

Association of *IL13* with total IgE: Evidence against an inverse association of atopy and diabetes

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Background: Atopic illnesses, related to high circulating IgE levels, and the autoimmune disease type 1 diabetes, have been reported to be inversely associated. One possible explanation is that susceptibility alleles for one disease provide protection for the other.

Objective: Using the largest sample sizes reported so far for the identification of genetic determinants of circulating IgE levels, we investigated associations between total serum IgE (log-transformed) and single nucleotide polymorphisms in 8 genes that are candidate susceptibility loci for IgE levels/atopic illness (*IL13*, *IL4*, *IL4RA*, *FCER1B*, *IL12B*, *TBET*) and/or type 1 diabetes (*CTLA4*, *PTPN22*, *IL2RA*).

Methods: As many as 4570 DNA samples obtained from members of the British 1958 Birth Cohort were genotyped for 51 candidate variants, and the associations of alleles and genotypes with log-transformed serum IgE levels were evaluated by regression modeling.

Results: We obtained evidence of association between *IL13* variants and total serum IgE levels ($P = .00002$, explaining 0.59% of phenotypic variance). However, there was no evidence of association of the confirmed type 1 diabetes susceptibility genes *CTLA4* and *PTPN22* and the candidate gene *IL2RA* with IgE levels.

Conclusion: Allelic variation in the *IL-13* gene is robustly confirmed as a contributor to the variance of IgE levels but has no detectable effect in type 1 diabetes.

Clinical implications: Although the allelic variation at the confirmed *IL-13* locus explains too little of the between-individual variation of circulating IgE to be of use for clinical prediction on its own, the discovery of additional susceptibility loci in the future may aid in the stratification of atopic subjects and improve risk assessment. (J Allergy Clin Immunol 2006;117:1016-1024.)

Key words: *IL-13*, gene, IgE, type 1 diabetes, atopy

The familial aggregation of asthma and allergic diseases is well recognized, and twin studies strongly suggest a heritable component to both asthma and allergic disease.¹ The heritability of asthma has been estimated as approximately 60%,² and a similar figure can be derived for the heritability of total circulating IgE.^{1,2} Several studies have reported an inverse relationship between atopic diseases, characterized by a T_H2 -associated pathway, and type 1 diabetes, which involves cell-mediated, T_H1 destruction of the insulin-producing cells of the pancreas.³⁻⁶ The possibility of an inverse association between the 2 immune-mediated disorders stems from the T_H1/T_H2 paradigm: in response to exposure to different kinds of pathogens, counterregulatory activities in the immune system control the T_H1 cells (secreting IL-2 and IFN- γ) and T_H2 cells (secreting IL-4, IL-5, and IL-13) while maintaining immunologic self-tolerance and suppression of autoimmune disease. Some pathways driving T_H1 or T_H2 differentiation can be mutually exclusive in that activators of one pathway are considered to be negative regulators of the other.⁷ For example, in experiments in animal models used to study allergic inflammatory diseases and in human beings with chronic inflammatory disease, T_H1 T-cell-induced actions such as those via the T_H1 cytokine IFN- γ ameliorated T_H2 T-cell responses.^{8,9}

A meta-analysis of studies investigating the association between type 1 diabetes and atopic disease suggested a significant, albeit small, reduction in the frequency of asthma among children with type 1 diabetes (OR, 0.82;

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Abbreviations used

DIP: Deletion/insertion polymorphism
 SNP: Single nucleotide polymorphism

95% CI, 0.68-0.99).¹⁰ However, a study of as many as 20,050 American adults found no evidence of an inverse association between atopy and autoimmune disorders.¹¹ The discovery of genes with alleles exerting opposite effects in the 2 disorders would provide support for some of the clinical and epidemiologic observations.

Therefore, we investigated whether the T_H2-related phenotype total circulating IgE and the T_H1-mediated disease type 1 diabetes share genetic loci. Testing whether total circulating IgE and type 1 diabetes share genetic loci requires that susceptibility loci for both phenotypes have unequivocal statistical support for their associations. Aside from various associations in the HLA region, both diseases have been associated with several non-HLA loci. In type 1 diabetes, 2 immunoregulatory genes, *CTLA4* and *PTPN22*, both of which influence T-cell activity and expansion, are established susceptibility loci for the disease. Another candidate gene region, containing *IL2RA*, which encodes an essential surface receptor (CD25) for the differentiation and activity of T-regulatory cells, has recently been associated with type 1 diabetes.¹² In atopic illness and IgE production, several genes have been implicated, most notably those encoding the cytokine IL-13 (Table I), which is a positive regulator of IgE production, and also IL-4 and its receptor IL-4R α . Our study aims to find genetic markers that are associated with total serum IgE levels. We note that such markers, although useful in aiding prediction of total serum IgE levels that are commonly observed in atopy, may not be sufficient in the prediction of incidence of clinical disease on their own.

Polymorphisms in *IL13* were considered major candidates for our association study, not only because 10 investigations have published associations between IgE levels and *IL13* variants (Table I), but also because of the availability of some functional data.¹³⁻¹⁵ IL-13 plays a crucial role in T_H2-cytokine-mediated immune responses, and its signaling pathway is important in the pathogenesis of allergic disease.^{16,17} Previous studies showed that the Arg110Gln variant in *IL13* is associated with raised total serum IgE level¹⁸ and asthma.¹⁹ *In vitro* studies show that the Gln110 variant represents a more active form than the Arg110 variant in promoting effector pathways of allergic inflammation.^{13,14} Functional properties have also been attributed to the *IL13* promoter single nucleotide polymorphism (SNP) at position -1024 C>T (also referred to as -1055, -1111, -1112), located in a region containing a nuclear factor of activated T cells transcription factor binding site; the T allele of this -1024 C>T SNP is associated with greater IL-13 production by stimulated T cells.²⁰

Polymorphisms in *IL4RA* have been associated with atopic phenotypes: the Ile50Val (+148 A>T), Cys406Arg (+1216 T>C), Ser478Pro (+1682 T>C), and Gln551Arg (+1902 G>T) variants have shown association with increased risk for asthma^{21,22} and atopy,^{21,23} but a large study cohort of German children reported that *IL4RA* polymorphisms only have a minor effect on total serum IgE levels.²⁴

We were also interested in whether the previously published association between IgE levels and the +49A>G/rs231775 SNP in *CTLA4* using a Dutch population with asthma²⁵ could be replicated in an independent data set. The same group also studied *CTLA4* -1147C>T/rs16840252 SNP, which was previously associated with bronchial hyperresponsiveness and asthma.²⁵ In contrast with the study by Howard et al,²⁵ Munthe-Kaas et al²⁶ could not detect associations between IgE levels and the +49A>G/rs231775 and -1147C>T/rs16840252 variants, but found *P* values < .05 between IgE levels and the *CTLA4* variants MH30/C (rs231806), CT60/A (rs3087243), JO31/T (rs11571302), JO30/A (rs7565213), and JO27_1/C (rs11571297). Intriguingly, the alleles positively associated in the study by Munthe-Kaas et al²⁶ are negatively associated with type 1 diabetes and other autoimmune-mediated diseases,²⁷ providing suggestive evidence of a genetic basis for the proposed inverse relationship between atopic illness and type 1 diabetes.

We also analyzed a further 4 loci for association with total IgE levels: the β -chain of the high-affinity receptor for IgE (Fc ϵ RI- β) gene (*FCER1B*, *MS4A2*) on chromosome 11q13 was previously associated with atopy²⁸ and asthma²⁹; variants in *IL12B* on chromosome 5q31-q33 have also been studied for association with asthma and IgE levels³⁰⁻³², and a nonsynonymous SNP in the *TBX21* (also called *TBET*) gene (His33Glu, rs2240017), the gene product of which is implicated in the initiation of T_H1 lineage development and is capable of switching cytokine profiles from T_H2 polarized immune responses toward a T_H1-like profile.³³

We also included the coding SNP in the hematopoietic-specific tyrosine phosphatase LYP (*PTPN22*; Arg620Trp, rs2476601), associated with several autoimmune diseases, including type 1 diabetes.^{34,35} In addition, we speculate that the major regulatory mechanisms of the peripheral immune system, T regulatory lymphocytes, could well influence susceptibility of both type 1 diabetes and atopy illness. Therefore, we have also tested allelic variation in the *IL2RA/CD25* gene¹² for association with IgE levels.

METHODS

Description of the British 1958 Birth Cohort

The British 1958 Birth Cohort (also known as the National Child Development Study) includes all births in England, Wales, and Scotland during 1 week in 1958 (<http://www.cls.ioe.ac.uk/studies.asp?section=000100020003>). Survivors have been followed up by parental interview and school medical examination at ages 7, 11, and 16 years, and by cohort member interview at 23, 33, and 41 years. Immigrants of the same dates of birth were identified at ages 7, 11,

TABLE I. Polymorphisms in *IL13* previously found to be associated with allergic disease, IgE levels, skin test (ST) reactivity, or bronchial hyperresponsiveness

SNP	Reference	Association study	Sample size and population
–1512A>C (rs1881457)	18	Associated with higher IgE levels in skin test positive children ($P \leq .005$)	1399 white children (United States, Germany)
–1024C>T (rs1800925)	18	Associated with higher IgE levels in skin-test positive children ($P \leq .005$)	1399 white children (United States, Germany)
	65	T allele associated with asthma ($P = .004$), bronchial hyperresponsiveness ($P = .003$), positive ST ($P = .03$)	184 probands and spouses (The Netherlands)
	45	NA	188 patients with allergic rhinitis (China)
	44	T allele associated with higher total serum IgE ($P = .0002$)	823 children and 382 trios (Germany)
	20	TT genotype with allergic asthma ($P = .002$), TT genotype associated with reduced inhibition of IL-13 production ($P < .002$)	101 patients with allergic asthma (The Netherlands)
	66	T allele associated with atopy ($P = .0087$)	238 atopic patients and 104 controls (Denmark)
	67	NA	329 children (Denmark)
	46	NA	207 European American children
	46	NA	207 European American children
+1923C>T (rs1295686)	46	NA	207 European American children
+2044G>A Arg110Gln (rs20541)	18	A allele associated with higher IgE levels in skin test positive ($P = .00002$) and skin test negative children ($P = .05$)	1399 white children (United States, Germany)
	65	NA	184 probands and spouses (The Netherlands)
	68	NA	83 nuclear families (Costa Rica)
	44	A allele associated with higher IgE levels ($P < .0001$ for AA) in 823 children and in 382 trios ($P = .02$)	823 children and 382 trios (Germany)
	19	A allele associated with higher IgE levels ($P = .006$) and bronchial asthma ($P = .04$)	267 patients with atopy and 228 with bronchial asthma (Germany)
	45	A allele associated with higher IgE levels ($P = .04$)	188 patients with allergic rhinitis (China)
	67	AA genotype associated with atopic dermatitis ($P = .01$) and positive ST ($P = .05$)	326 children (Denmark)
	46	A allele associated with increased total IgE ($P = .0026$)	207 European American children

NA, Not associated.

and 16 years and followed into adulthood, but adult immigrants (after age 16 years) have not been included. The cohort has previously been influential in advancing understanding of the natural history^{36,37} and epidemiologic features^{36,38–40} of allergic diseases.

This work relates to analyses of DNA from as many as 4570 white subjects recruited in the first half of the fieldwork period. These were equally distributed by sex. Forty-six percent have always been nonsmokers, 26% were exsmokers, 4% were occasional smokers, and 24% were current daily smokers. Subjects were distributed throughout England, Wales, and Scotland. Because of changing DNA resources in the laboratory over time, certain DNA samples were unavailable for some SNP assays and available for others.

A biomedical assessment of cohort members at age 44 to 45 years included measurement of total serum IgE. Total IgE was assayed by the HYTEC automated enzyme immunoassay⁴¹ (Hycor Biomedical, Edinburgh, United Kingdom) in a single laboratory (Royal Victoria Hospital, Newcastle). Specimens were processed in monthly batches

over a period of 18 months of fieldwork, and the results are adjusted for batch in the statistical analysis. The lower limit of detection was 1 kU/L (14 individuals), and the upper limit was 2000 kU/L (17 individuals).

Genotyping

Invader (Third Wave Technologies, Madison, Wis) and TaqMan (Applied Biosystems, Warrington, United Kingdom) genotyping technologies were used. All genotyping data were scored by 2 independent researchers. Sequences of primers and probes used for genotyping are available on request. Note that the *IL12B* deletion/insertion polymorphism (DIP) originally described by Morahan et al³² is not a 4-bp deletion but a CTCTAA/CG 6 or 2-bp polymorphism as determined by direct sequencing of this complex variant (Payne F, Todd JA, Unpublished data, March 2006). All SNP genotypes were in Hardy-Weinberg equilibrium ($P > .05$).

TABLE II. Association of *IL13* candidate SNPs with total serum IgE levels in as many as 3615 British adults

Variant	DNA tested	MAF (%)	Genotypes	N (%)	Mean log ₁₀ (IgE)	SD	P (1 df)*	P (2 df)*
5'-1512A>C rs1881457	3615	17.8	AA	2451 (67.8)	1.43	0.62	.00003	.00002
			AC	1047 (29.0)	1.48	0.66		
			CC	117 (3.2)	1.67	0.64		
5'-1024C>T rs1800925	3595	18.0	CC	2428 (67.5)	1.44	0.63	.0009	.0009
			CT	1049 (29.2)	1.48	0.66		
			TT	118 (3.3)	1.65	0.62		
+1923C>T rs1295686	3576	18.1	CC	2416 (67.6)	1.43	0.63	.0007	.0011
			CT	1052 (29.4)	1.47	0.65		
			TT	108 (3.0)	1.62	0.68		
+2044G>A Arg110Gln rs20541	3493	18.0	GG	2427 (67.6)	1.44	0.63	.0003	.0005
			GA	1047 (29.1)	1.48	0.65		
			AA	119 (3.3)	1.61	0.67		

MAF, Minor allele frequency.

*1 df and 2 df test of heterogeneity, adjusted for batch, sex, region, and smoking status.

Statistical analysis

Statistical analyses were performed within STATA version 8 (www.stata.com). Particular use was made of routines available from <http://www-gene.cimr.cam.ac.uk/clayton/software/stata>. Reported *P* values are not corrected for multiple testing because of a previous hypothesis and nonindependence of tests.

Total IgE was log₁₀ transformed, thus ensuring it was approximately normally distributed. The censored values (*n* = 31) were set to the mean of their expected values given the observed data, assuming a log normal distribution for log₁₀(total circulating IgE).

Regression models were used to test for an association between log-transformed total IgE levels and genotype at each locus. Analyses were restricted to white subjects and adjusted for sex, laboratory batch, geographic region (12 categories), and smoking habit (4 categories).

For most purposes, analyses included genotype at each locus as 3 groups, deriving 1 df and 2 df significance tests. The 1 df test assumes a linear relationship between the number of minor alleles and log total IgE, whereas the 2 df test allows for dominant or recessive effects. For *IL12B* and *FCER1B*, sets of tag SNPs were used to capture the common variation throughout the gene (Payne F, Todd JA, Unpublished data, March 2006), and a multilocus test was used to assess statistical significance.^{12,42}

Stepwise regression allowed us to test whether a single SNP could explain the association of other SNPs in the same gene.⁴³ To investigate haplotype effects at *IL13*, haplotypes were generated by using snphap (www-gene.cimr.cam.ac.uk/clayton/software/) and then analyzed by regression using the posterior probabilities as weights.

RESULTS

Association of total serum IgE levels and *IL13* variants

Genotyping was performed in as many as 3615 DNA samples of subjects with total IgE level measurements. We genotyped 4 SNPs in *IL13*: 5'-1512A>C/rs1881457, 5'-1024C>T/rs1800925, +1923C>T/rs1295686, and +2044G>A/Arg110Gln/rs20541. Evidence for associations of log IgE with all 4 *IL13* SNPs was obtained, with *P* values in the range of .001 to .00002 (Table II). For all 4 SNPs, the minor allele was associated with higher IgE levels than the major allele. The frequencies for the 4 main haplotypes (AG, AA, CG, CA) correspond to

75.6%, 6.6%, 6.4%, and 11.3%, respectively. Tests investigating the possibility of a haplotype that shows stronger association than either of the 2 most associated SNPs (5'-1512A>C/rs1881457 and +2044G>A/Arg110Gln/rs20541) did not provide evidence of a haplotype effect (*P* = .76). The 5'-1512/rs1881457 SNP alone accounted for 0.59% of the phenotypic variance, compared with 0.69% for all 4 SNPs combined, and in multiple regression analyses explained the associations of the other 3 SNPs with log IgE.

Association of IgE levels with variation in atopy-related candidate genes *IL4*, *IL4RA*, *IL12B*, *FCER1B*, and *TBET*

We genotyped 8 SNPs in the *IL4RA* gene on chromosome 16p11-p12 (*IL4RA*; 5'-3223/rs2057768, Ile50Val/rs1805010, Glu375Ala/rs1805011, Cys406Arg/rs1805012, Ser411Leu/rs1805013, Ser478Pro/rs1805015, Gln551Arg/rs1801275, Ser761Pro/rs1805014), 1 SNP in the *IL-4* gene on chromosome 5q31 (*IL4*; 5'-524/rs2243250), and 2 previously studied variants in *IL12B* (promoter/5' DIP/ss28514857, 3' untranslated region/*TaqI* site/rs3212227). In addition, 6 tag SNPs were genotyped in *IL12B* and 5 tag SNPs for *FCER1B/MS4A2* (Payne F, Todd JA, Unpublished data, March 2006).

The Gln551Arg variant in *IL4RA* showed some evidence of association with IgE levels (Table III; *P* = .01), but there was no support for an association of *IL12B* and *TBET* polymorphisms with IgE levels (Table III). Multilocus tests for association of log IgE with variation in *IL12B* and *FCER1B* provided no support for association: *IL12B*, 6 tag SNPs, *P* = .74, (6 df); *FCER1B*, 5 tag SNPs, *P* = .61 (5 df).

Association of IgE levels with variation in type 1 diabetes-associated genes, *CTLA4* and *PTPN22*, and the type 1 diabetes candidate gene *IL2RA/CD25*

There was no evidence of association of the type 1 diabetes susceptibility genes *CTLA4* and *PTPN22* with

TABLE III. Association of variants in atopy candidate genes with IgE levels

Gene and variant	DNAs tested	MAF (%)	Genotypes	N (%)	Mean log ₁₀ (IgE)	SD	P (1 df)*	P (2 df)*
<i>IL4</i>	1612	13.4	TT	1225 (76.0)	1.44	0.64	.09	.24
5'-524T>C			TC	354 (22.0)	1.50	0.67		
rs2243250			CC	33 (2.0)	1.56	0.61		
<i>IL4RA</i>	1613	29.2	CC	784 (48.6)	1.45	0.64	.57	.55
5'-3223C>T			CT	656 (41.97)	1.45	0.67		
rs2057768			TT	126 (8.18)	1.51	0.54		
<i>IL4RA</i>	1569	45.0	AA	460 (29.3)	1.43	0.66	.33	.62
Ile50Val			AG	805 (51.3)	1.46	0.65		
rs1805010			GG	304 (19.4)	1.49	0.60		
<i>IL4RA</i>	1604	11.9	AA	1248 (77.8)	1.47	0.65	.18	.35
Glu375Ala			AC	331 (20.6)	1.43	0.66		
rs1805011			CC	25 (1.6)	1.45	0.55		
<i>IL4RA</i>	1665	11.2	TT	1309 (78.6)	1.47	0.65	.20	.33
Cys406Arg			TC	334 (20.1)	1.42	0.66		
rs1805012			CC	22 (13.2)	1.46	0.59		
<i>IL4RA</i>	1642	5.8	CC	1491 (90.8)	1.46	0.65	.62	.70
Ser411Leu			CT	146 (8.9)	1.45	0.66		
rs1805013			TT	5 (0.3)	1.35	0.29		
<i>IL4RA</i>	1624	17.2	TT	1103 (67.9)	1.47	0.65	.24	.50
Ser478Pro			TC	478 (29.4)	1.44	0.65		
rs1805015			CC	43 (2.7)	1.42	0.58		
<i>IL4RA</i>	1660	22.6	AA	984 (59.3)	1.49	0.65	.01	.04
Gln551Arg			AG	604 (36.4)	1.42	0.65		
rs1801275			GG	72 (4.3)	1.40	0.63		
<i>IL4RA</i>	1632	0.8	TT	1606 (98.45)	1.46	0.65	.69	.69
Ser761Pro			TC	26 (1.55)	1.50	0.60		
rs1805014			CC	0 (0)	—	—		
<i>IL12B</i>	4570	49.7	6bp/6bp	1124 (24.6)	1.44	0.61	.36	.09
Promoter DIP			6bp/2bp	2297 (50.3)	1.49	0.63		
rs17875299			2bp/2bp	1149 (25.1)	1.46	0.64		
<i>IL12B</i>	4568	19.8	AA	2950 (64.6)	1.46	0.62	.17	.39
3' UTR/TaqI (A>C)			AC	1446 (31.7)	1.49	0.63		
rs3212227			CC	172 (3.8)	0.64	0.64		
<i>TBET</i>	3647	2.6	CC	3463 (94.9)	1.45	0.64	.91	.45
His33Glu			CG	182 (5.0)	1.48	0.67		
rs2240017			GG	2 (0.1)	1.00	0.06		

MAF, Minor allele frequency; UTR, untranslated region.

*1 df and 2 df test of heterogeneity, adjusted for batch, sex, region, and smoking status.

IgE levels (Table IV). In addition, genotyping of 20 tag SNPs in the type 1 diabetes candidate gene *CD25*¹² did not provide evidence of association ($P = .12$; 20 df).

DISCUSSION

Our results confirm the association of the IL-13 gene with IgE levels (Table II) and concur with previous reports of the variant allele being associated with higher IgE levels.^{18,19,44-46} For the first time, we provide evidence that the 5'-1512A>C/rs1881457 SNP might alone account for the association of the IL-13 gene with total IgE in this national sample of the middle-aged British population. We note that functional studies that investigated *IL13* polymorphisms have not studied the potential effects of the

5'-1512A>C/rs1881457 SNP. The SNPs with functional data, namely 5'-1024C>T/rs1800925 and Arg110Gln/rs20541,^{13-15,20} are associated with total IgE in our study but do not appear to have genetic effects in addition to that of 5'-1512A>C/rs1881457. However, in the absence of functional studies investigating all *IL13* SNPs, it is impossible to exclude the presence of several functional SNPs in the region. It is also possible that the causal variant remains untyped or even undiscovered, lying elsewhere in this chromosome region, perhaps regulating IL-13 expression, but is in linkage disequilibrium with the currently typed SNPs. This possibility can be evaluated only by assembling and analyzing a complete polymorphism and linkage disequilibrium map of the entire region in a large sample set, thereby defining subregions in which a causal variant is unlikely to exist. Nevertheless,

TABLE IV. Association of variants in type 1 diabetes-related genes, *CTLA4* and *PTPN22*

Gene and variant	DNA tested	MAF (%)	Genotypes	N (%)	Mean log ₁₀ (IgE)	SD	P (1 df)*	P (2 df)*
<i>CTLA4</i>	1607	14.7	CC	1178 (73.3)	1.46	0.65	.98	.44
–1147C>T			CT	388 (24.1)	1.44	0.65		
rs16840252			TT	41 (2.6)	1.58	0.70		
<i>CTLA4</i>	1631	38.3	GG	622 (38.1)	1.43	0.65	.24	.35
+49G>A			AG	763 (46.8)	1.48	0.65		
rs231775			AA	246 (15.1)	1.47	0.64		
<i>CTLA4</i>	3598	46.1	GG	1077 (29.9)	1.46	0.63	.42	.65
+6230G>A			GA	1731 (48.1)	1.45	0.63		
rs3087243			AA	790 (22.0)	1.44	0.64		
<i>PTPN22</i>	3562	9.7	CC	2898 (81.4)	1.45	0.64	.79	.83
Arg620Trp (C>T)			CT	631 (17.7)	1.44	0.64		
rs2476601			TT	33 (0.9)	1.47	0.56		

MAF, Minor allele frequency.

*1 df and 2 df test of heterogeneity, adjusted for batch, sex, region, and smoking status.

the previous functional studies of *IL13* and its SNPs strongly suggest that *IL13* is the causal gene for raised IgE levels and atopy risk.

We hypothesized that *IL13* alleles predisposing to high IgE levels, which are part of T_H2 responses, might show an inverse effect on T_H1-associated type 1 diabetes. Our data do not lend support for such a mechanism, because we had previously shown that these *IL13* SNPs are not associated with type 1 diabetes in as many as 3266 type 1 diabetes cases and controls and as many as 748 type 1 diabetes families, including taking into account genotypes at the *IL4* and *IL4RA* loci.⁴⁷

Similarly, there is unequivocal evidence that *CTLA4* and *PTPN22* are associated with type 1 diabetes (and indeed many other autoimmune diseases, because both loci have been identified as autoimmune loci rather than being specific to type 1 diabetes).^{27,35,48-50} Neither of these genes or their SNPs showed any effect on IgE levels in our sample, nor did the type 1 diabetes candidate gene *IL2RA/CD25*. Furthermore, the genes encoding IL-4 and its receptor, IL-4R α , are not associated with IgE levels (Table III) or with type 1 diabetes.⁴⁷ This also applied to the 3 other candidate genes, *IL12B*, *TBET*, and *FCER1B* (Table III). It is noted that associations between type 1 diabetes and SNPs in *IL12B* have been reported,⁵¹⁻⁵³ but these failed to be replicated by others,⁵⁴⁻⁵⁷ including our unpublished observations. *TBET* and *FCER1B* are also not associated with type 1 diabetes (Bailey R, Todd JA, Unpublished data, March 2006; Payne F, Todd JA, Unpublished data, March 2006).

Inconsistencies with previously published studies of the role of *IL4RA*,²⁴ *CTLA4*,^{25,26} and *IL12B*³⁰⁻³² variants in the control of total serum IgE levels may be caused by several factors. Twin studies on the heritability of total IgE levels have reported that 40% of the variance in IgE is a result of environmental factors,¹ implying that allergens and pharmacologic, dietary, and infectious stimuli are likely to present parameters to consider for comparisons of studies investigating different populations in which environmental factors are important. In addition, heterogeneity in allele frequencies, true differences in IgE

associations between populations, ascertainment bias, and methods of analyses present plausible explanations for nonreplication of genetic association studies, because these were performed in different countries across Europe, Asia, and North America. Potentially the most important factor described in the literature, however, is the use of small sample sizes that are statistically inadequate to detect a true genetic effect.^{54,58-63} For a complex trait such as total serum IgE levels, studies using small sample sizes have minimal statistical power to detect plausible genetic effects. An effect size of a 5% increase in log(IgE), for example, will need a sample size in excess of 2000 individuals to ensure a low type 2 error probability. A convincing genetic susceptibility finding requires replication in independent, large sample sizes from the same population and ethnic group. With a size of as many as 4570 samples, our study is the largest so far attempting replication of previously reported SNPs associated with total serum IgE levels.

Finally, we remark on the size of the *IL13* effect and its implications. The *IL13* association with IgE and atopic illness is widely published, and is confirmed here, indicative that it is a true effect and not a false-positive result,^{59,61,64} and that SNPs within or very near the IL-13 gene are causal. Yet despite their prominence in the literature and their known direct biological effects on IgE levels, the *IL13* SNPs account for only 0.69% of the phenotypic (ie, log IgE) variance. We would, therefore, not recommend too large an investment in exploratory genotyping in sample sizes smaller than were used here.

In conclusion, our results have significant implications for the design of reliable studies to locate and confirm other genes regulating IgE levels and contributing to atopy, not least that sample sizes will have to be large, consisting of several thousands of subjects. Because we have previously reported that *IL13* variants are not associated with type 1 diabetes, and as shown here, other type 1 diabetes susceptibility variants do not influence IgE levels, we conclude that the proposed inverse association of atopic illness and type 1 diabetes is not explained by these genetic variants. We note that our negative

findings for other cytokine genes do not rule out the possibility that application of these molecules clinically might have therapeutic benefits.

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REFERENCES

- Strachan DP, Wong HJ, Spector TD. Concordance and interrelationship of atopic diseases and markers of allergic sensitization among adult female twins. *J Allergy Clin Immunol* 2001;108:901-7.
- Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. *J Allergy Clin Immunol* 1984;73:265-70.
- Constant SL, Bottomly K. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu Rev Immunol* 1997;15:297-322.
- Imagawa A, Hanafusa T, Tamura S, Moriaki M, Itoh N, Yamamoto K, et al. Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type 1 diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. *Diabetes* 2001;50:1269-73.
- Pipeleers D, Ling Z. Pancreatic beta cells in insulin-dependent diabetes. *Diabetes Metab Rev* 1992;8:209-27.
- Todd JA, Wicker LS. Genetic protection from the inflammatory disease type 1 diabetes in humans and animal models. *Immunity* 2001;15:387-95.
- Ansel KM, Lee DU, Rao A. An epigenetic view of helper T cell differentiation. *Nat Immunol* 2003;4:616-23.
- Huang TJ, MacAry PA, Eynott P, Moussavi A, Daniel KC, Askenase PW, et al. Allergen-specific Th1 cells counteract effector Th2 cell-dependent bronchial hyperresponsiveness and eosinophilic inflammation partly via IFN-gamma. *J Immunol* 2001;166:207-17.
- Ellis CN, Stevens SR, Blok BK, Taylor RS, Cooper KD. Interferon-gamma therapy reduces blood leukocyte levels in patients with atopic dermatitis: correlation with clinical improvement. *Clin Immunol* 1999;92:49-55.
- Cardwell CR, Shields MD, Carson DJ, Patterson CC. A meta-analysis of the association between childhood type 1 diabetes and atopic disease. *Diabetes Care* 2003;26:2568-74.
- Sheikh A, Smeeth L, Hubbard R. There is no evidence of an inverse relationship between TH2-mediated atopy and TH1-mediated autoimmune disorders: lack of support for the hygiene hypothesis. *J Allergy Clin Immunol* 2003;111:131-5.
- Vella A, Cooper JD, Lowe CE, Walker N, Nutland S, Widmer B, et al. Localization of a type 1 diabetes locus in the *IL2RA/CD25* region by use of tag single-nucleotide polymorphisms. *Am J Hum Genet* 2005;76:773-9.
- Arima K, Umeshita-Suyama R, Sakata Y, Akaiwa M, Mao XQ, Enomoto T, et al. Upregulation of IL-13 concentration *in vivo* by the *IL13* variant associated with bronchial asthma. *J Allergy Clin Immunol* 2002;109:980-7.
- Vladich FD, Brazille SM, Stern D, Peck ML, Ghittoni R, Vercelli D. IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation. *J Clin Invest* 2005;115:747-54.
- Chen W, Ericksen MB, Levin LS, Khurana Hershey GK. Functional effect of the R110Q IL13 genetic variant alone and in combination with *IL4RA* genetic variants. *J Allergy Clin Immunol* 2004;114:553-60.
- Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004;202:175-90.
- Holgate ST. The epidemic of allergy and asthma. *Nature* 1999;402:B2-4.
- Graves PE, Kabisch M, Halonen M, Holberg CJ, Baldini M, Fritzsche C, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;105:506-13.
- Heinzmann A, Mao XQ, Akaiwa M, Kreomer RT, Gao PS, Ohshima K, et al. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet* 2000;9:549-59.
- van der Pouw Kraan TC, van Veen A, Boeije LC, van Tuyt SA, de Groot ER, Stapel SO, et al. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun* 1999;1:61-5.
- Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. *N Engl J Med* 1997;337:1720-5.
- Ober C, Leavitt SA, Tsalkenko A, Howard TD, Hoki SK, Daniel R, et al. Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am J Hum Genet* 2000;66:517-26.
- Hackstein H, Hecker M, Kruse S, Bohnert A, Ober C, Deichmann KA, et al. A novel polymorphism in the 5' promoter region of the human interleukin-4 receptor alpha-chain gene is associated with decreased soluble interleukin-4 receptor protein levels. *Immunogenetics* 2001;53:264-9.
- Woitsch B, Carr D, Stachel D, Schmid I, Weiland DM, Fritzsche C, et al. A comprehensive analysis of interleukin-4 receptor polymorphisms and their association with atopy and IgE regulation in childhood. *Int Arch Allergy Immunol* 2004;135:319-24.
- Howard TD, Postma DS, Hawkins GA, Koppelman GH, Zheng SL, Wysong AK, et al. Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 2002;110:743-51.
- Munthe-Kaas MC, Carlsen KH, Helms PJ, Gerritsen J, Whyte M, Feijen M, et al. CTLA-4 polymorphisms in allergy and asthma and the TH1/TH2 paradigm. *J Allergy Clin Immunol* 2004;114:280-7.
- Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene *CTLA4* with susceptibility to autoimmune disease. *Nature* 2003;423:506-11.
- Hill MR, James AL, Faux JA, Ryan G, Hopkin JM, le Souef P, et al. Fc epsilon RI-beta polymorphism and risk of atopy in a general population sample. *BMJ* 1995;311:776-9.
- Shirakawa T, Mao XQ, Sasaki S, Enomoto T, Kawai M, Morimoto K, et al. Association between atopic asthma and a coding variant of Fc epsilon RI beta in a Japanese population. *Hum Mol Genet* 1996;5:1129-30.
- Randolph AG, Lange C, Silverman EK, Lazarus R, Silverman ES, Raby B, et al. The IL12B gene is associated with asthma. *Am J Hum Genet* 2004;75:709-15.
- Khoo SK, Hayden CM, Roberts M, Horak E, de Klerk N, Zhang G, et al. Associations of the *IL12B* promoter polymorphism in longitudinal data from asthmatic patients 7 to 42 years of age. *J Allergy Clin Immunol* 2004;113:475-81.
- Morahan G, Huang D, Wu M, Holt BJ, White GP, Kendall GE, et al. Association of *IL12B* promoter polymorphism with severity of atopic and non-atopic asthma in children. *Lancet* 2002;360:455-9.
- Lametschwandtner G, Biedermann T, Schwarzer C, Gunther C, Kund J, Fassl S, et al. Sustained T-bet expression confers polarized human TH2 cells with TH1-like cytokine production and migratory capacities. *J Allergy Clin Immunol* 2004;113:987-94.
- Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat Genet* 2004;36:337-8.
- Smyth D, Cooper JD, Collins JE, Heward JM, Franklin JA, Howson JM, et al. Replication of an association between the lymphoid tyrosine phosphatase locus (*LYP/PTPN22*) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 2004;53:3020-3.
- Strachan DP, Butland BK, Anderson HR. Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. *BMJ* 1996;312:1195-9.
- Williams HC, Strachan DP. The natural history of childhood eczema: observations from the British 1958 birth cohort study. *Br J Dermatol* 1998;139:834-9.
- Strachan DP. Hay fever, hygiene and household size. *BMJ* 1989;299:1259-60.
- Strachan DP. The epidemiology of childhood asthma. *Allergy* 1999;54(suppl 49):7-11.
- Williams HC, Strachan DP, Hay RJ. Childhood eczema: disease of the advantaged? *BMJ* 1994;308:1132-5.
- Nolte H, DuBuske LM. Performance characteristics of a new automated enzyme immunoassay for the measurement of allergen-specific IgE: summary of the probability outcomes comparing results of allergen skin testing to results obtained with the HYTEC system and CAP system. *Ann Allergy Asthma Immunol* 1997;79:27-34.

42. Chapman JM, Cooper JD, Todd JA, Clayton DG. Detecting disease associations due to linkage disequilibrium using haplotype tags: a class of tests and the determinants of statistical power. *Hum Hered* 2003;56:18-31.
43. Cordell HJ, Clayton DG. A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. *Am J Hum Genet* 2002;70:124-41.
44. Liu X, Beaty TH, Deindl P, Huang SK, Lau S, Sommerfeld C, et al. Associations between total serum IgE levels and the 6 potentially functional variants within the genes IL4, IL13, and IL4RA in German children: the German Multicenter Atopy Study. *J Allergy Clin Immunol* 2003;112:382-8.
45. Wang M, Xing ZM, Lu C, Ma YX, Yu DL, Yan Z, et al. A common IL-13 Arg130Gln single nucleotide polymorphism among Chinese atopy patients with allergic rhinitis. *Hum Genet* 2003;113:387-90.
46. Hoffjan S, Ostrovskaja I, Nicolae D, Newman DL, Nicolae R, Gangnon R, et al. Genetic variation in immunoregulatory pathways and atopic phenotypes in infancy. *J Allergy Clin Immunol* 2004;113:511-8.
47. Maier LM, Chapman J, Howson JM, Clayton DG, Pask R, Strachan DP, et al. No evidence of association or interaction between the IL4RA, IL4, and IL13 genes in type 1 diabetes. *Am J Hum Genet* 2005;76:517-21.
48. Torres B, Aguilar F, Franco E, Sanchez E, Sanchez-Roman J, Jimenez Alonso J, et al. Association of the CT60 marker of the CTLA4 gene with systemic lupus erythematosus. *Arthritis Rheum* 2004;50:2211-5.
49. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004;75:330-7.
50. Hinks A, Barton A, John S, Bruce I, Hawkins C, Griffiths CE, et al. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. *Arthritis Rheum* 2005;52:1694-9.
51. Morahan G, Huang D, Ymer SI, Cancilla MR, Stephen K, Dabadghao P, et al. Linkage disequilibrium of a type 1 diabetes susceptibility locus with a regulatory IL12B allele. *Nat Genet* 2001;27:218-21.
52. Davoodi-Semiromi A, Yang JJ, She JX. IL-12p40 is associated with type 1 diabetes in Caucasian-American families. *Diabetes* 2002;51:2334-6.
53. Windsor L, Morahan G, Huang D, McCann V, Jones T, James I, et al. Alleles of the IL12B 3' UTR associate with late onset of type 1 diabetes. *Hum Immunol* 2004;65:1432-6.
54. Dahlman I, Eaves IA, Kosoy R, Morrison VA, Heward J, Gough SC, et al. Parameters for reliable results in genetic association studies in common disease. *Nat Genet* 2002;30:149-50.
55. McCormack RM, Maxwell AP, Carson DJ, Patterson CC, Middleton D, Savage DA. The IL12B 3' untranslated region DNA polymorphism is not associated with early-onset type 1 diabetes. *Genes Immun* 2002;3:433-5.
56. Nistico L, Giorgi G, Giordano M, Galgani A, Petrone A, D'Alfonso S, et al. IL12B polymorphism and type 1 diabetes in the Italian population: a case-control study. *Diabetes* 2002;51:1649-50.
57. Bergholdt R, Ghandil P, Johannesen J, Kristiansen OP, Kockum I, Luthman H, et al. Genetic and functional evaluation of an interleukin-12 polymorphism (*IDDM18*) in families with type 1 diabetes. *J Med Genet* 2004;41:e39.
58. Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. *Lancet* 2001;358:1356-60.
59. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med* 2002;4:45-61.
60. Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;361:865-72.
61. Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG. Genetic associations in large versus small studies: an empirical assessment. *Lancet* 2003;361:567-71.
62. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33:177-82.
63. Thomas DC, Clayton DG. Betting odds and genetic associations. *J Natl Cancer Inst* 2004;96:421-3.
64. Wang WY, Barratt BJ, Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet* 2005;6:109-18.
65. Howard TD, Whittaker PA, Zaiman AL, Koppelman GH, Xu J, Hanley MT, et al. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001;25:377-84.
66. Hummelshoj T, Bodtger U, Datta P, Mallng HJ, Oturai A, Poulsen LK, et al. Association between an interleukin-13 promoter polymorphism and atopy. *Eur J Immunogenet* 2003;30:355-9.
67. He JQ, Chan-Yeung M, Becker AB, Dimich-Ward H, Ferguson AC, Manfreda J, et al. Genetic variants of the IL13 and IL4 genes and atopic diseases in at-risk children. *Genes Immun* 2003;4:385-9.
68. Celedon JC, Soto-Quiros ME, Palmer LJ, Senter J, Mosley J, Silverman EK, et al. Lack of association between a polymorphism in the interleukin-13 gene and total serum immunoglobulin E level among nuclear families in Costa Rica. *Clin Exp Allergy* 2002;32:387-90.