**Brief Genetics Report**

**Insulin Receptor Substrate-2 Amino Acid Polymorphisms Are Not Associated With Random Type 2 Diabetes Among Caucasians**

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The full-length coding region for hIRS2 was sequenced from two individual clones obtained from a human genomic fibroblast library and a human placenta cosmid library. Restriction mapping and sequence analysis revealed that the entire coding region occurred in a single exon containing 4,065 base pair. The coding region of hIRS2 is 82.3% identical to that of mIRS2, but only 57.5% identical to that of hIRS1. The deduced amino acid sequence of human IRS-2 encodes a protein of 1,354 amino acids. The human and mouse IRS-2 protein sequence was 81.3% identical; however, hIRS-2 was only 44.8% identical to hIRS-1 and 27.9% identical to hIRS-4 (Fig. 1). The NH$_2$-terminal of IRS-2 contains a pleckstrin homology (PH) domain and a phosphotyrosine binding (PTB) domain, which is similar to the other IRS protein. These NH$_2$-terminal regions are ~90.8% identical between human and mouse IRS-2; however, this region of hIRS-2 is only 64% identical to that of hIRS-1 and 45% identical to that of hIRS-4. These differences suggest that the PH and PTB domains may have unique functions in each IRS protein.

In the COOH-terminal, similarity between hIRS-2 and either hIRS-1 or hIRS-4 is largely restricted to a few common tyrosine phosphorylation motifs (Fig. 1). Phosphorylation of these tyrosine residues creates binding sites that recruit various signaling proteins into an activated complex. Human IRS-2 contains 22 potential tyrosine phosphorylation sites, and each site is conserved in mouse and rat IRS-2. However, only 13 tyrosine motifs are located in similar positions in both IRS-2 and IRS-1, and the relative position of six tyrosine residues is conserved when hIRS-4 is added to the alignment (Fig. 1). Moreover, the amino acid sequences of these common motifs are not completely identical, suggesting that the signals generated by each IRS protein may be somewhat different. Interestingly, two motifs that bind fluorescence in situ hybridization (FISH) are conserved in IRS-1 and IRS-2, but are absent from IRS-4. Thus, each IRS protein is likely to mediate unique signals during activation by the insulin receptor.

To further characterize the hIRS-2 gene, we determined its chromosomal location and tissue distribution. In situ hybridization of normal human metaphase spreads with hIRS2 DNA revealed one labeled site at the distal end of the long arm of chromosome 13; this position corresponded to band q34, as determined by the protein tyrosine phosphatase analysis (15,16). RNA blot analysis revealed IRS-2 and IRS-1 mRNA in a broad spectrum of fetal and adult tissues (Fig. 2). Both were relatively abundant in insulin-sensitive tissues, including skeletal and cardiac muscle, and IRS-2 was relatively more abundant than IRS-1 in the liver and...
pancreas (86.7 and 68%, respectively, for IRS-2 versus 50.7 and 34.1% for IRS-1). By contrast, hIRS-4 was detected strongly in the pituitary gland, but weakly in the salivary gland and barely at all in the stomach, the brain, the kidney thymus, and the lungs (data not shown, T. Uchida et al., unpublished observations). The unique occurrence of hIRS-2 mRNA in liver muscle and pancreas suggests that hIRS-2 may play an important role in peripheral insulin sensitivity and β-cell proliferation of function, as suggested by our results with the IRS2Δmt mice (14).

We next investigated the possible association between hIRS-2 mutations and type 2 diabetes. Since the entire coding region of hIRS-2 exists in a single exon, we analyzed it by single-strand conformation polymorphism (SSCP) technique (9). The primary mutation screening by single SSCP analysis was carried out on 71 Danish type 2 diabetic patients of Caucasian origin selected from a population of 252 unrelated Danish Caucasian type 2 diabetic patients. These patients tested negative for anti-GAD antibodies and were consecutively recruited from the outpatient clinic at the Steno Diabetes Cen-
Association studies were performed on the 252 unrelated Danish Caucasian type 2 diabetic patients and 267 age-matched, unrelated, and glucose-tolerant Danish Caucasian control subjects traced in the Danish Central Population Register and living in the same area of Copenhagen as the type 2 patients. Before participation in the study, informed consent was obtained from all subjects. A relatively frequent polymorphism was detected at base 3170 that replaces Gly with Asp (Fig. 1). However, its allelic frequency is similar in glucose-tolerant (33.9%) and type 2 diabetic patients (33.8%). Thus, there is no association of this polymorphism with type 2 diabetes. A much less frequent polymorphism was also detected at base 2635 that replaces Gly with Ser (Fig. 1); however, this polymorphism also displays equivalent frequency in the normal and patient populations.

Although the possibility that mutations in the hIRS2 coding region contribute to type 2 diabetes is not supported by our genetic analysis, IRS-2 remains an attractive candidate because deletion of the mIRS2 gene causes type 2 diabetes in mice (14). Taken together with our animal studies, our results suggest that attention should focus on the search for the mechanisms that reduce hIRS-2 expression or function in type 2 diabetic individuals. While dysfunction of IRS-1 may cause insulin resistance in patients, this deficit alone is unlikely to impair the β-cell. By contrast, based on our work with the IRS2(−/−) mouse, dysfunction of IRS-2 caused by environmental or metabolic stress may reduce both peripheral insulin sensitivity and expansion of the β-cell mass required for adequate compensation. Confirmation of this hypothesis in humans could simplify our understanding of type 2 diabetes and facilitate the discovery of safe and efficacious therapies.

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