Brief Genetics Report

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

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Type 2 diabetes is a complex disease characterized by insulin resistance in target tissues and a reduced capacity to produce compensatory levels of insulin in pancreatic β-cells (1). Not surprisingly, a single molecular defect causing type 2 diabetes has been difficult to identify. A considerable body of evidence suggests that several genetic factors contribute to the pathogenesis of type 2 diabetes, given its familial clustering and prevalence in certain ethnic groups (2,3). Mutations in several genes have been linked to rare monogenic forms of type 2 diabetes, including those encoding insulin, the insulin receptor, glucokinase, hepatocyte nuclear factors, insulin-promoting factor 1 (IPF-1, also known as IDX-1 and STF-1), and some mitochondrial proteins (4–8). In addition, genetic variations have been found in the sequence of insulin receptor substrate (IRS)-1 in a small percentage of patients with type 2 diabetes (9–12). Disruption of IRS-1 in mice causes insulin resistance, but does not impair the compensatory insulin production (13). In fact, islets are larger in IRS1$^{(-/-)}$ mice, suggesting that the increased requirement for insulin is accommodated, at least partially, by an increased number of β-cells (14). Thus, dysfunction of IRS-1 alone is unlikely to cause type 2 diabetes.

Recently, we found that homozygous disruption of the *mirs2* gene causes peripheral insulin resistance and relative β-cell failure (14). Consequently, IRS2$^{(-/-)}$ mice display progressive deterioration of glucose homeostasis culminating with profound fasting hyperglycemia between 8 and 10 weeks of age. These results suggest that dysfunction of IRS-2 may contribute to the pathophysiology of type 2 diabetes. To establish a genetic basis for this hypothesis in humans, we cloned the human IRS-2 gene and characterized it in normal individuals and patients with type 2 diabetes.

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IRS, insulin receptor substrate; PH, pleckstrin homology; PTB, phosphotyrosine binding; SSCP, single-strand conformation polymorphism.

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

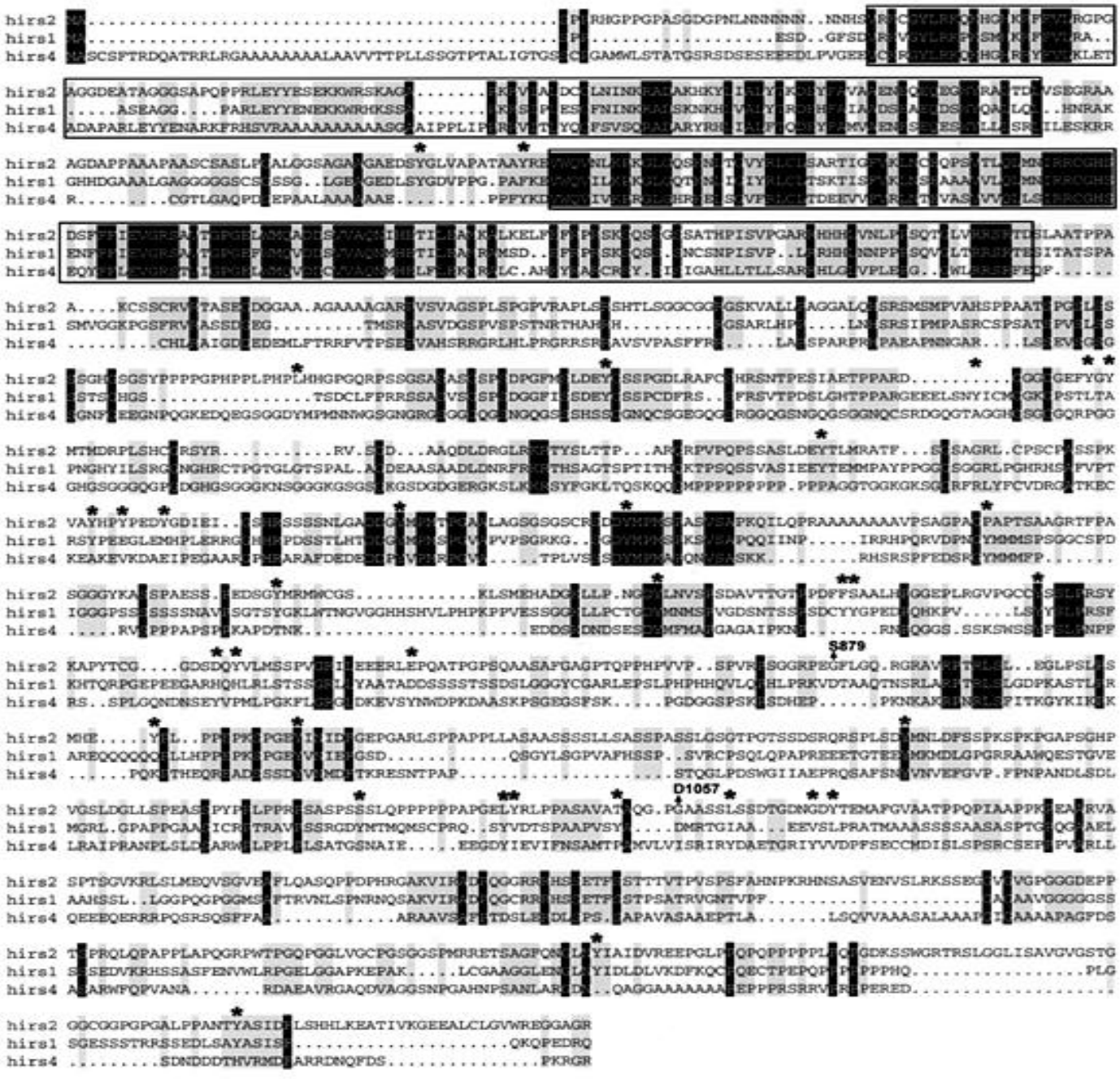

FIG. 1. Amino acid sequence alignment of the IRS proteins. Identical amino acids are highlighted in black and similar amino acids are shaded in gray. The box around residues on lines 1 and 2 outlines the PH domain, and the box around residues on lines 3 and 4 outlines the PTB domain. A star (*) identifies a potential tyrosine phosphorylation site. In each case, the rare polymorphism detected in hIRS-2 is indicated above the sequence. The alignment was determined with the PILEUP program, and similarity was calculated using the PLOTSIMILARITY program (Genetics Computer Group).

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 IRS2$^{(-/-)}$ 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-

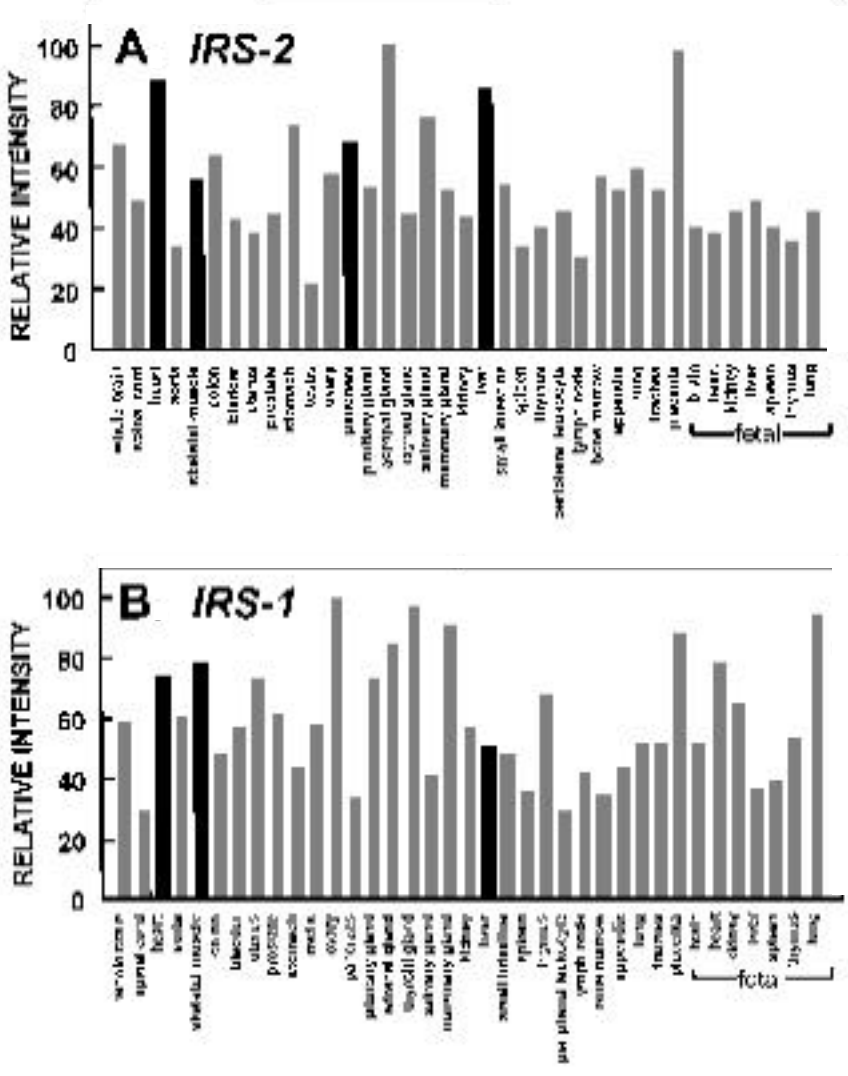

FIG. 2. Distribution of IRS-1 and IRS-2 mRNAs in various human tissues. The RNA blots were performed using a commercially available membrane (Clontech, Palo Alto, CA), in which the loading of RNA was normalized to eight different housekeeping genes to compensate for the tissue-specific variations in transcription levels (Human RNA Master Blot, Clontech). The filter was hybridized first with hIRS-2 (nucleotides 1230–2620) labeled with [α^{32}P]dCTP and washed according to the manufacturer's instructions. After removing the hIRS-2 probe, the membrane was hybridized with hIRS-1 probe (nucleotides 1080–2625). Quantitation was determined by Phosphorimager analysis, and the data are expressed relative to the maximum signal for IRS-2 and IRS-1.

ter. 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_{1057} 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_{879} 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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REFERENCES

1. DeFronzo RA: Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Reviews* 5:177–269, 1997
2. Hitman GA, McCarthy MI: Genetics of non-insulin dependent diabetes mellitus. *Baillieres Clin Endocrinol Metab* 5:455–476, 1991
3. Turner RC, Hattersley AT, Shaw JTE, Levy JC: Type II diabetes: clinical aspects of molecular biological studies. *Diabetes* 44:1–10, 1995
4. Taylor SI, Cama A, Accili D, Barbetti F, Imano E, Kadowaki H, Kadowaki T: Genetic basis of endocrine disease. 1. Molecular genetics of insulin resistant diabetes mellitus. *J Clin Endocrinol Metab* 73:1158–1163, 1991
5. Gerbitz KD, Gempel K, Brdiczka D: Mitochondria and diabetes: genetic, biochemical, and clinical implications of the cellular energy circuit. *Diabetes* 45:113–126, 1996
6. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boiriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Lebeau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Turner RC, Velho G, Chevre JC, Froguel P, Bell GI: Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young. *Nature* 384:455–458, 1996
7. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4α gene in maturity-onset diabetes of the young. *Nature* 384:458–460, 1996
8. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF: Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet* 15:106–110, 1997
9. Almind K, Bjorbaek C, Vestergaard H, Hansen T, Echwald SM, Pedersen O: Amino acid polymorphisms of insulin receptor substrate-1 in non-insulin-dependent diabetes mellitus. *Lancet* 342:828–832, 1993
10. Laakso M, Malkki M, Kekalainen P, Kuusisto J, Deeb SS: Insulin receptor substrate-1 variants in non-insulin-dependent diabetes. *J Clin Invest* 94:1141–1146, 1994
11. Armstrong M, Haldane F, Taylor RW, Alberti KG, Turnbull DM, Walker M: Human insulin receptor substrate-1: variant sequences in familial non-insulin-dependent diabetes mellitus. *Diabet Med* 13:133–138, 1996
12. Zouali H, Vaxillaire M, Lesage S, Sun F, Velho G, Vionnet N, Chiu K, Passa P, Permutt MA, Demenais F, Cohen D, Beckmann JS, Froguel P: Linkage analysis and molecular scanning of glucokinase gene in NIDDM families. *Diabetes* 42:1238–1245, 1993
13. Bruning JC, Winnay J, Bonner-Weir S, Taylor SI, Accili D, Kahn CR: Development of a novel polygenic model of NIDDM in mice heterozygous for *IR* and *IRS-1* null alleles. *Cell* 88:561–572, 1997
14. Withers DJ, Sanchez-Gutierrez JC, Towery H, Ren JM, Burks DJ, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF: Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391:900–903, 1998
15. Pinkel D, Straume T, Gray JW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 83:2934–2938, 1986
16. Blennow E, Telenius H, Larsson C, de Vos D, Bajalica S, Ponder BA, Nordenskjold M: Complete characterization of a large marker chromosome by reverse and forward chromosome painting. *Hum Genet* 90:371–374, 1992