (1125) | 0.26 (964) | 0.18 (355) | 0.67 (243) | 0.52 | – | – | 0.48 |
| Eosinophils | – | 0.17 (1362) | 0.32 (898) | 0.17 (230) | 0.59 (113) | 0.28 | – | 0.14 | 0.58 |

*Tetrachoric (affection traits) and Pearson (continuous traits) correlations, uncorrected for ascertainment. Continuous traits were normalized and, with the exception of test–retest, adjusted for significant covariates (see Methods).

†Estimated from univariate variance components analyses with ascertainment correction. All traits were adjusted for significant covariates (see Methods). Variance components estimates are reported according to the best fitting model.

‡253 participants were tested in both phases (mean age 36, range: 19–61). The mean timespan between test and retest was 4.8 years (range: 4.2–6.2).

AHR, airway histamine responsiveness; *D. pter*, *Dermatophagoides pteronyssinus*; $FEV_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, immunoglobulin E; DZ, dizygotic; MZ, monozygotic.

$\sigma_A^2$, additive polygenic variance; $\sigma_D^2$, dominance genetic variance; $\sigma_C^2$, variance due to environmental factors shared between siblings; $\sigma_E^2$, variance due to environmental factors not shared between relatives.

Table 3. Environmental (above main diagonal) and genetic (below main diagonal) correlations between asthma traits

| | Environmental and genetic correlations* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Asthma | AHR | Atopy | *D. pter* | $FEV_1$ | $FEV_1/FVC$ | IgE | Eosinophil |
| Asthma | | **0.63** | **0.44** | **0.49** | **−0.31** | **−0.28** | **0.23** | **0.30** |
| AHR | 0.24 | | **0.67** | **0.60** | −0.32 | **−0.38** | **0.57** | **0.60** |
| Atopy | **0.46** | 0.35 | | **0.89** | 0.02 | −0.22 | **0.71** | **0.55** |
| *D. pter* | **0.38** | 0.43 | 0.76 | | −0.06 | **−0.27** | **0.58** | **0.52** |
| $FEV_1$ | −0.24 | −0.20 | −0.05 | −0.11 | | **0.42** | 0.04 | 0.11 |
| $FEV_1/FVC$ | −0.24 | **−0.43** | **−0.22** | −0.22 | 0.33 | | **−0.25** | −0.09 |
| IgE | **0.41** | 0.33 | 0.61 | 0.58 | −0.18 | −0.07 | | **0.38** |
| Eosinophils | 0.24 | 0.13 | 0.27 | 0.19 | −0.21 | −0.16 | **0.44** | |

*Estimated from bivariate variance components analyses with ascertainment correction.

Correlations significantly different from zero are highlighted in bold ($P < 0.0018$). A negative correlation indicates that environmental or genetic factors induced a negative phenotypic correlation between the two traits. Affection traits were coded as 0 (unaffected) and 1 (affected).

All traits were adjusted for significant covariates (see Methods).

AHR, airway histamine responsiveness; *D. pter*, *Dermatophagoides pteronyssinus*; $FEV_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, immunoglobulin E.

IgE was mostly due to environmental factors shared between traits.

## Discussion

Asthma has long been recognized to be heritable (27). Large population-based twin studies have estimated the heritability of asthma to be between 60% and 73% (28–30). Similarly, most asthma intermediate phenotypes, such as atopy and total serum IgE, have been shown to be moderately to highly heritable in population-based samples (14, 31). The present study involved selective ascertainment of families through twins who had reported asthma or wheezing in previous screening questionnaires. Approximately 70% of subjects were found to be atopic, 44% had previously been diagnosed with asthma

by a doctor and 40% had a positive AHR test. As a result of this selection procedure, self-reported asthma displayed very low familial correlations. On the contrary, the bias introduced by the ascertainment did not extend to any of the other asthma-related phenotypes tested. This has been shown previously (32, 33) and it is likely to reflect the low to moderate cross-trait correlations observed between asthma and related phenotypes (range: −0.25 to 0.42). Also, the ascertainment bias observed for asthma was largely adjusted by the correction used when fitting variance components models. Heritabilities corrected for ascertainment ranged from 28% (eosinophils) to 71% ($FEV_1$).

We performed bivariate variance components analyses to estimate the genetic and environmental correlation between asthma traits. Similar analyses have been performed previously to estimate the genetic and
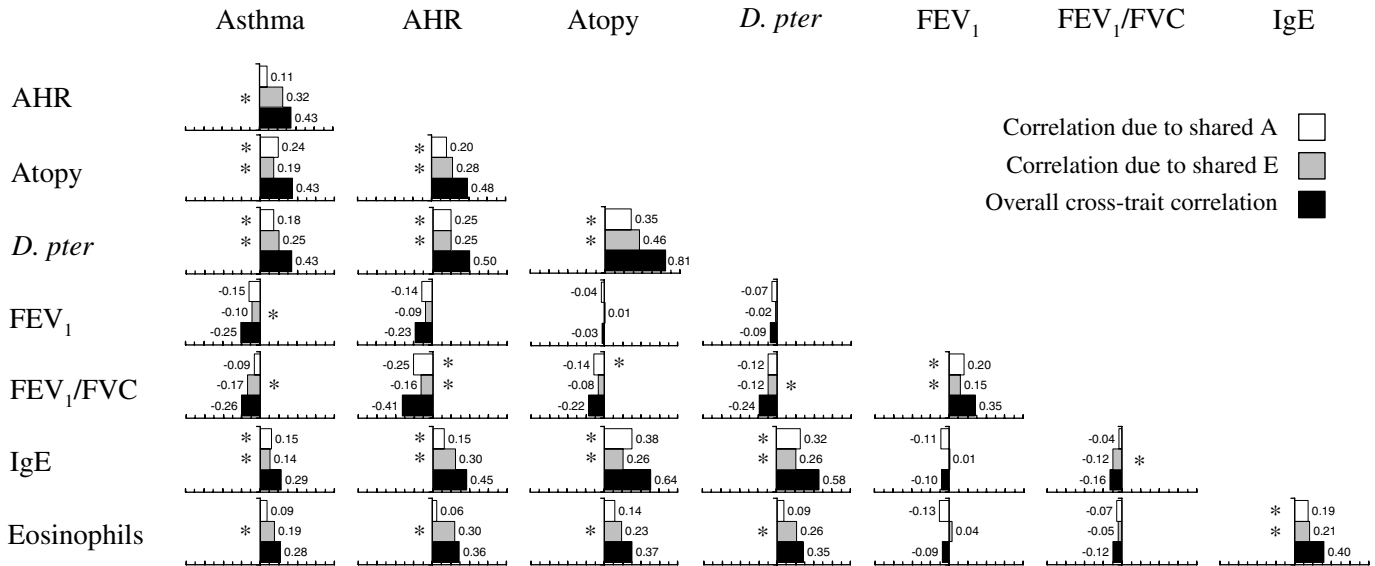
*Figure 2.* Maximum likelihood cross-trait correlations for eight asthma phenotypes, corrected for ascertainment. The correlation between any two traits was decomposed into a component due to both traits sharing the same set of latent additive genetic factors (A) and to a component due to both traits sharing the same set of latent-specific environmental factors (E). The cross-trait correlations estimated by this maximum likelihood approach were highly correlated (0.98) with the corresponding Pearson (continuous–continuous traits), tetrachoric (affection–affection traits) or biserial correlations (continuous–affection traits) estimated with SAS (not shown). *Indicates traits with environmental or genetic correlations significantly different from zero.

AHR, airway histamine responsiveness; *D. pter*, *Dermatophagoides pteronyssinus*; FEV, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, Immunoglobulin E.

environmental overlap between different allergic diseases (28, 34) and between different allergic diseases and their symptoms (15), but not between different asthma intermediate phenotypes. Our analyses showed that many asthma traits had significant environmental correlations with each other, often $> 0.5$. This is consistent with the hypothesis that the same set of environmental factors regulates the variation of many asthma traits. This hypothesis is also supported by the observation that MZ twin pairs discordant for asthma are also likely to be discordant for other traits and that the case for the different traits is often the same twin (11, 12). The main exception in our study was $FEV_1$, which showed very low environmental correlations with atopy, *D. pter* and IgE. This strongly suggests that the environmental factors not shared between relatives that regulate variation in $FEV_1$ are entirely distinct from the environmental factors that regulate variation in allergy status.

The bivariate analyses also showed that only a comparatively smaller number of traits had genetic correlations significantly different from zero. As expected, the most significant of these were between atopy, *D. pter* and IgE, although there was no evidence for complete pleiotropy. This implies that although a large proportion of genetic factors were shared between these traits, there were still specific genetic pathways that regulated the expression of each trait individually. Palmer et al. (14) reported similar results when comparing the genetic overlap between total and specific IgE with regression

models. They also found that the genetic determinants of total IgE and airway responsiveness exhibited $< 30\%$ sharing, which is consistent with our estimate of the genetic correlation between these two traits (0.33). In addition, in the present study, both asthma and AHR showed some degree of genetic overlap with atopy, thus supporting the concept that these three traits share a group of genetic risk factors (10). Interestingly, Eosinophils and IgE were also found to have a significant genetic correlation, which could explain the recent report of genetic linkage of eosinophilia to 2q33 (35), a region previously linked to atopy (36). Finally, there was very limited or no genetic overlap between $FEV_1$ and any other trait other than $FEV_1$/FVC. Thus, to a great extent, baseline $FEV_1$ was genetically distinct from Asthma, AHR and Atopy, which is also consistent with previous findings (13).

How can these results be used to guide the choice of traits for multivariate linkage analysis? The joint analysis of multiple correlated traits has been demonstrated to increase the power of linkage analysis, both with real (4–6) and simulated (7, 37) data sets. However, not all correlated traits provide the same improvement in power when analysed with multivariate methods. Traits with strong genetic correlations provide increased power only when the pleiotropic QTL and the residual sources of variation induce cross-trait correlations in opposite directions (7, 8, 38). As this may be uncommon, multivariate analysis of traits such as atopy and *D. pter* is

unlikely to be justified. Similarly, traits with minimal genetic correlations, such as $FEV_1$ and Atopy, are unlikely to have common QTL and so are also unlikely to benefit from being analysed with multivariate methods. Therefore, the traits most likely to provide increased power for multivariate linkage are traits with low to moderate genetic correlations, such as Asthma, AHR, Atopy, IgE and $FEV_1/FVC$. The observed genetic correlations between these imply that although each trait is regulated to great extent by specific genetic factors, there is still some degree of genetic overlap that could be exploited by multivariate approaches. Nonetheless, we note that the moderate to high environmental correlations observed between some of these traits may prove counterproductive.

Finally, a number of limitations need to be born in mind when examining the conclusions of the present study. First, the clinical protocol differed in a few aspects between the two rounds of testing. However, these differences were minor and are unavoidable when pooling data from different samples or from different studies. Secondly, the definition of asthma used here may not fully represent the clinical entity of asthma. Nonetheless, it was our intention to contrast a self-reported asthma phenotype with more objective clinical phenotypes. Thirdly, all subjects that were tested twice in this study were over the age of 18. As the majority of our sample (75%) was within this age group, this is unlikely to have greatly overestimated the reliability of the asthma traits measured. Lastly, the Bonferroni correction applied when testing the significance of genetic and environmental correlations may have been conservative. The parameter estimates were nonetheless unaffected by this correction and should be assessed in conjunction with the reported significance.

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