Brief Genetics Report

Association of *IL4R* Haplotypes With Type 1 Diabetes

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We have investigated, in 282 multiplex Caucasian families (the Human Biological Data Interchange Repository), the association of type 1 diabetes with polymorphisms in the *IL4R* gene. *IL4R* encodes a subunit of the interleukin-4 receptor, a molecule critical to T-helper cell development. By genotyping eight different *IL4R* single-nucleotide polymorphisms (SNPs) and identifying haplotypes (complex alleles) in the multiplex type 1 diabetic families who were stratified for *HLA* genotype, we have observed significant evidence of linkage and association of the *IL4R* gene to type 1 diabetes. In particular, we have identified a specific haplotype that appears to be protective and observed that this protective effect is strongest among individuals not carrying the *HLA* DR3/DR4 genotype (which confers the strongest genetic risk for type 1 diabetes). These findings suggest an important role for the *IL4R* gene in immune-related disease susceptibility and illustrate the value of using multi-SNP haplotype information in association studies. *Diabetes* 51:3336–3341, 2002

Type 1 diabetes, an early-onset autoimmune disease of glucose homeostasis, is caused by destruction of insulin-producing cells of the pancreas and involves a cellular T-helper response characteristic of the Th1 (cell-mediated) pathway (1,2). One molecule critical to T-helper cell differentiation and commitment is the interleukin-4 (IL4) receptor heterodimer, and in particular, the subunit encoded by the *IL4R* gene located at 16p11.2–12.1. Many of the known single-nucleotide polymorphisms (SNPs) in *IL4R* change the protein primary structure (reviewed in 3). Changes to IL4R amino acid residues, particularly at Ile50Val, Ser478Pro, and Gln551Arg, are suggested to be functionally important and impact IL4 receptor signaling (ref. 4 and citations therein). *IL4R* SNPs have been associated with susceptibility to immune-related diseases, including (but not limited to) asthma and atopy (4–7). However, a consistent pattern of allele or genotype associations with specific disease-related phenotypes has not emerged. Furthermore, the genotyping of single *IL4R* SNP alleles appears insufficient to capture the genetic diversity of the *IL4R* locus (8–13), and this may in part explain the discrepancies cited. Here, by genotyping eight different *IL4R* SNPs and identifying haplotypes (complex alleles) in multiplex type 1 diabetic families stratified with respect to high-risk HLA genotypes, we have observed significant evidence of linkage and association of the *IL4R* gene to type 1 diabetes.

We genotyped 282 type 1 diabetic multiplex families at eight SNPs in the *IL4R* gene (Table 1) using a multiplex PCR assay coupled with sequence-specific hybridization to an immobilized probe array (Fig. 1). The 282 Human Biological Data Interchange (HBDI) families used in this study are nuclear families of Caucasian ethnicity, with unaffected parents and two ascertained affected siblings (14). The two-generation family structure and the absence of recombination among SNPs enabled us to deduce *IL4R* haplotypes for all individuals and assess transmission patterns. Herein, we will use the term "haplotype" to represent the ordered set of *IL4R* SNP alleles present on a single chromosome. The "haplotype" notation is representative of the primary sequence of the protein and is consistent with terminology used by others (8,11–13).

There is substantial evidence that many genetic loci, as well as environmental factors, are involved in predisposition to type 1 diabetes (15). However, the genetic locus with the largest contribution by far to familial clustering is the *HLA* region on chromosome 6p21.3 (*IDDM1*). This region is thought to contribute at least 50% of the overall genetic risk (16). Multiple loci within the *HLA* region (e.g., *HLA-DRB1*, *-DQA1*, *-DQB1*, *-DPB1*, and *-A*), and specific *HLA* locus genotypes and haplotypes, all contribute to the genetic risk (15–18). Some *HLA* alleles are positively associated with type 1 diabetes (conferring increased risk), while others are negatively associated (conferring reduced risk). One heterozygous genotype (*DRB1**0301-*DQB1**0201/*DRB1**0401-*DQB1**0302) confers a significantly increased risk, with an odds ratio (OR) of 20–30. The identification of type 1 diabetes risk loci outside the *DR-DQ* region in some cases has required stratification of DR3/4 and non-DR3/4 genotypes (17).

In our analyses of the *IL4R* data, we stratified the families into two groups: those in which either or both of the affected sibs are DR3/4 versus those in which neither of the sibs is DR3/4 (see RESEARCH DESIGN AND METHODS). Since the *HLA* DR3/4 genotype confers such a large

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HBDI, Human Biological Data Interchange; IBD, identity by descent; IL4, interleukin-4; LD, linkage disequilibrium; LOD, logarithm of odds; OR, odds ratio; SNP, single-nucleotide polymorphism; TDT, transmission disequilibrium test.

TABLE 1
Identification of *IL4R* SNPs used and pairwise LD

SNP	Amino acid variation	*IL4R* SNP position in X52425.1	*IL4R* SNP position in AC004525.1	SNP 1	2	3	4	5	6	7	8
1	I50V	398A>G	94272T>C	—	**1.00**	0.37	0.37	0.33	0.52	0.36	**1.00**
2	N142N	676C>T	92548A>G	0.35	—	**−1.00**	**−1.00**	**−1.00**	0.41	0.38	**1.00**
3	E375A	1374A>C	76608T>G	0.16	−0.15	—	**1.00**	**1.00**	**0.97**	**1.00**	**−1.00**
4	L389L	1417G>T	76565C>A	0.16	−0.15	**1.00**	—	**1.00**	**0.97**	**1.00**	**−1.00**
5	C406R	1466T>C	76516A>G	0.13	−0.14	**0.96**	**0.96**	—	**1.00**	**1.00**	**−1.00**
6	S478P	1682T>C	76300A>G	0.26	0.28	0.79	0.79	0.78	—	**1.00**	**1.00**
7	Q551R	1902A>G	76080T>C	0.21	0.23	0.72	0.72	0.69	0.88	—	**1.00**
8	S761P	2531T>C	75451A>G	0.10	0.29	−0.04	−0.04	−0.04	0.20	0.18	—

Information identifying the *IL4R* SNPs is presented in the first four columns of the table. Each SNP is described by its position in the GenBank cDNA reference accession sequence X52425.1, and in the reference genomic sequence for chromosome 16 BAC clone AC004525.1 (these two sequences are antiparallel). The amino acid variation numbering refers to that of the mature IL4R protein (4,11). The remaining eight columns present two measures of linkage disequilibrium (LD) between all pairs of *IL4R* SNPs used in this study. D' values are shown in the upper-right triangle; Δ values in the lower-left triangle. Bold text indicates LD measures whose absolute values are near 1.0, indicating "complete" or "absolute" pairwise LD.

genetic risk for type 1 diabetes, our approach was to look in families without the high-risk *HLA DR-DQ* genotypes so that other less strong linkage and association signals might be detected. Since below we present results together for the full dataset and for the two subgroups, we apply a conservative Bonferroni adjustment to the unadjusted *P* values to take account of these multiple comparisons.

The transmission disequilibrium test (TDT) (19) was performed on the *IL4R* genotype data to test for the association of the individual alleles of the eight *IL4R* SNPs to type 1 diabetes. The results of the SNP locus TDT are shown in Table 2. Nearly statistically significant evidence of excess transmission is only seen at SNP 6 (S478P) and only in "neither sib DR3/4" families (unadjusted $P = 0.03$, adjusted $P = 0.10$), although among all families, the data show a strong trend for SNP 6. The transmissions of the "reference" allele (Table 1) of SNPs 3, 4, 5, and 7 are elevated to the same extent (~58%) as for SNP 6. We note that the comparisons of the eight SNPs presented are not independent statistical tests, as the SNPs are in very strong linkage disequilibrium (LD) (Table 1).

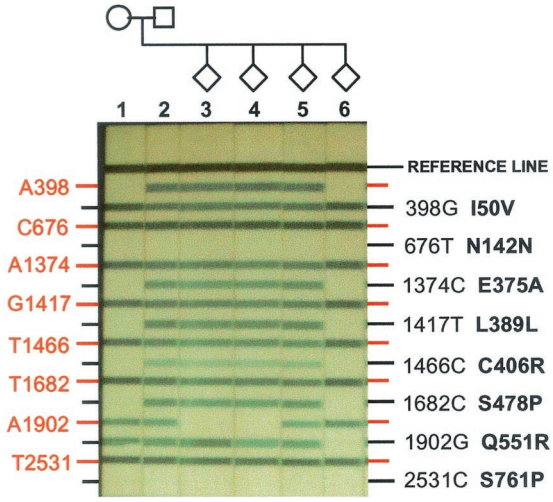

FIG. 1. Example of *IL4R* SNP genotyping linear array. The results of six individuals from a single family are shown. The linear array strips are arranged vertically, with a reference ink line at the top. The presence of a blue horizontal line on each linear array indicates hybridization to the sequence-specific probe at that position and thus the presence of that allele. The two sequence-specific probe lines for each SNP are shown; the reference allele is listed on the left in red, and the variant allele and the SNP name are on the right in black. The positions of SNPs in GenBank accession X52425.1 are shown (Table 1). By inspection, one can conclude in this example that individual 6 is homozygous for haplotype H-2 ("21111111," Table 3), whereas individual 1 (mother) possesses haplotypes H-2 and H-8 ("21111121"). One can thus deduce that individual 2 (father) possesses haplotypes H-2 and H-5 ("11222221").

Evidence for linkage of *IL4R* to type 1 diabetes was assessed by the haplotype sharing method (16), following inference of haplotypes and DR3/4 stratification (see RESEARCH DESIGN AND METHODS). The distribution of haplotype identity-by-descent (IBD) sharing is shown in the online appendix. A nearly statistically significant deviation from the expected distribution of IBD sharing (unadjusted $P = 0.03$, adjusted $P = 0.08$) was observed in the "neither sib DR3/4" subgroup (online appendix), providing evidence for linkage of the *IL4R* locus to type 1 diabetes, but only in that subgroup. A $\lambda_s = 1.16$ for *IL4R* can be calculated for the complete dataset (20).

Genome-wide linkage scans have identified many potential regions of linkage of type 1 diabetes in addition to the *HLA* region (*IDDM1*) and *INS* (*IDDM2*). Whereas evidence exists for a linked locus at 16q22-q24 (logarithm of odds [LOD] = 3.93) in U.K. and U.S. families, support for linkage has also been reported at 16p11-q12 (LOD = 1.74), where *IL4R* resides (21). Our IBD sharing data (online appendix) provide evidence consistent with those reports for linkage of *IL4R* with type 1 diabetes in the "neither sib DR3/4" group. One explanation for the lower LOD score near *IL4R* in those other genome-wide scans is that they may not have had sufficient power to detect loci with a $\lambda_s < 1.3$.

Association of *IL4R* haplotypes with type 1 diabetes was assessed using a modified TDT haplotype association analysis (Table 3). We evaluated the nine most frequent eight-locus haplotypes using a two-sided modified TDT for the null hypothesis of no association and no linkage (see RESEARCH DESIGN AND METHODS). The TDT analyses (Table 3) revealed significant evidence of undertransmission of a single eight-locus *IL4R* haplotype (labeled "21222221" or H-3) in the full cohort (unadjusted $P = 0.007$, adjusted $P =$

TABLE 2
TDT analysis of individual *IL4R* SNPs

SNP	All families (n = 282)				Either/both sib DR3/4 (n = 135)				Neither sib DR3/4 (n = 147)			
	Number of informative transmission events	% Transmission of reference allele to affecteds	McNemar χ^2 statistic	P	Number of informative transmission events	% Transmission of reference allele to affecteds	McNemar χ^2 statistic	P	Number of informative transmission events	% Transmission of reference allele to affecteds	McNemar χ^2 statistic	P
1	564	47.3%	1.60	0.21	256	51.6%	0.25	0.62	308	53.6%	1.57	0.21
2	188	54.8%	1.72	0.19	88	52.3%	0.18	0.67	100	57.0%	1.96	0.16
3	216	56.0%	3.13	0.08	104	53.8%	0.62	0.43	112	58.0%	2.89	0.09
4	216	56.0%	3.13	0.08	104	53.8%	0.62	0.43	112	58.0%	2.89	0.09
5	214	55.1%	2.26	0.13	104	53.8%	0.62	0.43	110	56.4%	1.78	0.18
6	314	55.4%	3.68	0.06	138	52.2%	0.26	0.61	176	58.0%	4.45	0.03
7	368	54.1%	2.45	0.12	170	52.9%	0.59	0.44	198	55.1%	2.02	0.16
8	24	50.0%	0.00	1.00	14	42.9%	0.29	0.59	10	60.0%	0.40	0.53

The table lists the observed transmissions of the reference allele (as defined in Table 1) at each of the eight *IL4R* SNP loci individually. The TDT analysis was performed on the complete HBDI cohort and after stratification for *HLA*-DR3/4 status, as described in the text. The number of informative transmissions for each SNP and the percent transmission are listed, as are the associated McNemar χ^2 statistic and the significance level. P values are shown before adjustment.

0.021) and the "neither sib DR3/4" group (unadjusted P = 0.005, adjusted P = 0.015). In the full cohort, the H-3 haplotype has an OR of 0.63 (unadjusted 95% CI = 0.44–0.89), and in the "neither sib DR3/4" group, the OR = 0.49 (unadjusted 95% CI = 0.28–0.81) (Table 3). The haplotype H-3 can thus be regarded as conferring protection to type 1 diabetes.

The ratio of transmissions to nontransmissions of H-3 to affected sibs is 28:44 from H-1/H-3 parents and 17:25 from H-2/H-3 parents, among unambiguously determined haplotypes (data not shown). These transmission proportions of ~40% differ from the observed 50% transmission of H-1 or H-2 from H-1/H-2 parents expected under the null hypothesis (data not shown). The effect of H-3 is thus specific and not due to an apparent overtransmission of any other haplotype.

The use of multiplex families such as the HBDI cohort increases the power to detect a genetic effect precisely because sibships are more likely to share a genetic factor. Because of this ascertainment method, the observed association of *IL4R* in type 1 diabetes might apply only to a subset of the general population.

A recent report by Reimsnider et al. (7) investigated the association of *IL4R* and type 1 diabetes using the same HBDI family cohort (among others), but concluded that there was none. There are several possible explanations for the differences between the results of that report and ours. First, our IBD sharing results revealed marginal evidence of linkage only when DR3/4 stratification was applied. Second, in that previous study, only a single *IL4R* SNP (no. 7, Q551R) was genotyped (7), and it was not the SNP (no. 6, S478P) for which we detected the best evidence for a transmission bias (Table 2). More importantly, the observation of eight-SNP haplotypes increases the informativeness of the locus by converting biallelic SNPs into one multiallelic locus (Table 3).

The association of haplotype H-3 with type 1 diabetes may reflect the combined effect of the eight SNPs that compose the haplotype. Alternatively, the eight *IL4R* SNPs studied here may not capture all the variation defining the type 1 diabetes–protective haplotype, and other SNPs on or in LD with haplotype H-3 may be involved. We have found that haplotype H-3 is associated with protection from type 1 diabetes, with a similar OR, in a Filipino population (T. Bugawan, D.B.M., A.M.V., A. Panelo, C. Solfelix, P. Pozzilli, R. Buzetti, H.A.B., manuscript in preparation) and favor the hypothesis that this specific variant of the IL4R protein confers protection to type 1 diabetes. An alternative hypothesis, in which H-3 acts as a marker in LD with other causal variation(s), seems less likely because the LD patterns and haplotype distributions among Filipinos are different from those of the Caucasian HBDI population.

Our results suggest that a specific combination of the eight SNPs, rather than any individual SNP, is responsible for the observed protection from type 1 diabetes (Table 3). The evidence for the negative association of the H-3 haplotype with type 1 diabetes is stronger than that seen with any of the individual SNPs (33 vs. 42% transmission in the "neither DR3/4" group; unadjusted P = 0.005 vs. 0.03, respectively). The effect of individual SNPs in a haplotype seems to depend on the nature of the other SNPs, which is

TABLE 3
TDT analysis of *IL4R* haplotypes

Haplotype ID	Haplotype	All families			Either/both sib DR3/4				Neither sib DR3/4			
		Number of informative transmission events	% transmitted	OR	95% CI	Number of informative transmission events	% transmitted	OR	95% CI	Number of informative transmission events	% transmitted	OR	95% CI
H-1	11111111	550	52%	1.09	0.92–1.30	269	52%	1.08	0.85–1.38	281	53%	1.11	0.86–1.40
H-2	21111111	498	51%	1.06	0.90–1.25	226	50%	1.01	0.80–1.28	272	52%	1.10	0.86–1.42
H-3	21222221	122	39%*	0.63	0.44–0.89	61	44%	0.80	0.50–1.27	62	33%†	0.49	0.28–0.81
H-4	22111111	93	47%	0.89	0.61–1.30	50	45%	0.81	0.47–1.36	43	50%	0.99	0.56–1.71
H-5	11222221	86	49%	0.95	0.59–1.50	37	40%	0.68	0.30–1.42	49	55%	1.24	0.67–2.36
H-6	22111221	77	44%	0.78	0.45–1.24	27	52%	1.07	0.49–2.40	50	40%	0.65	0.31–1.23
H-7	11111121	77	57%	1.33	0.90–2.06	43	55%	1.23	0.71–2.20	33	60%	1.48	0.75–3.19
H-8	21111121	27	43%	0.75	0.38–1.60	12	38%	0.61	0.18–2.22	15	47%	0.88	0.38–3.09
H-9	22111222	16	45%	0.83	0.25–2.68	10	50%	1.00	—‡	6	38%	0.60	—‡

Each haplotype is listed by the ordered set of alleles present at each of the eight *IL4R* SNPs. A "1" denotes the presence of the reference allele, and a "2" indicates the presence of the variant allele for each SNP, as described in Table 1. Haplotypes are also given arbitrary haplotype ID numbers (H-1 through -9). The table shows only haplotypes that were observed in at least 15 effective transmission events in the affected children (representing 1,546/1,658 = 93% of observed haplotypes in informative transmission events). For each haplotype, percent transmitted is the ratio of actual transmissions to possible transmissions to affected children of informative parents. The ORs compare actual with Mendelian segregation ratios. The 95% CI column lists the upper and lower bounds of the 95% CI of the OR, calculated based on the number of families (not shown) informative for that haplotype. *P* values are based on permutation. *Significant after adjustment at $P = 0.021$; †significant after adjustment at $P = 0.015$; ‡CIs are not reported in instances where 10 or fewer families were informative for the haplotype.

biologically plausible, as these SNPs encode allelic variants of the same IL4R protein. For example, haplotypes H-1 and -2 differ only at SNP 1 and have the same OR (Table 3); H-3 and -5 also differ only at SNP1, although H-5 does not appear to influence susceptibility to type 1 diabetes, whereas H-3 does. The role of SNP 1 in susceptibility to type 1 diabetes is therefore context dependent, highlighting the value of using haplotype data.

How might haplotype H-3 confer protection to type 1 diabetes given the known role of *IL4R* variation in Th2-related disease? Although numerous studies report associations of individual *IL4R* SNP alleles and genotypes with asthma and atopy, the results are not consistent. In some studies, the variant allele is associated with a disease-related phenotype, while in others it is the reference allele. If, in fact, the variant alleles (or haplotypes bearing them, such as H-3) are associated with a Th2 phenotype, then the protection to type 1 diabetes reported here may reflect a shift of the balance between Th1 and Th2 responses toward Th1. If, on the other hand, the reference alleles (or haplotypes bearing them) are associated with asthma and atopy, then this simple model of Th1 versus Th2 balance does not fit the data. Perhaps, therefore, variations in *IL4R* influence the regulation of both Th1 and Th2 responses, as elaborated further below.

The SNPs we have used to define *IL4R* haplotypes do not fully coincide with those used by others in assessing haplotypic association to asthma and atopy phenotypes. Nonetheless, what Ober et al. (11) called haplotype "222122" and Hackstein et al. (8) called IL4RA*03 both correspond to our haplotype H-3. IL4RA*03 was reported to be associated with lower total IgE levels in nonatopics than were more common haplotypes (equivalent to our haplotypes H-1, -2, and -4) (8). Also, haplotype "222122" was observed by TDT to be significantly undertransmitted to asthmatics in an isolated Hutterite population, although in that study the TDT was used as a test of linkage (11). These observations suggest that H-3 might be associated with a dampened T-helper cell response in either the Th1 or Th2 direction. In vitro and in vivo studies comparing the functions of the receptor molecules expressed from fully defined *IL4R* haplotypes will be instructive. Whatever the mechanism, allelic variants of the IL4R protein, one of which is encoded by haplotype H-3, are likely to be modifiers of immune disease susceptibility.

RESEARCH DESIGN AND METHODS

Subjects. The family-based samples were provided as purified genomic DNA from the HBDI, a repository for cell lines from families affected with type 1 diabetes (14). All of the 282 HBDI families used in this study are nuclear families with unaffected parents and at least two affected siblings. In addition to the 564 offspring (2 sibs in each of 282 families in the affected sib pairs on which ascertainment was based), there were 26 other affected children and 270 unaffected offspring among these families. Further description of these samples and details of *HLA* class II genotyping can be found elsewhere (16,17).

Definition of DR3/4, stratification. Families were stratified into two groups based on the *HLA* genotype of the two ascertained affected children. A child possesses the high-risk *HLA*-DR3/DR4 genotype if he/she carries a DRB1*0301-DQB1*02 haplotype and a DR4 allele that is not DRB1*0403 (a protective allele) in a haplotype with DQB1*02 or DQB1*0302 (but not *0301 or *0304). In "either/both sib DR3/4" families ($n = 135$), one or two children possess the DR3/DR4 genotype. In "neither sib DR3/4" families ($n = 147$), neither child possesses the DR3/DR4 genotype.

IL4R genotyping

List of SNPs detected. The eight SNPs in the human *IL4R* gene are listed in Table 1. The SNPs will subsequently be referred to by the SNP number listed in Table 1. The "reference" allele for each SNP is that present in GenBank accession X52425.1 and is denoted by a "1" in subsequent tables; the variant allele for each SNP is denoted by a "2."

PCR primers and amplification. Primers were purchased from Operon (Alameda, CA) and were modified at the 5′ phosphate by conjugation with biotin. Pairs of primers were designed and used to amplify the regions encompassing the eight SNPs. Other SNPs not relevant to this report were genotyped concurrently by coamplification within the same multiplex PCR and hybridization to an immobilized probe array (data not shown).

PCR amplification was performed in a total reaction volume of 50–100 μl containing the following reagents: 70 mmol/l KCl, 12 mmol/l Tris-HCl (pH 8.3 at 25°C), 3 mmol/l $MgCl_2$, 0.2 ng/μl purified human genomic DNA, 0.2 μmol/l each primer, 200 μmol/l each of dATP, dTTP, dCTP, and dGTP, and 0.25 units/μl AmpliTaq Gold DNA polymerase. Amplification was carried out in a GeneAmp PCR System 9600 thermal cycler (PE Biosystems, Foster City, CA) using the following specific temperature cycling profile: an initial hold at 94°C for 12.5 min; 33 cycles of 95°C for 45 s, 61°C for 30 s, and 72°C for 45 s; and a final extension step of 72°C for 7 min.

Allele-specific detection by probe hybridization. The detection of allelic variants at each SNP is performed by stringent hybridization of the biotinylated PCR products to the immobilized sequence-specific probes, followed by visualization using an enzyme-catalyzed color development step. The procedure is essentially as described in Cheng et al. (22), except that hybridization and washing were performed at 55°C. An example of linear array results is shown in Fig. 1. Haplotypes were determined as described below.

Inference of haplotypes. We inferred *IL4R* haplotypes by a combination of two methods: *1*) using the GeneHunter program (Falling Rain Genomics, Palo Alto, CA), which calculates haplotypes from genotype data from pedigrees; and *2*) inspecting each HBDI family pedigree individually using the Cyrillic program (Cherwell Scientific Publishing, Palo Alto, CA) to resolve any ambiguous or unsupported haplotype assignments. Haplotypes could be confidently and unambiguously assigned in all but 6 of the 282 families; in the remainder, the phase of SNP 1 could not be determined.

Statistical analysis, methods, and algorithms. Evidence for linkage of *IL4R* to type 1 diabetes was assessed by the haplotype sharing method. IBD values of parental *IL4R* haplotypes in the affected sibs could be determined unambiguously in 256 families. The locus-specific sibling relative risk was calculated using the formula $\lambda_s = 0.25/(\text{proportion of families IBD} = 0)$ (20). CIs were calculated using the standard error of the IBD proportions. The TDT was performed for individual SNPs by the method of Spielman (19). Association of *IL4R* haplotypes with type 1 diabetes was assessed using the modified TDT haplotype association analysis (described below).

LD among the eight *IL4R* SNPs was assessed using the pool of AFBAC haplotypes deduced for the entire cohort. The AFBAC pool represents an unbiased estimate of the population haplotype frequencies (23). We calculated the values of two distinct but related metrics for LD, namely D' and Δ, by standard methodology.

Modified TDT haplotype association analysis. We evaluated the nine most frequent eight-locus haplotypes using a two-sided modified TDT for the null hypothesis of no association and no linkage. As obtaining haplotypes for parents based on data from the children can introduce bias (24), we used a statistic that does not rely on this information. The statistic is the proportion of times a haplotype was transmitted among informative transmissions where it was available. When both parents and a child are heterozygous at $n \geq 1$ loci, the statistic gives weight = $(0.5)^n$ to each haplotype arrangement consistent with the observed diplotypes. We used 2,000 randomly permuted datasets to assess significance (25). A child's complementary diplotype is defined as the entire set of alleles remaining when the child's observed diplotype is subtracted from the combined diplotypes of the two parents. The permutation procedure randomly and independently switches the roles of each child's observed and complementary diplotypes to create 2,000 permuted datasets. This procedure is valid when phase is unknown, even if recombination is present. Empirical CIs were based on 2,000 bootstrap samples, created by resampling the observed families with replacement. Intervals for the proportion transmitted were converted to equivalent intervals for the reported ratio, which can be interpreted as an OR for observed segregation relative to Mendelian segregation. The haplotype analysis was performed using the S-PLUS software package (Insightful, Seattle, WA), and S-PLUS bootstrap functions were used for both resampling methods.

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REFERENCES

1. Glimcher LH, Murphy KM: Lineage commitment in the immune system: the T helper lymphocyte grows up. *Genes Dev* 14:1693–1711, 2000
2. Azar ST, Tamim H, Beyhum HN, Habbal MZ, Almawi WY: Type I (insulin-dependent) diabetes is a Th1- and Th2-mediated autoimmune disease. *Clin Diagn Lab Immunol* 6:306–310, 1999
3. Pan PY, Rothman P: IL-4 receptor mutations. *Curr Opin Immunol* 11:615–620, 1999
4. Shirakawa I, Deichmann KA, Izuhara I, Mao I, Adra CN, Hopkin JM: Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. *Immunol Today* 21:60–64, 2000
5. Hackstein H, Bitsch A, Bohnert A, Hofmann H, Weber F, Ohly A, Linington C, Maurer M, Poser S, Rieckmann P, Bein G: Analysis of interleukin-4 receptor alpha chain variants in multiple sclerosis. *J Neuroimmunol* 113:240–248, 2001
6. Youn J, Hwang SH, Cho CS, Min JK, Kim WU, Park SH, Kim HY: Association of the interleukin-4 receptor alpha variant Q576R with Th1/Th2 imbalance in connective tissue disease. *Immunogenetics* 51:743–746, 2000
7. Reimsnider SK, Eckenrode SE, Marron MP, Muir A, She JX: IL4 and IL4Ralpha genes are not linked or associated with type 1 diabetes. *Pediatr Res* 47:246–249, 2000
8. Hackstein H, Hofmann H, Bohnert A, Bein G: Definition of human interleukin-4 receptor alpha chain haplotypes and allelic association with atopy markers. *Hum Immunol* 60:1119–1127, 1999
9. Kruse S, Japha T, Tedner M, Sparholt SH, Forster J, Kuehr J, Deichmann KA: The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. *Immunology* 96:365–371, 1999
10. Lozano F, Places L, Vila JM, Padilla O, Arman M, Gimferrer I, Suarez B, Lopez de la Iglesia A, Miserachs N, Vives J: Identification of a novel single-nucleotide polymorphism (Val554Ile) and definition of eight common alleles for human IL4RA exon 11. *Tissue Antigens* 57:216–220, 2001
11. Ober C, Leavitt SA, Tsalenko A, Howard TD, Hoki DM, Daniel R, Newman DL, Wu X, Parry R, Lester LA, Solway J, Blumenthal M, King RA, Xu J, Meyers DA, Bleecker ER, Cox NJ: Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am J Hum Genet* 66:517–526, 2000
12. Olavesen MG, Hampe J, Mirza MM, Saiz R, Lewis CM, Bridger S, Teare D, Easton DF, Herrmann T, Scott G, Hirst J, Sanderson J, Hodgson SV, Lee J, MacPherson A, Schreiber S, Lennard-Jones JE, Curran ME, Mathew CG: Analysis of single-nucleotide polymorphisms in the interleukin-4 receptor gene for association with inflammatory bowel disease. *Immunogenetics* 51:1–7, 2000
13. Wu X, Di Rienzo A, Ober C: A population genetics study of single nucleotide polymorphisms in the interleukin 4 receptor alpha (IL4RA) gene. *Genes Immun* 2:128–134, 2001
14. Lernmark A, Ducat L, Eisenbarth G, Ott J, Permutt MA, Rubenstein P, Spielman R: Family cell lines available for research. *Am J Hum Genet* 47:1028–1030, 1990
15. Tisch R, McDevitt H: Insulin-dependent diabetes mellitus. *Cell* 85:291–297, 1996
16. Noble JA, Valdes AM, Cook M, Klitz W, Thomson G, Erlich HA: The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. *Am J Hum Genet* 59:1134–1148, 1996
17. Noble JA, Valdes AM, Thomson G, Erlich HA: The HLA class II locus DPB1 can influence susceptibility to type 1 diabetes. *Diabetes* 49:121–125, 2000
18. Valdes AM, Thomson G, Erlich HA, Noble JA: Association between type 1 diabetes age of onset and HLA among sibling pairs. *Diabetes* 48:1658–1661, 1999
19. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
20. Risch N: Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 40:1–14, 1987
21. Cox NJ, Wapelhorst B, Morrison VA, Johnson L, Pinchuk L, Spielman RS,

Todd JA, Concannon P: Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am J Hum Genet* 69:820–830, 2001
22. Cheng S, Grow MA, Pallaud C, Klitz W, Erlich HA, Visvikis S, Chen JJ, Pullinger CR, Malloy MJ, Siest G, Kane JP: A multilocus genotyping assay for candidate markers of cardiovascular disease risk. *Genome Res* 9:936–949, 1999
23. Valdes AM, Thomson G: Detecting disease-predisposing variants: the haplotype method. *Am J Hum Genet* 60:703–716, 1997
24. Dudbridge F, Koeleman BP, Todd JA, Clayton DG: Unbiased application of the transmission/disequilibrium test to multilocus haplotypes. *Am J Hum Genet* 66:2009–2012, 2000
25. Lazzeroni LC, Lange K: A conditional inference framework for extending the transmission/disequilibrium test. *Hum Hered* 48:67–81, 1998