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Lack of association of type 1 diabetes with the *IL4R* gene

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Abstract *Aims/hypothesis:* The association between *IL4R* and type 1 diabetes has been tested in many studies in recent years, with contradictory results. The aim of this study was to re-evaluate the genetic association in type 1 diabetic nuclear families of mixed European background. *Subjects, materials and methods:* We genotyped six non-synonymous single-nucleotide polymorphisms (SNPs) of the *IL4R* gene in 830 nuclear families as specified above, including a French Canadian subset. *Results:* No association between type 1 diabetes and any SNP or haplotype was found by the transmission disequilibrium test. *Conclusions/interpretation:* Previous positive reports may be due to population stratification as, according to HapMap data, allele frequencies in the *IL4R* region vary considerably by ethnicity.

Keywords Interleukin 4 receptor · Population stratification · Transmission disequilibrium test · Type 1 diabetes

Abbreviations ESM: Electronic Supplementary Material · *IL4R*: interleukin 4 receptor · LD: linkage disequilibrium · nsSNP: non-synonymous single-nucleotide polymorphism · SNP: single-nucleotide polymorphism · TDT: transmission disequilibrium test

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Introduction

The *IL4R* gene (GeneID 3566) encodes the α -chain of the IL-4 receptor, a type I transmembrane protein that can promote differentiation of Th2 cells through binding with IL-4 [1]. The role of Th2-mediated mechanisms in type 1 diabetes has attracted much attention in recent years [2]; given the importance of genetic susceptibility in the aetiology of type 1 diabetes [3] it is reasonable to hypothesise a role for genetic variation of *IL4R* in disease development. Some other autoimmune diseases or allergic conditions, such as asthma [4, 5], primary Sjogren's syndrome [6], penicillin allergy [7] and atopic dermatitis [8], have been reported to be associated with the *IL4R* polymorphisms in recent years. However, the association between *IL4R* polymorphisms with type 1 diabetes has been addressed in several reports [9–14] with contradictory results. Two independent case-control studies [9, 11] reported associations of the same three single-nucleotide polymorphisms (SNPs), E375A (rs1805011), L389L (rs2234898) and C406R (rs1805012), with type 1 diabetes. The majority of the subjects (case $n=102$, control $n=198$) in one of these studies [9] were non-Hispanic white (80%). The other positive case-control study was performed in a population from the island of Luzon in the Philippines (case $n=90$, control $n=94$) [11]. However, the association of the two non-synonymous SNPs (nsSNPs) were not found in a large case-control study (case $n=1,616$, control $n=1,829$) in a White British population sample [5]. One possible explanation for the discrepant results is population stratification. It was also found that allele frequencies in six of the eight *IL4R* SNPs (except for E375A and C406R) were different among European populations, with high statistical significance ($p \leq 2 \times 10^{-4}$) [12], and it is not impossible that the Filipino population might also contain genetic diversity leading to population stratification. Population stratification may bias the real type 1 diabetes association and cause either false positive or false negative results [15]. An unequivocal answer to this question can only be given by a study design immune to population stratification, such as a family-based study employing the

Table 1 TDT test of the six *IL4R* nsSNPs in 830 type 1 diabetic nuclear families

SNP (dbSNP)	Hardy–Weinberg <i>p</i> value ^a	Minor allele (frequency)	Overtransmitted	T:U	χ^2	<i>p</i> value
I50V (rs1805010)	0.052	C (0.443)	T	383:364	0.5	0.487
E375A (rs1805011)	0.507	C (0.116)	C	160:153	0.2	0.692
C406R (rs1805012)	0.410	C (0.106)	C	163:145	1.1	0.305
S411L (rs1805013)	0.655	T (0.048)	T	80:64	1.8	0.182
P478S (rs1805015)	0.572	C (0.167)	C	235:207	1.8	0.183
Q551R (rs1801275)	0.258	G (0.222)	G	283:253	1.7	0.195

^aCalculated in parents only

transmission disequilibrium test (TDT) [16]. However, even the available TDT studies on the association between *IL4R* and type 1 diabetes are contradictory [10, 12–14]. In this study, we tested the association between type 1 diabetes and each of six *IL4R* nsSNPs in a DNA collection from 830 families of European descent with one affected child; the study had a statistical power of >99% to detect the *IL4R* association with the effect size as most recently reported [9].

Subjects, materials and methods

We investigated 830 nuclear family trios (one affected child and two parents). The average age-at-onset of the type 1 diabetic children was 9.49 ± 0.36 years (mean $\pm$ SD). Ethnic backgrounds were of mixed European descent, with the largest single subset (409 families) being French Canadian. The Research Ethics Board of the Montreal Children's Hospital and other participating centres approved the study, and written informed consent was obtained from all subjects. The SNPs were genotyped by AcycloPrime FP SNP Detection Kit (Perkin Elmer, Boston, MA, USA). PCR primers, designed by Primer3 webtool [17], and fluorescence polarisation probes are listed in Table 1 of the Electronic Supplementary Material (ESM). Amplification primers were used at 100 nmol/l each (all concentrations are final). PCR was performed in a Dual 384-well GeneAmp PCR system 9700 in clear 384-well microplates (Greiner Labortechnik, Frickenhausen, Germany). After the PCR reaction, unincorporated primers and dNTPs were removed and final extension with allele-specific fluorescent dNTPs was performed in by Dual 384-well GeneAmp PCR system 9700 (Greiner Labortechnik) in black microplates (MJ Research, Waltham, MA, USA). Incorporated allele-specific fluorochrome was detected by fluorescence polarisation in the Criterion Analyst HT System (Molecular Devices, Sunnyvale, CA, USA). All genotypes were confirmed with probes on both strands. All six SNPs had unambiguous clusters corresponding to the three genotypes and call rates >98.0%. Mendelian error rates (after removing families with discrepancies at multiple independent SNPs) were: 0% for rs1805010, rs1805011, rs1805012 and rs1805013; 0.24% for rs1805015; and 0.12% for rs1801275. No genotype distribution of the parents significantly deviated from Hardy–Weinberg equilibrium. The TDT, and linkage and

linkage disequilibrium (LD) analysis were performed by Haploview software (available at <http://www.broad.mit.edu/personal/jcbarret/haploview>) [18]. Haplotypic association was tested by the Family-Based Association Test software (available at <http://www.biostat.harvard.edu/~fbat/fbat.htm>) [19].

Results and discussion

Our results show no association between type 1 diabetes and the six nsSNPs (Table 1). This result is concordant with two independent family-based studies [10, 12, 14]. In the study of 309 US diabetic families, one nsSNP, rs1801275 (NP_000409/R576Q, deduced from the genotyping protocol of that study), was tested for type 1 diabetes association and no association was found [14]. In the other study [12], one promoter SNP and seven nsSNPs were tested in 3,475 families, with the two largest subsets being 999 UK families and 1,231 Finnish families. None of the nsSNPs in that study was significantly associated with type 1 diabetes

Fig. 1 The LD map of the six *IL4R* nsSNPs based on the 830 type 1 diabetic nuclear family genotyping. The haplotype map was made by Haploview v3.2 software (available at <http://www.broad.mit.edu/personal/jcbarret/haploview>). *D'* values (%) are shown in the boxes (*D'*=100% for the empty boxes). Five nsSNPs from the second to the sixth SNP are in one LD block

Table 2 Haplotypic association of the three *IL4R* nsSNPs

Haplotype (rs1805012–rs1805013–rs1801275)	Frequency	Informative family number ^a	<i>S</i> ^b	<i>E(S)</i> ^c	<i>Var(S)</i> ^d	<i>Z</i> value	<i>p</i> value
TCA	0.776	401	521.9	530.9	129.0	−0.8	0.428
CCG	0.105	240	150.0	138.0	78.0	1.4	0.174
TCG	0.072	181	89.1	97.6	45.7	−1.3	0.209
TTG	0.045	126	71.9	64.4	32.7	1.3	0.190

^aNumber of nuclear families informative for (with a non-zero contribution to) Family-Based Association Test Analysis

^bObserved haplotype number in the affected offspring

^cExpected haplotype number in the affected offspring

^dThe variance of haplotype distribution among the affected offspring

in the total family subjects, but in the Finnish subset the nsSNP rs1805012 was associated with type 1 diabetes, with $p=0.03$ and odds ratio 0.77 (95% CI 0.62–0.97) [12]. This value for the odds ratio was estimated from the transmission ratio based on the reported method [20]. The minor allele (cytosine) of the same SNP was also found to be protective of type 1 diabetes in Filipinos, with a borderline p value (0.05) and odds ratio 0.56 (95% CI 0.31–0.99) [11]. Allele frequencies at rs1805012 were similar between the parents in our study and those in the Finnish subset (0.106 vs 0.097, respectively) [12], but our study did not replicate the association. If anything, the cytosine allele tends to be transmitted in the opposite direction (our present results 163/145 vs 135/175 in a Finnish population [12]).

As well as the individual SNPs' association, haplotypic association of *IL4R* has also been reported in type 1 diabetes and multiple sclerosis [11, 13, 21], conceivably due to another variant not tested in any of the studies. LD analysis of the six nsSNPs that we genotyped showed that, except for rs1805010, the nsSNPs are located in one solid LD block (Fig. 1). Three of these five SNPs, rs1805012, rs1805013 and rs1801275, are identified as the tag SNPs that can represent four common haplotypes with frequency >0.01 in this region with tight LD. Again, association analysis of these four common haplotypes showed no association with type 1 diabetes (Table 2). Thus, our study shows no association of *IL4R* with type 1 diabetes in our European family collection, in agreement with the study that showed no association in the reported European family collection [12]. The finding of an association of one SNP in both the Finnish subset and the Filipino study may be due to genetic heterogeneity resulting in a peculiarity shared by these two populations. However, it seems more likely that the borderline statistical significance of these associations was an artefact of population stratification (in the Filipino study) and multiple analysis without correction of the alpha level in the Finnish subset. Population stratification can occur when populations with different allele frequencies are unequally represented in cases vs controls. HapMap data (<http://www.hapmap.org>) are available for six of the *IL4R* SNPs tested for association with type 1 diabetes. Except for one very rare SNP (rs1805014), the other five (rs2057768, rs1805011, rs2234898, rs1805013 and rs1805015) all showed significant differences ($p<1\times 10^{-10}$) in allele frequencies in the *IL4R* gene region between

African, East Asian and European sets (ESM, Table 2). This makes diversity within each of these continents also seem likely. Indeed, three (rs2057768, rs1805013 and rs1805015) of these five SNPs were found to have dramatically significant differences among European populations [12]. In the Philippines, the population also encompasses more than 100 distinct native ethnic groups. Luzon island, where the studied subjects originated, contains the country's capital, Manila, and its most populous city, Quezon City, with a total population of 42.811 million (<http://en.wikipedia.org/wiki/Luzon>). Thus, population stratification is a distinct possibility in the Filipino set as well; in our opinion this is the most likely explanation for the findings. These considerations underscore the importance of generating study designs that are impervious to population stratification whenever possible, or alternatively, of using genomic controls to rule that out [22].

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