Type II Diabetes Mellitus and Polymorphism of Insulin-Receptor Gene in Mexican Americans

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Resistance to insulin action is a well-established feature of type II (non-insulin-dependent) diabetes and is believed by many to contribute to the etiology of this condition. We therefore characterized restriction-fragment–length polymorphisms of the insulin-receptor gene with the restriction enzyme *Rsa* I in 242 Mexican Americans and non-Hispanic Whites with type II diabetes and 202 age-, sex-, and ethnicity-matched control subjects who participated in a population-based study in San Antonio. Alleles of 6.7 kilobases (kb) (*A* allele), 6.2 kb (*B* allele), and 3.4 kb (*C* allele) were identified. The *C* allele was observed in Mexican Americans only, where its frequency among nondiabetic control subjects was 17.7%. Diabetic Mexican Americans were twice as likely as control subjects to be homozygous for the *C* allele. The crude odds ratio for diabetes in *CC* homozygotes compared with the other two genotypes was 2.22, although this result was not statistically significant (χ^2 = 1.57, *P* = .21). The Mantel-Haenszel odds ratio, adjusting for age, however, indicated a 4.71-fold increased risk of diabetes among Mexican Americans with the *CC* genotype compared with Mexican Americans without this genotype (χ^2_{M-H} = 5.38, *P* = .020). The age of onset of diabetes was also slightly younger in *CC* homozygote cases (45.4 ± 9.2 yr) than in *CX* or *XX* cases (47.7 ± 9.0 and 48.6 ± 9.6 yr, respectively), although this difference was not statistically significant (*P* .467). The *C* allele may be involved directly in the etiology of type II diabetes, or it may be in linkage disequilibrium with a functionally significant region of the insulin receptor gene. *Diabetes* 38:975–80, 1989

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A strong genetic component to the etiology of type II (non-insulin-dependent) diabetes is suggested by the marked racial and ethnic differences in prevalence (1–4), the association between prevalence and genetic admixture in hybrid populations (5–8), and the high degree of concordance (60–90%) for this condition in monozygotic twins (9,10). Despite this evidence, the search for specific susceptibility genes for type II diabetes has thus far been disappointing. The recent rapid advances in identifying and characterizing restriction-fragment–length polymorphisms (RFLPs) of various genes have made it attractive to search for associations between type II diabetes and RFLPs in candidate genes, i.e., genes that code for proteins known to be involved in carbohydrate metabolism. Among such candidate genes are insulin, insulin-receptor, and glucose-transporter genes. Although initial reports seemed to suggest an association between type II diabetes and polymorphism of the insulin gene (11,12), later reports did not bear this out (13,14).

Resistance to insulin action is a well-established feature of type II diabetes and is believed by many to play an important role in the etiology of this condition (15,16). Moreover, insulin resistance has been shown to be familial in Pima Indians (17), which suggests that it may have a genetic basis. Bogardus et al. (18) have recently shown that insulin resistance assessed by the euglycemic insulin-clamp technique is trimodally distributed in Pima Indians, which is consistent with an autosomal, codominant mode of inheritance. We recently reported that the proportion of nondiabetic Mexican Americans who are hyperinsulinemic, an indirect indicator of insulin resistance, increases in a stepwise fashion according to whether they have zero, one, or two diabetic parents (19). Because pedigrees with progressively stronger family histories of diabetes may be expected to contain progressively greater numbers of prediabetic subjects, these data lend further support to the concept that prediabetic subjects are hyperinsulinemic (and, therefore, possibly in-

sulin resistant) and that this defect is at least familial if not inherited.

Although it has been claimed that insulin resistance in type II diabetes is due to a post–insulin-receptor defect, this concept is based primarily on studies of receptor number and binding (20,21). Recent data, however, have clearly established that the intracellular portion of the β-subunit of the insulin receptor exhibits tyrosine kinase activity, which appears to be involved in receptor function (22). Therefore, insulin-receptor function related to postbinding events could contribute to in vivo insulin resistance. Moreover, tyrosine kinase activity has been reported to be reduced in obese type II diabetic subjects compared to obese nondiabetic subjects in hepatic (23) and adipose (24) tissue.

In this article , we examine the association between type II diabetes and RFLPs of the insulin-receptor gene in Mexican Americans, a high-risk population for diabetes (2,5,25).

RESEARCH DESIGN AND METHODS

The study subjects are derived from the San Antonio Heart Study, a population-based study of cardiovascular disease and diabetes in Mexican Americans and non-Hispanic Whites. Detailed descriptions of the study design, sampling procedures, response rates, and field procedures have appeared elsewhere (2,5,6,25,26). The study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and informed consent was obtained from all subjects. A total of 3302 Mexican Americans and 1857 non-Hispanic Whites have participated in the San Antonio Heart Study. This report concerns a case-control study of diabetic subjects and matched control subjects nested within the larger parent study (26). The subjects with diabetes thus represent a population-based case series. Diabetes was diagnosed according to the National Diabetes Data Group criteria (27), as modified for epidemiologic studies (2,5). Only type II diabetic subjects are considered in this report (2,5). Age at diagnosis among previously diagnosed cases was ascertained by patient self-report. For subjects who were newly diagnosed at the time of their survey examination, age at diagnosis was taken to be their attained age at the time of the survey. A total of 376 Mexican Americans and 87 non-Hispanic Whites with type II diabetes were identified, of whom 300 (79.8%) Mexican Americans and 69 (79.3%) non-Hispanic Whites participated in a diabetic-complications examination (26). Gene-polymorphism studies were performed on a random subset of these individuals that consisted of 208 Mexican Americans and 34 non-Hispanic Whites. Controls were selected at random from among the nondiabetic members of the study population. The sampling of controls was stratified by sex, ethnicity, and decade of age, with stratum-specific sampling fractions chosen to match the age, sex, and ethnic distribution of the diabetic subjects. Gene-polymorphism studies were performed on a total of 202 control subjects, of whom 186 were Mexican American.

Whole blood specimens were obtained in heparinized containers and were kept frozen in sterile polypropylene tubes until analyzed. DNA was extracted as previously described (28) and digested with the restriction endonuclease *Rsa* I according to the manufacturer's directions (International Biotechnology, New Haven, CT). The DNA (10 μg/lane) was then separated by size on 0.8% agarose gel and blotted onto a nylon membrane by the method of Southern (29). Prehybridization and hybridization conditions have been described previously (30). RFLPs were detected by use of a 1.4 kb *Bgl* I fragment of the insulin receptor cDNA (kindly provided by G.I. Bell; 31). This fragment corresponds with bases 1601–2963 from the 5′ end of the insulin-receptor cDNA described by Ullrich et al. (32). For hybridization, DNA was oligolabeled (33) to a specific activity of $1–3 \times 10^9$ cpm/μg with [^{32}P]dCTP (New England Nuclear, Boston, MA). After washing under stringent conditions (30), the filters were subjected to autoradiography by use of Kodak XAR film with an intensifying screen at 80°C for 19–60 h. Assessment of insulin-receptor gene RFLPs was performed blind i.e., without knowledge of whether the specimen came from a diabetic or a control subject.

Body mass index (BMI), defined as kg/m^2, was used as an indicator of overall adiposity, and the ratio of the subscapular to the triceps skinfold (centrality index) was used as an index of body-fat distribution. Anthropometric methods have been described previously (2,34,35).

Differences in age, anthropometric indexes, and age at diagnosis (cases only) according to genotype were evaluated by analysis of variance (36). The odds ratio for diabetes according to genotype, adjusted for age, was computed by the Mantel-Haenszel procedure and evaluated for statistical significance with the Mantel-Haenszel χ^2 statistic (37). The 95% confidence interval of the odds ratio was computed by the method of Robins et al. (38).

RESULTS

Hybridization of the 1.4-kb insulin-receptor–cDNA probe to *Rsa* I–digested human genomic DNA revealed three alleles of 6.7, 6.2, and 3.4 kb (Fig. 1). For purposes of this report,

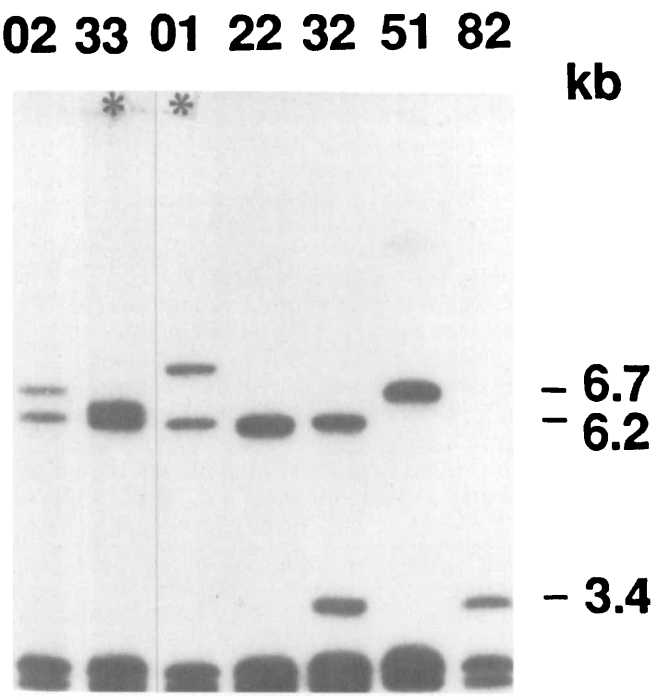

FIG. 1. Hybridization of 1.4-kilobase (kb) probe to *Rsa* I–digested human DNA. Individuals are indicated by number at top of each lane, and sizes of fragments are indicated at right in kb. *Individuals with variant alleles of 7.5 kb (V_1, sample 01) and 6.4 kb (V_2, sample 33).

these alleles have been designated *A*, *B*, and *C*, respectively. Two variant alleles of 7.5 and 6.4 kb, designated as V_1 and V_2, respectively, were also observed (Fig. 1). The *A* and *B* alleles were observed in Mexican Americans and non-Hispanic Whites, whereas the *C* allele was observed exclusively in Mexican Americans, among whom its frequency in nondiabetic control subjects was 17.7%, calculated from Table 1 as

$$\frac{22 + 33 + 5(2) + 1}{2 \times 186} = 0.177$$

The two variant alleles were also observed in Mexican Americans only. The remaining analyses are confined to Mexican Americans.

The distribution of genotypes in Mexican-American diabetic and control subjects is presented in Table 1. The genotype frequencies conformed to Hardy-Weinberg equilibrium ($3\chi^2 = 1.32$, $P = .72$). Few differences were observed between diabetic and control subjects except for the *CC* genotype, which was more than twice as common in diabetic as in control subjects. For this reason, and because the *C* allele was observed exclusively in Mexican Americans, the genotypes were aggregated into three categories: *CC* homozygotes (2 *C* alleles); *CX* heterozygotes (1 *C* allele); and *XX* (no *C* alleles). The crude odds ratio for diabetes in *CC* homozygotes versus the other two genotypes was 2.22, although this result was not statistically significant ($\chi^2 = 1.57$, $P = .21$). This crude analysis, however, does not take into account possible differences between diabetic and control subjects in other diabetes-related risk factors.

Table 2 shows the mean age, BMI, and centrality index according to genotype for diabetic and control subjects. There were no significant differences in the anthropometric indexes according to genotype in either group. Mean age, however, was significantly younger in control subjects with the *CC* genotype than in control subjects with the other two genotypes. (Note that although the overall group of diabetic and control subjects were matched by age, there is no guarantee that this matching will persist within each genotype.) Because type II diabetes usually does not develop until the 5th or 6th decade of life, the *CC* control subjects are, by virtue of their younger age, at greater risk of becoming diabetic than the *CX* or *XX* control subjects. The effect of such differential misclassification would be to underestimate the effect of the *C* allele on diabetes risk.

To take into account the greater risk of future diabetes among *CC* control subjects, we adjusted the odds ratio for age by the Mantel-Haenszel procedure. The results indicate that the odds of diabetes are 4.71 times greater in individuals with the *CC* genotype than in individuals with the other two genotypes ($P = .020$; Table 3). Note that three of the five *CC* control subjects were <30 yr of age. Diabetic subjects with the *CC* genotype also had a younger age of onset (45.4 ± 9.2 yr) than diabetic subjects with the *CX* or *XX* genotype (47.7 ± 9.0 and 48.6 ± 9.6 yr, respectively), although this difference was not statistically significant ($P = .467$).

TABLE 1
Distribution of observed genotypes in Mexican-American diabetic and control subjects

	Diabetic		Control	
Genotype	*n*	%	*n*	%
AA	21	10.1	21	11.3
AB	61	29.3	57	30.6
AC	30	14.4	22	11.8
BB	47	22.6	46	24.7
BC	33	15.9	33	17.7
CC	12	5.8	5	2.7
BV_1	1	0.5	0	
BV_2	0		1	0.5
AV_2	1	0.5	0	
CV_2	2	1.0	1	0.5

DISCUSSION

With the restriction endonuclease *Rsa* I in a study of 51 unrelated non-Hispanic Whites, Shaw and Bell (31) described a two-allele polymorphism at the insulin-receptor locus with bands of 6.7 and 6.2 kb. The presence of a 3.4 kb RFLP after digestion with *Rsa* I, identical to our *C* allele, has been previously reported in Pima Indians but not in non-Hispanic Whites or Blacks (39). No association between the *C* allele and type II diabetes was found in this study, although only 54 Pima subjects were studied, which raises the possibility of a type II error.

Although we have reported an association between the *C* allele and type II diabetes, some caution is in order, because this association was not statistically significant in the crude analysis and was detected only after adjustment for age. The case for an association is strengthened, however, by the fact that the *C* allele has thus far been detected only in Pima Indians and Mexican Americans, two high-risk populations for diabetes that share Native American ancestry (25,40). The absence of the *C* allele in other populations is based on studies involving 58 Blacks (39) and 193 non-Hispanic Whites (50 non-Hispanic Whites in this study, 51 in the study by Shaw and Bell [31], and 92 in the study by Elbein et al. [39]).

The frequency of the *C* allele in Pima Indians was reported to be 34% (39). The 17.7% frequency of this allele in Mexican Americans is compatible with the 15–45% Native American admixture that we previously reported in this population (5,6). It is also compatible with the lower prevalence of type II diabetes in Mexican Americans compared with Pima Indians (~15 vs. 40%; 25,40). Although these ecological associations argue for an association between the *C* allele and diabetes, it is also possible that the presence of this allele in Mexican-American diabetic subjects simply reflects their greater degree of Native American genetic admixture relative to nondiabetic Mexican Americans (6). Nevertheless, that this allele occurs in a gene that codes for a protein of obvious relevance to carbohydrate metabolism (the insulin-receptor gene) suggests that it may have functional significance with respect to the etiology of diabetes or may be in linkage disequilibrium with a functionally significant region of the insulin-receptor gene.

If alterations of the insulin-receptor gene confer increased susceptibility to type II diabetes, this might arise as a result of mutations in the structural or regulatory portions of the gene. Further studies on the nucleotide sequence of this

TABLE 2
Age and anthropometric variables according to genotype in diabetic and control subjects

	Diabetic			Control		
	CC	*CX*	*XX*	*CC*	*CX*	*XX*
n	12	65	131	5	56	125
		P = .726			*P* = .560	
Age (yr)	50.2 ± 9.7	52.1 ± 8.4	52.6 ± 8.6	36.0 ± 13.0	50.5 ± 10.6	51.4 ± 9.0
	P = .352			*P* = .001		
		P = .955			*P* = .446	
Body mass index (kg/m²)	33.2 ± 5.9	31.3 ± 6.7	31.2 ± 6.1	29.7 ± 6.6	28.8 ± 5.5	29.5 ± 5.7
	P = .294			*P* = .948		
		P = .167			*P* = .432	
Centrality index	1.54 ± 0.76	1.35 ± 0.61	1.47 ± 0.54	1.22 ± 0.29	1.37 ± 0.47	1.32 ± 0.45
	P = .690			*P* = .655		

Values are means ± SD.

gene among individuals with *C* alleles may help clarify this issue. The *C* allele is apparently the result of an alteration in the nucleotide sequence giving rise to an additional *Rsa* I restriction-endonuclease–recognition site. Because the total nucleotide sequence of the insulin-receptor gene has not yet been established, we cannot determine if this *Rsa* I site falls in an exon or intron. If it falls in an exon, it could give rise to an altered insulin receptor as a result of a missense or nonsense mutation. If the *Rsa* I site of the *C* allele produces a missense mutation, it would fall in the region of cDNA bases 1928–2478, described by Ullrich et al. (32), because the coding sequences homologous to the *C* allele are located in this region (39). It is unlikely to be a nonsense mutation because this would result in the absence of some or all of the β-subunit of the insulin receptor.

Another possibility is that the *Rsa* I restriction-endonuclease–cleavage site falls in an intron but is associated with a mutation in the structural portion of the gene, resulting in the production of an abnormal insulin receptor. An example of an RFLP that is in linkage disequilibrium with a mutation in the structural portion of the gene occurs in sickle cell anemia (41).

The mechanism(s) whereby alterations of the insulin-receptor gene might contribute to the development of type II diabetes is unknown. Recently, a patient with type A insulin resistance was found to be homozygous for a point mutation within the processing site of the insulin proreceptor, which resulted in a conversion from Arg-Lys-Arg-Arg to Arg-Lys-Arg-Ser (42). The resulting insulin receptors are not cleaved to mature α- and β-subunits and are therefore unable to bind insulin efficiently. In another case, a patient with insulin resistance secondary to leprechaunism was found to have inherited a different insulin-receptor mutant from each parent (43). One of the alleles contained a missense mutation, resulting in the replacement of Lys with Glu at amino acid 460 in the α-subunit. The other allele contained a nonsense mutation, resulting in premature termination of the protein after amino acid 671.

Two studies have reported associations between an *Sst* I RFLP of the insulin-receptor gene and type II diabetes (44,45). In one study, a negative association was reported between a 5.8-kb RFLP and type II diabetes in Blacks (44), whereas in the other study (45), a positive association was found in a predominantly White population. Although opposite in direction, both associations are compatible with linkage disequilibrium between an endonuclease-restriction site and a diabetes-susceptibility site on the insulin-receptor gene. Interestingly, the second study included six Hispanics, four of whom were diabetic. However, because *Rsa* I polymorphisms were not reported in this study, it is not known whether any of these subjects had the *C* allele. Also, in the second study, the four nondiabetic White subjects with the *Sst* I 5.8-kb RFLP (the nominal susceptibility genotype) were considered to be hyperinsulinemic, suggesting that they might be prediabetic. Two pedigree studies have attempted to document possible linkage between *Rsa* I and *Sst* I poly-

TABLE 3
Distribution of diabetic and control subjects by age and genotype

Age (yr)		Genotype *CC*	*CX*	*XX*	Total
25–29	Diabetic	0	0	1	1
	Control	3	4	2	9
30–34*	Diabetic	2	3	5	10
	Control	0	3	5	8
35–39*	Diabetic	0	3	7	10
	Control	0	3	7	10
40–44	Diabetic	1	6	13	20
	Control	0	2	9	11
45–49	Diabetic	2	11	14	27
	Control	1	11	23	35
50–54	Diabetic	4	13	27	44
	Control	1	8	31	40
55–59*	Diabetic	0	13	28	41
	Control	0	14	19	33
60–64*	Diabetic	3	16	36	55
	Control	0	11	29	40
Total	Diabetic	12	65	131	208
	Control	5	56	125	186

Mantel-Haenszel odds ratio and 95% confidence interval for *CC* homozygotes vs. *CX* and *XX* combined = 4.71 (1.19–18.6); χ^2_{M-H} = 5.38, *P* = 0.20.
*To avoid 0 marginals, the age groups 30–34 and 35–39 were combined, as were the age groups 55–59 and 60–64. Mantel-Haenszel analysis was also performed with 10-yr strata to avoid necessity of combining strata. Results remained statistically significant (*P* = .05), although the odds ratio decreased to 3.08.

morphisms of the insulin-receptor gene and type II diabetes (46,47). However, too few pedigrees were studied to confirm or exclude genetic linkage. Finally, an *Xba* I RFLP of a glucose-transporter gene was reported to be associated with type II diabetes in a study of Europeans and Japanese (48). Unlike our study, however, none of these studies were population based.

In conclusion, the *C* allele appears to be associated with type II diabetes in Mexican Americans. Moreover, this allele is observed only in Pima Indians and Mexican Americans, two high-risk populations for type II diabetes (25,40), and not in non-Hispanic Whites or Blacks. The explanation for the association with diabetes remains to be elucidated. Further studies on the nucleic-acid sequence of insulin-receptor genes among individuals with *C* alleles and family studies to determine if the *C* allele cosegregates with diabetes should help clarify the role of this gene in type II diabetes among Mexican Americans.

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