

Exclusion of Insulin Receptor Substrate 2 (IRS-2) As a Major Locus for Early-Onset Autosomal Dominant Type 2 Diabetes

Arsun Bektas, James H. Warram, Morris F. White, Andrzej S. Krolewski, and Alessandro Doria

We investigated whether variability at the insulin receptor substrate (IRS)-2 locus plays a role in the etiology of early-onset autosomal dominant type 2 diabetes. By means of radiation hybrid mapping, we placed the human IRS-2 gene on 13q at 8.6 cRays from SHGC-37358. Linkage between diabetes and two polymorphic markers located in this region (D13S285 and D13S1295) was then evaluated in 29 families with early-onset autosomal dominant type 2 diabetes. Included were 220 individuals with diabetes, impaired glucose tolerance, or gestational diabetes (mean age at diabetes diagnosis 36 ± 17 years) and 146 nondiabetic subjects. Overall, strongly negative logarithm of odds (LOD) scores for linkage with diabetes were obtained by multipoint parametric analysis (LOD score -45.4 at D13S285 and -40.9 at D13S1295). No significant evidence of linkage was obtained under the hypothesis of heterogeneity or by nonparametric methods. Fourteen pedigrees for which linkage could not be excluded (LOD score > -2.0) were screened for mutations in the IRS-2 coding region by dideoxy fingerprinting. However, no mutations segregating with diabetes could be detected in these families. These data indicate that IRS-2 is not a major gene for early-onset autosomal dominant type 2 diabetes, although a role of mutations in the promoter region cannot be excluded at this time. *Diabetes* 48:640-642, 1999

Genetic factors play a crucial role in the development of type 2 diabetes. However, efforts to identify type 2 diabetes genes have been mostly unsuccessful due to the complexity and heterogeneity of the disease (1). As a strategy for finding genes for type 2 diabetes, we are investigating early-onset autosomal

dominant type 2 diabetes, a subtype of diabetes that is more strongly determined by genetic factors than common type 2 diabetes (2,3), but, like common type 2 diabetes, is frequently characterized by the presence of insulin resistance (4). Insulin exerts its action through the insulin receptor, which is a tyrosine kinase that is activated when insulin binds to it (5). This insulin signal initiates a cascade of events involving phosphorylation and dephosphorylation of several substrates, the best known of which is insulin receptor substrate (IRS)-1 (6). Previous studies have shown that genetic variability at the insulin-receptor and IRS-1 loci does not play a major role in type 2 diabetes (1,7-9). However, several other substrates of the insulin receptor have been recently identified. Because of their homology with IRS-1, these proteins have been termed IRS-2, IRS-3, and IRS-4 (10-12). IRS-1 and IRS-2 are broadly expressed, and IRS-2 is the predominant IRS protein in β -cells. In contrast, IRS-3 is restricted to adipocytes, and IRS-4 appears to be expressed mainly in the pituitary and thyroid (M.F.W., unpublished observations). Homozygous disruption of the IRS-2 gene in mice results in a phenotype similar to human type 2 diabetes, characterized by peripheral insulin resistance and accompanied by a failure of the β -cell to compensate for the defect of insulin action by appropriately increasing insulin secretion (13). In contrast with these exciting results, polymorphisms in the IRS-2 gene do not appear to contribute to the etiology of common type 2 diabetes in humans (14). However, because of the heterogeneity of type 2 diabetes, it is still possible that mutations in the IRS-2 gene are responsible for selected forms of type 2 diabetes, especially those transmitted with a Mendelian mode of inheritance.

In the present study, we investigated whether the IRS-2 locus is involved in the etiology of early-onset type 2 diabetes transmitted with an autosomal dominant mode of inheritance. To identify polymorphic markers suitable for linkage studies, the IRS-2 gene was mapped by screening the Stanford G3 Radiation Hybrid panel (15) with a sequence tagged site from the 3' end of its cDNA. Screening was performed by standard polymerase chain reaction (PCR), using the primers and the conditions specified in the on-line appendix, which can be found at www.diabetes.org/diabetes/appendix.htm. Scores were entered into the Stanford on-line RH Server (<http://www.shgc.stanford.edu/rhserver2>) for a two-point statistical analysis with markers in the $v2.0$ Stanford Human Genome Center G3 map. Consistent with its previous mapping by fluorescence in situ hybridization (14), IRS-2 was mapped

From the Sections on Genetics and Epidemiology (A.B., J.H.W., A.S.K., A.D.) and Molecular Physiology (M.F.W.), Research Division, Joslin Diabetes Center; and the Department of Medicine (A.B., M.F.W., A.S.K., A.D.), Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Alessandro Doria, MD, PhD, Section on Genetics and Epidemiology, Joslin Diabetes Center, One Joslin Pl., Boston, MA 02215. E-mail: adoria@joslin.harvard.edu.

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ddF, dideoxy fingerprinting; IRS, insulin receptor substrate; LOD, logarithm of odds; PCR, polymerase chain reaction.

to chromosome 13q at 8.6 cRay (~250 kb) from marker SHGC-37358. The logarithm of odds (LOD) score for this location was 7.8. The closest polymorphic marker on the v2.0 SHGC G3 map was identified as Genethon marker AFM309va9 (D13S285), located at 28 cRays (~800 kb) from SHGC-37358

The presence of linkage between diabetes and the IRS-2 chromosomal region on 13q was evaluated in 29 multigenerational families with an autosomal dominant pattern of occurrence of early-onset type 2 diabetes. Diabetes in these families is unlinked to HNF-1 α , HNF-4 α , and glucokinase genes and is frequently characterized by insulin resistance rather than a defect of insulin secretion alone (4). Recruitment procedures have been described in detail previously (4). Briefly, families were ascertained at the Joslin Diabetes Center according to the following screening criteria: 1) a proband and at least one first-degree relative with type 2 diabetes diagnosed before age 35, 2) three or more generations affected by diabetes, and 3) diabetes entering the family on only one side. Included were 220 individuals with diabetes, impaired glucose tolerance, or gestational diabetes, and 146 nondiabetic subjects. All families were of Caucasian origin with the exception of three Hispanic and two African-American pedigrees. The mean age at diagnosis of diabetes among affected members was 36 ± 17 years. A table with the clinical characteristics of the study subjects is provided in an on-line appendix at www.diabetes.org/diabetes/appendix.asp. In addition to marker D13S285, family members were genotyped at D13S1295, which is located at the same position as D13S285 on the Genethon map (16). Genotyping was performed by means of 32 P-labeled PCR followed by denaturing gel polyacrylamide electrophoresis and autoradiography (17). Linkage with diabetes was analyzed by both parametric and nonparametric methods, using the MLINK program of the LINKAGE package and the GENEHUNTER software (version 1.1) (18,19). The parametric analysis assumed an autosomal dominant model, with a rare disease allele ($D = 0.001$) and four age-related liability classes, similar to the model used by Vaxillaire et al. for MODY3 on chromosome 12 (20).

The results of linkage analysis are reported in Table 1. In the two-point parametric analysis, both markers in the IRS-2 regions produced strongly negative total LOD scores at any recombination fraction. Results did not change significantly when the five non-Caucasian families were considered separately. Similarly, negative LOD scores were obtained by considering the two markers together in a multipoint parametric analysis using the GENEHUNTER software. Multipoint LOD scores smaller than -2.0 , excluding linkage, were observed at one or the other marker position in 15 families. Another eight families showed negative LOD scores between 0.0 and -2.0 . Of the six families with positive LOD scores, two had values

>1.0 (1.2 and 1.4). However, no significant evidence of linkage heterogeneity was detected by the program HOMOG (21), and the maximum heterogeneity LOD score was only $+0.3$, with a proportion of linked family (α) of 8%. Results were comparable when the D allele frequency was set to 0.01 rather than 0.001. Negative results were also obtained by nonparametric analysis, which considers only affected individuals and is therefore independent of the penetrance of the disease allele. The overall Z_{all} score calculated by GENEHUNTER was $+0.43$ at marker D13S285 and $+0.44$ at marker D13S1295, with a P value of 0.24 for both. Since nonparametric methods do not take into account genetic heterogeneity, the analysis was also performed after stratifying the families into two equal groups according to whether the within-family average insulin increment during oral glucose tolerance testing was lower or higher than $30 \mu\text{U/ml}$ at 120 min. At both marker positions, the Z_{all} score was higher in the subset of families with a lower insulin response ($Z_{\text{all}} = +1.55$) than in pedigrees with higher insulin secretion ($Z_{\text{all}} = -0.23$), but this value, corresponding to a LOD score of only $+0.5$, was not significant or even suggestive of linkage ($P = 0.06$).

The 14 families in which linkage with diabetes could not be excluded were screened for mutations in the coding region of IRS-2. Mutations were searched by dideoxy fingerprinting (ddF), a powerful modification of single-strand conformational polymorphism analysis (22). Five 800- to 900-bp overlapping fragments, covering the whole IRS-2 cDNA (4,065 bp), were amplified by PCR from one proband per family using the primers and conditions described in the on-line appendix. Each PCR product was then subjected to unidirectional ddF per the protocol described by Sarkar et al. (22) with modifications, using ddF and the primers used for the primary PCR plus two nested primers (each ddF primer screens 200–250 bp). Samples with changes in band patterns were subcloned and sequenced. In addition to the previously described Gly1057Asp polymorphism, we detected a silent polymorphism (C to T) at position 2448 of the IRS-2 cDNA. However, no mutations segregating with diabetes could be identified in these families.

Our results indicate that genetic variability in IRS-2 does not play a major role in the etiology of early-onset autosomal dominant type 2 diabetes. One must consider, however, the limitations of these findings. The fact that no significant linkage or mutations were identified in 32 pedigrees does not absolutely exclude the existence of families with early-onset type 2 diabetes due to IRS-2. It only indicates that if these families exist, they are extremely rare. It is also important to consider that our results apply only to forms of type 2 diabetes that can be identified through the ascertainment criteria that were used in this study. The case might be different for forms of type 2 diabetes with a later onset, although the results of

TABLE 1

Result of two-point and multipoint analyses for linkage between early-onset NIDDM and the IRS-2 locus

Marker	Two-point analysis: LOD score at $\theta =$						Multipoint analysis						
	0.00	0.05	0.10	0.20	0.30	0.40	HET	INF	LOD	HLOD	α	Z_{all} score	P value
D13S285	-56.2	-30.6	-20.0	-9.1	-3.9	-1.3	0.81	0.67	-45.4	+0.34	0.08	+0.43	0.24
D13S1295	-37.9	-22.7	-15.6	-7.7	-3.6	-1.3	0.75	0.64	-40.9	+0.35	0.08	+0.44	0.24

HET, marker heterozygosity; HLOD, heterogeneity LOD score; INF, proportion of genetic information that was extracted by markers.

a recent study showing a lack of association between two IRS-2 polymorphisms and type 2 diabetes in a Danish population argue against this possibility (14). Finally, in our study, as well as in the one from Denmark, the mutation screening was confined to the coding sequence of IRS-2. The possibility exists that sequence differences in the IRS-2 promoter region may cause diabetes by impairing IRS-2 expression. This hypothesis will need to be thoroughly investigated as soon as the IRS-2 promoter sequence becomes available.

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