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Human insulin receptor substrate-1 gene *(IRS1)*: chromosomal localization to 2q35-q36.1 and identification of a simple tandem repeat DNA polymorphism

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Summary. The protein designated as insulin receptor substrate-1 (IRS-1) is a major substrate for the insulin receptor tyrosine kinase. Since post-receptor defects in the insulin signalling pathway are a common feature of Type 2 (non-insulin-dependent) diabetes mellitus, we have cloned the human IRS-1 gene in order to study the role of genetic variation in this gene in the pathogenesis of diabetes mellitus. As a first step in these studies, we have mapped the IRS-1 gene to chromosome 2, bands q35- q36.1 and identified a simple tandem repeat DNA polymorphism in this gene that will be useful for genetic studies.

Key words: DNA polymorphism, genetics, chromosome 2.

Insulin resistance and beta-cell malfunction account for the complex clinical presentation of Type 2 (non-insulin-dependent) diabetes mellitus [1]. Candidate sites for genetic defects of insulin resistance include the insulin receptor, its signal transduction pathway, and insulin-regulated genes involved in peripheral utilization of glucose or hepatic glucose production. Mutations in the insulin receptor have been identified in patients with syndromes of severe insulin resistance [2]; however, they are rare in patients with Type 2 diabetes. Similarly, rare variants of the GLUT4/muscle- adipocyte glucose transporter have been identified but they are not known to impair the function of this protein [3, 4].

Recently, several groups reported the isolation of cDNAs encoding a 160–185 kDa phosphotyrosyl protein that is a substrate of the insulin receptor tyrosine kinase and a putative participant in insulin signalling [5] (S. R. Keller, R. Aebersold, C. W. Garner and G. E. Lienhard, unpublished observations). This protein, designated as insulin receptor substrate 1 or IRS-1, is found in a variety of insulin responsive cells and tissues. It exhibits no intrinsic enzymatic activity but is believed to serve as a docking protein involved in binding and activating other signal transduction molecules after being phosphorylated on tyrosine by the insulin receptor kinase. Because of its central role in the signal transduction pathway, IRS-1 is a candidate for the site of the defect in insulin action seen in patients with Type 2 diabetes.

As a first step in studying the role of genetic variation in IRS-1 in the aetiology of Type 2 diabetes, we have cloned the human IRS-1 gene *(IRS1)*, determined its chromosomal localization and identified a highly polymorphic simple tandem repeat DNA polymorphism in this gene that will be useful for genetic studies.

Materials and methods

General methods

Standard methods were carried out as described in Sambrook et al. [6]. DNA sequencing was done by the dideoxynucleotide chain termination procedure after subcloning appropriate DNA fragments into M13 mp18 or M13 mp19. The sequence was confirmed on both strands.

Isolation of the human IRS-1 gene

A human male placenta genomic library in λFIX II (Stratagene, LaJolla, Calif., USA; catalogue no. 946203) was screened by hybridization with a ^{32}P-labelled fragment of the mouse pp160, IRS-1, cDNA corresponding to nucleotides 594–1562 of the mouse cDNA sequence (S. R. Keller, R. Aebersold, C. W. Garner and G. E. Lienhard, unpublished observations). Five clones were obtained and designated λhIRS1–1 to –5.

Chromosomal mapping using a human-hamster somatic cell hybrid panel

The chromosomal localization of *IRS1* was determined using PCRable DNA from a somatic cell hybrid mapping panel (Bios, New Haven, Conn., USA) and the polymerase chain reaction (PCR)

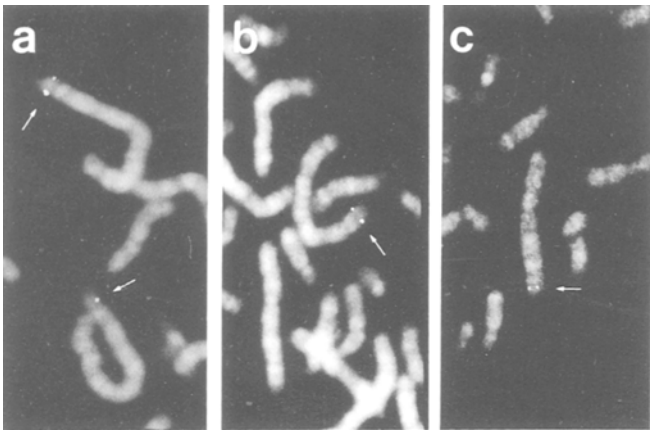

Fig. 1a–c. In situ hybridization of digoxigenin-labelled λhIRS1–4 DNA to human metaphase chromosomes from phytohaemagglutinin-stimulated peripheral blood lymphocytes. **a–c**, Partial metaphase spreads; the chromosome 2 homologues are identified with arrows. Specific labelling was observed at 2q35–q36.1. Images were obtained using a Zeiss Axioplan microscope coupled to a thermoelectrically cooled charge coupled device camera. Separate images of DAPI-stained chromosomes and the hybridization were acquired and merged using image analysis software (IPLab Spectrum and GeneJoin)

with oligonucleotide primers IRS1–2, 5′-GTTCATTAATATTG-TTCAACTGTGG-3′, and IRS1–3, 5′-AATTAATTTGAAACCC-GTTTGATGG-3′.

Fluorescence in situ chromosomal hybridization

Human metaphase cells were prepared from phytohaemagglutinin-stimulated peripheral blood lymphocytes. Fluorescence in situ hybridization was performed as described previously [7] using the clone λhIRS1–4 which has an insert of 12.5 kilobase pairs (kb). Probes were prepared by nick-translation using Biotin-11-dUTP (Enzo Diagnostics, New York, NY, USA) or digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, Ind., USA). Hybridization with biotin- labelled probes was detected with fluorescein-conjugated avidin (Vector Laboratories, Burlingame, Calif., USA) and digoxigenin- labelled probes were detected by incubation with rhodamine-conjugated sheep anti-digoxigenin antibodies (Boehringer Mannheim). Chromosomes were identified by staining with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI).

Identification of simple tandem repeat DNA polymorphism

One microgramme of DNA from the five *IRS1* genomic clones was digested with *Sau*3 AI and after electrophoresis blotted onto a nitrocellulose filter and hybridized with ^{32}P-labelled poly (dA- dC)-poly (dG-dT) (Pharmacia LKB Biotechnology, Piscataway, NJ, USA) as described previously [8]. A 1 kb fragment of λhIRS1–4 showed a strong hybridization signal. This fragment was isolated, subcloned into the *Bam*HI site of M13 mp18 and sequenced.

Amplification of the simple tandem repeat DNA polymorphism

Two primers flanking the GT repeat-rich region identified in λhIRS1–4, IRS1–2 and IRS1–3, were selected by inspection and used to amplify the ≈ 138 base pair region containing this repetitive sequence. The PCR was performed using ^{32}P-labelled IRS1–2 and unlabelled IRS1–3. DNA was denatured at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, extension at 72°C for 90 s and a final extension step of 10 min. The PCR products were analysed on a 5% denaturing polyacrylamide gel. The PCR reactions were carried out in a volume of 25 µl containing 50 mmol/l KCl, 10 mmol/l Tris-HCl pH 8.3, 1.5 mmol/l $MgCl_2$, 200 µmol/l each of dATP, dCTP, dGTP, dTTP, 0.1 µg of DNA, 10 pmoles of each primer and 1.5 units of Taq polymerase (Perkin Elmer Cetus, Norwalk, Conn., USA) in a GeneAmp 9600 PCR System (Perkin Elmer Cetus).

Results

Localization of the human IRS-1 gene

To localize *IRS1*, we analysed the segregation of the human gene in DNA samples prepared from a panel of human-hamster somatic cell hybrids retaining different human chromosomes by PCR using primers IRS1–2 and IRS1–3. These studies indicated that *IRS1* was on human chromosome 2 (data not shown). We confirmed this assignment and obtained a regional localization of *IRS1* by fluorescence in situ hybridization of biotin and digoxigenin-labelled λhIRS1–4 DNA to normal human metaphase chromosomes. Hybridization of λhIRS1–4 DNA resulted in specific labelling only of chromosome 2 (Fig. 1). In an analysis of 25 cells, specific labelling of 2q35–q36.1 was observed on four (20 cells), three (4 cells), two (1 cell) and one (0 cells) chromatids of the chromosome 2 homologues. Similar results were obtained in three additional experiments using these probes. Thus, *IRS1* is localized to chromosome 2, bands q35–q36.1.

Characterization of a simple tandem repeat DNA polymorphism

The human *IRS-1* genomic clones were screened for the presence of simple tandem repeat DNA polymorphisms (STRP) such as $(GT)_n$ by hybridization with ^{32}P-labelled poly (dA-dC)-poly (dG-dT). One such sequence of the form $(GT)_{11}CTGTGCAT(GT)_{12}$ was identified in λhIRS1–4 (Fig. 2). Oligonucleotide primers flanking this repeat element were selected (Fig. 2) and used to amplify this sequence in unrelated individuals of Caucasian, African-American and Japanese origin. This sequence was polymorphic (Fig. 2) and five alleles were observed in Caucasians and African-Americans, whereas four were noted in a smaller sample of unrelated Japanese subjects (Table 1). The heterozygosity (i.e. the fraction of individuals in whom the two alleles at this locus differed in size) varied from 0.53 to 0.75 indicating that this STRP will be very useful for linkage studies. Co-dominant inheritance of this STRP was demonstrated in two large families.

Discussion

We have mapped the human *IRS1* gene to chromosome 2, bands q35–q36.1. In addition, we have identified a highly informative STRP that will facilitate genetic studies of *IRS1* in diabetes-prone families especially those with the late-onset Type 2 diabetes in which insulin-resistance is a feature.

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                                                        IRS1-2
TTAACCCAAGCATTTTGTTTCCTTGGAAAAATTTCTAATTCCATGTTCATTAATATTGTT  60
AATTGGGTTCGTAAAACAAAGGAACCTTTTTAAAGATTAAGGTACAAGTAATTATAACAA

CAACTGTGGATGGTGTGTGTGTGTGTGTGTGTGTCTGTGCATGTGTGTGTGTGTGTGT 120
GTTGACACCTACCACACACACACACACACACACAGACACGTACACACACACACACACA

GTGTGTCTGTAAGGCTCAAGGAAAAGTCCCCATCAAACGGGTTTCAAATTAATTTGTACA 180
CACACAGACATTCCGAGTTCCTTTTCAGGGGTAGTTTGCCCAAAGTTTAATTAAACATGT
                                                IRS1-3
TTAATTCTGTGTAGGAAGCTTTCTTTATTTGATAAAGA 218
AATTAAGACACATCCTTCGAAAGAAATAAACTATTTCT
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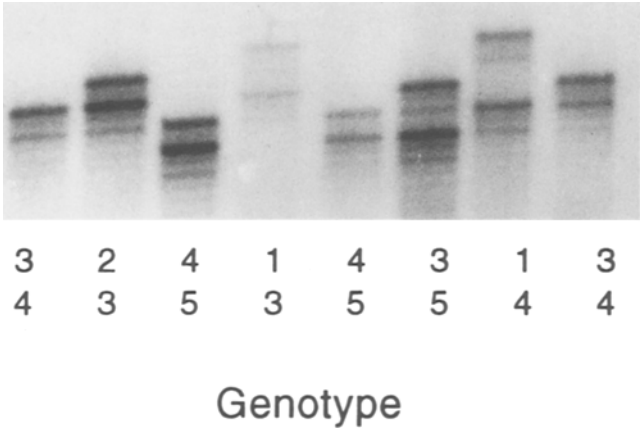

Fig. 2. Simple tandem repeat DNA polymorphism in human insulin receptor substrate-1 gene. Upper panel: Nucleotide sequence of the polymorphic region. The sequences of the primers used to amplify this region are shown in bold-face type. Lower panel: Polymerase chain reaction amplification of simple tandem repeat DNA polymorphism. The genotypes of the unrelated individuals studied here are shown at the bottom of the figure. The DNA sequence shown in this figure has been deposited in the GenBank data base with accession number L05198

Table 1. Characterization of simple tandem repeat DNA polymorphism in the insulin receptor substrate-1 gene

		Racial Group		
		Caucasian	African-American	Japanese
Allele Frequencies		(n = 28)	(n = 30)	(n = 20)
Allele	Size (bp)			
1	142	0.054	0.033	–
2	140	0.018	0.350	0.025
3	138	0.357	0.350	0.50
4	136	0.482	0.217	0.450
5	134	0.089	0.050	0.025
Heterozygosity		0.67	0.53	0.75

bp, base pairs

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