No evidence for SEL1L as a candidate gene for IDDM11-conferred susceptibility

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Abstract

Background The SEL1L gene is located on human chromosome 14q24.3-31 close to D14S67 which has been previously proposed to be a type 1 diabetes mellitus locus (IDDM11). Sel-1 is a negative regulator of the Notch signalling pathway and SEL1L is selectively expressed in adult pancreas and islets of Langerhans. This suggests that SEL1L may be a candidate gene for IDDM11.

Methods We have analysed two newly identified CA-repeat polymorphisms within the genomic sequence of the SEL1L locus for association with type 1 diabetes mellitus (T1DM) in 152 Danish T1DM-affected sib-pair families and in 240 Sardinian families (229 simplex and 11 sib-pair families).

Results No evidence for association of the two SEL1L markers with T1DM was observed in either the Danish or the Sardinian families. We have also used allelic sharing methods to analyse linkage with T1DM in the IDDM11 region using the same markers and the Danish collection of affected sib-pair families. No evidence of linkage was observed (Zmax = 0.86).

Conclusion Although several lines of evidence suggest that SEL1L might be a candidate for IDDM11-conferred susceptibility to T1DM the present study does not support this hypothesis. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords type 1 diabetes; IDDM11; candidate gene; genetics; pancreas development

Introduction

Type 1 diabetes mellitus (T1DM) results from an immune-mediated destruction of the insulin-producing β cells of the pancreas. Predisposition to the disease is polygenic. It is now clear that while HLA region genes contribute the major predisposition to the disease, there are numerous other genes with smaller effects on susceptibility. With the exception of IDDM2, these ‘minor’ predisposing genes have only been localized, not yet identified.

In the present study, we have evaluated SEL1L as a candidate gene for IDDM11-conferred susceptibility. SEL1L, which was recently cloned [1,2], is the human homologue of the Caenorhabditis elegans gene sel-1. Sel-1 is an important negative regulator of the Notch signalling pathway [3] that acts as a key regulator of the cellular differentiation processes. Notch signalling was recently shown to be important for proper development of pancreatic endocrine cells [4–6]. SEL1L is ubiquitously expressed in human fetal tissues, but it exhibits high mRNA levels only in adult pancreas [7] and in islets of Langerhans [8]. Furthermore, a series of recent studies revealed that apart from the well-documented involvement of Notch in differentiation [9], both
proliferation [10] and apoptotic [11] events can be affected by Notch signalling. Preliminary studies on pancreatic sections indicate accumulation of SEL1L protein in the acini and in a subpopulation of cells in the islet of Langerhans (I. Biunno, unpublished data). SEL1L is located on human chromosome 14q24.3-31 about 4.7 Mb proximal to D14S67 [2], a marker for the putative type 1 diabetes mellitus susceptibility locus, IDDM11 (OMIM 601208) [12]. The SEL1L gene comprises 21 exons and spans 70 kb of genomic DNA [2]. Two new and significantly polymorphic (CA)n repeats positioned in intron 2 (GenBank Accession No. G44759) and intron 20 (GenBank Accession No. G44758) of the SEL1L gene, respectively, were recently reported [2].

We have analysed 152 Danish Caucasoid T1DM multiplex families and 240 Sardinian T1DM simplex families for these two microsatellite markers as possible candidate markers for IDDM11 in addition to D14S67, the original marker of IDDM11 [12].

Subjects and methods

Subjects

The 152 Danish families comprised 311 affected and 137 unaffected offspring. In 108 families both parents and in 44 families one parent only were available for genotyping. All diabetic offspring were diagnosed according to WHO criteria before the age of 30 years (mean age at onset ± SD: 13.6 ± 9.3 years) [13,14]. HLA conditioning was performed as follows: HLA DR3/4 heterozygous patients were assigned highest risk, whereas non-HLA DR3/4 were defined as lower risk. The 240 Sardinian families comprised 253 affected and 194 unaffected offspring. The appropriate ethics committees approved the study.

Genotyping

Genotyping for D14S67, G44759 and G44758 was performed using the following primers: F: 5'-ttcacatatgcctctacaattctatg, R: 5'-TAGTCAGGGTTTGCCAGAGA, F: 5'-TGGGCTTGGTTAGTACTTGG, R: 5'-AAAATTACTGACCTACAAGAGGG, F: 5'-CGTATTGGATTACTGGTGAAAG, and R: 5'-GGCAAGGAACTGGGAAAGTTAC, respectively. All forward primers were 5'-fluorescence labelled. The PCR reactions were performed under standard conditions in 20 μl final volume using 0.5–1.0 μM of each primer and 0.5 U Taq polymerase (Gibco BRL, Paisley, UK) in a thermal cycler 9700 (Applied Biosystems, Foster City, CA, USA). After 95°C for 5 min and 33 cycles at 95°C for 30 s, 62°C for 1 min and 72°C for 1 min followed by 72°C for 10 min, PCR products were analysed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) using standard software.

Statistical analysis

Transmission of microsatellite marker alleles was assessed from heterozygous parents to both affected and unaffected offspring using the transmission disequilibrium test (TDT) [15,16]. The TDT statistics (Tsp) described by Martins et al. [17] was used, which combines sib-pair and simplex families in a single test. In particular, the Tsp version of the TDT takes into account the presence of linkage in a proportion of the sib-pairs and allows the data from the second sib to be included, thereby giving a completely valid test of association. Linkage was analysed using the Genehunter software package version 1.2 [18].
Results

No evidence of association to T1DM of the SEL1L G44758 and G44759 microsatellites was found by use of the TDT in either the Danish families or the Sardinian families (Table 1).

In both the Danish and Sardinian data sets the same four alleles of each marker were the most common (Table 1). Alleles 2, 5, 9 and 8 of G44759 comprised 90.0% of all transmissions in the Danish families and 95.0% in the Sardinian families. Alleles 2, 7, 6 and 8 of G44758 accounted for 77.3% of all transmissions in the Danish and 95.4% in the Sardinian families. Since no significant differences were seen between the Danish and Sardinian data between affected and non-affected individuals the two data sets were combined for analysis of association. No significant differences in transmission patterns were observed in the combined data set. Conditioning for HLA risk did not reveal association in either the Danish or the Sardinian data set or in the data combined (data not shown). Also, no significant differences were observed for comparing transmission patterns of the two markers to affected versus unaffected offspring in either the Danish or the Sardinian data sets \( p=0.2 \) (DK G44759), \( p=0.9 \) (SAR G44759), \( p=0.2 \) (DK G44758) and \( p=0.5 \) (SAR G44758). In an attempt to replicate the original observation by Field et al. [12], D14S67 was tested in the Danish affected sib-pairs. However, no evidence for linkage of T1DM to D14S67 (single point NPL = 1.22; \( p=0.11 \)) was found. Also, TDT provided negative results at D14S67 (\( p=0.25 \)) (data not shown). Multipoint analysis including G44759, G44758 and D14S67 resulted in a \( Z_{\text{max}} \) of 0.86 (\( p=0.2 \)). Thus, also testing the IDDM11 region [12] with allelic sharing methods revealed negative results and a gene effect equivalent to \( Z_s = 1.5 \) could be excluded at Lod –2.

Discussion

We have identified SEL1L as a positional and possible functional candidate gene for IDDM11-conferred susceptibility to T1DM [2]. In the present study no evidence was found for association of the two tested SEL1L microsatellite markers to T1DM in either the Danish or the Sardinian the data set or in the combined data set. In the Danish data set no evidence for linkage was observed for D14S67 in the affected sib-pair analysis nor for association using the TDT. HLA conditioning did not reveal association in either the Danish or the Sardinian families. Interest-

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