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Association and interaction analyses of eight genes under asthma linkage peaks

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**SUPPLEMENTARY INFORMATION**

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**Outline**

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1. Supplementary Methods

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2. Supplementary Tables

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3. Supplementary References

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## 15 1. Supplementary Methods

### 17 **Ascertainment of families for the asthma VU study**

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

22 Twin families were recruited by asking all city councils in The Netherlands for addresses of twins  
23 aged 13-22 years. An initial positive response was received from 252 city councils that supplied 3859  
24 addresses; 177 addresses were available from other sources. After contacting these 4036 families by letter,  
25 2375 twin families indicated that they were willing to complete a questionnaire on health and lifestyle and  
26 1700 families returned these questionnaires in 1991. Data from 3 families were entered twice by mistake,  
27 leaving a total of 1697 families. In 1993 a second questionnaire was mailed to the 4036 families that had  
28 been contacted before and to 1987 new families. Additional addresses of new twin families were obtained  
29 from city councils which had reacted positively to our request, but were not able to furnish addresses in  
30 time for the first wave of data collection. The new addresses included several of the larger cities in the  
31 Netherlands. At the second measurement occasion we obtained questionnaires from 1974 families; 959  
32 families participated for the second time; 877 families came from the new addresses; 138 families were  
33 contacted before in 1991 but had not responded at the time. In total we have studied 2712 families  
34 measured at two different occasions, with 959 families participating twice.

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

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

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

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

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

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

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

#### 66 **Clinical protocol used in the asthma VU study**

67 *Questionnaire administration.* The questionnaire is based on the WHO and CARA-TNO-questionnaires.

68 Both were extensively investigated in the Netherlands. Questions concerned symptoms such as cough,

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5 69 periods of bronchitis, wheezing, dyspnoe, attacks of asthma, rhinitis, pneumoniae (doctor diagnosis) and  
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7 70 asthma (doctor diagnosis). Information about smoking habits, medication, work, and family were  
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9 71 recorded. Most questions have a good reproducibility ( $\kappa > 0.5$ ). For the present analyses,  
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11 72 participants with a self-reported doctor diagnosis of asthma were considered affected for the Asthma trait.  
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14 73  
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16 74 *Lung function test.* FVC and FEV<sub>1</sub> are measured with the VMAX22 pneumotachograph of Sensor  
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18 75 Medics. The measurements are according guidelines of the ATS. Twice a day, calibration was carried out  
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21 76 with a calibration pump of 3 ltr and an automatic calibration program. Calibration reports are available.  
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23 77  
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26 78 *Airway methacoline challenge test.* The test used the method of tidal breathing for 2 minutes and meets  
27  
28 79 the guidelines of standardization. After baseline measurements of pulmonary function, individuals inhaled  
29  
30 80 nebulized NaCl 0.9% from a DEVILLBISS 646 nebulizer (output 0.13 ml/min, flow of 5.5 l/min,  
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33 81 aerosol about 5 micron ). If the decrease in FEV<sub>1</sub> was less than 10%, the test was performed.  
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35 82 Sequential aerosols of metacholine was given in concentrations of 0.15, 0.3, 0.6, 1.25, 2.5, 5, 10, 20, 40,  
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37 83 80 and 160 mg/ml. Each concentration was inhaled for 2 minutes, after each challenge FEV<sub>1</sub> maneuvers  
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39 84 were performed. The metacholine concentration at which there was a decrease in FEV<sub>1</sub> of 20%  
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42 85 (compared to baseline) was taken as the threshold value (PC<sub>20m</sub>). The determination of PC<sub>20m</sub> is  
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44 86 calculated by linear interpolation of the last two points of the concentration-dose curve (4).  
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47 87 For the present analyses, a bronchial hyperresponsiveness (BHR) test was considered positive if the  
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49 88 participant experienced a drop in FEV<sub>1</sub> of 20% after the last dose of methacoline given.  
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54 90 *Bronchial Reversibility Test.* Following the methacoline challenge test, a bronchial reversibility test with  
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56 91 Salbutamol was performed after FEV<sub>1</sub> returned to baseline value (mostly after one hour). Subjects  
57  
58 92 inhaled four times 200mcg of salbutamol (MDI) through a chamber (volumatic). After 15 minutes FEV<sub>1</sub>  
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60 93 maneuvers were performed. When there is an improvement of FEV<sub>1</sub> of 200 ml or 12%, reversibility is  
94 significant according to ATS criteria.  
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4 96 *Skin Prick Test*. Tests were done to grass, trees, weeds, house dust-mite, other mites, cat, dog, horse, other  
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7 97 animals (rabbit), feathers (birds), *Aspergillus fumigatus*, *Cladosporium herbarum*, *Alternaria alternarium*,  
8  
9 98 and *Candida albicans*. The test was considered positive when a response was > 2mm greater than the  
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11 99 negative control. A participant was defined as atopic if one or more of the following criteria were met: (1)  
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14 100 total IgE > 2 SD above normal of the Dutch population; (2) raised (>100 U/l) serum IgE against molds,  
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16 101 cat, grass or English rawweed; or (3) positive skin prick test.  
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21 103 *Measurement of total and specific IgE levels*. Total IgE was measured using Phadezym Prist, Pharmacia  
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23 104 ltd. Specific IgE against molds, cat, grass and English rawweed was detected by Pharmacia Cap Sweden.  
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5 105 **2. Supplementary Tables**  
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11 **Supplementary Table 1.** Characteristics of the study participants for the four individual studies.

	AS	VU	BH	TM	
	<i>N individuals</i>				
Genotyped	2493	353	532	2092	
Clinically tested	1703	360	457	660	
Both	1697	353	457	660	
	<i>Clinical characteristics<sup>a</sup></i>				
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	-	
	<i>N families analysed<sup>b</sup></i>				
N Parents	N offspring				
2	3 or more	139	4	0	0
2	2	146	67	0	152
2	1	37	6	0	69
1	3 or more	29	1	0	0
1	2	66	10	0	98
1	1	38	1	0	41
0	3 or more	28	0	0	0
0	2	146	1	156	17
0	1	106	9	147	50
	Total	735	99	303	427

54 108 <sup>a</sup> Figures are based on individuals that were both genotyped and clinically tested.

55 109 <sup>b</sup> Figures consider founders in a family if genotyped, and non-founders if both genotyped and clinically  
56 110 tested.

57 111 AS: QIMR asthma study. VU: VU University study. BH: Bispebjerg Hospital study. TM: QIMR twin  
58 112 moles study. BHR: positive bronchial hyperresponsiveness. Dpter: positive skin-prick response to house  
59 113 dust mite.

114 **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	
	<i>SNP Individuals</i>		<i>SNP Individuals</i>		<i>SNP Individuals</i>		<i>SNP Individuals</i>		<i>SNP Individuals</i>	
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 $P < 0.0001$	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.

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4 118 **Supplementary Table 3.** Multivariate association results between four asthma traits and *CD28*, *CTLA4*  
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7 119 and *ICOS* SNPs on chromosome 2q33, for the combined analysis of the AS, VU and BH studies.  
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Gene	SNP	Position, bp	N Families	N individuals	P	Dpter	Loadings <sup>a</sup>			Minor allele	
							FEV <sub>1</sub> /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

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

<sup>a</sup>Loadings 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. FEV<sub>1</sub>/FVC: forced expiratory volume in 1 s (FEV<sub>1</sub>) divided by forced vital capacity (FVC).

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4 127 **Supplementary Table 4.** Univariate association results for the TM study between eosinophil levels and  
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7 128 *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

47 129 SNP: single nucleotide polymorphism.  
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4 130 **Supplementary Table 5.** Univariate association results for the AS study between IgE levels and  
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7 131 *ADAMTSL1* SNPs on chromosome 9p21.  
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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

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4 133 **Supplementary Table 6.** Multivariate association results for the AS study between *MS4A2* SNPs and  
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7 134 Atopy and total IgE levels.

SNP	Position, bp	N families	N individuals	P	Loadings <sup>a</sup>		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

19135 <sup>a</sup>Loadings for each trait are reported to guide the identification of phenotypes contributing to the  
20136 multivariate result. Loadings correspond to the correlation between each individual trait and the first  
21137 canonical variate extracted for the trait set (5). From our experience, traits with loadings > |0.5| can be  
22138 interpreted as showing evidence for association. Individual associations suggested by the multivariate  
23139 analysis should then be confirmed by univariate approaches.  
24140 SNP: single nucleotide polymorphism.

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4 141 **Supplementary Table 7.** Multivariate association results for the AS study between 20q13 SNPs and six  
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7 142 asthma traits.

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

28 143 <sup>a</sup>Loadings for each trait are reported to guide the identification of phenotypes contributing to the  
29 144 multivariate result. Loadings correspond to the correlation between each individual trait and the first  
30 145 canonical variate extracted for the trait set (5). From our experience, traits with loadings > |0.5| can be  
31 146 interpreted as showing evidence for association. Individual associations suggested by the multivariate  
32 147 analysis should then be confirmed by univariate approaches.  
33 148 SNP: single nucleotide polymorphism. Dpter: skin-prick response to house dust mite. BHR: bronchial  
34 149 hyperresponsiveness. FEV<sub>1</sub>/FVC: forced expiratory volume in 1 s (FEV<sub>1</sub>) divided by forced vital capacity  
35 150 (FVC).  
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4 151 **Supplementary Table 8.** Gene-gene interaction results for Atopy in the AS study between all eight genes  
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7 152 tested.

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

60 153 SNP: single nucleotide polymorphism.

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4 154 **Supplementary Table 9.** Summary of main results from published candidate-gene association studies of  
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7 155 asthma traits and *CD28*, *CTLA4* and *ICOS* polymorphisms.  
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Reference (ref. #)	Population	N cases	N controls	<i>CD28</i>		<i>CTLA4</i>		<i>ICOS</i>		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	

59 157 BHR: bronchial hyperresponsiveness. SPT: skin-prick test. NS: not significant ( $P > 0.05$ ). NA: not  
60 158 available.

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