DIABETES/METABOLISM RESEARCH AND REVIEWS
Diabetes Metab Res Rev 2001; **17**: 292–295.
DOI: 10.1002/dmrr.201

RESEARCH ARTICLE

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

Flemming Pociot[1]*
Zenia M. Larsen[1]
Patrizia Zavattari[2]
Elisabetta Deidda[2]
Jørn Nerup[1]
Monica Cattaneo[3]
Raffaella Chiaramonte[3]
Paola Comi[3]
Marta Sabbadini[3]
Massimo Zollo[4,5]
Ida Biunno[3]
Francesco Cucca[2]

[1]*Steno Diabetes Center, Niels Steensensvej 2, DK-2820 Gentofte, Denmark*

[2]*Dipartimento di Scienze Biomediche e Biotecnologie, University of Cagliari, 09121 Cagliari, Italy*

[3]*Dipartimento di Scienze Biomediche e Biotecnologie, University of Milan, 20090 Segrate, Italy*

[4]*TIGEM, HSR Biomedical Science Park, 20132 Milan, Italy*

[5]*TIGEM, 80131 Napoli, Italy*

*Correspondence to: Flemming Pociot, Steno Diabetes Center, Niels Steensensvej 2, DK-2820 Gentofte, Denmark.
E-mail: fpoc@novo.dk

Received: 6 February 2001
Revised: 26 March 2001
Accepted: 6 April 2001
Published online: 16 May 2001

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 ($Z_{max} = 0.86$).

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

Table 1. Transmission of the four most common alleles of G44759 (intron 2) and G44758 (intron 20) of the SEL1L locus to affected and unaffected offspring in Danish and Sardinian type 1 diabetes families

	Affected offspring					Unaffected offspring					
	Allele	T	NT	%T	p^*	Allele	T	NT	%T	χ^2	p^*
DK											
G44759	2	79	81	49	0.911	2	29	25	54	0.296	0.586
	5	61	60	50	0.931	5	23	25	48	0.083	0.773
	9	40	42	49	0.869	9	19	21	48	0.100	0.752
	8	14	16	47	0.756	8	4	3	57	0.143	0.705
SAR											
G44759	2	78	56	58	0.057	2	61	60	50	0.008	0.928
	5	52	68	43	0.144	5	52	57	48	0.229	0.632
	9	26	32	45	0.431	9	29	27	52	0.071	0.789
	8	14	15	48	0.853	8	15	13	54	0.143	0.705
DK											
G44758	2	58	50	54	0.492	2	22	21	51	0.023	0.880
	7	53	50	52	0.801	7	19	17	53	0.111	0.739
	6	44	47	48	0.779	6	22	14	61	1.778	0.182
	8	9	20	31	0.044	8	1	9	10	6.400	0.011
SAR											
G44758	2	63	67	49	0.726	2	55	52	51	0.084	0.772
	7	53	54	50	0.923	7	53	42	56	1.274	0.259
	6	55	48	53	0.490	6	46	48	49	0.043	0.837
	8	17	20	46	0.622	8	13	21	38	1.882	0.170

DK, Danish data set; SAR, Sardinian data set; T, transmitted (*n*); NT, not transmitted; p^*, uncorrected *p* values.

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 $\pm$SD: 13.6$\pm$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′-ttcac tacgcctctacaattctatg, R: 5′-TAGTCAGGGTTTGCCAGAGA, F: 5′-TGGGCTTGGTTAGTACTTGG, R: 5′-AAAATTACT GACCTACAAGAGGG, F: 5′-CGTATTGGATTACTGGTG GAAAG, and R: 5′-GGCAAGGAACTGGGAAAGTTAC, respectively. All forward primers were 5′-fluorescense 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 a 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 (T_{sp}) described by Martins *et al.* [17] was used, which combines sib-pair and simplex families in a single test. In particular, the T_{sp} 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_{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 $\lambda_s = 1.5$ 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 evidence for association of any of the *SEL1L* markers. Given the well-known results on the insulin VNTR [19] in T1DM and of calpain-10 in type 2 diabetes [20], a true association could be present even when linkage analysis failed to show evidence of linkage, and we cannot rule out that specific variation(s) in the *SEL1L* gene sequence can be associated with T1DM. Interestingly, several correlations between mutations in Notch-like genes and human diseases have been reported. These include cancer, stroke, late-onset neurological disease (CADASIL) and Alagille syndrome [21,22], highlighting the broad spectrum of Notch activity in humans. Detailed mutation scanning of the SEL1L sequence (Z. Larsen *et al*., unpublished observations) revealed a number of inconsistencies with earlier reported sequences [1,2], and could not confirm the presence of previously reported polymorphisms of the cDNA sequence. However, alteration(s) in genes such as *SEL1L* could cause disruption of the normal pancreatic organogenesis developmental program(s) that, in turn, may cause defects in the genes that regulate pancreatic islet development and insulin biosynthesis. In addition, studies have indicated that Notch plays a fundamental role in the lineage decision of T-cell development [23]. It may be that Notch confers selective survival and/or cell proliferation on cells that have already made a lineage decision. Hence, data exist to imply an interaction (direct or indirect) between SEL1L and Notch proteins that presently are unknown. In *C. elegans*, mutations in *sel-1* leads to a reduction in lin-12 activity [24]; in rodent and man this association has not as yet been established.

In 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. However, it cannot be excluded that specific mutations in the gene could be of etiological relevance for T1DM development.

Acknowledgements

The expert technical skills of Bodil Bosmann Jørgensen are greatly appreciated. We are grateful to Drs Jesper Johannesen and Ole Peter Kristiansen for collecting most of the Danish families on behalf of the Danish Study Group for Diabetes in Children (DSGD) and the Danish IDDM Epidemiology and Genetics Group (DIEGG). We also wish to thank Drs Efisio Angius, Mario Silvetti, Margi Chessa, Paola Frongia and Rossella Ricciardi for help in collecting the Sardinian T1DM families. The study was financially supported by the Danish Medical Research Council (#9802011), the 'Regione Autonoma Sardegna' (L.R.11, 30-4-90), the Telethon Grant (TIGEM 96-99), and by MURST (Grant No. 5911).

References

1. Harada Y, Ozaki K, Suzuki M, *et al*. Complete cDNA sequence and genomic organization of a human pancreas-specific gene homologous to *Caenorhabditis elegans sel-1*. *J Hum Genet* 1999; **44**: 330–336.
2. Biunno I, Bernard L, Dear P, *et al*. SEL1L, the human homolog of *C-elegans sel-1*: refined physical mapping, gene structure and identification of polymorphic markers. *Hum Genet* 2000; **106**: 227–235.
3. Weinmaster G. Notch signal transduction: a real Rip and more. *Curr Opin Genet Dev* 2000; **10**: 363–369.
4. Apelqvist A, Li H, Sommer L, *et al*. Notch signalling controls pancreatic cell differentiation. *Nature* 1999; **400**: 877–881.
5. Jensen J, Pedersen EE, Galante P, *et al*. Control sf endodermal endocrine development by Hes-1. *Nat Genet* 2000; **24**: 36–44.
6. Jensen J, Heller RS, Funder-Nielsen T, *et al*. Independent development of pancreatic alpha- and beta-cells from

Neurogenin3-expressing precursors – a role for the notch pathway in repression of premature differentiation. *Diabetes* 2000; **49**: 163–176.
7. Biunno I, Appierto V, Cattaneo M, *et al*. Isolation of a pancreas-specific gene located on human chromosome 14q31 – expression analysis in human pancreatic ductal carcinomas. *Genomics* 1997; **46**: 284–286.
8. Donoviel DB, Donoviel MS, Fan E, Hadjantonakis AK, Bernstein A. Cloning and characterization of Sel-11, a murine homolog of the *C-elegans sel-1* gene. *Mech Dev* 1998; **78**: 203–207.
9. Lewis J. Notch signalling and the control of cell fate choices in vertebrates. *Semin Cell Dev Biol* 1998; **9**: 583–589.
10. Miele L, Osborne B. Arbiter of differentiation and death: notch signaling meets apoptosis. *J Cell Physiol* 1999; **181**: 393–409.
11. Shelly LL, Fuchs C, Miele L. Notch-1 inhibits apoptosis in murine erythroleukemia cells and is necessary for differentiation induced by hybrid polar compounds. *J Cell Biochem* 1999; **73**: 164–175.
12. Field LL, Tobias R, Thomson G, Plon S. Susceptibility to insulin-dependent diabetes mellitus maps to a locus (*IDDM11*) on human chromosome 14q24.3-q31. *Genomics* 1996; **33**: 1–8.
13. Pociot F, Nørgaard K, Hobolth N, *et al*. A nationwide population-based study of the familial aggregation of insulin-dependent diabetes in Denmark. *Diabetologia* 1993; **36**: 870–875.
14. Lorenzen T, Pociot F, Stilgren L, *et al*. Predictors of IDDM recurrence risk in offspring of Danish IDDM patients. *Diabetologia* 1998; **41**: 666–673.
15. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993; **52**: 506–516.
16. Spielman RS, Ewens WJ. A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. *Am J Hum Genet* 1998; **62**: 450–458.
17. Martin ER, Kaplan NL, Weir BS. Test for linkage and association in nuclear families. *Am J Hum Genet* 1997; **61**: 439–448.
18. Kruglyak L, Daly MJ, Reevedaly MP, Lander ES. Parametric and nonparametric linkage analysis – a unified multipoint approach. *Am J Hum Genet* 1996; **58**: 1347–1363.
19. Bennett ST, Wilson AJ, Esposito L, *et al*. Insulin VNTR allele-specific effect in type 1 diabetes depends on identity of untransmitted paternal allele. *Nat Genet* 1997; **17**: 350–352.
20. Horikawa Y, Oda N, Cox NJ, *et al*. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 2000; **26**: 163–175.
21. Gridley T. Notch signaling in vertebrate development and disease. *Mol Cell Neurosci* 1997; **9**: 103–108.
22. Weinmaster G. The ins and outs of notch signaling. *Mol Cell Neurosci* 1997; **9**: 91–102.
23. Hayday AC, Barber DF, Douglas N, Hoffman ES. Signals involved in gamma/delta T cell versus alpha/beta T cell lineage commitment. *Semin Immunol* 1999; **11**: 239–249.
24. Grant B, Greenwald I. The *Caenorhabditis elegans sel-1* gene, a negative regulator of lin-12 and glp-1, encodes a predicted extracellular protein. *Genetics* 1996; **143**: 237–247.