Familial NIDDM

Molecular-Genetic Analysis and Assessment of Insulin Action and Pancreatic β-Cell Function

STEVEN C. ELBEIN, W. KENNETH WARD, JAMES C. BEARD, AND M. ALAN PERMUTT

Although the hereditary nature of non-insulin-dependent diabetes mellitus (NIDDM) is well recognized, the nature of the predisposing defect remains elusive. Individuals with a history of gestational diabetes had shown a reduced insulin-sensitivity index (S_I) in the absence of fasting hyperglycemia. To determine whether this finding could result from an inherited defect of the insulin receptor, an NIDDM pedigree was ascertained through a former gestational-diabetic proband. The proband, her siblings, and her first cousins were clinically characterized for insulin sensitivity with the minimal-model–based S_I from a modified glucose tolerance test. Islet function was characterized by the incremental insulin response to 5 g i.v. arginine at baseline and at a plasma glucose level of 500–600 mg/dl. Genetic studies included linkage analyses for the insulin gene and the insulin-receptor gene with DNA polymorphisms (restriction-fragment–length polymorphisms, RFLPs) previously described. The pattern of inheritance in this large pedigree appeared to follow autosomal-dominant transmission. No defect in islet function was found, but as a group, third-generation family members had an S_I that was significantly lower than that of weight-matched control individuals, suggesting an inherited defect in insulin action. Genetic studies showed no sharing of insulin gene, insulin-receptor–gene alleles among the diabetic individuals, or insulin-receptor alleles among third-generation individuals with insulin insensitivity. The genetic analyses thus suggest that this pedigree has an inherited defect that is not linked to the insulin gene or the insulin-receptor gene. The diminished S_I may nonetheless suggest an inherited defect in insulin action. *Diabetes* 37:377–82, 1988

Despite considerable study of the pathophysiology of non-insulin-dependent diabetes mellitus (NIDDM), the primary defect remains obscure. A major genetic component is evident from the >90% concordance in identical twins and the markedly increased incidence of NIDDM among siblings of a diabetic individual (1,2), but with the exception of maturity-onset diabetes of the young (MODY) (3), a simple pattern of inheritance has not been identified. In diabetic individuals, defects in islet function (4) and tissue sensitivity to insulin (5,6) are well documented, but secondary changes in both insulin secretion and insulin action are also well described (7,8) and are likely to obscure the primary defect in overtly diabetic individuals. Clinical and physiological studies have seldom been performed in the families of a diabetic proband, where prediabetic changes might be identifiable in individuals at high risk for NIDDM. However, attempts have been made to identify a prediabetic lesion in former gestational-diabetic women, another group at high risk for NIDDM (9). These studies uncovered defects of both insulin sensitivity (decreased insulin-sensitivity index, S_I) and islet function (diminished first-phase insulin release).

Recent advances in human genetics have enabled researchers to use the tools of molecular biology to study NIDDM families. Both the insulin gene (10) and the insulin-receptor gene (11) have been cloned, and for both genes these clones have helped to identify DNA-sequence polymorphisms (restriction-fragment–length polymorphisms, RFLPs) (12–14). Sufficient polymorphism existed at both loci to permit informative analysis of the cosegregation of haplotypes for each gene with NIDDM. Studies have included analyses of the insulin gene (15,16) and insulin-receptor gene (17) in several MODY pedigrees and examination of the insulin gene in several NIDDM pedigrees (18,19). This

From the Department of Medicine, Division of Endocrinology, Veterans Administration Medical Center and University of Utah, Salt Lake City, Utah; Department of Medicine, Division of Endocrinology and Metabolism, University of Washington and Veterans Administration Medical Center, Seattle, Washington; and Department of Medicine, Metabolism Division, Washington University School of Medicine, St. Louis, Missouri.
Address correspondence and reprint requests to Dr. Steven C. Elbein, Division of Endocrinology, Room 4C216, University of Utah, 50 North Medical Drive, Salt Lake City, UT 84132.
Received for publication 26 May 1987 and accepted in revised form 16 September 1987.

approach avoids the dependence on measurements that are subject to secondary fluctuations but is nonetheless limited by the late and incomplete penetrance of the disease, and thus extending the analysis to younger and potentially prediabetic individuals is difficult. The use of physiological studies to characterize family members with regard to islet function and insulin sensitivity may permit the identification of specific lesions of insulin action or insulin secretion in individuals at risk. Such studies could provide an early marker for the genetic defect, a means to identify potential heterogeneity among NIDDM families, and an important clue to the precise genetic defect.

This study combines the molecular-genetic approach with physiological studies of the members of one generation in an attempt to minimize the limitations of purely genetic analyses and to fully characterize one family. This family was ascertained through an individual who had had gestational diabetes, thus permitting the examination of nondiabetic siblings at risk for the same defects that characterize gestational diabetes (9). Additionally, this inclusion of young individuals at high risk permitted the extension of linkage analysis to a younger generation than is usually possible in genetic studies of NIDDM. Finally, the analyses of the insulin and insulin-receptor genes in this family provided an opportunity to examine the relationship between genotype and phenotype for two genes that code for proteins that are pivotal in glycemic control. To our knowledge, this analysis represents the first attempt to combine studies of β-cell function and insulin sensitivity with the molecular-genetic approach to study familial diabetes.

MATERIALS AND METHODS

Subjects. Members of the pedigree were ascertained through a former gestational-diabetic proband in the third generation (III-8) (Fig. 1), who at the time of the study was not diabetic. That individual subsequently developed NIDDM with fasting hyperglycemia (plasma glucose 130–150 mg/dl). Individual III-9 became pregnant while the pedigree was under study, and she underwent a 50-g glucose tolerance test late in her 2nd trimester, which resulted in a plasma glucose level of 140 mg/dl (after 1 h). For all available family members, weight, height, and fasting plasma glucose were measured, and blood was drawn for DNA analysis. Determination of insulin sensitivity and glucose potentiation was limited to individuals III-8 through III-12 (Fig. 1), who were

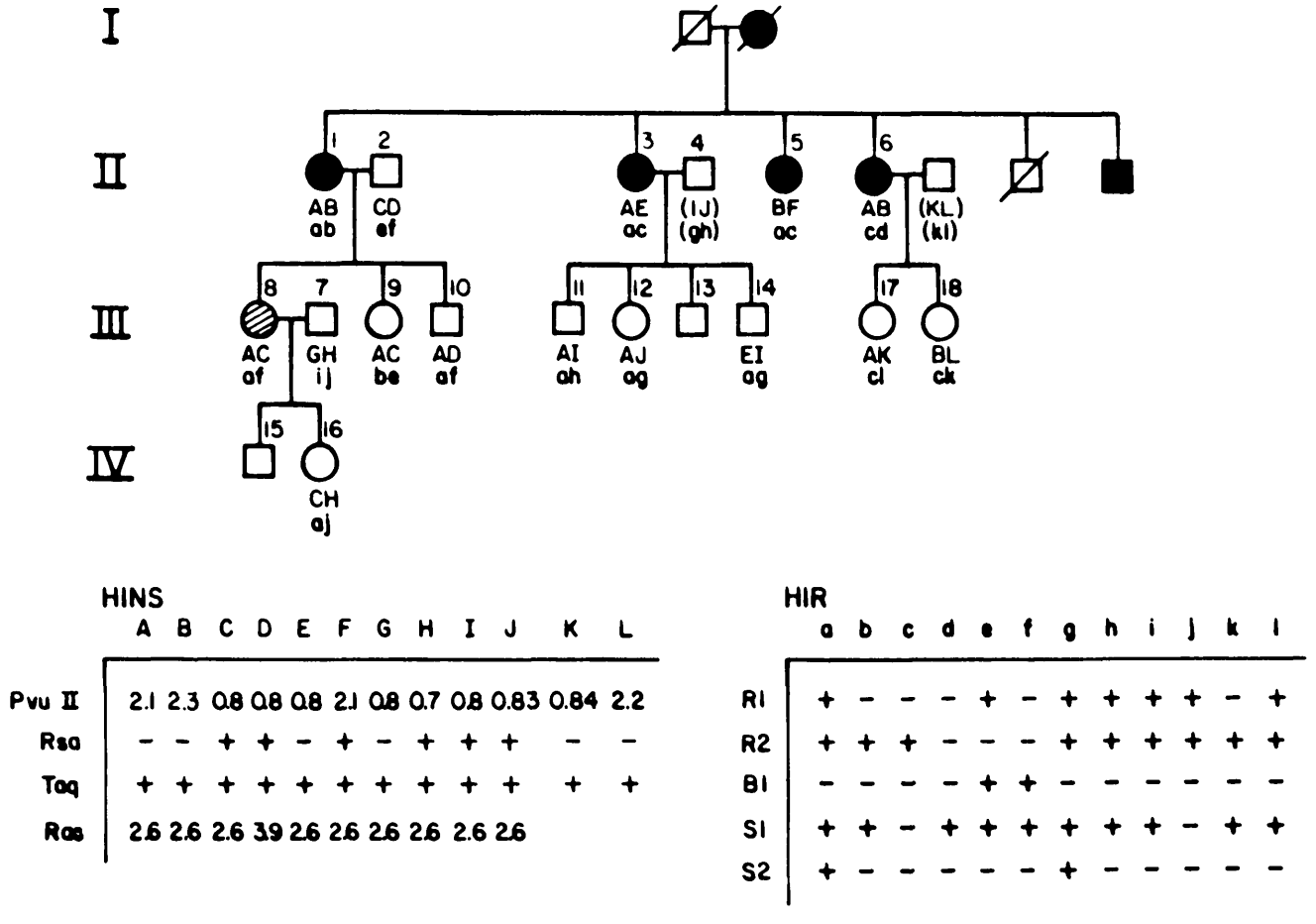

FIG 1. Pedigree of NIDDM family with linkage analysis. Four generations of pedigree are shown. □, Men; ○, women; *closed symbols*, individuals with NIDDM. III-8 (⊘) is proband and had had gestational diabetes before ascertainment of pedigree. Linkage analysis is shown for insulin gene (*Hins*) and insulin-receptor gene (*Hir*). For all available individuals, haplotypes were established as described, and each allele was assigned a letter, as shown in *bottom panel*, whether distinguished by haplotype or inheritance pattern. Haplotypes inferred from offspring are shown in parentheses.

available and not known to be diabetic at the time of study. No individuals were pregnant at the time of S_I determination.

Genetic analysis. For each member studied, lymphocytes were prepared from 10 ml of blood, as previously described (20), and transported to Washington University, St. Louis, MO, on dry ice for further analysis. High-molecular-weight DNA was prepared from lysed lymphocytes by proteinase K digestion, phenol extraction, and ethanol precipitation (20,21). Analyses for both insulin- and insulin-receptor–gene studies involved digestion of 5 μg DNA with 25 U of the appropriate restriction enzyme (obtained from New England Biolabs, Beverly, MA; Bethesda Research, Gaithersburg, MD; or IBI, New Haven, CT), followed by separation of the fragments by electrophoresis on 0.8–1.2% agarose gels and Southern transfer to nylon membrane (Zetabind, AMF Cuno, Marck, France). Appropriate probes were labeled by nick translation (20) to specific activities of 5×10^8 to 2×10^9 with ^{32}P-labeled dCTP (New England Nuclear, Boston, MA; or ICN, Covina, CA). Southern blots were hybridized according to manufacturers' specifications and were subsequently exposed to X-ray film (Eastman-Kodak, Rochester, NY) for 7–10 days as previously described (12,14,20).

Studies of the insulin gene included the region of tandem repeats, known as the 5′-flanking polymorphism (5′FP) (12), as analyzed with the restriction endonuclease *Pvu*II and the specific probe p*Pvu*II 1.3 previously described (12). Additionally, RFLPs at *Rsa*I and *Taq*I sites [−13- and −11-kilobase (kb) pairs, respectively] were detected with a second probe, pHINS 6.0 (12), and a region of tandem repeats at the *c-Ha-Ras* oncogene locus (HRAS) near the insulin gene was a fourth RFLP (22). For that analysis, Southern blots of *Taq*I digests were hybridized with the HRAS probe pTBB2. These four polymorphisms may be treated as one locus for genetic analysis, although a small recombination frequency (3%) has been observed between the insulin and *HRAS* loci (23). Heterozygosity for this locus, a measure of the informativeness for genetic studies, approaches 100% (unpublished data).

Insulin-receptor–gene studies were performed with two probes constructed from the cDNA probe pHIR 12.1 (provided by A. Ullrich, Genentech, South San Francisco, CA). Probe 12.1B1.3 helped to detect two RFLPs with *Rsa*I, which have been called R1 and R2. The second probe, 12.1P1.6, helped to reveal two RFLPs with the restriction endonuclease *Sac*I (S1 and S2) and one RFLP with the restriction endonuclease *Bgl*II (B1), as previously described (14). Although the exact location of these RFLPs is not known, they appear to be within 20 kb of each other, and no recombination between these markers has been observed (S.C.E., unpublished data). Heterozygosity is 0.86 for this locus in Caucasians, and allelic frequencies for this group have been reported (14).

Linkage analysis. For both insulin and insulin-receptor loci, haplotypes were established with the RFLPs described and mendelian inheritance to establish marker phase. Linkage analysis was performed under the assumption of no recombination between the candidate gene and the disease locus ($\theta = 0$). For both insulin-gene and insulin-receptor–gene studies, all alleles could be determined from the haplotypes without ambiguity. Thus, linkage was considered to be disproved if the affected individuals (known to be diabetic) failed to share any haplotypes. This analysis did not include any penetrance function or sporadic frequency, and for purposes of the study, linkage was determined without the inclusion of potentially prediabetic individuals in the third generation.

Determination of S_I. Tissue sensitivity to insulin was estimated with an intravenous glucose tolerance test (IVGTT) from which S_I was calculated with the minimal model of insulin action developed by Bergman et al. (24). Since 1985, the test has been modified by the addition of a bolus of tolbutamide given 20 min after the initial glucose bolus; this change causes a second rise in endogenous insulin secretion and improves parameter identification without altering the fundamental S_I value (25). Four of 5 members of the current pedigree and 27 of 46 controls received the tolbutamide modification.

The procedure for the tolbutamide-modified IVGTT was as follows: a glucose bolus (11.4 i.v. g/m^2 body surface area) was given at time 0, and a tolbutamide bolus (Upjohn, Kalamazoo, MI) of 150 mg/m^2 i.v. was given at 20 min. Blood samples (4 ml) for measurement of glucose and insulin were obtained from a wrist vein (arterialized by a 60°C hand-warming box) at −20, −15, 0, 2, 3, 4, 5, 6, 8, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. The glucose was measured in all samples by the glucose oxidase method, and insulin was measured by a modification of the double-antibody method of Morgan and Lazarow (26). Individual S_I values were calculated as previously described (24,25).

In addition to analysis of pedigree members, S_I values were obtained from 64 subjects without family histories of diabetes. The age of these subjects averaged 30 ± 7 yr (means ± SD), which was similar to the age of the pedigree members (28 ± 5 yr). S_I values for the controls were plotted as a function of percentage of ideal body weight (relative body weight, RBW), and the inverse correlation between S_I and RBW in this group was highly significant ($P < .00005$; Fig. 2). Analysis of covariance helped to assess whether S_I in the pedigree members was significantly lower than expected for their RBW. Because the wide range of S_I with obesity made comparison of individual values impossible, analyses were performed by comparing pedigree members as a group with the control population.

Islet function. Studies of islet function were performed as previously described (9,27). After an overnight fast, subjects were given 5 g i.v. arginine hydrochloride (Aginomoto, Los Angeles, CA), and the incremental insulin response at 2–5 min was determined. An intravenous glucose challenge of 300 mg/kg was administered 30 min later, and the 2- to 5-min incremental acute insulin response (AIR) to glucose was determined. After an additional 30 min, the glucose level was clamped at 500–600 mg/dl, and a final bolus of arginine was administered. This final AIR (the AIR_{max}) represents the maximal potentiation by glucose of the insulin response to a nonglucose stimulus (arginine) (27,28). The AIR to glucose was also calculated as a percentage of the basal insulin level, because individuals with higher basal insulin levels would be expected to have higher insulin responses to glucose. All values were compared with those obtained from nondiabetic individuals of similar age who had no family history of diabetes (Table 1).

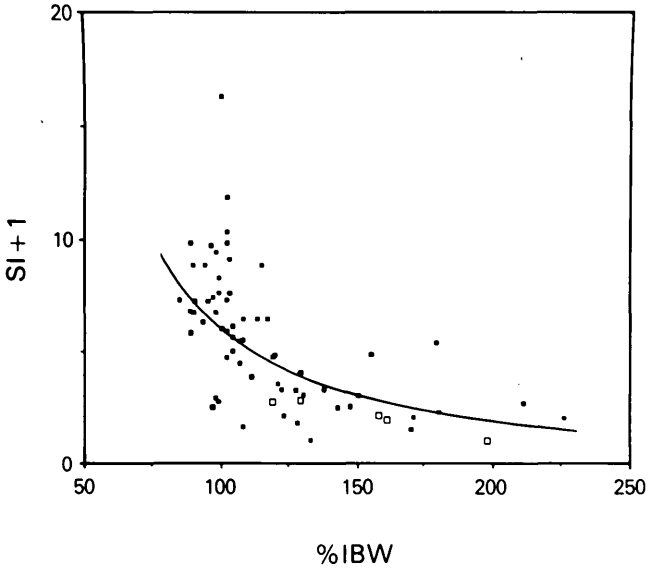

FIG 2. Insulin-sensitivity index (S_I) values of pedigree members (□) and control individuals (■) relative to regression line of normal S_I values. Although individual pedigree members did not fall outside normal limits, S_I of pedigree members studied was significantly lower than expected for their degree of adiposity when compared as group (P = .01).

RESULTS

Insulin sensitivity and islet cell function. The clinical data and the results of the glucose-potentiation studies are shown in Table 1 for six members of the third generation whose ages range from 23 to 30 yr. The S_I data are shown in Fig. 2 relative to control individuals. Individual III-8, the proband, had a history of gestational diabetes and subsequently developed fasting hyperglycemia. Individual III-9 became pregnant during the study and had a normal 50-g IVGTT in her 2nd trimester (MATERIALS AND METHODS). The results show that when analyzed as a group, the five family members tested had a significantly lower S_I compared with control individuals (P = .01; Fig. 2), although Fig. 2 shows that the individual S_I values for the pedigree members could not be distinguished from those of the control individuals and could not be directly compared with each other. Nonetheless, these data suggest that the family had primary insulin resistance out of proportion to obesity when nondiabetic members were compared as a group with the control subjects.

In contrast, AIR_{max}, which has proved to be a good measure of islet function (27,28), was indistinguishable from normal in this family (Table 1). However, two individuals (8 and 9) had a reduced first-phase insulin response, both when the AIR to glucose was expressed as an absolute insulin level and when expressed as a percentage of the basal insulin level.

With the exception of the first-phase insulin response, the physiological studies did not provide evidence for different defects among the two siblings and three first cousins. AIR_{max} did not differ significantly from normal in the pedigree members studied. Because AIR_{max} has been shown to be inversely related to S_I (9), an analysis of variance was performed to examine the AIR_{max} corrected for S_I. This analysis also did not show significant differences among siblings or from the normal population. Whether the inability to identify differences in the physiological (phenotypic) parameters in these family members, and thus patterns of segregation in these measurements, was the result of identical underlying genotypes or of the inability of these measurements to accurately distinguish differing individual genotypes could not be evaluated.

Linkage analysis. Analyses of linkage to both the insulin gene and the insulin-receptor gene were performed with previously described RFLPs for both loci (12,14) and are shown with the pedigree in Fig. 1. For the insulin locus, the two regions of length polymorphism (the insulin gene 5′FP, designated *Pvu*II in Fig. 1, and the HRAS polymorphism) are denoted by the size of the allele in kilobase pairs, and the *Taq*I and *Rsa*I polymorphisms are marked for the presence (+) or absence (−) of the site. For the insulin-receptor gene, all but the S1 polymorphism represent the presence or absence of a restriction enzyme site and are similarly denoted + or −. The S1− allele represents an insertion of 0.5 kb (S.C.E., unpublished data), and the S1+ allele represents the absence of that insertion.

For the insulin gene, 12 haplotypes were identified; A, B, E, and F originated in the first generation (Fig. 1). Were linkage of the insulin gene and diabetes present, one of these four haplotypes (representing one of the first-generation alleles) would have been present in all of the affected individuals, including the proband (diabetic) and probably individual III-9 (apparently prediabetic). In contrast, diabetic individuals II-1 and II-5 shared only allele B, whereas only allele A was shared by the other diabetic pedigree members.

TABLE 1
Clinical data and islet function

Pedigree no.	Age (yr)	Percent IBW	FPG (mg/dl)	Basal IRI (μU/ml)	AIR_{arg} (μU/ml)	AIR_{max} (μU/ml)	AIR to glucose (absolute/% of basal)
8	30	198	112	30	69	540	48/160
9	24	158	108	23		305	7/30
10	36	139	95	10	27	494	78/780
11	23	119	86	13	69	387	107/823
12	26	129	88	23	94	415	126/548
14	28	161	91	12	46	413	141/1175
Normal range	24–38	96–221	82–103	2–19	27–125	125–645	23–324/196–2083
Means ± SD	33 ± 4	139 ± 41	91 ± 6	11 ± 6	63 ± 25	344 ± 152	100 ± 80/1066 ± 511

Insulin secretion measurements in pedigree members and in a group of normal subjects without family histories of diabetes (n = 17). IBW, ideal body weight; FPG, fasting plasma glucose; IRI, immunoreactive insulin; AIR_{arg}, acute insulin response to arginine obtained at euglycemia; AIR_{max}, AIR to arginine obtained at a maximally potentiating glucose level of 500–600 mg/dl.

Thus, the analysis is inconsistent with any major role for insulin-gene defects in the etiology of diabetes in this family.

Similar analysis of the insulin-receptor gene likewise identified 12 haplotypes, of which a–d distinguish the alleles of the first generation (Fig. 1). Once again, if an inherited insulin-receptor defect were responsible for the familial insulin resistance, the same defective allele, as denoted by the haplotype (a, b, c, or d), would be shared by all affected individuals. No haplotype met this condition, because diabetic individuals II-1 and II-6 shared no allele (Fig. 1). This study is thus not compatible with a mutation of the insulin receptor causing the predisposition to NIDDM in this pedigree.

DISCUSSION

Although the genetic predisposition to NIDDM is well recognized (1,2,29), most studies of the pathophysiology of this disease have been done with unrelated individuals. Consequently, any abnormalities of insulin secretion or insulin action in NIDDM have been studied in individuals who fully express the diabetes phenotype. The limitations of such studies become clear when the secondary changes of insulin-receptor number, postreceptor insulin action, and islet function are considered (7,8,30–32). Thus, although studies of diabetic individuals may adequately describe the events that characterize the fully developed disease, they are unlikely to determine the primary lesion. Alternatively, the study of the genetics of NIDDM without clinical characterization avoids the confusion caused by secondary changes in the physiological parameters but is nonetheless limited by the inability to identify nondiabetic individuals at risk and to characterize families according to islet dysfunction or insulin resistance.

A possible solution to these dilemmas lies in the combination of physiological and genetic studies to examine the clinical characteristics of individuals at risk but who are less likely to have the primary defect obscured by secondary changes. The genotype of specific candidate genes may thus be correlated with the phenotype of potentially diabetic individuals, and linkage analysis may be extended to prediabetic individuals. Additionally, the study of physiological parameters in pedigrees may permit the identification of defects that segregate as genetic traits, suggesting primary rather than secondary changes.

The pedigree we studied is of particular interest because the proband formerly had gestational diabetes. In the setting of a striking family history of NIDDM, the occurrence of gestational diabetes probably represents the early evidence of an underlying genetic predisposition to NIDDM. The presence of diabetes in three generations and in over half of the individuals in the second generation strongly suggests autosomal-dominant inheritance. It is unknown whether pedigrees such as this one, ascertained through a gestational or former gestational-diabetic proband, actually represent a unique subset of NIDDM or whether they are typical NIDDM pedigrees that demonstrate a unique pattern of inheritance only as the result of a bias introduced by selecting young individuals at risk for NIDDM. A demonstration of transmission through three generations may be possible only when an early onset of NIDDM is detected. On the other hand, the former possibility is suggested by the evidence for a defect in insulin action found in this study and of the similar defect found in the previous study of individuals with a history of gestational diabetes (9). An autosomal-dominant pattern of inheritance has been noted in MODY (3) and in four Caucasian pedigrees not ascertained for gestational diabetes but in which an individual with gestational diabetes was present (unpublished data).

The presence of insulin resistance as determined by decreased S_I in individuals who had developed gestational diabetes and yet did not have overt diabetes at the time of study (9) may suggest a primary defect in insulin action. When examined as a group, the individuals in the pedigree we studied demonstrated a statistically diminished S_I out of proportion to their obesity, and thus may have a similar defect. Unfortunately, the normal range of S_I values, adjusted for obesity, was sufficiently large to prevent the determination of the degree to which an individual value represented normal insulin sensitivity (Fig. 2). Thus, we could not identify individuals with impaired insulin action or the segregation of such a defect. Additionally, although we suspect that the low S_I values detected in this pedigree or in other studies represent a predisposition to NIDDM, this belief remains unproved, and a low S_I would probably not be predictive for a given individual. The biological significance of these findings in the present pedigree is therefore uncertain.

Some defects in β-cell function (diminished first-phase insulin release) were noted in the study of unrelated former gestational-diabetic subjects (9), and similar defects were noted in the proband (individual III-8) and her sister (individual III-9) in this study. Acute-phase insulin release (AIR to glucose) is known to be a sensitive indicator of early NIDDM or β-cell dysfunction from a variety of causes and appears to be reversible with insulin treatment (27). Thus, this finding may indicate only early β-cell decompensation, but not a primary defect. The AIR_{max}, which is arguably a better measurement of β-cell reserve than of stored insulin (27,28), was not significantly different from normal in the individuals of this pedigree, as in the study of women with histories of gestational diabetes (9). We thus found little evidence for a primary defect of insulin secretion. Nonetheless, the failure to distinguish pedigree members with a normal S_I could be the result of a similar defect causing insulin resistance in all family members, with the altered first-phase insulin release representing the primary predisposing lesion.

The linkage studies demonstrated no linkage to either the insulin gene or the insulin-receptor gene in the current pedigree and thus eliminate these two loci as possible causes of the diabetes in this pedigree. These conclusions are independent of the physiological studies. If the diminished S_I is indeed evidence of inherited insulin resistance, the linkage data thus suggest a mutation in one of the genes necessary for the postreceptor chain of insulin action or in a gene that regulates the insulin receptor or the other events of insulin action. The linkage conclusions rest only on the analysis of the overtly diabetic individuals in the second generation, who share no alleles for either locus (insulin or insulin receptor). Nonetheless, these conclusions could be wrong if the second-generation diabetic members who did not share an allele represented sporadic (noninherited) cases of NIDDM or if they represented inherited diabetes from a different defect or locus. Because the gene frequency

and rate of sporadic cases in this disease are unknown, particularly in familial NIDDM, the probability of erroneously rejecting the linkage hypothesis is uncertain and, we would expect, very low.

Lack of linkage of diabetes and the insulin locus has been noted in MODY (15,16) and in 10 other large NIDDM pedigrees (17,19; unpublished observations), although none of these studies included pedigrees ascertained through a gestational-diabetic individual. Interestingly, individuals III-8 and III-9, both of whom have reduced first-phase insulin secretion, share both insulin alleles, but this sharing would occur by chance, with a probability of .25. Defects in insulin action have been described in families with extreme insulin resistance, such as leprechaunism and type A insulin resistance associated with acanthosis nigricans (30,31). In many of those families, the defect appears to result from a homozygous mutation of the insulin receptor resulting in diminished receptor number or affinity (31). Much of the insulin resistance in NIDDM is clearly secondary (5,6,31,32), and inherited insulin resistance in typical NIDDM pedigrees has not been convincingly demonstrated. This study demonstrates the difficulty in distinguishing individual values of S_I and thus of proving inheritance or segregation of physiological parameters. Nonetheless, although the study of one pedigree at best provides only a clue to the etiology of NIDDM in the majority of families, the combination of methods we have used suggests an approach that may succeed in the larger endeavor.

ACKNOWLEDGMENTS

We thank Lynn Corsetti for excellent assistance in DNA extractions and restriction endonuclease analyses, Gina Wade for performing insulin assays, and Dave McCullough and Brad Wallum for their contribution of data to the control insulin-sensitivity ranges.

S.C.E., J.C.B., and W.K.W. are supported by the research service of the Veterans Administration Medical Center. S.C.E. is also supported by a grant from the Juvenile Diabetes Foundation. This work was supported by National Institutes of Health Grants AM-31866 and AM-16746 (M.A.P.) and AM-12829 and AM-17047 (J.C.B., W.K.W.).

REFERENCES

1. Barnett AH, Eff C, Leslie RDG, Pyke DA: Diabetes in identical twins. *Diabetologia* 20:87–93, 1981
2. Rotter JI, Rimoin DL: Genetics. In *Handbook of Diabetes Mellitus*. Brownlee M, Ed. New York, Garland, 1980, p. 3–93
3. Tattersall RB, Fajans SS: A difference between the inheritance of classical juvenile-onset and maturity-onset diabetes in young people. *Diabetes* 24:44–53, 1975
4. Fajans SS, Cloutier MC, Crowther RL: Clinical and etiologic heterogeneity of idiopathic diabetes mellitus. *Diabetes* 27:1112–25, 1978
5. Reaven GM: Non-insulin-dependent diabetes mellitus: metabolic characteristics. *Metabolism* 29:445–54, 1980
6. DeFronzo RA, Ferrannini E: The pathogenesis of non-insulin dependent diabetes. *Medicine* 61:125–40, 1980
7. Garvey WT, Olefsky JM, Griffin J, Hamman RF, Kolterman OG: The effect of insulin treatment on insulin secretion and insulin action in type II diabetes mellitus. *Diabetes* 34:222–34, 1985
8. Hidaka H, Nagulesparan M, Klimes I, Clark R, Sasaki H, Aronoff SL, Vasquez B, Rubenstein AH, Unger RH: Improvement of insulin secretion but not insulin resistance after short-term control of plasma glucose in obese type II diabetics. *J Clin Endocrinol Metab* 54:217–22, 1982
9. Ward WK, Johnston CLW, Beard JC, Benedetti TJ, Halter JB, Porte D Jr: Insulin resistance and impaired insulin secretion in subjects with histories of gestational diabetes mellitus. *Diabetes* 34:861–69, 1985
10. Bell GI, Pictet RL, Rutter WJ, Cordell B, Tischer E, Goodman HM: Sequence of the human insulin gene. *Nature* (Lond) 284:26–32, 1980
11. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao Y-C, Tsubokawa M, Mason A, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J: Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* (Lond) 313:756–61, 1985
12. Elbein SC, Corsetti L, Permutt MA: New polymorphisms at the insulin locus increase its usefulness as a genetic marker. *Diabetes* 34:1139–44, 1985
13. Chakravarti A, Elbein SC, Permutt MA: Evidence for increased recombination near the human insulin gene: implication for disease association studies. *Proc Natl Acad Sci USA* 83:1045–49, 1986
14. Elbein SC, Corsetti L, Ullrich A, Permutt MA: Multiple restriction fragment length polymorphisms at the insulin receptor locus: a highly informative marker for linkage analysis. *Proc Natl Acad Sci USA* 83:5223–27, 1986
15. Owerbach D, Thomsen B, Johansen K, Lamm LU, Nerup J: DNA insertion sequences near the insulin gene are not associated with maturity-onset diabetes of young people. *Diabetologia* 25:18–20, 1983
16. Andreone T, Fajans S, Rotwein P, Skolnick M, Permutt MA: Insulin gene analysis in a family with maturity-onset diabetes of the young. *Diabetes* 34:108–14, 1985
17. Elbein SC, Corsetti L, Permutt MA: Linkage analysis of the insulin gene and insulin receptor gene in diabetic families (Abstract). *Diabetes* 35 (Suppl. 1):76A, 1986
18. Elbein SC, Borecki I, Corsetti L, Fajans SS, Hansen AT, Nerup J, Province M, Permutt MA: Linkage analysis of the human insulin receptor gene and maturity onset diabetes of the young. *Diabetologia* 30:641–47, 1987
19. Hitman GA, Jowett NI, Williams LG, Humphries WS, Winter RM, Galton DJ: Polymorphisms in the 5′ flanking region of the insulin gene and non-insulin dependent diabetes. *Clin Sci* 66:383–88, 1984
20. Elbein S, Rotwein P, Permutt MA, Bell GI, Sanz N, Karam JH: Lack of association of the polymorphic locus in the 5′-flanking region of the human insulin gene and diabetes in American Blacks. *Diabetes* 34:433–39, 1985
21. Maniatas T, Fritsch F, Sambrook J (Eds.): *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY, Cold Spring Harbor, 1982
22. Goldfarb M, Shimizu K, Perucho M, Wigler M: Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. *Nature* (Lond) 296:404–409, 1982
23. White R, Leppert M, Bishop DT, Barber D, Berkowitz J, Brown C, Callahan P, Holm T, Jerominski L: Construction of linkage maps with DNA markers for human chromosomes. *Nature* (Lond) 313:101–105, 1985
24. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and β-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest* 68:1456–67, 1981
25. Beard JC, Bergman RN, Ward WK, Porte D Jr: The insulin sensitivity index in nondiabetic man, correlation between clamp-derived and IVGTT-derived values. *Diabetes* 35:362–69, 1986
26. Morgan CR, Lazarow A: Immunoassay of insulin: two antibody system: plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes* 12:115–26, 1963
27. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D Jr: Diminished β cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Invest* 74:1318–28, 1984
28. Ward WK, Beard JC, Halter JB, Pfeifer MA, Porte D Jr: Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. *Diabetes Care* 7:491–502, 1984
29. Rotter JI, Rimoin DL: The genetics of the glucose intolerance disorders. *Am J Med* 70:116–26, 1981
30. Grunberger G, Taylor SI, Dons RF, Gordon P: Insulin receptors in normal and disease states. *Clin Endocrinol Metab* 12:191–219, 1983
31. Taylor SI: Receptor defects in patients with extreme insulin resistance. *Diabetes Metab Rev* 1:171–205, 1985
32. Truglia JA, Livingston JN, Lockwood DH: Insulin resistance: receptor and post-binding defects in human obesity and non-insulin-dependent diabetes mellitus. *Am J Med* 979 (Suppl. 2B):13–21, 1985