Molecular and Cellular Biochemistry **232:** 159–161, 2002.

Short communication

Allele frequency of two intragenic microsatellite loci of *SEL1L* gene in Northern Italian population

Raffaella Chiaramonte,[1] Marta Sabbadini,[1] Francesca Balordi,[1] Paola Comi[1] and Gajanan V. Sherbet[2]

[1]*Department of Biomedical Sciences and Technologies, University of Milano, Segrate, Italy;* [2]*Institute for Molecular Medicine, Huntington Beach, CA, USA and Communications and Signal Processing Research Group, Department of Electrical and Electronic Engineering, University of Newcastle upon Tyne, Newcastle upon Tyne, UK*

Received 31 August 2001; accepted 20 November 2001

Abstract

Two cytosine-adenine (CA) repeats CAR/CAL and RepIN20 occur in the human *SEL1L* gene, which is regarded as a candidate gene for insulin-dependent diabetes mellitus (IDDM) and Grave's disease. We have characterized these repeats to determine if they might serve as effective microsatellite markers for linkage analysis to clarify whether *SEL1L* gene plays a role in the pathogenesis of these autoimmune diseases. The allele frequencies and average heterozygosity of the microsatellite repeats were analysed in 94 DNA samples from peripheral blood mononuclear (PBMC) cells from adults of Northern Italy. The average heterozygosity was 0.68 for CAR/CAL polymorphism and 0.85 for RepIN20. The size of PCR fragments of CAR/CAL ranged from 207–225 bp and the most frequent allele was 207 bp (40.4%). The size of the fragments of RepIN20 ranged from 237–255 bp and the most frequent allele was 249 bp (30.8%). In the light of the highly polymorphic nature of both microsatellites and their intragenic location in *SEL1L* gene, we suggest that they could provide a means for linkage analysis to clarify the potential role of *SEL1L* in conferring susceptibility to IDDM or Grave's disease. (Mol Cell Biochem **232**: 159–161, 2002)

Key words: *SEL1L*, microsatellite, CA repeat, IDDM, Grave's disease

Introduction

Microsatellites are repeat sequences that occur within or between gene sequences. Cellular genes that contain microsatellites sequences become susceptible to replication error, which could lead to allelic alterations, loss of heterozygosity (LOH), loss or gain of function mutations, and abnormal gene expression. Microsatellite markers are also valuable tools in mapping genetic linkage and recombination. Allelic diversity and heterozygosity are important features for the establishment of microsatellite markers for linkage studies.

The Notch signalling pathway plays a major role in the differentiation of many cell types as well as in the pathogenesis of haematological malignancies such as leukaemias and lymphomas, and in certain congenital autosomal dominant conditions. The deregulation of notch signalling leads to inhibition of differentiation, maintenance of the undifferentiated or precursor state and enhancement of cell proliferation [1, 2].

The human *SEL1L* gene is a homologue of *C. elegans sel-1*, which is a negative regulator of notch signalling pathway in *C. elegans* [3]. *SEL1L* is located on human chromosome 14q24.3–q31 [4], proximal to the locus that is believed to confer susceptibility to IDDM11. Also, *SEL1L* is highly expressed only in adult pancreatic cells, both acinar and β-cells, and it is virtually undetectable in the other tissues [5–7]. Furthermore, *sel-1* in *C. elegans* [8] and *Hdr*3 in *S. cerevisiae* [9] have putatively been assigned a role in protein processing and degradation. These findings have raised the intriguing possibility that defects in *SEL1L* in IDDM patients could lead to inappropriate processing of or failure to degrade an islet

Address for offprints: R. Chiaramonte, L.I.T.A., via Fratelli Cervi 93, 20090 Segrate, Italy (E-mail: Raffaella.Chiaramonte@unimi.it)

antigen. *SEL1L* has been implicated also in Grave's disease. Tomer *et al.* [10] have reported evidence that the susceptibility gene for Grave's disease is located in proximity of IDDM11 locus. Although SEL1L expression seems to be specific for the pancreas, a recent report has excluded the involvement of *SEL1L* in autoimmune thyroid disease [11]. This leads to the hypothesis that this region could represent a clustering of different genes related to different autoimmune phenomena, albeit without being responsible for associated pathogenesis.

Here we report the allele set frequency of two intragenic cytosine-adenosine microsatellite repeats, CAR/CAL and RepIN20, which we recently identified in the course of a structural analysis of *SEL1L* gene (GenBank accession number NT_026437). These are located in intron 2 and 20 respectively [12].

Materials and methods

Genomic DNA was extracted from PBMCs from 94 healthy people using a standard method [13]. Polymerase chain reaction was performed in a 5 μl reaction mixture containing 30 ng of DNA, 10 × PCR Buffer II, 1.25 mM $MgCl_2$, 200 μM dNTPs, 0.125 units of AmpliTaq Gold DNA Polymerase (Perkin–Elmer Corporation). The primers used for PCR are as follows: CAR/CAL set of primers: Upper 5′-AAAATTACTGACCTACAAGAGGG-3′; lower 5′-TGGGCTTGGTTAGTACTTGG-3′. The RepIN20 set of primers: Upper 5′-CGTATTGGATTACTGGTGGAAAG-3′; lower 5′-GGCAAGGAACTGGGAAAGTTAC-3′.

For amplification with the CAR/CAL set of primers, 0.5 μM of unlabelled upper primer, 0.4 μM of unlabelled and 0.1 μM ^{32}P-labelled lower primer were added. For the RepIN20 set of primers, the upper/lower primers ratio was maintained, and the upper primer was labelled.

Amplifications were performed, after an initial denaturation at 95°C for 12 min, for 30 cycles according to the following regime: denaturation at 95°C for 30 sec, annealing at 60°C (CAR/CAL) and 65°C (RepIN20) for 30 sec and extension at 72°C for 30 sec. PCR products were electrophoresed in denaturing sequencing gel electrophoresis apparatus for 2–3 h and autoradiographed on Kodak Biomax MS-1 Film.

The PCR products were directly sequenced using the T7 Sequenase PCR product sequencing KIT (USB) with the same primers used for the PCR reactions.

Results and discussion

The average heterozygosity and the levels of allele set frequency of CAR/CAL and RepIN20 microsatellite loci were determined in healthy subjects from Northern Italy (Table 1).

Table 1. Allele frequency of CAR/CAL and RepIN20 polymorphisms in 94 normal individuals from Northern Italy

CAR/CAL	Allele frequency	RepIN20 alleles	Allele frequency
207-bp	40.43	237-bp	3.19
209-bp	1.08	239-bp	2.13
211-bp	0	241-bp	26.6
213-bp	29.79	243-bp	1.06
215-bp	2.13	245-bp	1.06
217-bp	1.06	247-bp	8.51
219-bp	2.13	249-bp	30.85
221-bp	22.34	251-bp	18.09
223-bp	0	253-bp	5.32
225-bp	1.06	255-bp	3.19

The average heterozygosity for CAR/CAL polymorphism was 0.68 (64/94) and for RepIN20 was 0.85 (80/94). Fragment size was determined by direct sequencing. In healthy individuals the size of CAR/CAL PCR fragments ranged from 207–225 bp and the most frequent allele was the 207-bp fragment (40.4%), followed by 213-bp (29.7%) and 221-bp (22.3%) fragments. The size of RepIN20 PCR fragments ranged from 237–255 bp and the most frequent allele was the 249-bp fragment (30.8%) followed by 241-bp (26.6%), 251-bp (18.0%) and 247-bp (8.5%) fragments.

Dinucleotide microsatellite repeats tend to be more polymorphic than tri- or tetranucleotide repeats. In the light of the highly polymorphic nature of both CAR/CAL and RepIN20 microsatellite repeats and their the intragenic location, we suggest that they could provide an effective means for linkage analysis to clarify the potential role of *SEL1L* in conferring susceptibility to IDDM or Grave's disease. However, there is a report that *SEL1L* might not be related to IDDM [14]. Nonetheless, our findings emphasise the importance of investigating the integrity of the notch signalling pathways in these disease conditions.

Acknowledgment

This work was partially supported by grants 'Cofin 1999' and MURST to PC.

References

1. Joutel A, Tournier-Vasserve E: Notch signalling pathway and human diseases. Semin Cell Dev Biol 9: 619–625, 1998
2. Swiatek PJ, Lindsell CE, del Amo FF, Weinmaster G, Gridley T: Notch1 is essential for post-implantation development in mice. Genes Dev 8: 707–719, 1994
3. Sundaram M, Greenwald I: Suppressor of a *lin*-12 hypomorph defines genes that interact with both *lin*-12 and *glp*-1 in *Caenorhabditis elegans*. Genetics 135: 765–783, 1993

4. 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 33: 1–8, 1996
5. Biunno I, Appierto V, Cattaneo M, Leone BE, Balzano G, Socci C, Saccone S, Letizia A, Della Valle G, Sgaramella V: Isolation of a pancreas-specific gene located on human chromosome 14q31: Expression analysis in human pancreatic ductal carcinomas. Genomics 46: 284–286, 1997
6. Donoviel LL, Donoviel MS, Fan E, Hadjantonakis AK, Bernastain A: Cloning and characterization of *Sel-1l*, a murine homologue of the *C. elegans sel-1* gene. Mech Dev 78: 207–211, 1998
7. Harada Y, Ozaki K, Suzuki M, Fujiwara T, Takahashi E, Nakamura Y, Tanigami A: Complete cDNA sequence and genomic organization of a human pancreas-specific gene homologous to *Caenorhabditis elegans* sel1. J Hum Genet 44: 330–336, 1999
8. Grant B, Greenwald I: Structure, function, and expression of *SEL-1*, a negative regulator of *LIN-12* and *GLP-1* in *C. elegans*. Development 124: 637–644, 1997
9. Hampton RY, Gardner RG, Rine J: Role of 26S proteasome and *HRD* genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein. Mol Biol Cell 7: 2029–2044, 1996
10. Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davies TF: Mapping the major susceptibility loci for familial Graves' and Hashimoto's diseases: Evidence for genetic heterogeneity and gene interactions. J Clin Endocrinol Metab 84: 4656–4664, 1999
11. Ban Y, Taniyama M, Tozaki T, Yanagawa T, Tomita M, Ban Y: SEL1L microsatellite polymorphism in Japanese patients with autoimmune thyroid disease. Thyroid 11: 335–338, 2001
12. Biunno I, Bernard L, Dear P, Cattaneo M, Volorio S, Zannini L, Bankier A, Zollo M: *SEL1L*, the human homolog of C.elegans *sel-1*: Refined physical mapping, gene structure and identification of polymorphic markers. Hum Genet 106: 227–235, 2000
13. Blin N, Stafford DW: A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res 3: 2303–2308, 1976
14. Pociot F, Larsen ZM, Zavattari P, Deidda E, Nerup J, Cattaneo M, Chiaramonte R, Comi P, Sabbadini M, Zollo M, Biunno I, Cucca F: No evidence of SEL1L as a candidate gene for IDDM11-conferred susceptibility. Diabetes Metab Res Rev 17: 292–295, 2001