

OAS1 Splice Site Polymorphism Controlling Antiviral Enzyme Activity Influences Susceptibility to Type 1 Diabetes

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Both genetic and nongenetic factors contribute to the development of type 1 diabetes. Many investigations, including prospective studies of high-risk children, have implicated virus infections as predisposing environmental agents. We previously reported that basal activity of the key antiviral enzyme 2'5'-oligoadenylate synthetase (2'5'AS) was significantly elevated in type 1 diabetic patients compared with healthy control subjects. Recently, we showed that an A/G splice site single nucleotide polymorphism (SNP) in the *OAS1* gene encoding 2'5'AS is strongly associated with basal 2'5'AS activity. Basal enzyme activity was highest in individuals with GG genotype and lowest in those with AA genotype. In the present study, we genotyped 835 type 1 diabetic and 401 healthy siblings at the *OAS1* splice site polymorphism and (for comparison) at an A/C SNP of the insulin (*IDDM2*) locus. Results showed that *OAS1* GG and GA were significantly increased in diabetic compared with healthy siblings ($P = 0.0023$). The strength of association was similar to that at *IDDM2*, where, as expected, the C/C (variable number tandem repeat class I homozygote) genotype was increased in affected compared with healthy siblings ($P = 0.0025$). The results suggest that host genetic response to virus infection could influence susceptibility to type 1 diabetes. *Diabetes* 54: 1588–1591, 2005

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Type 1 diabetes is caused by some combination of multiple genetic and environmental factors that precipitate autoimmune destruction of the insulin-producing β -cells of the pancreas. It is known that a preclinical period of several years often precedes development of overt diabetes, during which time autoantibodies to various β -cell components appear. Although susceptibility genes in numerous regions have been suggested by linkage and association studies (1), the best documented and most widely accepted predisposing genetic factors are located in the HLA-DR,DQ class II region (*IDDM1*) and at a variable number tandem repeat (VNTR) in the 5' region of the insulin gene (*IDDM2*). Recent long-term prospective Finnish studies have strongly suggested that infections with enteroviruses such as Coxsackievirus may trigger the autoimmune process (2–5). For example, increased frequencies of serum enterovirus antigens and antibodies toward enterovirus were observed during the preclinical phase in children who subsequently developed diabetes (2,4,5). Finnish researchers also demonstrated a temporal association between appearance of antiviral antibodies and islet cell autoantibodies in prediabetic siblings of type 1 diabetic patients (3,5). Similarly, children who were positive for islet autoantibodies had significantly higher levels of IgG antibodies toward Coxsackie B4 virus than control children (6), and nondiabetic children with islet cell autoantibodies had stronger T-cell reactivity against enterovirus proteins than children without such autoantibodies (7).

Virus infections induce interferon- α , which, through a complex signal transduction pathway, induces the OAS genes encoding the key antiviral enzyme 2'5'-oligoadenylate synthetase (2'5'AS). This enzyme synthesizes oligoadenylates that activate a latent RNase (RNaseL), which then degrades viral and cellular RNA, inhibiting virus replication and promoting the death of infected cells. The innate 2'5'AS-RNaseL antiviral defense system is critical for controlling infection with novel viruses before development of an adaptive (antigen-specific) immune response. We previously showed (8) that basal activity of 2'5'AS was significantly increased in patients with type 1 diabetes compared with healthy control subjects ($P < 0.001$). Furthermore, new analysis of data from our earlier study

TABLE 1
OAS1 splice acceptor site genotypes in diabetic and healthy siblings

	<i>OAS1</i> genotype			Low risk AA
	GG	High risk GA	GG + GA	
Diabetic siblings (<i>n</i> = 835)	104 (12.5)	386 (46.2)	490 (58.7)	345 (41.3)
Healthy siblings (<i>n</i> = 401)	39 (9.7)	162 (40.4)	201 (50.1)	200 (49.9)

Data are *n* (%). High versus low risk, $\chi^2 = 8.05$, 1 df, one-sided *P* = 0.0023.

(9) of 2'5'AS response to vaccination with yellow fever virus revealed that basal and virus-stimulated activity are strongly correlated (correlation coefficient 0.65, *P* < 0.0001). To determine whether variation in basal (and by inference, virus-stimulated) activity is under genetic control, we recently examined the relationship between basal activity and genetic markers distributed across the *OAS1-OAS3-OAS2* gene cluster on chromosome 12q24.2. We found a highly significant association between basal 2'5'AS activity and an A/G splice acceptor site single nucleotide polymorphism (SNP) in the *OAS1* gene: enzyme activity was highest in individuals with the GG genotype, intermediate in those with the GA genotype, and lowest in those with the AA genotype (ANOVA *P* = 1×10^{-14}) (10). Exon splicing requires the sequence AG at the splice acceptor site. We showed that the A/G splice site polymorphism generates different isoforms of the 2'5'AS enzyme, since the G allele (sequence AG at the acceptor site) enables exon 7 splicing at the SNP (generating the p46 isoform), whereas the A allele (sequence AA at the acceptor site) ablates the splice site and results in splicing at alternate sites further downstream (generating the p48 isoform and a novel p52 isoform). Thus, only individuals possessing at least one G allele can produce the p46 isoform. Those who only have A alleles cannot make p46; instead, they produce other isoforms such as p48 and p52. It is unknown why the p46 isoform (G allele) is associated with higher basal enzyme activity than the p48/p52 isoforms (A allele). However, the p48 isoform was previously shown (11) to have proapoptotic activity that is independent of its synthetase activity (since enzymatically inactive p48 mutants could still induce cellular apoptosis) and independent of RNaseL activation (since transfected p48 caused apoptosis in RNaseL^{-/-} cells). This proapoptotic activity of p48 was localized to a BH3 (Bcl-2 homology-3) domain encoded by exon 7 that binds specifically to antiapoptotic proteins of the Bcl-2 family. Therefore, p48 is a dual-function protein with distinct 2'5' AS and apoptotic capabilities. Thus, the A/G splice site polymorphism may orchestrate functionally distinct responses to virus infection.

To date, there is only limited information on the relation of genetic variation at the *OAS* genes and susceptibility to virus infection. However, recent mouse studies have shown that the *Oas1b* gene, the murine equivalent of *OAS1*, controls host susceptibility/resistance to West Nile virus and other flaviviruses (12–14). In humans, persistent hepatitis C virus infection was recently shown (15) to be associated with genotype GG at *OAS1* SNP rs2660, which we have shown to be in almost complete linkage disequilibrium with genotype GG at the *OAS1* splice site polymorphism (10). Thus, it is possible that inability to clear

hepatitis C virus is influenced by one's *OAS1* splice site genotype.

In the current study, we tested the hypothesis that individuals with type 1 diabetes have higher frequencies of *OAS1* GG and GA genotypes (associated with elevated 2'5'AS activity) than nondiabetic control subjects. For comparison, we also genotyped an A/C SNP (rs3842753) in the 3' untranslated region of the insulin gene whose C and A alleles were previously shown (16,17) to be in virtually complete linkage disequilibrium with *IDDM2* VNTR class I and class III alleles, respectively. Many studies have shown that class I homozygotes (SNP genotype CC) are increased in type 1 diabetes. We genotyped a total of 835 diabetic patients and 401 healthy sibling control subjects at the *OAS1* splice site SNP and the *IDDM2* SNP. Subjects came from 574 families: 83 Danish families previously assessed for 2'5'AS activity, 206 additional Danish diabetes-discordant sibling pairs, 156 Canadian families, and 128 American families. DNA from the latter was purchased from the Human Biological Data Interchange (18). Markers were genotyped on an Applied Biosystems 7000 real-time thermocycler using *Taq*-Man probes and primers designed and purchased from Applied Biosystems. To test for association between *OAS1* or *IDDM2* genotype and type 1 diabetes, we compared genotype frequencies in diabetic versus healthy siblings by contingency table χ^2 testing. Significance was assessed using one-sided *P* values, since prior studies predicted the direction of genotype frequency differences. Healthy siblings constitute conservative control subjects, since diabetic children will tend to be genetically more similar to their siblings than to unrelated control subjects. Thus, given that a true association exists between a genetic marker and a disease, it will generally be more difficult to detect a significant difference in marker allele frequencies between case and sibling control subjects than between case and unrelated control subjects.

Tables 1 and 2 show the genotype frequencies at the *OAS1* splice site polymorphism and the *IDDM2* polymorphism in diabetic and healthy siblings. At the splice site polymorphism, diabetic patients had higher frequencies of both GG and GA genotypes and a lower frequency of AA genotype than their healthy siblings (GG + GA genotype: diabetic patients 0.59, siblings 0.50; one-sided *P* = 0.0023) (Table 1). All four datasets showed an increased frequency of GG + GA genotypes in diabetic versus healthy siblings (original Danish: 0.61 vs. 0.50; additional Danish: 0.56 vs. 0.52; Canadian: 0.59 vs. 0.49; American: 0.59 vs. 0.42). Both sexes showed a significantly increased frequency of GG + GA genotypes in diabetic compared with healthy siblings (data not shown). As expected, diabetic patients had a higher frequency of the *IDDM2* SNP genotype CC (VNTR

TABLE 2
IDDM2 (SNP rs3842753) genotypes in diabetic and healthy siblings

	<i>IDDM2</i> genotype			
	High risk CC	CA + AA	Low risk CA	AA
Diabetic siblings (<i>n</i> = 835)	591 (70.8)	244 (29.2)	205 (24.5)	39 (4.7)
Healthy siblings (<i>n</i> = 401)	252 (62.8)	149 (37.2)	131 (32.7)	18 (4.5)

Data are *n* (%). High versus low risk, $\chi^2 = 7.87$, 1 df, one-sided *P* = 0.0025.

class I homozygote) than their healthy siblings (CC genotype: diabetic patients 0.71, siblings 0.63; one-sided *P* = 0.0025) (Table 2). Thus, the magnitude of the splice site association was similar to that of the *IDDM2* association in these sibling subjects. The risk of developing diabetes for siblings with splice site GG and GA genotypes was increased 1.55 and 1.38 times, respectively, compared with siblings with the lowest-risk genotype (AA). These relative risks mirror the dose-dependent effect of genotype on 2'5'AS activity (described above). The relative risk of diabetes for combined GG + GA genotypes was 1.41. As a comparison, the relative risk of diabetes for siblings with *IDDM2* genotype CC was 1.43 times that for siblings with CA or AA genotypes. (It should be noted that these relative risks may differ from those that would be obtained using unrelated control subjects.)

A subset of 636 diabetic and 195 nondiabetic siblings was genotyped for additional markers across the *OAS1-OAS3-OAS2* gene cluster (D12S1329, rs3741981, rs2010549, D12S2397, rs2240185, D12S811, and D12S129), but only the splice site polymorphism and another *OAS1* marker (rs3741981) showed significant allele frequency differences between diabetic and nondiabetic siblings (data not shown). However, compared with the splice site polymorphism, the association with rs3741981 was weaker and of only borderline significance (*P* = 0.047). Thus, susceptibility to type 1 diabetes mapped to the same genetic marker (the splice site polymorphism) that we previously showed to be the marker most strongly associated with elevated 2'5'AS enzyme activity (10).

In summary, compared with their healthy siblings, individuals with autoimmune diabetes had an increased frequency of the splice site genotypes GG and GA, a result consistent with our other studies using largely independent datasets showing higher basal 2'5'AS activity in type 1 diabetic patients than in healthy unrelated control subjects (8) and higher basal activity in individuals with GG and GA genotypes than in those with AA genotype (10).

It is unclear why the heightened antiviral enzyme activity of individuals with GG and GA would increase their risk of type 1 diabetes. One explanation is that increased enzyme activity, particularly during virus infection, directly damages small numbers of sensitive β -cells by RNaseL-mediated degradation of cellular RNA. In children possessing additional strongly predisposing genes (such as high-risk HLA alleles), this minor damage could initiate an aggressive autoimmune attack on β -cells by exposing normally sequestered antigens to the immune system. AA individuals with lower enzyme activity would be less prone to this minor-but-critical initial β -cell damage. A second possible explanation focuses on the unique dual function of p48 (discussed above). This is that individuals

with the AA genotype could induce rapid p48-related apoptosis of enterovirus-infected β -cells, inhibiting virus spread to neighboring cells. Individuals who produce less or possibly no p48 (GA and GG genotypes, respectively) would be less able or unable to mount this important antiviral response through the p48 pathway, resulting in more extensive β -cell damage and, if they also carry additional diabetes-susceptibility genes, initiation of autoimmune destruction.

Although these two possible explanations are not mutually exclusive, it would be of interest to know which might be more likely. To attempt to address this question, we analyzed transmission of the G and A alleles from parents to diabetic children (one per family) and healthy siblings using the AFBAC (Affected-Family-Based-Controls) program to compare the frequencies of transmitted and nontransmitted alleles (19). Parental splice site genotypes were available for 368 families. Results showed that the frequency of the G allele was only weakly increased among alleles transmitted to the 368 diabetic children compared with alleles not transmitted to these children (G allele frequency: 0.36 in transmitted vs. 0.33 in nontransmitted alleles, *P* = 0.271). Surprisingly, however, the frequency of the A allele was significantly increased among alleles transmitted to 198 unaffected siblings compared with alleles not transmitted (A allele frequency: 0.71 in transmitted vs. 0.61 in nontransmitted alleles, *P* = 0.003). These findings suggest that A allele may have a protective effect, perhaps particularly in the context of other diabetes-predisposing genes (in the present case, as a result of being genetically related to type 1 diabetic individuals). This protective effect appears to be strongest in homozygous form (AA genotype), since GA genotypes are still increased in diabetic compared with healthy siblings.

In future studies, it will be important to further elucidate the role of 2'5'AS in type 1 diabetes because this could assist in designing preventative therapies for children at high risk for developing this disease. In addition, the *OAS1* splice site polymorphism needs to be assessed for its possible role in susceptibility to infection with pathogenic viruses and to other autoimmune diseases with possible viral triggers.

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