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SHORT COMMUNICATION

The association between the *IFIH1* locus and type 1 diabetes

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Abstract
Aims/hypothesis We set out to validate a recently reported type 1 diabetes association from the *IFIH1* gene variation in an independent cohort from a population of mixed European descent.
Methods We genotyped five single-nucleotide polymorphisms in the *IFIH1* locus, i.e. rs2111485, rs1990760, rs3747517, rs17783344 and rs984971589, in 589 type 1 diabetes nuclear family trios (1,767 individuals).
Results This study independently replicated the reported genetic association using a family-based approach.
Conclusions/interpretation The reported type 1 diabetes association is from a linkage disequilibrium region including three candidate genes, i.e. *FAP*, *IFIH1* and *GCA*. Further variant discovery and fine mapping could help clarify a novel type 1 diabetes mechanism.

Keywords Autoimmune disease · *IFIH1* · Genetic susceptibility · Genotype · Interferon induced with helicase C domain 1 · Transmission disequilibrium test · Type 1 diabetes · Picornavirus infection · Single-nucleotide polymorphism · Validation

Abbreviations

LD	linkage disequilibrium
OR	odds ratio
SNP	single-nucleotide polymorphism

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Introduction

Recently, a large-scale screening of 12,000 non-synonymous single-nucleotide polymorphisms (SNPs) across the human genome [1] found an association between type 1 diabetes and a non-synonymous SNP (Ala946Thr) at the *IFIH1* gene, with the evidence coming from both a case–control approach and a family-based approach. *IFIH1* is a putative RNA helicase, upregulated by interferons, especially β-interferon [2]. *Ifih1*-deficient mice are highly susceptible to picornavirus infection, which suggests that *IFIH1* is critical for innate antivirus responses [3]. This is of particular interest given evidence for a role of viral infection in type 1 diabetes [4]. In addition to a proven causal link between type 1 diabetes and congenital rubella [5], there is evidence suggesting an association with enterovirus and picornavirus infections [6]. Although the genetic effect size found was relatively small [odds ratio (OR)=0.85], it suggests a potentially important role of innate immunity and interferon responses in the pathogenesis of type 1 diabetes, which may reveal therapeutic targets. The purpose of the current study was to replicate the genetic finding in an independent, family-based Canadian population sample.

Methods

Participants In this study, 589 type 1 diabetes nuclear family trios (1,767 individuals) were genotyped. Genomic DNA was obtained after informed consent. The Research Ethics Board of the Montreal Children's Hospital and other participating centres approved the study. Ethnically, participants were of mixed European descent, with the largest single group being of Quebec French-Canadian origin (40% of total cohort). All patients were diagnosed with type 1 diabetes while younger than 18 years old and had required insulin treatment continuously from the time of diagnosis.

Table 1 Five SNPs from the *IFIH1* locus in this study

SNP	Chr2 position	Gene
rs2111485	162,936,043	3′ flanking of *IFIH1*
rs1990760	162,949,558	nsSNP (Ala946Thr) of *IFIH1*
rs3747517	162,954,331	nsSNP (His848Arg) of *IFIH1*
rs17783344	163,034,400	nsSNP (Ala80Ser) of *GCA*
rs984971	163,050,028	3′ flanking of *GCA*

ns, non-synonymous

SNP selection Five SNPs were genotyped in our study [Table 1; Electronic supplementary material (ESM) Fig. 1]. Four of these, i.e. rs2111485, rs1990760, rs3747517 and rs984971, were found to be associated with type 1 diabetes [1], the most significant being rs1990760. The remaining three were in tight linkage disequilibrium (LD) with the condition (r^2=0.61–0.92) [1]. The fifth SNP, rs17783344, is flanked by the type 1 diabetes-associated SNPs in this locus (80 kb to the telomeric side of rs3747517 and 16 kb to the centromeric side of rs984971), but was not associated with type 1 diabetes [1]. It is a non-synonymous SNP (Ala80Ser) of the *GCA* gene and maps to a conserved functional domain (calcium binding). We decided to include it because, according to HapMap data (http://www.hapmap.org/), rs17783344 is common in Europeans but non-polymorphic in East Asians and Africans. This dramatic population differentiation may cause a biased estimation in a case–control study (including false negative results) because of potential population stratification. Our study on the type 1 diabetes association of rs17783344 is immune to population stratification.

Genotyping Genotypes for this study were obtained using the Sequenom iPLEX assay (Sequenom, Cambridge, MA, USA), which has been described in detail in a previous study [7]. The remaining SNPs in the panel were chosen to replicate some candidate-gene findings. One Mendelian error (0.17%) was found in the genotyping of rs2111485; two Mendelian errors (0.34%) were found in the genotyping of rs1990760. None were found in the genotyping of the other three SNPs.

Statistics Linkage disequilibrium analyses were performed by Haploview software (http://www.broad.mit.edu/personal/jcbarret/haploview) [8]. Genetic association was tested by the family-based association test software (http://www.biostat.harvard.edu/~fbat/fbat.htm) [9]. Using logistic regression, the OR was estimated based on the transmission disequilibrium test and the UNPHASED software package (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/) [10].

Results and discussion

The family-based association test in our study is shown in Table 2. The four SNPs reported to be associated with type 1 diabetes, i.e. rs2111485, rs1990760, rs3747517 and rs984971, showed a trend of association with type 1 diabetes in the same direction as previously reported [1]. Two SNPs, rs2111485 and rs984971, have replicable type 1 diabetes association in our dataset, with statistical significance at α=0.05. The lack of statistical significance of the other two SNPs, rs1990760 and rs3747517, may be due to the lack of statistical power, which is ~61% for rs1990760 with OR=0.85 at α=0.05 level and ~49% for rs3747517 with OR=0.86 at α=0.05 level. No association was found for the non-synonymous SNP rs17783344 from the *GCA* gene.

The type 1 diabetes-associated SNPs, rs2111485 and rs984971, locate in a LD block containing four genes: *FAP*, *IFIH1*, *GCA* and *KCNH7* (ESM Fig. 2). The *FAP* gene product has been identified as a human stromal antigen, which can stimulate cytotoxic T cell responses [11]. The *GCA* gene product is abundant in neutrophils and macrophages, and is associated with degranulation and conse-

Table 2 Family-based association tests of the *IFIH1* SNPs

SNP	Minor allele (frequency)	Hardy–Weinberg *p* value	Genotyping success rate (%)	Informative family number[a]	*z* value (single-sided *p* value)	Transmitted/untransmitted	OR (95% CI)[b]
rs2111485 (A/G)	A (0.404)	0.480	98.0	355	−1.97 (0.0244)	212:252	0.84 (0.70–1.00)
rs1990760 (C/T)	C (0.401)	0.291	98.1	346	−1.53 (0.0630)	212:248	0.87 (0.73–1.04)
rs3747517 (A/G)	A (0.260)	0.490	98.2	319	−0.80 (0.2119)	186:201	0.92 (0.76–1.12)
rs17783344 (G/T)	G (0.147)	0.338	99.9	224	−0.19 (0.4247)	119:122	0.98 (0.77–1.25)
rs984971 (A/G)	G (0.362)	0.912	99.6	369	−1.69 (0.0455)	220:257	0.85 (0.71–1.02)

[a] Number of nuclear families informative for (with a non-zero contribution to) family-based association test analysis
[b] The OR of the minor allele, with the major allele as reference, was estimated by the transmission disequilibrium test using the UNPHASED software package (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/)

quent immune reaction [12]. Beside the antivirus-related type 1 diabetes mechanism of *IFIH1*, *FAP* and *GCA* may participate in the autoimmune or inflammatory destruction of pancreatic beta cells. Therefore, all of these three genes are candidates for type 1 diabetes. However, further variant discovery and fine mapping are required to confirm that the *IFIH1* SNP is actually causative. Because of the tight LD and the small effect size of type 1 diabetes association, this may not be easy. However, if the antivirus-related genetic effect exists, an association between the SNPs and enterovirus infection could be found by a case–control study of host susceptibility. Moreover, if an autoimmune effect were found to exist, association between the SNPs and other cytotoxic autoimmune diseases could be expected, bearing in mind that other autoimmune diseases could also be associated with virus infection. A combined genetic effect of these genes, i.e. antivirus-associated effect and cytotoxic autoimmune reactions, is possible.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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