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Report

No Evidence of Association or Interaction between the IL4RA, IL4, and IL13 Genes in Type 1 Diabetes

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Attempts to identify susceptibility loci that, on their own, have marginal main effects by use of gene-gene interaction tests have increased in popularity. The results obtained from analyses of epistasis are, however, difficult to interpret. Gene-gene interaction, albeit only marginally significant, has recently been reported for the interleukin-4 and interleukin-13 genes (*IL4* and *IL13*) with the interleukin-4 receptor A gene (*IL4RA*), contributing to the susceptibility of type 1 diabetes (T1D). We aimed to replicate these findings by genotyping both large family and case-control data sets and by using previously published data. Gene-gene interaction tests were performed using linear regression models in cases only. We did not find any single-locus associations with T1D and did not obtain evidence of gene-gene interaction. Additional support from independent samples will be even more important in the study of gene-gene interactions and other subgroup analyses.

The genetic analysis of common, multifactorial diseases, such as type 1 diabetes (T1D [MIM 222100]), that are highly clustered in families is proving to be a challenging task (Altmuller et al. 2001; Hirschhorn et al. 2002; Ioannidis et al. 2003; Wang et al. 2005). There are many reasons for this, including statistically underpowered small sample sizes; lack of coverage of the genome due to technical, and thus cost, limitations in genotyping; and statistical issues, such as subgroup analyses and very low prior probability of obtaining a true result (Dahlman et al. 2002; Thomas and Clayton 2004; Wang et al. 2005). One possibility, which is gaining popularity among some authors (Culverhouse et al. 2002; Moore 2003; Hoh and Ott 2004), is that susceptibility gene effects may be only identified in analyses of statistical interaction, since the individual marginal effects may be close to null. However, these extreme models of epistasis are difficult to explain biologically. Furthermore, the interpretation of statistical interaction in terms of "epistatic" mechanisms is problematic (Cordell 2002). Whether testing for interactions or subgroup analyses increases the power to detect disease susceptibility genes or not, such approaches may exacerbate the problem of false positives due to the even lower prior probability of detecting a true positive (Thomas and Clayton 2004; Wang et al. 2005). Subsequently, for reliable gene-gene interaction results, very small *P*-value thresholds, less than $P < 10^{-6}$, and larger sample sizes, in addition to replication in independent samples, may be required.

Recently, in a sample of 90 cases of T1D and 94 Filipino population-based controls, Bugawan et al. (2003) reported evidence of an interaction between 10 SNPs in the interleukin-4 receptor A gene (*IL4RA* [MIM 147781]) on chromosome 16p11-p12 (SNPs 5′ −3223C→T [*rs2057768*], 5′ −1914C→T [*rs2107356*], I50V [*rs1805010*], N142N [*rs2234895*], E375A [*rs1805011*], L389L [*rs2234898*], C406R [*rs1805012*], S478P [*rs1805015*], Q551R [*rs1801275*], and S761P [*rs1805014*]) and 5 SNPs in the adjacent interleukin-4 and interleukin-13 genes (*IL4* [MIM 147780] and *IL13* [MIM 147683]) on chromosome 5q31 (SNPs 5′ −524T→C [*rs2243250*] in *IL4* and 5′ −1512A→C [*rs1881457*], 5′ −1112C→T [*rs1800925*], +1923C→T/intron 3 [*rs1295686*], and R110Q [*rs20541*] in *IL13*).

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Table 1

Association Analysis of *IL4, IL13,* and *IL4RA* SNPs in a T1D Case-Control Sample

Gene and SNP	dbSNP	Nucleotide Change	No. of Controls with Genotype	No. of Cases with Genotype	MAF for Controls	MAF for Cases	*P*
IL4:							
5′ −524	*rs2243250*	T→C	1,622	1,557	.13	.14	.47
IL13:							
5′ −1512	*rs1881457*	A→C	1,655	1,578	.19	.17	.87
5′ −1112	*rs1800925*	C→T	1,660	1,583	.18	.17	.83
+1923	*rs1295686*	C→T	1,624	1,559	.18	.18	.43
R110Q	*rs20541*	G→A	1,653	1,559	.17	.18	.41
IL4RA:							
5′ −3223	*rs2057768*	C→T	1,578	1,592	.30	.29	.46
I50V	*rs1805010*	A→G	1,582	1,583	.46	.45	.48
E375A	*rs1805011*	A→C	1,622	1,565	.12	.12	.79
C406R	*rs1805012*	T→C	1,674	1,575	.11	.11	.49
S411L	*rs1805013*	C→T	1,653	1,587	.05	.05	.16
S478P	*rs1805015*	T→C	1,639	1,584	.18	.17	.35
Q551R	*rs1801275*	A→G	1,673	1,590	.22	.22	.73
S761P	*rs1805014*	T→C	1,644	1,590	.01	.01	.87

Note.—To correct for regional variation in allele frequencies, the analyses were stratified according to 12 broad geographical regions within Great Britain (D.G.C., unpublished data). Note that, for every SNP, genotyping of DNA samples from 1,829 controls and 1,616 patients with T1D was attempted. The numbers of cases and controls, MAFs of cases and controls, and *P* values were obtained by association analyses with T1D.

Of these 15 SNPs, 4 showed some evidence of primary disease association—namely, E375A ($P = .02$), L389L ($P = .001$), and C406R ($P = .05$) in *IL4RA* and the 5′ −1512A→C SNP in *IL13* ($P = .05$). Furthermore, at *IL4RA,* a 7-locus haplotype ($P = .005$) and a 10-locus haplotype ($P = .001$) showed some evidence of association. Additional effects of five-locus haplotypes at *IL4* and *IL13* (smallest *P* value = .004) were observed, as well as gene-gene interaction of the *IL4* and *IL13* loci with *IL4RA* ($P < .045$) (Bugawan et al. 2003). Note that, to correct for multiple testing, Bugawan et al. (2003) permutated genotypes at chromosomes 5 and 16 within patients and controls, keeping genotype frequencies constant. The *P* values were <.05, but, nevertheless, if the reported effect were true, then a much larger study should confirm the result. Given the linkage and association results from studies of T1D and other diseases that are inherited in a similarly complex way, it is likely that non–human leukocyte antigen T1D-susceptibility loci will have effect sizes with odds ratios (ORs) <2.0 (Wang et al. 2005). However, since the sample size used by Bugawan et al. (2003) is very small (90 cases and 94 controls) for purposes of detecting a true effect, the effect size would need to be in considerable excess of OR 2.0 for the minor allele. Since the effects reported here for minor alleles are much smaller than this, the posterior probability that Bugawan et al. (2003) have detected a true effect is very small. In this study, we attempted replication of the main findings reported by Bugawan et al., using a larger sample of up to 748 multiplex families and up to 1,616 cases and 1,829 controls.

First, we tested the primary, single-locus association of the 15 SNPs from *IL4, IL13,* and *IL4RA* in a white British case-control sample. The case-control DNA set consisted of 1,616 white individuals with T1D who were recruited from across Britain for the Juvenile Diabetes Research Foundation/Wellcome Trust–funded U.K. GRID study (see U.K. GRID Study Web site), and 1,829 population-based controls from the 1958 British Birth Cohort (see National Child Development Study Web site). The mean age at onset of the patients, who were all <16 years old at diagnosis, is 7.5 years (SD 4 years). We obtained no evidence of association with T1D (table 1). The SNPs included *IL13* R110Q, which showed some evidence of association in previous studies (Bugawan et al. 2003). Genotyping of *IL4RA* L389L was not performed, since this variant is in very strong linkage disequilibrium with its neighboring SNPs E375A, C406R, and Q551R (Bugawan et al. 2003). We employed the Invader (Third Wave Technologies) and TaqMan (Applied Biosystems) genotyping technologies. Primer and probe sequences are shown in tables A1 and A2 (online only). The *IL4RA* results were consistent with our previous study of 3,475 families with T1D (Maier et al. 2003), in that no *P* values <.05 were obtained. There was also no evidence for the previously suggested *IL13* R110Q and E375A genotype associations: the OR was 0.90 (95% CI 0.77–1.06) for the *IL13* R110Q AG heterozygote and 1.05 (95% CI 0.88–1.26) for the E375A AC heterozygote. Furthermore, no evidence was obtained for the previously reported associations of the *IL4RA* −3223C→T SNP ($P > .05$) (Maier

et al. 2003) or for that of a six-locus *IL4RA* haplotype ($P > .05$) (Mirel et al. 2002; Bugawan et al. 2003; Maier et al. 2003). We have analyzed two haplotypes, one consisting of *IL4RA* I50V (*rs1805010*), E375A (*rs1805011*), C406R (*rs1805012*), S411L (*rs1805013*), S478P (*rs1805015*), and Q551R (*rs1801275*) and the other consisting of *IL13* −1512A→C (*rs1881457*), *IL13* −1112C→T (*rs1800925*), *IL13* +1923C→T (*rs1295686*), *IL13* R110Q (*rs20541*), and *IL4* −524C→T (*rs2243250*). However, no results with $P < .05$ were obtained, and all 95% CIs overlapped 1. Results from haplotype analyses for *IL4RA* and *IL4/IL13* are given in tables A3 and A4 (online only).

We note that the minor-allele frequencies (MAFs) of all SNPs except for *IL4RA* I50V and S478P are lower in our U.K. and U.S. populations than in the 184 Filipino subjects studied elsewhere (Bugawan et al. 2003). However, the *IL4RA* S761P SNP, which was monomorphic in Filipinos, was polymorphic in our population (MAF 0.01). Nevertheless, our larger data set and, hence, increased statistical power should compensate for the loss of power due to the lower MAFs.

Second, we tested for evidence of an interaction (i.e., deviation from a multiplicative model of epistasis) between eight *IL4RA* SNPs and the one *IL4* and four *IL13* SNPs in a case-only analysis (table 2) (Piegorsch et al. 1994; Umbach and Weinberg 1997). We assume markers are in linkage equilibrium in the general population (i.e., the controls). A convenient and powerful approach is to test the correlation coefficient between the genotypes at the two loci, scored 0, 1, and 2 (Piegorsch et al. 1994; Umbach and Weinberg 1997). We genotyped a DNA collection consisting of 1,616 cases and 1,829 controls from Great Britain, for the previously associated candidate SNPs. The candidate SNPs for *IL4* and *IL13* were *IL4* −524C→T (*rs2243250*), *IL13* −1512A→C (*rs1881457*), *IL13* −1112C→T (*rs1800925*), *IL13* +1923C→T (*rs1295686*), and *IL13* R110Q (*rs20541*). We did not observe any evidence of interaction between single SNPs in our data set ($P > .05$) (table 2).

Third, we considered the possibility that there is a true association and that the etiological variant has not been identified yet. To ensure that we captured the information in the *IL4* and *IL13* regions, we genotyped tagSNPs for both genes, using 748 white families with T1D from the United Kingdom and the United States, to perform further interaction analyses with *IL4RA*. The families with T1D each included two parents and at least one affected child. The 748 families with T1D comprised 472 multiplex families from the U.K. Warren 1 repository (Bain et al. 1990) and 276 multiplex families from the Human Biological Data Interchange ascertained in the United States (Lernmark 1991), with inclusion criteria described elsewhere (Vella et al. 2004). The sequencing data and genotypes of a sequencing panel required for the tagging approach were obtained from the University of Washington–Fred Hutchinson Cancer Research Center (UW-FHCRC) Cancer Variation Discovery Resource (SeattleSNPs), published at the UW-FHCRC Web site. From this resource, we downloaded data on common polymorphisms of exons and introns from 23 white individuals (from Centre d'Etude du Polymorphism Humain [CEPH]) and used these to select tagSNPs. For *IL4* and *IL13,* 12 and 6 tagSNPs, respectively, with MAFs >0.05, were selected as described elsewhere (Chapman et al. 2003; Clayton et al. 2004) and are listed in tables A5 and A6 (online only). With the chosen subset of tagSNPs, the remaining SNPs were required to be predicted with a minimum locus R^2 of 0.80. In total, 18 tagSNPs were selected and genotyped in the same 748 families with T1D investigated for *IL4RA* association by Maier et al. (2003). Note that, in the selection of tagSNPs for *IL4,* the *IL4* −524 variant was selected as a tagSNP. Similarly, for *IL13,* the *IL13* −1512A→C (*rs1881457*), *IL13* −1112C→T (*rs1800925*), and *IL13* +1923C→T (*rs1295686*) variants were selected as tagSNPs.

The multilocus test (Chapman et al. 2003; Clayton et al. 2004) *P* values for *IL4* and *IL13* were .58 and .74, respectively, indicating that neither locus was associated

Table 2

***P* Values Obtained by Regression Analyses of Interactions of *IL13* and *IL4* SNPs with Eight *IL4RA* SNPs for Susceptibility to T1D**

	IL4RA SNP (dbSNP)							
Gene and SNP (dbSNP)	5′ 3223 (*rs2057768*)	I50V (*rs1805010*)	E375A (*rs1805011*)	C406R (*rs1805012*)	S411L (*rs1805013*)	S478P (*rs1805015*)	Q551R (*rs1801275*)	S761P (*rs1805014*)
IL4:								
5′ −524 (*rs2243250*)	.92	.56	.98	.96	.45	.93	.61	.55
IL13:								
5′ −1512 (*rs1881457*)	.63	.87	.95	.54	.80	.70	.34	.43
5′ −1112 (*rs1800925*)	.48	.68	.99	.52	.98	.81	.52	.43
+1923 (*rs1295686*)	.41	.37	.98	.47	.75	.39	.61	.79
R110Q (*rs20541*)	.38	.43	.26	.53	.64	.35	.62	.45

Note.—Results are shown for a sample of 1,616 white patients. To correct for regional variation in allele frequencies, the analyses were stratified according to 12 broad geographical regions within Great Britain (D.G.C., unpublished data).

Table 3

***P* Values for Interaction Tests Obtained from Regression Analyses of *IL13* and *IL4* tagSNPs with Eight *IL4RA* SNPs**

IL4RA SNP (dbSNP)	*P* VALUE FOR	
	IL13 tagSNPs	*IL4* tagSNPs
5′ −3223 (*rs2057768*)	.72	.97
I50V (*rs1805010*)	.14	.37
E375A (*rs1805011*)	.21	.62
C406R (*rs1805012*)	.55	.20
S411L (*rs1805013*)	.39	.43
S478P (*rs1805015*)	.71	.97
Q551R (*rs1801275*)	.65	.51
S761P (*rs1805014*)	.55	.24

NOTE.—Results are shown for a U.K. and U.S. family collection of up to 748 families with T1D.

with T1D in this U.K. and U.S. family collection, which is consistent with the data obtained in our U.K. case-control collection. We tested for interaction, a deviation from the model of multiplicative effect, between single SNPs in one region or gene and a set of tagSNPs in a second region, by regressing the genotype at the single SNP on all tag genotypes in cases. For each *IL4RA* locus, we used this regression technique to test for an interaction between specific *IL4RA* loci and the *IL4* region, as well as the *IL13* region. As shown in table 3, neither *IL4* nor *IL13* tagSNPs showed *P* values <.05 with eight *IL4RA* SNPs for an interaction effect. For completeness, we also performed the same test employed by Bugawan et al. (2003) in both a case-control and case-only design for interaction between *IL4RA* SNPs and *IL13* tagSNPs and between *IL4RA* SNPs and *IL4* tagSNPs, but obtained no evidence of interaction ($P > .05$).

We conclude that there is no evidence of interaction between the *IL4RA* and the *IL13* or *IL4* loci in susceptibility to T1D, with regard to the investigated variants in our populations of European descent. The previously published result obtained in 184 individuals from the Philippines may be specific to that sample population, but a study of such small size has minimal power to detect a true interaction effect. In comparing the study of Bugawan et al. (2003) with our study, it is evident that the present study has substantially greater statistical power. This is because we not only used a larger sample size but also employed regression analyses that use only cases, and this approach has been shown elsewhere to be more powerful when the assumption of independence is true (Piegorsch et al. 1994; Umbach and Weinberg 1997). Failure to replicate genetic association studies is well documented in the literature and, even though several reasons have been reported—including allelic heterogeneity, true variation in disease association between populations, modifying genetic and/or environmental factors, and misclassification of outcome—the most important factor is probably insufficient and/or inadequate sample sizes in the context of a very-low prior probability of detecting a true effect (Clayton and McKeigue 2001; Dahlman et al. 2002; Hirschhorn et al. 2002; Colhoun et al. 2003; Ioannidis et al. 2003; Lohmueller et al. 2003; Thomas and Clayton 2004; Wang et al. 2005). Such problems will be much more extreme for reported gene-gene interactions; power to detect interaction is low and the problems of low prior probability and subgroup analyses are more extreme. These results highlight the necessity of attempting replication in independent samples before drawing conclusions about a potential disease-susceptibility locus or gene-gene interaction.

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Electronic-Database Information

The URLs for data presented herein are as follows:

National Child Development Study, http://www.cls.ioe.ac.uk/Cohort/Ncds/mainncds.htm
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for T1D, *IL4R, IL4,* and *IL13*)
U.K. GRID Study, http://www-gene.cimr.cam.ac.uk/ucdr/grid.shtml
UW-FHCRC, http://pga.mbt.washington.edu (for SeattleSNPs)

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