The *IRF5* polymorphism in type 1 diabetes

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The interferon regulatory factor 5 gene (*IRF5*) has been shown to play a crucial role in harmful immune responses by induction of proinflammatory cytokines. Functional genetic variants associated with increased *IRF5* expression of specific isoforms are associated with systemic lupus erythematosus (SLE) and it is possible that they may also predispose to other autoimmune disorders. We tested the association of two *IRF5* SNPs, correlated with *IRF5* expression and SLE risk, in 947 nuclear family trios type 1 diabetes (T1D) using the transmission disequilibrium test. Our results suggest that the functional *IRF5* variations do not confer an obvious risk for T1D.

The interferon regulatory factor 5 (*IRF5*; OMIM 607218) gene maps to Chr7q32, and encodes a member of the interferon regulatory factor (IRF) family. The IRFs are a group of transcription factors that mediate virus-induced signalling pathways by the induction of type 1 interferon genes (interferons α and β).¹ Specifically, IRF-5 is involved downstream of the Toll-like receptor (TLR)-MyD88 pathway and plays a crucial role in some potentially harmful immune responses by inducing proinflammatory cytokines such as interleukin (IL)-6, IL-12 and tumour necrosis factor- α .² The role of proinflammatory cytokines in the autoimmune destruction of pancreatic β -cells is well established.³

Multiple functional genetic variations in the *IRF5* gene have been reported recently. In a genome-wide study with gene expression as the phenotype, the single nucleotide polymorphism (SNP) rs2280714, downstream of *IRF5*, was found to correlate with *IRF5* mRNA level.⁴ The T allele of rs2280714 is correlated with higher expression of the gene.⁵ Additionally, association between the rs2004640 SNP at intron 1 of the *IRF5* gene and systemic lupus erythematosus (SLE) was identified in various European populations.^{5–8} According to the study by Graham *et al.*,⁵ susceptibility to SLE depends on the presence of exon 1B, a result of *IRF5* alternative splicing modulated by rs2004640. Exon 1B is found only in people with the rs2004640 T allele. In this study, we investigated whether the two SNPs (rs2280714, associated with the gene expression level, and rs2004640, associated with alternative splicing and SLE), are also susceptibility loci for type 1 diabetes (T1D).

METHODS

Participants

The DNA samples of 947 nuclear family trios (one affected child and two parents) type 1 diabetes (T1D) were collected in Canadian paediatric diabetes clinics. The mean (SD) age at onset of the children with T1D was 7.9 (4.0) years (median 8 years; 25th–75th centiles 4.6–11 years). All patients were diagnosed at <18 years old, had been treated with insulin since diagnosis and none had stopped treatment for any reason since commencement. The cohort was of mixed European descent, with the largest single subset (~40%) being French–Canadian. The research ethics board of Montreal Children's Hospital and

other participating centres approved the study, and written informed consent was obtained from all subjects.

Genotyping

Genotypes for this study were obtained using the Sequenom iPLEX assay (Sequenom, Cambridge, Massachusetts, USA). Locus-specific PCR primers and allele-specific detection primers were designed using MassARRAY Assay Design 3.0 software (Sequenom Inc., San Diego, California, USA). The sample DNAs were amplified in a 34-plex PCR reaction and labelled using a locus-specific single base extension reaction. The resulting products were desalted and transferred to a 384-element chip array (SpectroCHIP). Allele detection was performed using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. The mass spectrograms were analysed by MassARRAY TYPER software (Sequenom). The CEU set (90 European-descent individuals genotyped in HapMap) were included as accuracy controls. The genotyping calls are shown in table 1.

Statistics

Transmission disequilibrium tests (TDT) were performed using Haploview V.3.32 software (www.broad.mit.edu/personal/jcbarret/haploview/).⁹

RESULTS AND DISCUSSION

The association test for each individual SNP is shown in table 2. We found no T1D association with either SNP. For rs2004640, with minor allele frequency (MAF) = 0.50, our study had >80% statistical power to detect a genetic association conferring an odds ratio (OR) as low as 1.20. For rs2280714 (MAF = 0.36) our study had >80% statistical power to detect association with the effect size of OR as low as 1.21. By comparison, the pooled effect of the three studies on the SLE association had OR = 1.50 (95% CI 1.39 to 1.61).^{5–8}

To exclude combined effects from the two functional SNPs, we also tested haplotypic association. Because of the tight LD between rs2004640 and rs2280714 ($D' = 0.99$, $r^2 = 0.56$), three common haplotypes accounted for 99.8% of all chromosomes

Table 1 The genotyping assay of two *IRF5* single nucleotide polymorphisms

SNP	Chr	Position	Call rate (%)	Parent HWD (p value)	Mendelian error
rs2004640	7	128 365 536	99.2	0.37	2
rs2280714	7	128 381 960	99.5	0.71	0

Chr, chromosome; HWD, Hardy–Weinberg disequilibrium; SNP, single nucleotide polymorphism.

Abbreviations: IL, interleukin; *IRF5*, interferon regulatory factor 5; MAF, minor allele frequency; OMIM, Online Mendelian Inheritance in Man; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; T1D, type 1 diabetes; TLR, Toll-like receptor

Table 2 No T1D association of two *IRF5* single nucleotide polymorphisms

SNP	Frequency	Transmitted : untransmitted (overtransmitted allele)	χ^2 (p value)
rs2004640	G (0.50)	426:421 (T)	0.03 (0.86)
rs2280714	C (0.36)	402:390 (C)	0.18 (0.67)

Table 3 No association of the haplotypes of the two *IRF5* single nucleotide polymorphisms in type 1 diabetes.

Haplotype (rs2004640- rs2280714)	Minor allele frequency	Transmitted: untransmitted (overtransmitted allele)	χ^2 (p value)
T-T	0.50	441:428	0.17 (0.68)
G-C	0.36	403:390	0.22 (0.64)
G-T	0.14	201:225	1.30 (0.25)

(table 3). For the two *IRF5* SNPs, the major allele T of rs2004640 and the major allele T of rs2280714 are the ancestral alleles by human–chimpanzee alignment. The most common ancestral T–T haplotype (frequency = 0.50) of rs2004640 and rs2280714 correlates with higher expression level of the *IRF5* gene and the presence of exon 1B by alternative splicing,^{4,5} and is associated with higher SLE susceptibility.^{5,6} Two derived haplotypes, G–C (frequency = 0.36) and G–T (frequency = 0.14), encode the *IRF5* isoforms without exon 1B. The more common derived haplotype G–C also has markedly lower expression.⁵ Both derived haplotypes do not confer risk for SLE.⁵ In our study, none of these haplotypes was associated with T1D. In addition, no difference was found in age of onset for different genotypes or diplotypes was found. Our results suggest that the genetic variations correlated with *IRF5* expression and associated with SLE susceptibility do not confer an obvious risk of T1D.

Both T1D and SLE are autoimmune diseases with complex and unclear aetiology. T1D is caused by T cell-mediated pancreatic β -cell destruction.¹⁰ SLE is a disease with multi-system damage mediated by autoantibodies against a diverse

range of nuclear and cell surface autoantigens, and characterised by overactive B cells and abnormally activated T cells.¹¹ Genetic susceptibility plays a crucial role in both diseases,^{12,13} which share three common genetic susceptibility loci (*HLA*, *PTPN22*, and *CTLA4*), all three involving proteins crucial in immune function with effects on immune self-tolerance. As expected, however, not all loci are common to the two diseases. The association of SLE with *IRF5* has been strongly replicated in independent studies,^{5–8} but our study clearly shows that this genetic susceptibility is not shared by T1D. The increased risk of SLE is correlated with increased harmful immune responses mediated by the proinflammatory cytokines induced by the gene products of *IRF5*. The absence of T1D association suggests that immune responses in the pathway in which *IRF5* is involved do not play an important role in T1D. Another study has shown no association between the *IRF5* genetic variations and rheumatoid arthritis.¹⁴

These results indicate that the interferon-induced signalling mediated by *IRF5* might be a unique mechanism in SLE, but not in other autoimmune diseases such as T1D or rheumatoid arthritis.

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Key points

- In a genome scan in which microarray transcriptional profiling was examined as the phenotype in lymphoblastoid cell lines, *IRF5* was one of the genes with the strongest regulation of expression levels in cis.
- The locus responsible for this expression effect is also strongly associated with risk of SLE, as might be expected from the important role of *IRF5* in immune function.
- Because association of *IRF5* with other autoimmune diseases, such as T1D, has a high prior probability, we tested a large sample of patients with T1D and their parents for association to this locus, by TDT. No association was found.
- As the type 1 interferon response is strongly affected by this locus, as seen by its effect on SLE, we can conclude that this response has no important role in human T1D.

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