ORIGINAL ARTICLE

Type 1 diabetes and the OAS gene cluster: association with splicing polymorphism or haplotype?

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Background: The 2',5'-oligoadenylate synthetase genes (*OAS1*, *OAS2*, and *OAS3*) map to human chromosome 12q24 and encode a family of enzymes pivotal to innate antiviral defence. Recently, the minor allele of an *OAS1* single nucleotide polymorphism (SNP) that alters splicing (rs10774671) was found to be associated with increased enzymatic activity and, in a case-sibling control study, with type 1 diabetes (T1D).

Methods: We have confirmed this T1D association in 784 nuclear families (two parents and at least one affected offspring) by the transmission disequilibrium test (TDT; G:A=386:329, $p=0.033$). However, because of linkage disequilibrium within *OAS1* and with the other two *OAS* genes, functional attribution of the association to this SNP cannot be assumed. To help answer this question, we also genotyped two non-synonymous SNPs in *OAS1* exons 3 and 7.

Results: All three SNPs showed significant transmission distortion. Three of the eight possible haplotypes accounted for 98.4% of parental chromosomes and two of them carried the non-predisposing A allele at rs10774671. Parents heterozygous for these two haplotypes showed significant transmission distortion ($p=0.009$) despite being homozygous at rs10774671.

Conclusions: We confirm the T1D association with rs10774671, but we conclude that it cannot be attributed (solely) to the splicing variant rs10774671. A serine/glycine substitution in *OAS1* exon 3 is more likely a functional variant.

Genetic susceptibility to type 1 diabetes (T1D)¹ is a complex trait, less than half of which can be attributed to the major histocompatibility complex.² The remaining half must be accounted for by an unknown number of loci of much smaller effect, of which only a handful have been multiply confirmed.^{3–5} In search of the remaining loci, our group is pursuing a candidate polymorphism approach by evaluating 464 (thus far) single nucleotide polymorphisms (SNPs) with computationally estimated highest likelihood of functional effect on 146 immune function genes.

So far, several SNPs have shown transmission distortion (unpublished data). Among them was rs3741981, a non-synonymous (Ser162Gly) SNP in the 2',5'-oligoadenylate synthetase 1 gene (*OAS1*, MIM 164350). It showed association whose statistical significance ($p=0.021$) was lost after correction for multiple hypothesis testing. This finding acquires new importance given a recent report of T1D association with another *OAS1* SNP (Celera hCV2567433, dbSNP rs10774671) which changes mRNA splicing, increases enzymatic activity,⁶ and in a case-control study, was found to be associated with T1D.⁷

The purpose of the present study was to confirm the reported T1D association by a family based method, impervious to stratification artifacts, and to test the claim that this SNP entirely accounts for the genetic effect. We

genotyped rs10774671 as well an additional *OAS1* nsSNP, rs3177979, in our family based DNA set. We confirm the association but also present evidence that it cannot be solely accounted for functionally by rs10774671.

METHODS

Participants

Genomic DNA was obtained after informed consent from 784 nuclear family trios with one T1D affected offspring and two parents (2352 individuals). The Research Ethics Board of the Montreal Children's Hospital and other participating centres approved the study. Ethnic backgrounds were of mixed European descent, with the largest single group being of Quebec French-Canadian origin. All patients were diagnosed under the age of 18 years and required insulin treatment continuously from the time of diagnosis.

SNP genotyping

The SNPs were genotyped using the AcycloPrime™-FP SNP Detection Kit (PerkinElmer, Boston, MA). PCR primers,

Abbreviations: FBAT, Family Based Association Test; FP, fluorescence polarisation; LD, linkage disequilibrium; *OAS1*, 2',5'-oligoadenylate synthetase 1; OR, odds ratio; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test; T1D, type 1 diabetes

Table 1 Transmission disequilibrium tests of the *OAS1* SNPs

SNP	Minor allele (frequency)	Hardy-Weinberg p	Allele transmission counts (overtransmitted allele)	χ^2 (p value)	OR (95% CI)
rs3741981 (C/T)	C (0.463)	0.204	405:342 (C)	5.3 (0.021)	1.19 (1.03 to 1.37)
rs10774671 (A/G)	G (0.373)	1	386:329 (G)	4.5 (0.033)	1.18 (1.02 to 1.37)
rs3177979 (A/G)	G (0.358)	0.899	381:322 (G)	5.0 (0.026)	1.19 (1.03 to 1.38)

designed by the Primer3 webtool (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi),⁸ and fluorescence polarisation (FP) probes, designed using GeneRunner v3.05, are listed in supplementary table 1 (available at <http://www.jmedgenet.com/supplemental>). Reagents for each PCR reaction included 12 ng DNA, 5 mM MgCl₂, 25 μ M dNTPs, and 0.025 U/ μ l of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Amplification primers were used at 100 nM each (all concentrations are final). PCR was performed in a Dual 384-Well GeneAmp PCR System 9700 in clear 384 well microplates (Axygen Scientific, Union City, CA). Touch-up PCR conditions were: 95°C 5 min; five cycles at 97°C 30 s, 60°C 55 s, 72°C 30 s; 25 cycles at 95°C 30 s, (60°C+0.2°C/cycle) 55 s, 72°C 30 s; 15 cycles at 95°C 30 s, 65°C 55 s, 72°C 30 s; 72°C 7 min in a final volume of 10 μ l. The removal of unincorporated primers and dNTPs was achieved according to the PE AcycloPrime PCR Clean-Up protocol. Pyrophosphatase was added to the Clean-Up reaction mix. The final extension reaction was performed according to the PE AcycloPrime-FP protocol in the Dual 384-Well GeneAmp PCR System 9700 in black microplates (Axygen Scientific) for 35 cycles at 95°C 10 s; 55°C 30 s (except for rs3177979 sense reaction and rs3741981 antisense reaction which were performed at 45°C instead of 55°C). Final detection of the SNP was carried out using the Criterion Analyst HT System (Molecular Devices, Sunnyvale, CA). All genotypes were confirmed by re-testing with opposite strand probes with 100% concordance.

All three SNPs had unambiguous clusters corresponding to the three genotypes and call rates >99.5%. The Mendelian error rate was 0.0004 (1:2418) for rs10774671 and 0 for the other two (families with Mendelian errors in >5 unlinked loci have been removed). No genotype distribution significantly differed from Hardy-Weinberg equilibrium.

Statistics

Transmission disequilibrium test (TDT), linkage, and linkage disequilibrium (LD) analysis were performed using Haploview software (www.broad.mit.edu/personal/jcbarret/haploview).⁹ Genotypic and haplotypic association was tested using Family Based Association Test (FBAT) software (<http://www.biostat.harvard.edu/~fbat/fbat.htm>).

Parental specific association was assessed with the parent of origin likelihood ratio test.¹¹ To assess the effect size of T1D susceptibility from each SNP, the odds ratio (OR) was estimated by comparing the allele frequency of T1D offspring versus the untransmitted allele frequency of both parents in complete families using Mantel-Haenszel analysis.

RESULTS

All three SNPs show transmission distortion by TDT analysis (table 1). The OR calculated from these data (table 1) are similar for all three SNPs and indicate an effect stronger than that described for *CTLA4* but much weaker than that of the *INS*³ and *PTPN22* loci.^{5,12} On genotypic FBAT analysis (table 2), only homozygosity for the minor allele of each of the three SNPs significantly confers increased risk for T1D. Despite the much larger numbers, heterozygous genotypes show no significant differences. In fact, all three are slightly and non-significantly undertransmitted, suggesting a recessive effect.

D' was 1 for each pair of SNPs (supplementary table 2, available at <http://www.jmedgenet.com/supplemental>), which suggests no recombination in this region. In addition, the HapMap data show that the three *OAS* genes (*OAS1*, *OAS3*, and *OAS2*) tend to be in LD (supplementary fig 1, available at <http://www.jmedgenet.com/supplemental>). Because of allele frequency differences, the r^2 between rs10774671 and rs3741981 was only 0.69, despite which the statistical significance of the rs374198 distortion was, if anything, somewhat higher. This raises doubts as to whether rs10774671 is the (only) functional variant.

Haplotype analysis was used to explore this further. Of the eight possible haplotypes of these three SNPs, only four had a frequency >1% and three of these accounted for 98.4% of parental chromosomes (table 3). C-G-G (SNP order: rs3741981-rs10774671-rs3177979) is significantly overtransmitted and T-A-A undertransmitted, as expected from carrying predisposing and protective alleles, respectively, at all three SNPs. However, the mismatched C-A-A (protective at rs10774671 but predisposing at rs3741981) was not significantly undertransmitted; if anything, it was slightly

Table 2 Genotypic association analysis between *OAS1* SNPs and T1D

Genotype	Frequency	Family number*	S†	E(S)‡	Var(S)§	Z value	p
rs3741981							
T/T	0.304	401	143	156	89	-1.351	0.177
C/T	0.481	568	278	284	142	-0.504	0.615
C/C	0.215	346	147	128	75	2.161	0.031
rs10774671							
A/A	0.401	446	170	182	101	-1.193	0.233
A/G	0.464	551	271	276	138	-0.383	0.701
G/G	0.134	269	110	94	57	2.185	0.029
rs3177979							
A/A	0.419	443	168	182	101	-1.345	0.179
A/G	0.458	543	269	272	136	-0.215	0.830
G/G	0.123	260	106	90	55	2.157	0.031

*Number of nuclear families informative for (with a non-zero contribution to) FBAT analysis; †observed genotype number in the affected offspring; ‡expected genotype number in the affected offspring; §the variance of genotype distribution among the affected offspring.

Table 3 Haplotypic association of the 3 OAS1 SNPs

Haplotype (rs3741981-rs10774671-rs3177979)	Frequency	Informative family number*	S†	E(S)‡	Var(S)§	Z value	p
T-A-A	0.543	481	477	509	189	-2.325	0.020
C-G-G	0.352	461	399	369	173	2.281	0.023
C-A-A	0.089	233	126	122	60	0.515	0.606
C-G-A	0.015	34	21	22	10	-0.156	0.876

*Number of nuclear families informative for (with a non-zero contribution to) FBAT analysis; †observed haplotype number in the affected offspring; ‡expected haplotype number in the affected offspring; §the variance of haplotype distribution among the affected offspring.

overtransmitted. More importantly, transmission from C-A-A/T-A-A heterozygous parents is significantly distorted in favour of C-A-A (50:27, $p = 0.009$). Conditional on the C/T transmission distortion 405/342 of rs3741981, the corrected p value of the C-A-A/T-A-A transmission distortion is 0.030. This overtransmission of the rs3741981 C allele in parents homozygous for A at rs10774671 is compatible with (though not proof of) rs3741981 being the sole functional variant accounting for the genetic effect. The finding is incompatible with rs10774671 playing this role.

We also noticed that for all SNPs and haplotypes, the transmission distortion was almost entirely accounted for by heterozygous mothers. Distortion in inheritance from fathers was weak and not statistically significant (table 4). This was due to a smaller number of non-transmissions rather than a larger number of transmissions from heterozygous mothers compared to fathers (table 4). Indeed, as shown in supplementary table 3 (available at <http://www.jmedgenet.com/supplemental>), there was a trend towards fewer mothers carrying one or two copies of the predisposing allele that acquired a borderline significance for rs3177979: the number of mothers with G allele (G/G+A/G) is statistically less than that of fathers ($\chi^2 = 4.021$, $p = 0.045$).

DISCUSSION

The OAS1 gene encodes a protein of the 2',5'-oligoadenylate synthase family, a group of enzymes that play an important role in innate antiviral defence. Induced by interferon- α , they catalyze the synthesis of 2',5'-oligomers of adenosine, which consequently activates latent RNAase resulting in the degradation of viral and cellular RNA, thus inducing apoptosis of infected cells.¹³ The antiviral activity of *Oas1b*, the mouse ortholog of OAS1, is crucial in the inhibition of West Nile and other flavoviruses, both in vivo and in vitro.¹⁴⁻¹⁶ The human OAS1 has been shown to be an important part of the total constitutive activity of OAS enzymes.⁶ The A/G substitution at rs10774671 involves the last nucleotide of intron 6 of OAS1. G, the minor allele, retains the splice acceptor site with the consensus sequence AG, while the A allele ablates the splice site. This splicing SNP is shown to be

associated with OAS enzyme activity change, the G allele being associated with higher enzyme activity.⁶ Higher basal activity has also been reported in subjects with T1D compared to healthy siblings.¹⁷ Thus, our confirmation of the results of Field *et al*⁷ and the hypothesis that enhanced OAS activity might promote β -cell apoptosis, either upon viral infection or because of aberrant INF- α induction in the course of autoimmunity, has the potential to provide valuable novel insights into the pathogenesis of human T1D. However, several points made by Field *et al*⁷ need to be discussed.

First, the conclusion that rs10774671 is the functional variant in this association is very poorly supported in the study of Field *et al*⁷ and contradicted by our data. The failure to find significant T1D association in several other OAS1 polymorphisms despite the tight LD is obviously due to the much lower power to detect resulting from the smaller number of patients and (especially) controls tested in the study of Field *et al*.⁷ As such, these findings permit no conclusion. When we tested two additional SNPs with the same cohort size, the association for both of them was actually higher than that of rs10774671. More importantly, our finding of significant distortion in transmissions from parents homozygous for rs10774671, points to rs3741981 as a more likely functional variant. The serine/glycine substitution caused by this nsSNP occurs in an evolutionarily conserved DNA region and is close to a dsRNA binding domain of OAS1 protein. Alternatively, and perhaps more likely, these SNPs are merely markers for a haplotype containing a variant, or cluster of variants, that affects OAS levels/activity and, consequently, T1D susceptibility. As we have previously argued,¹⁸ the assumption that complex trait associations need always be reduced to a single polymorphism, as in monogenic diseases, is not supported by any evidence or theoretical consideration. The splicing site SNP rs10774671 may well be part of the functional effect, but our data clearly show that it cannot account for it exclusively. Because the association is in a long range LD region, there may be an additive effect of several functional variants (coding non-synonymous, regulatory, splicing) in these genes. In addition to the experimental evidence, the recessive effect suggested by the analysis in table 2 is hard to reconcile with a gain of function variant.

As new T1D loci are confirmed, a perspective on their relative contributions to T1D risk becomes important. In this respect, we are puzzled by the assertion that the OAS1 and INS loci are approximately equivalent in effect magnitude.⁷ The OR (95% CI) of the INS locus in the study of Field *et al* is 1.43 (1.11 to 1.84). In our hands, the transmission distortion from heterozygous parents is much higher at the INS rs689 SNP and, with the same sample size, seven orders of magnitude more significant: transmission ratio A/T = 258/133, $\chi^2 = 40.0$, $p = 2.59 \times 10^{-10}$, OR (95% CI) 1.96 (1.60 to 2.41), which is in accordance with a very large aggregate sample size in the published literature.³ Given that both population samples are of European descent and the markers used by us (rs689) and by Field *et al* (rs3842753) are in very strong LD ($r^2 = 1$) and both maximally associated with T1D,³ we have no explana-

Table 4 Parental specific TDT analysis

SNP (nucleotide substitution)	Transmission: untransmission (overtransmitted allele)	χ^2 (p value)
rs3741981 (C/T)		
Maternal transmission	160:112 (C)	8.5 (0.004)
Paternal transmission	158:143 (C)	0.7 (0.387)
rs10774671 (A/G)		
Maternal transmission	153:110 (G)	7.0 (0.008)
Paternal transmission	152:135 (G)	1.0 (0.316)
rs3177979 (A/G)		
Maternal transmission	148:104 (G)	7.7 (0.006)
Paternal transmission	153:135 (G)	1.1 (0.289)

tion for the much smaller effect magnitude for insulin found in their study.⁷

Finally, a potentially interesting finding of this study is the preferentially maternal transmission of T1D predisposing *OAS* haplotypes in our population sample. It may well be a statistical artifact but, if it is confirmed with larger numbers, it might provide functional clues. Parental imprinting is a possibility, but the smaller than expected number of mothers of T1D children with the predisposing haplotype and its excess transmission in their affected offspring may suggest effects of the intrauterine environment. If the T1D predisposing haplotype increases antiviral activity,⁶ mothers carrying it might better protect the fetus from T1D promoting perinatal infection, a possible explanation for the lower number of non-transmitting mothers. Once transmitted to the child, however, the same haplotype may increase T1D risk by enhancing the pro-apoptotic effects of IFN- α .⁷ In this respect, it is worth noting that the only viral infection proven to cause T1D in humans is congenital rubella acquired in utero.¹⁹ Investigating the effect of the genetic variations on the *OAS* enzymes against congenital rubella virus infection may address the problem directly.

In conclusion, we confirm the previously reported⁷ association of T1D with the *OAS* gene cluster but our data clearly show that the causative variant remains to be determined. Pending ascertainment of the maximally associated SNP in this LD block, the effect appears to be modest, much lower than that of the *INS* locus.

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ELECTRONIC-DATABASE INFORMATION

Information on Primer3 can be found at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi, on Haploview at www.broad.mit.edu/personal/jcbarret/haploview, and on FBAT at <http://www.biostat.harvard.edu/~fbat/fbat.htm>.

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