Brief Genetics Report

No Evidence for Association of *OAS1* With Type 1 Diabetes in Unaffected Siblings or Type 1 Diabetic Cases

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Type 1 diabetes is a common autoimmune disorder that is strongly clustered in families. As the sharing of alleles of the HLA class II genes cannot explain all of this aggregation, alleles of multiple other loci are involved. Recently, it was reported that an A/G splice-site single nucleotide polymorphism (SNP; rs10774671) in the OAS1 gene, encoding 2′5′-oligoadenylate synthetase, was associated with a protective effect against type 1 diabetes in unaffected siblings, and yet affected siblings showed random transmission. Since this finding is difficult to explain biologically, we genotyped the *OAS1* SNP in 1,552 type 1 diabetic families from the U.K., U.S., Romania, and Norway and in 4,287 type 1 diabetic cases and 4,735 control subjects from the U.K. We found no evidence of association in either unaffected (relative risk 1.00; $P = 0.94$) or affected (1.00; $P = 0.96$) siblings or in the case-control study (odds ratio 0.99; $P = 0.83$). These results suggest that additional evidence of association of a low penetrance effect in common disease should be sought when the primary result comes from unaffected siblings in the absence of any effect in cases. *Diabetes* 55:1525–1528, 2006

To date, four loci have been identified with convincing and reproducible statistical support that predispose to type 1 diabetes development: the HLA class II genes (1), the insulin gene on chromosome 11p15 (2,3), the *CTLA4* locus on 2q33 (4,5), and *PTPN22* (6,7). Most recently, strong evidence for association of the *IL2RA/CD25* locus with type 1 diabetes has been reported ($P = 1.3 \times 10^{-10}$) (8), but this finding requires independent replication. All of these genes are involved in T-cell activation, homeostasis, and repertoire formation.

Field et al. (9) recently reported that the A allele of an A/G splice-site single nucleotide polymorphism (SNP) (rs10774671) in the *OAS1* gene, encoding 2′5′-oligoadenylate synthetase, had a type 1 diabetes protective effect in 401 unaffected siblings but no effect in 835 affected siblings. The 401 unaffected and 835 affected siblings were from 574 families comprising 83 Danish Families, 206 Danish diabetes-discordant sibpairs, 156 Canadian families, and 128 U.S. families from the Human Biological Data Interchange (9). Field et al. compared the A/G splice-site SNP genotype frequencies between affected and unaffected siblings using a χ^2 test and found differences between the genotype frequencies of diabetic and unaffected siblings (derived: $\chi^2_2 = 8.31$, $P = 0.016$; reported: GG+GA [high-risk] versus AA [low-risk] $\chi^2_1 = 8.05$, one-sided $P = 0.0023$). However, as the statistical test of Field et al. was invalid in that interdependence between sibling genotypes was ignored and the P value is not very small, this difference between diabetic and healthy siblings may be a false-positive. A more important caveat regarding the interpretation of their data arises from their results using the AFBAC (affected-family based controls) method (10) to compare the frequencies of transmitted and untransmitted alleles from parents to 368 diabetic siblings (one per family) and to 198 unaffected siblings; they found no evidence of an association between type 1 diabetes and the A/G splice-site SNP in the affected siblings ($P = 0.27$) but did find evidence of an increased frequency of the A allele in unaffected siblings (A allele frequency 0.71 in transmitted vs. 0.61 in nontransmitted alleles; $P = 0.003$) (9). Consequently, Field et al. concluded that the A allele was having a protective effect in the unaffected siblings. Our interpretation of this published data, given the biological and genetic implausibility of obtaining an allelic association with low penetrance in unaffected siblings with absence of significant deviation in transmission to diabetic siblings, was that the reported increase in A allele frequency in the unaffected siblings was a chance event.

To further investigate the potential role of the *OAS1* A/G splice-site SNP in type 1 diabetes, we genotyped it in 455 U.K., 327 U.S. (which included ~128 families previously analyzed by Field et al. [9]), 360 Norwegian, and 410 Romanian multiplex and simplex families, providing 1,913 parent-affected sibling trio genotypes and 856 parent-unaffected sibling trio genotypes. In addition, we genotyped 4,287 type 1 diabetic cases and 4,735 geographically matched control subjects from the U.K. (11).

We found no evidence for a protective effect in unaffected siblings: we observed random transmission of the A allele to unaffected sibs, using the transmission/disequilbrium test (TDT) (12) (369 transmissions vs. 375 nontransmissions, 49.6% transmission; $P = 0.83$) (Table 1). We

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Received for publication 7 November 2005 and accepted in revised form 2 February 2006.

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

nsSNP, nonsynonymous SNP; SNP, single nucleotide polymorphism; TDT, transmission/disequilbrium test.

DOI: 10.2337/db05-1452

TABLE 1
Association analyses of *OAS1* A/G splice–acceptor site SNP (rs10774671) in 1,552 families

Population	Parent-child trios (*n*)	Parental MAF	TDT (G allele) T	NT	%T	RR	*P**
U.K.	706	0.34	340	322	51.4	1.06	0.48
U.S.	592	0.39	229	259	46.9	0.88	0.17
Norway	323	0.30	139	124	52.8	1.12	0.35
Romania	292	0.36	133	138	49.0	0.96	0.76
All families	1,913	0.34	841	843	49.9	1.00	0.96
Unaffected sibs	856	0.34	369	375	49.6	0.98	0.83

**P* values by TDT. MAF, minor allele frequency; NT, not transmitted; T, transmitted.

found, consistent with Field et al. (9), no evidence for an association with type 1 diabetes in the family collections from four populations (Table 1) (relative risk [RR] for allele G = 1.00 [95% CI 0.91–1.10]; $P = 0.96$). In addition, we found no evidence for association between the A/G splice-site SNP and type 1 diabetes in the case-control collection (odds ratio for allele G = 0.99 [0.93–1.06]; $P = 0.83$) (Table 2). We found virtually identical genotype frequencies in the diabetic offspring and pseudo-control subjects (13) for the "high-risk GG+GA genotype" (56.7 and 56.7%, respectively), as well as in the case-control collection (58.5 and 58.9%, respectively) (Table 2).

More recently, Tessier et al. (14) reported marginally significant evidence for an association between type 1 diabetes and the *OAS1* splice-site SNP, using the TDT in 784 families of mixed European descent, mainly French Canadian (RR for the G allele = 1.18 [95% CI 1.02–1.37]; $P = 0.033$). They did not test transmission to unaffected siblings. Tessier et al. also genotyped two nonsynonymous SNPs (nsSNPs) in *OAS1*, rs3741981 (C>T) and rs3177979 (A>G), obtaining an RR for allele C of 1.19 (1.03–1.37; $P = 0.021$) and an RR for allele G of 1.19 (1.03–1.38; $P = 0.026$), respectively. On examining haplotypes for the three SNPs, Tessier et al. found that transmission from C-A-A/T-A-A to diabetic siblings was overtransmitted in favor of C-A-A ($P = 0.009$). Consequently, they suggested that rs3741981 was the sole functional variant accounting for the genetic effect (14). They reported that maternal transmissions to affected offspring were increased for the three SNPs compared with transmissions from fathers.

We also genotyped the two nsSNPs from Tessier et al. (14) and found no evidence of association between rs3741981 or rs3177979 and type 1 diabetes in the family or case-control collections (supplementary Tables 1 and 2 [online appendix, available at http://diabetes.diabetesjournals.org]). We also tested for an association with the three–*OAS1* SNP haplotype (rs10774671-rs3741981-rs3177979) and found no evidence of association ($P = 0.28$ and 0.86 for the case-control and family collections, respectively). The linkage disequilibrium between the SNPs was consistent with that reported by Tessier et al. (14); in control subjects, the D′ was 1.00 for each pair of SNPs, and the r^2 was 0.74 between rs10774671 and rs3741981 and 0.98 between rs10774671 and rs3177979. Finally, despite no evidence of primary association, we tested for increased maternal or paternal transmission to affected siblings (one affected sibling per family) for the three *OAS1* SNPs but could not replicate the findings of Tessier et al. (14) (parent-of-origin test $\chi^2_1 = 2.86$, $P = 0.091$).

The RR observed by Tessier et al. (14) was 1.18 for the G allele for the A/G splice-site SNP and 1.19 for both rs3741981 and rs3177979, minor alleles C and G, respec-

TABLE 2
OAS1 A/G splice–acceptor site SNP (rs10774671) allele and genotype frequencies and association test results in the type 1 diabetes family and the case-control collection

Family collection	Transmitted	Untransmitted*	RR (95% CI)	*P*
Allele			TDT	
G (TDT)	841	843	1.00 (0.91–1.10)	0.96
Genotype			Conditional logistic regression	
AA	829 (43.3)	2,484 (43.3)	1.00 (ref.)	—
GA	848 (44.4)	2,554 (44.5)	1.00 (0.88–1.13)	0.95
GG	236 (12.3)	701 (12.2)	1.01 (0.82–1.24)	0.92
GG+GA	1,084 (56.7)	3,255 (56.7)	1.00 (0.88–1.13)	0.96
Case-control collection	**Cases (n = 4,287)**	**Control subjects (n = 4,735)**	**OR (95% CI)**	***P***
Allele			Logistic regression	
A	5,522 (64.4)	6,093 (64.3)	1.00 (ref.)	—
G	3,052 (35.6)	3,377 (35.7)	0.99 (0.93–1.06)	0.83
Genotype				
AA	1,779 (41.5)	1,945 (41.1)	1.00 (ref.)	—
GA	1,964 (45.8)	2,203 (46.5)	0.96 (0.88–1.05)	0.40
GG	544 (12.7)	587 (12.4)	1.01 (0.88–1.16)	0.86
GG+GA	2,508 (58.5)	2,790 (58.9)	0.97 (0.89–1.06)	0.52

Data are *n* (%) unless otherwise indicated. Families comprise U.K., U.S., Norway, and Romania. *Untransmitted (pseudo-control) data for genotypes in the type 1 diabetic family collection are estimated, as in Cordell and Clayton (13). OR, odds ratio.

tively. Using our family and case-control collections, we had 94% power in the family collections and 100% in the case-control collection to detect such effect sizes at the 5% significance level.

The inconsistencies between the two previous studies are further highlighted by the reply from Field et al. (15) to Tessier et al., in which Field et al. reanalyzed their own data but failed to find a haplotypic association or evidence for *OAS1* parental sex–specific effects on diabetes susceptibility, indicating that the results of Tessier et al. were probably false-positives; a conclusion consistent with the results found in the present report. Previously, we had reported evidence for an association of the LRP5 gene in type 1 diabetes, for which most of the statistical support came from a lack of transmission of a haplotype to unaffected siblings (16). This sort of effect, which can be described as a protective effect in unaffected siblings, is difficult to explain biologically, especially in the absence of any effect in affected siblings and when the effect size or penetrance of the potential susceptibility gene under analysis is very low. Hence, we subsequently analyzed much larger sample sizes, and the undertransmission of LRP5 gene region alleles to unaffected siblings disappeared, as one would expect (17). Nevertheless, it remains a possibility that there is an effect at or near *OAS1* in type 1 diabetes. However, if there is, then it must be very small and/or susceptible to population-specific effects, requiring replication in extremely large population-specific studies.

RESEARCH DESIGN AND METHODS

All families were Caucasian and of European descent and were composed of two parents and at least one affected child (supplementary Table 3). The population studied consisted of 455 multiplex families from the Diabetes U.K. Warren collection (18), 410 simplex families from Romania (19), 360 Norwegian simplex families (20), and 327 multiplex families from the Human Biological Data Interchange (U.S.) (21). The 4,287 case subjects are part of the Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory (U.K.) GRID (Genetic Resource Investigating Diabetes) study (http://www-gene.cimr.cam.ac.uk/ucdr/grid.shtml) and the 4,735 control subjects from the 1958 British Birth Cohort (http://www.cls.ioe.ac.uk/studies.asp?section=000100020003), as previously described (11). All DNA samples were collected after approval from the relevant research ethics committees, and written informed consent was obtained from the participants.

Genotyping. Genotyping was undertaken using TaqMan (Applied Biosystems, Warrington, U.K.), and probes and primers were designed by Applied Biosystems. All genotyping was double scored to minimize error (concordance was 100%). The nsSNP rs3177979 was also genotyped using a second independent technology (MegAllele technology: Affymetrix GeneChip Tag arrays using ParAllele's molecular inversion probes) (11,22). From 1,051 cases and control subjects, the concordance between MegAllele and Taqman genotypes was 99.99% (one discrepancy).

Statistical analysis. All statistical analyses were performed in the Stata statistical package (http://www.stata.com), using the Genassoc package. Allele frequencies for all subjects were in Hardy-Weinberg equilibrium ($P > 0.05$) for all three SNPs. The case-control data were stratified by broad geographical region within the U.K. in order to reduce to a minimum any confounding due to variation in allele frequencies across the U.K. (11). Haplotype analysis was performed in the family collection using TRANSMIT (13) and in the case-control collection using SNPHAP (http://www-gene.cimr.cam.ac.uk/clayton/software/) to derive the haplotypes and logistic regression to analyze.

ACKNOWLEDGMENTS

This work was funded by the Wellcome Trust and the Juvenile Diabetes Research Foundation International.

We gratefully acknowledge the participation of all of the patients, control subjects, and family members and thank the Human Biological Data Interchange and Diabetes U.K. for U.S. and U.K. multiplex families, respectively, and the Norwegian Study Group for Childhood Diabetes for the collection of Norwegian families (Dag Undlien and Kjersti Ronningen) and Constantin Ionescu-Tirgoviste and Cristian Guja for Romanian families. We acknowledge use of DNA from the 1958 British Birth Cohort collection, funded by the Medical Research Council Grant G0000934 and Wellcome Trust Grant 068545/Z/02, and thank David Strachan and Paul Burton for their help. We also thank The Avon Longitudinal Study of Parents and Children laboratory in Bristol, including Susan Ring, Wendy McArdle, and Richard Jones, for preparing DNA samples.

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