

Brief Genetics Report

A Common Stromal Cell-Derived Factor-1 Chemokine Gene Variant is Associated With the Early Onset of Type 1 Diabetes

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Type 1 diabetes results from the autoimmune destruction of pancreatic β -cells. Although the disease shows a strong association with HLA class II alleles, other genes may influence the initiation or the rate of progression of the autoimmune process. The recruitment of mononuclear cells within the islets of Langerhans is a critical step in the pathogenesis of the disease. Because chemokines are cytokines that promote migration of mononuclear cells, we hypothesized that polymorphisms in chemokine receptor or chemokine genes, *CCR5* and *SDF1*, may be involved in susceptibility to or clinical expression of type 1 diabetes. The frequencies of the *CCR5-Δ32* and *SDF1-3'A* (801G→A in the 3' untranslated region) variants were similar in 208 unrelated Caucasian patients with type 1 diabetes and in 120 Caucasian control subjects. They were not modified after stratification for the predisposing HLA-DR3 and -DR4 haplotypes. However, the *SDF1-3'A* variant was strongly associated with early onset (<15 years) of the disease (odds ratio 2.6, $P = 0.0019$). On average, the presence of the *SDF1-3'A* allele was associated with a 5-year reduction in the age at onset of diabetes ($P = 0.0067$). Our results suggest that stromal cell-derived factor-1 may be implicated in the aggressiveness of the autoimmune process leading to type 1 diabetes. These preliminary data require replication in other populations. *Diabetes* 50:1211–1213, 2001

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MHC, major histocompatibility complex; OR, odds ratio; PCR, polymerase chain reaction; SDF, stromal cell-derived factor.

Type one diabetes is an autoimmune disease that is clinically heterogeneous with regard to the great variability of age at onset, its possible association with organ-specific autoimmune diseases, and its occurrence as a sporadic or a familial disease. The genes involved in susceptibility to type 1 diabetes remain largely unidentified. Apart from major histocompatibility complex (MHC) class II genes (*IDDM1*) and the insulin gene region (*IDDM2*), many other putative loci have been proposed but not confirmed by recent genome scan studies (1,2). Genetics may also influence the rate of progression or the aggressiveness of the disease. HLA DR3/DR4 heterozygosity is more frequent among patients with early rather than late-onset type 1 diabetes (3), and patients bearing the HLA class I A24 allele have more complete (4) or rapid (5) pancreatic β -cell destruction than patients who do not express this allele. The recruitment of activated mononuclear cells within pancreatic islets (i.e., insulinitis) is a critical step in the pathogenesis of type 1 diabetes (6). Chemokines are cytokines that promote the migration of leukocytes into inflammatory sites and differentiation or activation. In recent years, their role in the pathogenesis of several immune-mediated diseases has been recognized (7). Moreover, polymorphisms in genes encoding chemokines or their receptors, such as stromal cell-derived factor (SDF)-1, CCR5, and CCR2 have been shown to influence the course of HIV infection or inflammatory diseases (8–10). We thus hypothesized that such polymorphisms may participate in susceptibility to or clinical presentation of type 1 diabetes.

The frequencies of the *CCR5-Δ32* and *SDF1-3'A* (801G→A in the 3' untranslated region) variants were assessed in 208 unrelated Caucasian patients with type 1 diabetes and 120 Caucasian control subjects. Allelic and genotypic frequencies of the *CCR5-Δ32* and *SDF1-3'A* variants were similar in the two groups (Table 1). The observed frequencies in control subjects were the same as that previously reported in French subjects (11). The *CCR5-Δ32* and the *SDF1-3'A* alleles were present in 11.5 and 34.1% of the patients, respectively. These frequencies were not modified when the patients were stratified according to presence or absence of the predisposing HLA-DR3 and/or -DR4 haplotypes (data not shown). To test whether the two

TABLE 1
SDF1 and CCR5 genotypes and allelic frequencies in 208 patients with type 1 diabetes and 120 control subjects

	Control subjects	Patients
<i>n</i>	120	208
SDF1		
Genotype frequencies		
G/G	77 (64.2)	137 (65.8)
G/A	36 (30)	58 (27.9)
A/A	7 (5.8)	13 (6.3)
Allelic frequencies		
G	190 (79.2)	332 (79.8)
A	50 (20.8)	84 (20.2)
CCR5		
Genotype frequencies		
wt/wt	98 (81.7)	184 (88.5)
wt/Δ32	22 (18.3)	24 (11.5)
Δ32/Δ32	0	0
Allelic frequencies		
wt	218 (90.8)	392 (94.2)
Δ32	22 (9.2)	24 (5.8)

Data are *n* (%). wt, Wild type.

variants may modulate the phenotypic expression of the disease, patients were stratified for age at diabetes onset. The CCR5 allelic and genotypic frequencies did not differ according to age at onset of diabetes (not shown). In contrast, the distribution of SDF1 genotypes significantly differed between the two age groups (Table 2). The SDF1-3'A allele frequency was 25.8% in patients with early onset diabetes compared with 15.8% in patients with age at onset >15 years ($P = 0.013$, odds ratio [OR] 1.85, 95% CI 1.14–3.00). The association between early onset diabetes and the SDF1-3'A variant was significant in a dominant model (A/A + A/G versus G/G, where A and G indicate the variant and wild type alleles, respectively; $P = 0.0019$, OR 2.6, 1.44–4.68) but not in a recessive one. This finding was confirmed when the population was divided into terciles of age at disease onset as follows: the SDF1-3'A variant frequency was 47.6% in patients with age at onset <13 years, 31.4% for age at onset between 13 and 26 years, and 25.3% for age at onset >26 years (χ^2 7.9, $P = 0.019$; χ^2 for

TABLE 2
SDF1 genotypes in 208 patients with type 1 diabetes according to age at disease onset

	Onset <15 years	Onset ≥15 years	<i>P</i> *
<i>n</i>	91	117	
Genotype frequencies			
G/G	49 (53.8)	88 (75.2)	
G/A	37 (40.7)	21 (17.9)	0.0014†
A/A	5 (5.5)	8 (6.8)	
Allele frequencies‡			
G	135 (74.2)	197 (84.2)	0.0137
A	47 (25.8)	37 (15.8)	
Dominant model§			
A/A + A/G	42 (46.2)	29 (24.8)	0.0019†
G/G	49 (53.8)	88 (75.2)	

Data are *n* (%). *By contingency table analysis; † $P < 0.02$ after correction for multiple comparisons; ‡OR for the presence of the A allele = 1.85 (95% CI 1.14–3.00); §OR for the A phenotype = 2.6 (1.44–4.68).

TABLE 3
Age at diabetes onset according to HLA DR and SDF1 genotypes

	DR3/DR4	non-DR3/DR4
<i>n</i>	57	151
All patients	18.2 ± 13.2	25.7 ± 18.7*
G/G	18.5 ± 12.6	27.7 ± 18.3
A/A + A/G	17.8 ± 14.1	21.2 ± 19†

Data are means ± SD. * $P = 0.015$ for DR3/DR4 vs. non-DR3/DR4; † $P = 0.012$ for A/A + A/G vs. G/G.

trend 7.4, $P = 0.0066$). On average, the presence of the SDF1-3'A variant was associated with a 5-year reduction in the age at diabetes onset (20.0 ± 17.4 years in patients expressing the SDF1-3'A allele vs. 25.5 ± 17.5 years in patient homozygotes for the wild-type allele, $P = 0.0067$). Because HLA DR3/DR4 heterozygosity has been associated with a younger age at onset, we also analyzed the interaction between the SDF1 variant and the HLA-DR phenotype. In DR3/DR4 patients, age at onset was not significantly altered by SDF1 phenotype. In contrast, among non-DR3/DR4 patients, the age at onset was lower in those who expressed the SDF1-3'A variant (Table 3).

Other endocrine autoimmune diseases may associate with type 1 diabetes and are characterized by infiltration of the gland by mononuclear cells. Thus, we stratified the patients according to the absence or presence of an associated autoimmune endocrinopathy, mainly thyroid diseases. There was no difference in the distribution of the two allelic variants between these two groups (data not shown).

Our results demonstrate an association between the SDF1-3'A allelic variant and the early onset of type 1 diabetes. This was observed in patients who do not express the HLA class II DR3/DR4 combination that confers the highest risk for type 1 diabetes and is associated with the early onset of the disease (3). SDF-1 is a highly potent chemoattractant for monocytes and naive T-cells (12) that is constitutively expressed by many cell types, including endothelial and dendritic cells (13). Few data are available concerning the natural history of insulinitis in humans. However, studies in the NOD mouse, a model for type 1 diabetes, have demonstrated the critical role of dendritic cells and macrophages in the initiation of insulinitis and β-cell destruction. Studies have also shown that the severity of pancreatic islet infiltration correlates with the rate of progression to diabetes (14). It has been recently demonstrated that the expression of CC-chemokines and the CCR5 chemokine receptor in the pancreas is associated with the development of insulinitis and the progression to diabetes in NOD mice (15). Whether SDF-1 is involved in the insulinitis process is not known at the present time, but in addition to its role in T-lymphocyte migration, SDF-1 is also a costimulator for CD4⁺ T-cells (16). In this context, one could hypothesize that the SDF1-3'A variant modulates the degree of islet infiltration by mononuclear cells and the age at onset of diabetes. However, the functional consequences of this variant at position 801 of the 3' untranslated region of SDF1 are not known. Although the 801G→A variant is quite close to a highly conserved sequence that may regulate expression of SDF1 at the protein level (9), no biological effect of the variant was observed in one in vitro transfection experiment (17).

SDF1 has not yet been implicated in the genetic susceptibility to type 1 diabetes. However, it could be a candidate gene because it is located in a region (10q11) (18) where two genome scan studies have shown linkage with type 1 diabetes susceptibility (1,19). This region is referred to as *IDDM10* (14). Alternatively, mutations in *SDF1* or other genes located in the same region may be involved in the observed association through linkage disequilibrium with *SDF1-3'A*.

Our results should be considered preliminary and need to be replicated in other populations. If confirmed, they may be of importance in prediction studies for at-risk individuals because islet autoantibodies and HLA class II typing fail to distinguish rapid from delayed progression to clinical diabetes (20). These results also show that clinical heterogeneity may be associated with genetic heterogeneity and that, even in the context of a well-defined disease such as autoimmune diabetes, refining phenotypic analysis is an important step to identify susceptibility genes.

RESEARCH DESIGN AND METHODS

A total of 208 unrelated Caucasian patients (mean age at diabetes onset 23.6 ± 17.6 years, range 1–88), all with serological markers of anti-islet cell autoimmunity (i.e., anti-islet cell antibodies and/or GAD autoantibodies and/or IA-2 autoantibodies), and 120 Caucasian control subjects were studied after giving consent.

The *CCR5-Δ32* deletion was identified by electrophoresis on 2% agarose gel after polymerase chain reaction (PCR) amplification (8). The *SDF1-3'A* variant (801G→A in the 3' untranslated region) was detected by electrophoresis on 2.5% agarose gel after PCR amplification and *MspI* digestion, as previously described (9).

Contingency table analysis was used to compare genotype and allele frequencies. Fisher's exact test was used in calculations involving small samples. *P* values were corrected for multiple comparisons by the Bonferroni method. ORs were computed when data were significantly different. The Mann-Whitney test was used to compare age at onset between groups.

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