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Association and interaction analyses of eight genes under asthma linkage peaks

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For Peer Review

1. Supplementary Methods

Ascertainment of families for the asthma VU study

The families who participate in the asthma VU study are a sample of a larger number of Dutch twin families who participate in an ongoing survey study of health-related behavior (1,2). The first two surveys on health and lifestyle were sent in 1991 and 1993 to adolescent twins and their parents. Subjects for the asthma study were selected from families who returned the first 2 questionnaires.

Twin families were recruited by asking all city councils in The Netherlands for addresses of twins aged 13-22 years. An initial positive response was received from 252 city councils that supplied 3859 addresses; 177 addresses were available from other sources. After contacting these 4036 families by letter, 2375 twin families indicated that they were willing to complete a questionnaire on health and lifestyle and 1700 families returned these questionnaires in 1991. Data from 3 families were entered twice by mistake, leaving a total of 1697 families. In 1993 a second questionnaire was mailed to the 4036 families that had been contacted before and to 1987 new families. Additional addresses of new twin families were obtained from city councils which had reacted positively to our request, but were not able to furnish addresses in time for the first wave of data collection. The new addresses included several of the larger cities in the Netherlands. At the second measurement occasion we obtained questionnaires from 1974 families; 959 families participated for the second time; 877 families came from the new addresses; 138 families were contacted before in 1991 but had not responded at the time. In total we have studied 2712 families measured at two different occasions, with 959 families participating twice.

Age of the twins was between 12-25 years. The mean age of the twins at the first measurement occasion was 17.7 years (SD = 2.3), 4% of this sample was younger than 14 years and 7% was 21 years or older. The mean age of the twins that participated for the first time in 1993 was 16.0 years (SD = 2.7). In this group 29% of the sample was younger than 14 years and 7% was 21 years or older. Zygosity of the twins was determined by DNA fingerprinting.

The questionnaires contained items about asthma, bronchitis and allergies, as well as questions about drinking, smoking, socio-economic status, religion and a number of personality factors.

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These questions were answered by the twin pair and by both their biological parents. The prevalence of asthma and allergies in twins based on first questionnaire was:

| | Asthma | Allergy | Correlation Asthma-allergy |
|-------|--------|---------|----------------------------|
| Boys | 13% | 14% | .51 |
| Girls | 10% | 17% | .52 |

The tetrachoric twin correlations (standard errors) were (see also Supplementary ref. 3):

| | N(pairs) | Asthma | Allergy |
|-----|----------|------------|------------|
| MZM | 243 | .77 (.075) | .64 (.100) |
| DZM | 233 | .19 (.155) | .15 (.148) |
| MZF | 324 | .77 (.072) | .43 (.110) |
| DZF | 300 | .44 (.126) | .22 (.117) |
| DOS | 448 | .25 (.116) | .19 (.095) |

Families were selected to participate in the asthma study if one of the twins or one of their parents indicated that they had asthma. The selection was also based on age of the twins (older than 18 years). Selection based on the first questionnaire indicated 146 families that met these criteria. A letter was sent to these families asking them to participate in the asthma study; 53 families were willing to participate and of these, 42 were studied in the hospital. The second selection was carried out on the new families who returned the second questionnaire. Based on the same criteria as before, 57 new families living near Amsterdam were tested.

Clinical protocol used in the asthma VU study

Questionnaire administration. The questionnaire is based on the WHO and CARA-TNO-questionnaires. Both were extensively investigated in the Netherlands. Questions concerned symptoms such as cough,

periods of bronchitis, wheezing, dyspnoe, attacks of asthma, rhinitis, pneumoniae (doctor diagnosis) and asthma (doctor diagnosis). Information about smoking habits, medication, work, and family were recorded. Most questions have a good reproducibility ($\kappa > 0.5$). For the present analyses, participants with a self-reported doctor diagnosis of asthma were considered affected for the Asthma trait.

Lung function test. FVC and FEV₁ are measured with the VMAX22 pneumotachograph of Sensor Medics. The measurements are according guidelines of the ATS. Twice a day, calibration was carried out with a calibration pump of 3 ltr and an automatic calibration program. Calibration reports are available.

Airway methacoline challenge test. The test used the method of tidal breathing for 2 minutes and meets the guidelines of standardization. After baseline measurements of pulmonary function, individuals inhaled nebulized NaCl 0.9% from a DEVILBISS 646 nebulizer (output 0.13 ml/min, flow of 5.5 l/min, aerosol about 5 micron). If the decrease in FEV₁ was less than 10%, the test was performed. Sequential aerosols of metacholine was given in concentrations of 0.15, 0.3, 0.6, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 mg/ml. Each concentration was inhaled for 2 minutes, after each challenge FEV₁ maneuvers were performed. The metacholine concentration at which there was a decrease in FEV₁ of 20% (compared to baseline) was taken as the threshold value (PC_{20m}). The determination of PC_{20m} is calculated by linear interpolation of the last two points of the concentration-dose curve (4). For the present analyses, a bronchial hyperresponsiveness (BHR) test was considered positive if the participant experienced a drop in FEV₁ of 20% after the last dose of methacoline given.

Bronchial Reversibility Test. Following the methacoline challenge test, a bronchial reversibility test with Salbutamol was performed after FEV₁ returned to baseline value (mostly after one hour). Subjects inhaled four times 200mcg of salbutamol (MDI) through a chamber (volumatic). After 15 minutes FEV₁ maneuvers were performed. When there is an improvement of FEV₁ of 200 ml or 12%, reversibility is significant according to ATS criteria.

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Skin Prick Test. Tests were done to grass, trees, weeds, house dust-mite, other mites, cat, dog, horse, other animals (rabbit), feathers (birds), *Aspergillus fumigatus*, *Cladosporium herbarum*, *Alternaria altenarium*, and *Candida albicans*. The test was considered positive when a response was > 2mm greater than the negative control. A participant was defined as atopic if one or more of the following criteria were met: (1) total IgE > 2 SD above normal of the Dutch population; (2) raised (>100 U/l) serum IgE against molds, cat, grass or English rawweed; or (3) positive skin prick test.

Measurement of total and specific IgE levels. Total IgE was measured using Phadezym Prist, Pharmacia ltd. Specific IgE against molds, cat, grass and English rawweed was detected by Pharmacia Cap Sweden.

For Peer Review

2. Supplementary Tables

Supplementary Table 1. Characteristics of the study participants for the four individual studies.

| | AS | VU | BH | TM |
|---------------------------------------|-------------|------|------|------|
| N individuals | | | | |
| Genotyped | 2493 | 353 | 532 | 2092 |
| Clinically tested | 1703 | 360 | 457 | 660 |
| Both | 1697 | 353 | 457 | 660 |
| Clinical characteristics ^a | | | | |
| Females, % | 54.5 | 53.3 | 59.1 | 51.7 |
| Mean age, years | 28.8 | 36.1 | 34.7 | 12 |
| Asthma, % | 54.7 | 19.3 | 58.9 | - |
| BHR, % | 40.0 | 22.1 | 26.3 | - |
| Atopy, % | 69.2 | 41.6 | 55.1 | - |
| Dpter, % | 56.5 | 23.8 | 24.5 | - |
| Ever smoker, % | 30.2 | 26.9 | 47.5 | - |
| N families analysed ^b | | | | |
| N Parents | N offspring | | | |
| 2 | 3 or more | 139 | 4 | 0 |
| 2 | 2 | 146 | 67 | 152 |
| 2 | 1 | 37 | 6 | 69 |
| 1 | 3 or more | 29 | 1 | 0 |
| 1 | 2 | 66 | 10 | 98 |
| 1 | 1 | 38 | 1 | 41 |
| 0 | 3 or more | 28 | 0 | 0 |
| 0 | 2 | 146 | 1 | 156 |
| 0 | 1 | 106 | 9 | 147 |
| Total | | 735 | 99 | 303 |
| | | | | 427 |

^a Figures are based on individuals that were both genotyped and clinically tested.

^b Figures consider founders in a family if genotyped, and non-founders if both genotyped and clinically tested.

AS: QIMR asthma study. VU: VU University study. BH: Bispebjerg Hospital study. TM: QIMR twin moles study. BHR: positive bronchial hyperresponsiveness. Dpter: positive skin-prick response to house dust mite.

Supplementary Table 2. Details of the QC filters applied to the SNP data.

| | Batch 1 | | Batch 2 | | Batch 3 | | Batch 4 | | Batch 5 | |
|--|--|------|---------------------------|------|-----------------|------|----------------------------------|------|------------------|------|
| Studies genotyped | AS,TM | | AS | | AS | | AS,VU,BH, others | | AS,VU,BH, others | |
| Region(s) targeted | 2q33, 9p21 | | 20p13, 11q13 | | 2q33 | | 2q33 | | 2q33 | |
| Genes targeted | <i>CD28, CTLA4, ICOS, ADAM23, ADAMTSL1</i> | | <i>CDH26, HRH3, MS4A2</i> | | <i>ADAM23</i> | | <i>CD28, CTLA4, ICOS, ADAM23</i> | | <i>ADAM23</i> | |
| N Sequenom assays | 2 | | 1 | | 1 | | 1 | | 1 | |
| | SNP Individuals | | SNP Individuals | | SNP Individuals | | SNP Individuals | | SNP Individuals | |
| Initial N | 39 | 4662 | 24 | 2530 | 25 | 2510 | 21 | 4638 | 20 | 4639 |
| Monomorphic SNP | 0 | - | 1 | - | 1 | - | 0 | - | 1 | - |
| SNP with call rate <0.9 | 3 | - | 0 | - | 3 | - | 0 | - | 0 | - |
| Individuals with call rate <0.9 | - | 501 | - | 40 | - | 36 | - | 91 | - | 211 |
| SNP with Mendel error rate >0.05 | 0 | - | 0 | - | 0 | - | 0 | - | 1 | - |
| Individuals from families with Mendel error rate >0.05 | - | 60 | - | 164 | - | 49 | - | 33 | - | 3 |
| SNP with H-W <i>P</i> <0.0001 | 0 | - | 1 | - | 0 | - | 0 | - | 0 | - |
| SNP with MAF<0.01 | 0 | - | 2 | - | 0 | - | 0 | - | 0 | - |
| Individuals from other studies | - | 0 | - | 0 | - | 0 | - | 1177 | - | 1119 |
| Final N | 36 | 4101 | 20 | 2326 | 21 | 2425 | 21 | 2273 | 18 | 2242 |

AS: QIMR asthma study. VU: VU University study. BH: Bispebjerg Hospital study. TM: QIMR twin moles study. SNP: single nucleotide polymorphism. H-W: Hardy-Weinberg equilibrium test. MAF: minor allele frequency.

Supplementary Table 3. Multivariate association results between four asthma traits and *CD28*, *CTLA4* and *ICOS* SNPs on chromosome 2q33, for the combined analysis of the AS, VU and BH studies.

| Gene | SNP | Position, bp | N Families | N individuals | P | Dpter | Loadings ^a | | | Minor allele | |
|--------------|------------|-----------------|---------------|------------------|-------|-------|-----------------------|-------|-------------|--------------|------|
| | | | | | | | FEV ₁ /FVC | IgE | Eosinophils | Allele | Freq |
| <i>CD28</i> | rs10211663 | 204271260 | 1125 | 2559 | 1.000 | - | - | - | - | T | 0.08 |
| <i>CD28</i> | rs1879877 | 204278245 | 1127 | 2566 | 0.643 | - | - | - | - | T | 0.22 |
| <i>CD28</i> | rs3181096 | 204278337 | 665 | 1532 | 0.028 | -0.13 | -0.88 | 0.05 | 0.41 | C | 0.36 |
| <i>CD28</i> | rs3181098 | 204278623 | 1125 | 2554 | 0.452 | - | - | - | - | A | 0.36 |
| <i>CD28</i> | rs3181100 | 204280251 | 1123 | 2556 | 0.625 | - | - | - | - | G | 0.40 |
| <i>CD28</i> | rs3181101 | 204280279 | 1121 | 2550 | 1.000 | - | - | - | - | G | 0.13 |
| <i>CD28</i> | rs2140148 | 204280385 | 1123 | 2558 | 0.520 | - | - | - | - | C | 0.17 |
| <i>CD28</i> | rs1181388 | 204284196 | 666 | 1539 | 0.049 | 0.28 | -0.59 | 0.45 | 0.86 | A | 0.13 |
| <i>CD28</i> | rs3769683 | 204287042 | 1125 | 2557 | 0.121 | - | - | - | - | A | 0.13 |
| <i>CD28</i> | rs7425641 | 204287845 | 1125 | 2561 | 0.333 | - | - | - | - | T | 0.08 |
| <i>CD28</i> | rs4675363 | 204298316 | 1124 | 2552 | 0.727 | - | - | - | - | C | 0.26 |
| <i>CD28</i> | rs3116496 | 204302757 | 667 | 1543 | 0.084 | -0.97 | 0.23 | -0.63 | -0.15 | C | 0.17 |
| <i>CD28</i> | rs3181113 | 204310155 | 1128 | 2586 | 0.375 | - | - | - | - | T | 0.03 |
| <i>CD28</i> | rs6435203 | 204319440 | 666 | 1539 | 0.192 | - | - | - | - | G | 0.26 |
| <i>CD28</i> | rs231385 | 204329736 | 664 | 1531 | 0.187 | - | - | - | - | A | 0.26 |
| <i>CTLA4</i> | rs231735 | 204402121 | 667 | 1536 | 0.089 | 0.51 | -0.64 | 0.53 | 0.78 | G | 0.48 |
| <i>CTLA4</i> | rs926169 | 204430997 | 1127 | 2567 | 1.000 | - | - | - | - | T | 0.41 |
| <i>CTLA4</i> | rs231770 | 204437398 | 665 | 1535 | 0.098 | 0.79 | -0.60 | 0.59 | 0.57 | T | 0.41 |
| <i>CTLA4</i> | rs5742909 | 204440592 | 1125 | 2566 | 0.579 | - | - | - | - | T | 0.08 |
| <i>CTLA4</i> | rs231775 | 204440959 | 1124 | 2561 | 0.778 | - | - | - | - | G | 0.38 |
| <i>CTLA4</i> | rs231779 | 204442732 | 1127 | 2566 | 1.000 | - | - | - | - | T | 0.39 |
| <i>CTLA4</i> | rs3087243 | 204447164 | 665 | 1531 | 0.054 | 0.70 | -0.56 | 0.48 | 0.73 | A | 0.44 |
| <i>CTLA4</i> | rs1365965 | 204460115 | 667 | 1533 | 0.167 | - | - | - | - | C | 0.33 |
| <i>CTLA4</i> | rs3096851 | 204472127 | 665 | 1522 | 0.288 | - | - | - | - | C | 0.33 |
| <i>ICOS</i> | rs3096851 | 204472127 | 665 | 1522 | 0.288 | - | - | - | - | C | 0.33 |
| <i>ICOS</i> | rs3116505 | 204487426 | 666 | 1530 | 0.159 | - | - | - | - | T | 0.33 |
| <i>ICOS</i> | rs3096859 | 204490820 | 667 | 1532 | 0.148 | - | - | - | - | C | 0.33 |
| <i>ICOS</i> | rs2033171 | 204496401 | 667 | 1531 | 0.226 | - | - | - | - | T | 0.48 |
| <i>ICOS</i> | rs1978594 | 204499714 | 667 | 1529 | 0.211 | - | - | - | - | G | 0.48 |
| <i>ICOS</i> | rs3096863 | 204500977 | 1125 | 2563 | 0.692 | - | - | - | - | C | 0.32 |
| <i>ICOS</i> | rs2352551 | 204503002 | 1121 | 2552 | 0.611 | - | - | - | - | T | 0.46 |

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|--------|------------|-----------|------|------|-------|-------|-------|-------|------|---|------|
| ICOS | rs11889031 | 204507639 | 1123 | 2563 | 1.000 | - | - | - | - | T | 0.05 |
| ICOS | rs11883722 | 204509090 | 1122 | 2555 | 0.857 | - | - | - | - | A | 0.20 |
| ICOS | rs4312468 | 204510350 | 1124 | 2561 | 0.857 | - | - | - | - | A | 0.09 |
| ICOS | rs4675374 | 204510823 | 1057 | 2408 | 1.000 | - | - | - | - | T | 0.21 |
| ICOS | rs7602383 | 204514697 | 1111 | 2530 | 1.000 | - | - | - | - | G | 0.13 |
| ICOS | rs4522587 | 204519868 | 664 | 1531 | 1.000 | - | - | - | - | G | 0.20 |
| ICOS | rs6728120 | 204527470 | 667 | 1530 | 0.545 | - | - | - | - | T | 0.45 |
| ICOS | rs1559931 | 204533974 | 663 | 1523 | 0.192 | - | - | - | - | A | 0.25 |
| ICOS | rs4675379 | 204534340 | 666 | 1533 | 0.643 | - | - | - | - | C | 0.15 |
| ICOS | rs3116534 | 204542014 | 662 | 1522 | 0.165 | - | - | - | - | T | 0.30 |
| ICOS | rs4675389 | 204543621 | 665 | 1529 | 0.327 | - | - | - | - | G | 0.38 |
| ICOS | rs933988 | 204545674 | 663 | 1521 | 0.070 | 0.41 | -0.31 | 0.01 | 0.87 | G | 0.23 |
| ADAM23 | rs10469652 | 207011064 | 1096 | 2435 | 0.135 | - | - | - | - | T | 0.44 |
| ADAM23 | rs1562660 | 207013899 | 728 | 1776 | 0.611 | - | - | - | - | C | 0.22 |
| ADAM23 | rs1448903 | 207017206 | 1122 | 2559 | 0.137 | - | - | - | - | G | 0.09 |
| ADAM23 | rs1448905 | 207020160 | 713 | 1685 | 0.082 | -0.38 | -0.08 | -0.69 | 0.46 | C | 0.41 |
| ADAM23 | rs3755224 | 207110679 | 728 | 1785 | 0.057 | -0.31 | -0.22 | -0.40 | 0.69 | G | 0.13 |
| ADAM23 | rs2300964 | 207112595 | 728 | 1785 | 0.237 | - | - | - | - | T | 0.05 |
| ADAM23 | rs4675615 | 207133339 | 726 | 1774 | 0.265 | - | - | - | - | C | 0.30 |
| ADAM23 | rs1013475 | 207135918 | 720 | 1753 | 0.298 | - | - | - | - | G | 0.21 |
| ADAM23 | rs3770978 | 207149833 | 725 | 1771 | 0.438 | - | - | - | - | C | 0.16 |
| ADAM23 | rs2276674 | 207161499 | 1120 | 2538 | 0.356 | - | - | - | - | T | 0.47 |
| ADAM23 | rs3821170 | 207161555 | 1113 | 2536 | 1.000 | - | - | - | - | T | 0.09 |
| ADAM23 | rs7565709 | 207166124 | 1094 | 2489 | 0.395 | - | - | - | - | A | 0.26 |
| ADAM23 | rs10497883 | 207167322 | 708 | 1689 | 0.327 | - | - | - | - | G | 0.19 |
| ADAM23 | rs3732079 | 207167712 | 1121 | 2553 | 1.000 | - | - | - | - | T | 0.09 |
| ADAM23 | rs10490744 | 207169528 | 1113 | 2535 | 0.857 | - | - | - | - | A | 0.05 |
| ADAM23 | rs3821172 | 207173209 | 1120 | 2546 | 0.520 | - | - | - | - | G | 0.16 |
| ADAM23 | rs10490745 | 207174805 | 1117 | 2534 | 1.000 | - | - | - | - | T | 0.08 |
| ADAM23 | rs4085933 | 207179129 | 728 | 1780 | 0.571 | - | - | - | - | C | 0.46 |
| ADAM23 | rs1991537 | 207180818 | 1120 | 2539 | 0.381 | - | - | - | - | G | 0.47 |
| ADAM23 | rs3821173 | 207186405 | 1118 | 2538 | 0.159 | - | - | - | - | G | 0.49 |
| ADAM23 | rs6732127 | 207187947 | 1113 | 2539 | 1.000 | - | - | - | - | T | 0.09 |
| ADAM23 | rs3770983 | 207188853 | 1117 | 2531 | 0.625 | - | - | - | - | C | 0.30 |
| ADAM23 | rs3770984 | 207190188 | 1117 | 2534 | 0.151 | - | - | - | - | T | 0.48 |
| ADAM23 | rs10186957 | 207195134 | 1119 | 2540 | 0.340 | - | - | - | - | C | 0.16 |
| ADAM23 | rs10932156 | 207197675 | 1121 | 2544 | 0.191 | - | - | - | - | C | 0.48 |

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|--------|------------|-----------|------|------|-------|---|---|---|---|---|------|
| ADAM23 | rs17786667 | 207199056 | 1112 | 2519 | 0.424 | - | - | - | - | C | 0.46 |
| ADAM23 | rs2163034 | 207199871 | 1117 | 2529 | 0.545 | - | - | - | - | G | 0.30 |

^aLoadings for each trait are reported for SNPs with a multivariate $P < 0.1$ to guide the identification of phenotypes contributing to the multivariate result. Loadings correspond to the correlation between each individual trait and the first canonical variate extracted for the trait set (5). From our experience, traits with loadings $> |0.5|$ can be interpreted as showing evidence for association. Individual associations suggested by the multivariate analysis should then be confirmed by univariate approaches.

SNP: single nucleotide polymorphism. Dpter: skin-prick response to house dust mite. FEV₁/FVC: forced expiratory volume in 1 s (FEV₁) divided by forced vital capacity (FVC).

For Peer Review

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Supplementary Table 4. Univariate association results for the TM study between eosinophil levels and *CD28*, *CTLA4* and *ICOS* SNPs on chromosome 2q33.

| Gene | SNP | Position, bp | Beta | P |
|---------------|-----------|--------------|--------|-------|
| <i>CD28</i> | rs1879877 | 204278245 | -0.013 | 0.810 |
| <i>CD28</i> | rs3181096 | 204278337 | -0.106 | 0.034 |
| <i>CD28</i> | rs3181098 | 204278623 | -0.116 | 0.020 |
| <i>CD28</i> | rs1181388 | 204284196 | 0.022 | 0.750 |
| <i>CD28</i> | rs3116496 | 204302757 | -0.044 | 0.450 |
| <i>CD28</i> | rs3181113 | 204310155 | 0.115 | 0.330 |
| <i>CD28</i> | rs6435203 | 204319440 | -0.035 | 0.500 |
| <i>CD28</i> | rs231385 | 204329736 | -0.035 | 0.510 |
| <i>CTLA4</i> | rs231735 | 204402121 | 0.014 | 0.910 |
| <i>CTLA4</i> | rs926169 | 204430997 | 0.006 | 0.810 |
| <i>CTLA4</i> | rs231770 | 204437398 | -0.012 | 0.920 |
| <i>CTLA4</i> | rs5742909 | 204440592 | -0.005 | 0.780 |
| <i>CTLA4</i> | rs231779 | 204442732 | 0.022 | 1.000 |
| <i>CTLA4</i> | rs3087243 | 204447164 | 0.000 | 0.980 |
| <i>CTLA4</i> | rs1365965 | 204460115 | 0.001 | 0.700 |
| <i>CTLA4</i> | rs3096851 | 204472127 | 0.019 | 0.620 |
| <i>ICOS</i> | rs3096851 | 204472127 | 0.025 | 0.620 |
| <i>ICOS</i> | rs3116505 | 204487426 | 0.003 | 0.960 |
| <i>ICOS</i> | rs3096859 | 204490820 | 0.001 | 0.990 |
| <i>ICOS</i> | rs2033171 | 204496401 | 0.016 | 0.750 |
| <i>ICOS</i> | rs1978594 | 204499714 | 0.022 | 0.650 |
| <i>ICOS</i> | rs4522587 | 204519868 | 0.042 | 0.470 |
| <i>ICOS</i> | rs6728120 | 204527470 | -0.063 | 0.180 |
| <i>ICOS</i> | rs1559931 | 204533974 | -0.115 | 0.036 |
| <i>ICOS</i> | rs4675379 | 204534340 | -0.123 | 0.083 |
| <i>ICOS</i> | rs3116534 | 204542014 | 0.072 | 0.170 |
| <i>ICOS</i> | rs4675389 | 204543621 | 0.011 | 0.820 |
| <i>ICOS</i> | rs933988 | 204545674 | 0.076 | 0.170 |
| <i>ADAM23</i> | rs3732079 | 207167712 | -0.122 | 0.122 |
| <i>ADAM23</i> | rs3770984 | 207190188 | -0.046 | 0.350 |

SNP: single nucleotide polymorphism.

Supplementary Table 5. Univariate association results for the AS study between IgE levels and *ADAMTSL1* SNPs on chromosome 9p21.

| SNP | Position, bp | Beta | <i>P</i> |
|-----------|--------------|-------|----------|
| rs1417037 | 18461746 | -0.03 | 0.540 |
| rs2383075 | 18525581 | -0.02 | 0.750 |
| rs1889007 | 18596825 | -0.09 | 0.103 |
| rs1412671 | 18628143 | -0.05 | 0.310 |
| rs1360567 | 18665325 | 0.01 | 0.820 |
| rs4977340 | 18708280 | -0.01 | 0.900 |

SNP: single nucleotide polymorphism.

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Supplementary Table 6. Multivariate association results for the AS study between *MS4A2* SNPs and Atopy and total IgE levels.

| SNP | Position, bp | N families | N individuals | P | Loadings ^a | | Minor allele | |
|------------|--------------|---------------|------------------|-------|-----------------------|-------|--------------|------|
| | | | | | Atopy | IgE | Allele | Freq |
| rs2583477 | 59610431 | 703 | 1696 | 0.097 | -0.89 | -0.04 | C | 0.43 |
| rs1441586 | 59612604 | 695 | 1677 | 0.019 | -0.18 | 0.77 | C | 0.43 |
| rs502581 | 59616754 | 703 | 1698 | 0.021 | -0.16 | 0.78 | T | 0.44 |
| rs502419 | 59622751 | 700 | 1692 | 0.016 | -0.19 | 0.76 | A | 0.44 |
| rs2855017 | 59622885 | 703 | 1700 | 0.012 | -0.29 | 0.69 | T | 0.41 |
| rs17528859 | 59623955 | 702 | 1696 | 0.018 | -0.29 | 0.69 | C | 0.41 |

^aLoadings for each trait are reported to guide the identification of phenotypes contributing to the multivariate result. Loadings correspond to the correlation between each individual trait and the first canonical variate extracted for the trait set (5). From our experience, traits with loadings > |0.5| can be interpreted as showing evidence for association. Individual associations suggested by the multivariate analysis should then be confirmed by univariate approaches.
SNP: single nucleotide polymorphism.

Supplementary Table 7. Multivariate association results for the AS study between 20q13 SNPs and six asthma traits.

| Gene | SNP | Position, bp | N families | N ind | P | Loadings ^a | | | | | | Minor allele | |
|-------|-----------|-----------------|---------------|----------|-------|-----------------------|-------|-------|------------------|--------|-----------------------|--------------|------|
| | | | | | | Dpter | Atopy | BHR | FEV ₁ | Asthma | FEV ₁ /FVC | Allele | Freq |
| CDH26 | rs6027217 | 57981747 | 696 | 1360 | 0.750 | - | - | - | - | - | - | A | 0.39 |
| CDH26 | rs194990 | 57992502 | 695 | 1359 | 0.556 | - | - | - | - | - | - | G | 0.16 |
| CDH26 | rs195001 | 58006979 | 687 | 1341 | 0.588 | - | - | - | - | - | - | T | 0.36 |
| CDH26 | rs910695 | 58021236 | 688 | 1346 | 0.857 | - | - | - | - | - | - | C | 0.28 |
| CDH26 | rs1576726 | 58026201 | 669 | 1299 | 0.857 | - | - | - | - | - | - | T | 0.04 |
| HRH3 | rs6061458 | 60214968 | 696 | 1363 | 1.000 | - | - | - | - | - | - | T | 0.28 |
| HRH3 | rs6062144 | 60215743 | 696 | 1366 | 0.036 | -0.910 | -0.68 | -0.12 | -0.20 | 0.04 | 0.23 | C | 0.16 |
| HRH3 | rs1760042 | 60220455 | 697 | 1361 | 0.524 | - | - | - | - | - | - | G | 0.18 |
| HRH3 | rs3787429 | 60224799 | 695 | 1360 | 0.524 | - | - | - | - | - | - | A | 0.39 |
| HRH3 | rs6587298 | 60229868 | 695 | 1358 | 1.000 | - | - | - | - | - | - | G | 0.35 |
| HRH3 | rs1614845 | 60231063 | 695 | 1364 | 0.857 | - | - | - | - | - | - | T | 0.19 |
| HRH3 | rs944887 | 60233194 | 696 | 1365 | 1.000 | - | - | - | - | - | - | T | 0.19 |
| HRH3 | rs6142998 | 60236858 | 689 | 1345 | 0.115 | - | - | - | - | - | - | A | 0.38 |

^aLoadings for each trait are reported to guide the identification of phenotypes contributing to the multivariate result. Loadings correspond to the correlation between each individual trait and the first canonical variate extracted for the trait set (5). From our experience, traits with loadings > |0.5| can be interpreted as showing evidence for association. Individual associations suggested by the multivariate analysis should then be confirmed by univariate approaches.

SNP: single nucleotide polymorphism. Dpter: skin-prick response to house dust mite. BHR: bronchial hyperresponsiveness. FEV₁/FVC: forced expiratory volume in 1 s (FEV₁) divided by forced vital capacity (FVC).

Supplementary Table 8. Gene-gene interaction results for Atopy in the AS study between all eight genes tested.

| Gene 1 | Gene 2 | N pairwise tests performed | Most significant interaction | | |
|----------|----------|----------------------------|------------------------------|------------|------------|
| | | | P | SNP1 | SNP2 |
| CD28 | CTLA4 | 135 | 0.00411 | rs6435203 | rs3087243 |
| CD28 | ICOS | 285 | 0.00746 | rs3116496 | rs4675374 |
| CD28 | ADAM23 | 405 | 0.04110 | rs6435203 | rs17786667 |
| CD28 | ADAMTSL1 | 90 | 0.02997 | rs3116496 | rs4977340 |
| CD28 | MS4A2 | 90 | 0.12560 | rs3181113 | rs17528859 |
| CD28 | CDH26 | 75 | 0.02188 | rs7425641 | rs194990 |
| CD28 | HRH3 | 120 | 0.02741 | rs3181098 | rs6142998 |
| CTLA4 | ICOS | 170 | 0.00451 | rs3087243 | rs4522587 |
| CTLA4 | ADAM23 | 243 | 0.01223 | rs3087243 | rs2300964 |
| CTLA4 | ADAMTSL1 | 54 | 0.02668 | rs3087243 | rs1417037 |
| CTLA4 | MS4A2 | 54 | 0.15560 | rs1365965 | rs1441586 |
| CTLA4 | CDH26 | 45 | 0.09339 | rs3087243 | rs194990 |
| CTLA4 | HRH3 | 72 | 0.08781 | rs231770 | rs6061458 |
| ICOS | ADAM23 | 513 | 0.01072 | rs6728120 | rs3821173 |
| ICOS | ADAMTSL1 | 114 | 0.09098 | rs4675389 | rs1412671 |
| ICOS | MS4A2 | 114 | 0.02232 | rs3116534 | rs2583477 |
| ICOS | CDH26 | 95 | 0.02906 | rs4675389 | rs1576726 |
| ICOS | HRH3 | 152 | 0.03159 | rs11889031 | rs3787429 |
| ADAM23 | ADAMTSL1 | 162 | 0.00160 | rs3770978 | rs1889007 |
| ADAM23 | MS4A2 | 162 | 0.02500 | rs1562660 | rs2583477 |
| ADAM23 | CDH26 | 135 | 0.00169 | rs3770984 | rs6027217 |
| ADAM23 | HRH3 | 216 | 0.00059 | rs1013475 | rs6587298 |
| ADAMTSL1 | MS4A2 | 36 | 0.17710 | rs1889007 | rs17528859 |
| ADAMTSL1 | CDH26 | 30 | 0.02937 | rs1417037 | rs910695 |
| ADAMTSL1 | HRH3 | 48 | 0.04545 | rs1417037 | rs3787429 |
| MS4A2 | CDH26 | 30 | 0.09139 | rs2583477 | rs1576726 |
| MS4A2 | HRH3 | 48 | 0.04053 | rs502419 | rs1760042 |
| CDH26 | HRH3 | 40 | 0.05979 | rs910695 | rs6142998 |

SNP: single nucleotide polymorphism.

Supplementary Table 9. Summary of main results from published candidate-gene association studies of asthma traits and *CD28*, *CTLA4* and *ICOS* polymorphisms.

| Reference (ref. #) | Population | N cases | N controls | CD28 | | CTLA4 | | ICOS | | Phenotype(s) |
|---------------------------|------------|---------|------------|-----------|-----------|-----------|-----------------|------------------|--|--------------|
| | | | | rs3181098 | rs3116496 | rs5742909 | rs231775 | rs11883722 | | |
| Nakao et al. 2000 (6) | Japanese | 120 | 200 | - | NS | NS | NS | - | Atopic asthma | |
| Heinzmann et al. 2000 (7) | German | 55-189 | 71-205 | - | NS | NS | NS | - | Asthma, atopy, IgE levels | |
| Hizawa et al. 2001 (8) | Japanese | 339 | 305 | - | - | NS | NS | - | Asthma, atopy | |
| | | 339 | - | - | - | 0.005 (C) | NS | - | IgE levels | |
| | | - | 305 | - | - | NS | NS | - | IgE levels | |
| Howard et al. 2002 (9) | American | 348-359 | - | NS | - | NS | 0.0005 (A) | - | IgE levels | |
| | | 173-218 | 129-162 | NS | - | NS | 0.012-0.042 (A) | - | Asthma, BHR, atopy | |
| Lee et al. 2002 (10) | Korean | 52-88 | 86-122 | - | - | NS | NS | - | Asthma, atopic asthma | |
| | | 88 | - | - | - | 0.037 (T) | NS | - | Asthma severity | |
| | | 45 | - | - | - | NS | 0.019 (G) | - | BHR | |
| Bourgain et al. 2003 (11) | Hutterites | 261 | 320 | - | - | 0.013 (T) | - | - | Atopy | |
| Shilling et al. 2005 (12) | Hutterites | 71-156 | NA | - | - | - | - | NS | Asthma, BHR, mold SPT | |
| | | 638 | - | - | - | - | - | 0.0043 (A) | IgE levels | |
| | | 148-311 | NA | - | - | - | - | 0.0002-0.049 (A) | Atopy, cockroach, mite and pollens SPT | |

BHR: bronchial hyperresponsiveness. SPT: skin-prick test. NS: not significant (*P* > 0.05). NA: not available.

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