Polymorphic Microsatellite Repeat Markers at the Glucokinase Gene Locus Are Positively Associated With NIDDM in Japanese

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To assess the possible role of glucokinase defects contributing to a genetic susceptibility to NIDDM in Japanese, allelic frequencies of two microsatellite repeat polymorphisms, one in the 3′-flanking region (GCK1) and the other in the 5′-flanking region (GCK2) of the human glucokinase gene, were analyzed in subjects with NIDDM and in nondiabetic control subjects. After typing 107 diabetic and 74 nondiabetic subjects, we found four GCK1 alleles (Z, Z2, Z4, Z6) and six GCK2 alleles (0, −4, −2, 2, 4, 8). The frequency distribution of GCK1 alleles was different between the two groups ($P = 0.005$), although not significant after correction for multiple comparisons. The Z4 allele was found more frequently in diabetic than in nondiabetic subjects (23 vs. 10%, $P = 0.002$). This was still significant after correction for multiple comparisons ($P < 0.05$). The frequency distribution of GCK2 alleles was not different between the two groups. However, the −2 allele was more common in diabetic than in nondiabetic subjects ($P = 0.044$), although not significant after adjusting for multiple comparisons. Clinical characteristics were compared between the diabetic subjects with Z4 and/or −2 allele and those without either of these two alleles. No differences were found in the age of diagnosis, positive family history, mode of therapy, current HbA_{1c}, or daily urinary C-peptide immunoreactivity excretion between the two groups. We demonstrated a significant association between GCK1 and GCK2 alleles and NIDDM. The results indicate that the polymorphic alleles GCK1 and GCK2 could be genetic markers in NIDDM in Japanese, suggesting a relationship between glucokinase defects and the susceptibility to NIDDM in this population.
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NIDDM, one of the most common metabolic diseases in humans, is a complex metabolic disorder that is characterized by abnormal insulin secretion and insulin resistance (1,2). NIDDM occurs in various racial groups with marked differences in prevalence: 2–4% in Caucasians, 4–6% in African Americans, 10–15% in Mexican Americans, and 35% in Pima Indians (3,4). The prevalence of NIDDM in Japanese is relatively low when living a traditional rural lifestyle but increases after migration and modernization (5–7). This evidence suggests that NIDDM occurs in a suitable genetic background, provoked by environmental factors. In addition, the high prevalence of NIDDM in those with a positive family history and the extremely high concordance (>90%) in monozygotic twins (8) support a hypothesis that genetic components play an important role in the pathogenesis of NIDDM. Nonetheless, the mode of inheritance and the primary pathophysiological defects remain uncertain because of genetic heterogeneity within the NIDDM phenotype, resulting from the interaction of multiple genetic and environmental factors.

One way to assess the genes involved in the predisposition to the disease would be the identification of the genetic marker at a particular locus through population studies (9). To search for a defective gene that contributes to the susceptibility to NIDDM, several candidate genes have been investigated in some racial groups using RFLPs, including insulin (10,11), insulin receptor (12,13), glucose transporter isoforms (14–17), and various apolipoprotein genes (18,19). Definitive conclusions, however, have not been obtained, suggesting that these loci are not major contributors to the disease.

The enzyme glucokinase (EC 2.7.1.1) is a unique

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NIDDM, non-insulin-dependent diabetes mellitus; CPR, C-peptide immunoreactivity; RFLP, restriction fragment-length polymorphism; G-6-P, glucose-6-phosphate; kb, kilobase; MODY, maturity-onset diabetes of the young; BMI, body mass index; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

member of the hexokinase family because of its functional characteristics; glucokinase differs functionally from other hexokinases in its greater specificity and higher K_m for glucose, and product inhibition by G-6-P (20–22). This gene is expressed only in liver and pancreatic islet β-cells, where glucokinase-dependent glucose phosphorylation is proportional to plasma glucose concentration (21–23). Thus, glucokinase probably plays important roles as a glucose sensor in glucose-induced insulin secretion in the pancreatic β-cell and as a key metabolic regulator in glucose utilization in the liver (22). The lack of glucokinase activity may induce the metabolic disorders of NIDDM characterized by impaired insulin secretion and deranged glucose metabolism, suggesting a possible contribution of this gene to the genetic susceptibility to NIDDM.

To study this candidate gene, we isolated human liver and islet cDNAs (24,25) and gene (26,27) and identified two microsatellite repeat regions (GCK1 and GCK2) composed of variable dinucleotide repeats: one is located at 6 kb upstream of islet exon 1 (GCK2) and the other ~8 kb downstream of exon 10 (GCK1) of the gene. These dinucleotide repeats were polymorphic and were used to map the gene to chromosome 7p (26). With the use of these two microsatellite polymorphisms, close linkage was first disclosed between the glucokinase locus and MODY, a subtype of NIDDM (28,29). Subsequently, mutations in the structural region of glucokinase gene were identified in these MODY pedigrees (30–33). In the common type of NIDDM, however, the role of glucokinase defects is less certain, although association between the microsatellites in the glucokinase locus and NIDDM was observed in two populations (34,35). In this study, we attempted to assess the contribution of the glucokinase gene defects to the genetic susceptibility to NIDDM in a Japanese population. The frequencies of these two polymorphisms at this locus were compared between the patients with NIDDM and nondiabetic individuals. We report a significant association between these markers and NIDDM in Japanese, suggesting contribution of possible glucokinase defects to the genetic susceptibility to NIDDM in this population.

RESEARCH DESIGN AND METHODS

Study samples and DNA preparation. A total of 107 individuals with NIDDM, according to the criteria of the Japanese Diabetes Association, were recruited from the outpatient clinics at Yamaguchi University Hospital and its affiliated hospitals. Nondiabetic subjects ($n = 74$) were recruited from the same clinics or from staff members of those hospitals according to the following criteria: >40 yr of age, no personal or family history of diabetes mellitus, and a random plasma glucose of <6.7 mM. Both diabetic and nondiabetic subjects were all unrelated Japanese living in the same region (Yamaguchi prefecture) where genetic admixture was not prevalent. At the time of recruitment, informed consent was obtained from each individual according to a protocol approved by the Human Studies Committee. The clinical information (age, sex, weight, height, and family history of diabetes) was collected directly from the subjects or from the medical records. A positive family history was defined as diabetes diagnosed in parents, grandparents, siblings, uncles, or aunts. Genomic DNA was extracted from white blood cells as reported previously (16).

PCR amplification of microsatellite repeat (CA)n regions. Both GCK1 and GCK2 were PCR amplified directly from genomic DNA and scored unambiguously on the autoradiogram as described previously (26,27). To confirm the molecular basis of GCK1 and GCK2, direct genomic sequencing was also conducted as described previously (26).

Statistical analysis. Differences in the frequencies of the quantitative variables, such as age, weight, height, and BMI between the diabetic and nondiabetic (control) samples were tested using unpaired, two-tailed Student's *t* tests. Genotypic and allelic frequencies for each group were estimated from the observed data. Genotypic frequencies were tested for Hardy-Weinberg equilibrium, i.e., disequilibrium coefficients were calculated as the differences between observed and expected genotypic frequencies and tested for departure from zero (36). Global tests for frequency differences of the alleles between the two groups were performed for 2 × 4 (GCK1) and 2 × 6 (GCK2) tables. In addition, individual allelic frequency differences were tested using 2 × 2 contingency tables for each allele by combining the remaining alleles into one category. All frequency differences between the two samples were tested using two-tailed Fisher exact tests.

To assess whether a difference existed in the pattern of allelic association between the two groups, two-locus haplotypes were formed between the GCK1 and GCK2 loci, and gametic frequencies were estimated and tested for group differences. Because it is not usually possible to determine the phase, and hence the haplotypes, of double heterozygotes, gametic frequencies were estimated using both the myriad haplotype algorithm (37) and the ASSOCIATE program (38). Both of these programs assume Hardy-Weinberg equilibrium. These estimated frequencies were also used to test for gametic disequilibrium within each group. The gametic disequilibrium coefficient is defined as the difference between an observed haplotype frequency and its expected frequency at gametic equilibrium (36).

Bonferroni's corrections are frequently used to protect against the possibility of obtaining a significant result purely by chance when performing multiple, independent comparisons. Under this method, if n comparisons are made and α is the type I error rate desired, a significant result for any one of the comparisons occurs if the P value is $\geq \alpha/n$. Hence, if α of 0.05 is desired and four tests of significance are performed, then the significance level for any one test should not be $>0.05/4 = 0.0125$. Note that use of this method increases the type II error as well as masking what may be a significant finding (39). Accordingly, we report the uncorrected P value of the statistic in the tables, and when discussing significance, report whether significance is attained using the Bonferroni correction; our subsequent analyses includes 26 comparisons. Thus, by reporting the uncorrected P values,

TABLE 1
Clinical characteristics of study subjects

	Diabetic subjects	Nondiabetic subjects
n	107	74
Age (*yr*)	59 ± 11*	49 ± 7.4
Age of diagnosis (*yr*)	50 ± 12	—
Female (%)	56	62
Weight (kg)	56.0 ± 9.0	57.7 ± 10.5
Height (cm)	156.0 ± 9.2	160.0 ± 7.4
BMI (kg/m^2)	23.0 ± 3.6	22.5 ± 3.2
Family history of diabetes (%)	28	0

Data are means ± SD.
*$P < 0.001$.

independent verification of a finding may be replicated without the need for such stringent criteria.

RESULTS

Clinical characteristics of study subjects. Clinical characteristics of the 107 diabetic and 74 nondiabetic subjects are summarized in Table 1. The average age in the diabetes group was significantly higher than that in the control group ($P < 0.001$). No differences were noted in sex distribution or BMI between the two groups. Of diabetic subjects, 28% had a family history of NIDDM.

Polymorphisms of the microsatellite repeat regions. Autoradiography of PCR products revealed four different alleles for GCK1 and six different alleles for GCK2 in Japanese (Table 2). DNA fragments of 195 nucleotides (Z allele) in GCK1 and 217 nucleotides (0 allele) in GCK2 were defined as the common alleles. The alleles varied in size from 0 to 6 nucleotides with respect to the Z allele (Z, Z2, Z4, and Z6), and from −4 to 8 nucleotides with respect to 0 allele (−4, −2, 0, 2, 4, and 8). We confirmed the molecular basis of dinucleotide repeat regions by direct sequencing of PCR products. Each repeat region was found to have a number of dinucleotide repeats that would be called a compound imperfect repeat according to the classification of Weber (40). By typing a total of 181 subjects, seven genotypes were identified for GCK1 and nine genotypes for GCK2. The frequencies of each genotype identified are shown in Table 2. No departure from Hardy-Weinberg equilibrium at either the GCK1 or GCK2 loci was detected (data not shown).

TABLE 2
Estimated genotypic frequencies at GCK1 and GCK2 loci

Alleles	Diabetic subjects	Control subjects
n	107	74
GCK1 marker		
Z/Z	0.38 (41)	0.38 (28)
Z/Z2	0.19 (20)	0.38 (28)
Z/Z4	0.21 (22)	0.15 (11)
Z2/Z2	0.05 (5)	0.03 (2)
Z2/Z4	0.10 (11)	0.05 (4)
Z2/Z6	0.00 (0)	0.01 (1)
Z4/Z4	0.08 (8)	0.00 (0)
GCK2 marker		
−4/0	0.01 (1)	0.01 (1)
−2/0	0.14 (15)	0.05 (4)
−2/2	0.07 (7)	0.03 (2)
0/0	0.47 (50)	0.53 (39)
0/2	0.29 (31)	0.37 (27)
0/4	0.01 (1)	0.00 (0)
0/8	0.01 (1)	0.00 (0)
2/2	0.01 (1)	0.01 (1)

n, total number of genotypes in samples at each locus. Observed genotypes in samples at each locus are in parenthesis.

TABLE 3
Estimated allele frequencies at GCK1 and GCK2 loci

Alleles	Diabetic subjects	Control subjects	*P* value
n	214	148	
GCK1 marker			
Z	0.58 (124	0.64 (95)	0.274
Z2	0.19 (41)	0.25 (37)	0.195
Z4	0.23 (49)	0.10 (15)	0.002*
Z6	0.00 (0)	0.01 (1)	0.409
GCK2 marker			
−4	0.01 (1)	0.01 (1)	1.000
−2	0.10 (22)	0.04 (6)	0.044*
0	0.70 (149)	0.74 (110)	0.346
2	0.19 (40)	0.21 (31)	0.593
4	0.01 (1)	0.00 (0)	1.000
8	0.01 (1)	0.00 (0)	1.000

n, total number of alleles in samples at each locus. Observed numbers of alleles in samples at each locus are in parentheses. Global $P = 0.005$ for GCK1; Global $P = 0.167$ for GCK2 (Fisher's exact test).
*$P = 0.05$ significant.

Frequencies of the microsatellite repeat alleles and haplotypes in NIDDM and control subjects. The frequencies of the alleles at GCK1 and GCK2 in the diabetic and control samples are estimated from genotypic frequencies (Table 2) and used for the analysis (Table 3). The global frequency distribution between the two groups differed at the GCK1 locus ($P = 0.005$, not significant after correction for multiple comparisons) but not at the GCK2 locus ($P = 0.167$). When the frequency of individual alleles was examined for group differences, the Z4 allele was found to occur more often than expected in the diabetic sample ($P = 0.002$). This result is still significant at the 0.05 level when adjusted for multiple comparisons. Examination of differences in allele frequencies between groups at the GCK2 locus revealed that the −2 allele occurred somewhat more frequently than expected in the diabetic sample ($P = 0.044$). This value is not significant when corrected for multiple comparisons.

We examined deviations from expected values between groups for each of the 14 haplotypes displayed in Table 4; slight differences occurred for the Z2/2 and Z4/0 haplotypes. Fewer Z2/2 haplotypes than expected were found in the diabetic group ($P = 0.034$) and somewhat more of the Z4/0 haplotype occurred in the diabetic sample ($P = 0.019$), although these were not significant after correcting for multiple comparisons. Because of the large number of haplotypes in this sample, performing an overall exact test was problematic even after collapsing or eliminating categories with small numbers of observations.

A distinction exists between the pattern of disequilibria in the diabetic and control subjects. Linkage disequilib-

TABLE 4
Estimated haplotype frequencies

Haplotypes	Diabetic subjects	Control subjects	*P* value
n	214	148	
Z/−4	0.00 (0)	0.01 (1)	0.409
Z/−2	0.03 (6)*	0.02 (3)	0.743
Z/0	0.42 (90)	0.52 (76)*	0.087
Z2	0.13 (27)	0.10 (15)	0.508
Z2/−2	0.02 (5)	0.00 (0)	0.082
Z2/0	0.13 (27)	0.16 (24)	0.359
Z2/2	0.03 (7)	0.09 (13)*	0.034†
Z2/4	0.01 (1)	0.00 (0)	1.000
Z2/8	0.01 (1)	0.00 (0)	1.000
Z4/−2	0.05 (11)*	0.01 (2)	0.083
Z4/0	0.15 (32)	0.07 (10)*	0.019†
Z4/2	0.03 (6)	0.02 (3)	0.743
Z4/−4	0.01 (1)	0.00 (0)	1.000
Z6/−2	0.00 (0)	0.01 (1)	0.410

n, total number of haplotypes in samples. Estimated numbers of haplotypes in samples are in parentheses.
*Linkage disequilibrium.
†$P = 0.05$ significant for group differences.

rium occurs for the Z/−2 and Z4/−2 haplotypes in the diabetic subjects ($P = 0.003$ and $P = 0.002$, respectively). Disequilibrium occurs in the control group for the Z/0, Z/2, and Z2/2 haplotypes ($P = 0.03$, $P = 0.04$, and $P = 0.01$, respectively). The observed linkage disequilibrium is significant at the 0.05 level after correcting for multiple tests in the diabetic group but not in the control group.

Clinical characteristics of NIDDM subjects with the −2 and/or Z4 allele. To determine whether Z4 and −2 alleles were associated with some specific clinical characteristics of NIDDM, the subjects with NIDDM were examined further. Of the 107 individuals in the NIDDM group, 81 were available for analysis of clinical data according to the presence or absence of these alleles. As shown in Table 5, no differences were observed in any clinical parameters between the subjects with Z4 and/or −2 alleles and the subjects with neither allele. Endogenous insulin secretion assessed by urinary excretion of CPR was compared between the two groups, and no significant difference was found. We examined the prevalence of hypertension, hyperlipidemia, and diabetic complications such as neuropathy, nephropathy, and retinopathy, and no differences were found between the two groups (data not shown). We also compared clinical features of 8 Z4 homozygous NIDDM patients (Table 2) with those of the patients who were not homozygous for Z4 allele, or those of the patients who did not have Z4 allele. No significant differences in the clinical characteristics were observed.

TABLE 5
Clinical characteristics of NIDDM patients with either Z4 or A−2 allele

	Z4 or A−2	Non-Z4/A−2
n	32	49
Female (%)	46.9	55.1
Age (yr)	57 ± 9	59 ± 11
Age of diagnosis (yr)	49 ± 10	50 ± 12
Positive family history of NIDDM (%)	29.2	28.6
BMI (kg/m^2)	22.6 ± 2.6	22.6 ± 3.5
HbA_{1c} (%)	8.8 ± 1.8	8.3 ± 1.7
Type of therapy		
Diet	8	22
Oral hypoglycemic agent	11	11
Insulin	13	16
Urinary CPR (μg/day)	62.1 ± 34.3 (7)	53.6 ± 40.2 (8)

Data are means ± SD (observed numbers).

DISCUSSION

Close linkage of the glucokinase locus and MODY was disclosed recently in French (28) and British (29) pedigrees, and subsequently, mutations of the glucokinase gene were identified in these pedigrees (30–33). It is now more clear that glucokinase defects are playing a role in this type of NIDDM. To assess the possible role of this gene in the genetic susceptibility to the common NIDDM in the Japanese population, we studied the association between the glucokinase locus and NIDDM using two polymorphic microsatellite markers.

In this study, we found a significant association of the markers of the glucokinase locus with NIDDM. The Z4 allele for GCK1 and the −2 allele for GCK2 were more common in NIDDM subjects than in control subjects (Table 2), and the association between Z4 allele and NIDDM remained significant even after adjusting for multiple comparisons. Haplotype frequencies were estimated and compared between the two groups to identify those haplotypes associated with the disease. Although the frequency of Z4/−2 haplotype was more than five times greater in the diabetic group than in the control group, this difference did not reach statistical significance ($P = 0.083$), possibly because of the small numbers of observations with this haplotype. However, note that the odds of having the Z4/−2 haplotype for those in the diabetic group versus those in the control group is ~4:1, whereas the ratios for having the Z4 allele or the −2 allele are ~2.5:1. Significant linkage disequilibrium between Z4 and −2 was demonstrated in the diabetic subjects. Thus the association observed between −2 allele and NIDDM might be a secondary effect of the linkage disequilibrium between these two alleles, and this haplotype may be in linkage disequilibrium with the putative mutation in diabetic subjects. Molecular analysis of the gene, by methods such as SSCP, will be necessary to clarify this possibility.

Population association studies are based on the assumption that a mutation causing a disease and a genetic marker are in linkage disequilibrium or that the marker is the disease locus (41). Positive associations do not establish linkage, however, and linkage analysis in families is required to firmly establish the role of a particular locus in a disease. A number of difficulties are involved in studying heterogeneous diseases like NIDDM in families, however (42–44). A population association study represents an alternative means of analysis. These studies can be performed without large multigenerational families that are often difficult to obtain in NIDDM. If

properly performed within an ethnic group with a closely matched control group, such a study could allow evaluation of a region of up to 100 kb of DNA from the marker (45) and may be able to identify susceptibility loci that have only minor effects on the development of multifactorial diseases and are therefore difficult to identify by linkage analysis (40).

Positive results of association studies must be interpreted cautiously, however, because numerous factors could give spurious associations (40). One very important factor would be lack of comparability of the cases and controls, a parameter often difficult to evaluate. Our patients and control subjects were recruited from the same region of Japan where genetic admixture was considered to be minimal. They were unrelated and matched for sex and BMI. Although control subjects were significantly younger, and some of these might develop diabetes later in life, this would be a factor that weighed against finding a difference in GCK allele frequencies between the two groups. Furthermore, in the control group, no difference was observed in the allelic frequencies for either GCK1 or GCK2 when compared between those >48 yr of age (the median age in the control group) and those <47 yr of age.

The recent linkage analyses and identification of mutations in families with MODY, a subtype of NIDDM, revealed the important role of defects in the glucokinase gene in the pathogenesis of this type of NIDDM (28–33). The phenotype of individuals who carry glucokinase mutations seems to be distinct, characterized by early-onset mild hyperglycemia, autosomal dominant mode of inheritance with high penetrance, low plasma insulin relative to the high blood glucose level, less requirement for medical intervention, and lower risk of developing diabetic complications (29,32,33,46), although a variety of presentations have been observed even in a single pedigree (29,32).

The role of glucokinase defects in the more common type of late-onset NIDDM is less clear. Recently, mutations in the glucokinase gene were identified in two so-called late-onset NIDDM pedigrees (32,47). Although the later age of onset (diagnosis) of the patients in these pedigrees does not meet the criteria for MODY (48), the phenotype is very similar to those described in the MODY pedigrees. In Caucasians, negative linkage (49,50) and negative association (51) between glucokinase locus and late-onset NIDDM were disclosed. Furthermore, mutations in the glucokinase gene structural region appeared to be very rare in late-onset NIDDM in this population when assessed by SSCP (S.C. Elbein, M. Hoffman, H. Qin, K.C. Chiu, Y.T., and M.A.P., unpublished observations). On the other hand, associations were observed in two other populations (34,35). This discrepancy may represent the genetic heterogeneities of NIDDM in different racial groups.

A previous report by Fujimoto et al. (7) revealed that the prevalence of NIDDM in the Japanese-American population is significantly higher than in Caucasians and much higher than their relatives in Japan. This result may suggest that an inherited risk for diabetes is higher in the Japanese population, although environmental and biological factors have marked effects on the incidence. The previous studies on the pathophysiology of impaired glucose tolerance in the Japanese population strongly suggested that impaired insulin secretion was a major component of the susceptibilities to NIDDM (52,53), contrasted with several studies suggesting that insulin resistance was a major component of pathogenesis of impaired glucose tolerance in Caucasian populations (54–58). If glucokinase gene mutations were common as a susceptibility factor of NIDDM in Japanese, this might explain the discrepancies in the pathophysiological studies. In fact, recently, a nonsense mutation was found in one Japanese late-onset NIDDM pedigree (46), although the frequency of this mutation is uncertain.

We have demonstrated that alleles of GCK1 and GCK2 at the glucokinase locus are significantly associated with NIDDM in the Japanese population. Although one should be cautious about the potential spurious observations in association studies, the significance of the results should also be recognized in the genetic analysis of heterogeneous disorders like NIDDM. It is therefore of interest to identify the molecular basis of this association.

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REFERENCES

1. Cerasi E, Efendic S, Luft R: Dose-response relationship between plasma insulin and blood glucose levels in prediabetic and diabetic subjects. *Lancet* 1:794–97, 1973
2. Reaven GM, Bernstein R, Davis B, Olefsky JM: Nonketotic diabetes mellitus: insulin deficiency or insulin resistance. *Am J Med* 60:80–88, 1976
3. Harris MI, Hadden WC, Knowler WC, Bennet PH: Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in US population aged 20–74 years. *Diabetes* 36:523–34, 1987
4. Knowler WC, Pettitt DJ, Saad MF, Bennet PH: Diabetes mellitus in the Pima Indians: incidence, risk factors, and pathogenesis. *Diabetes Metab Rev* 6:1–27, 1990
5. Kawate R, Yamakido M, Mishimoto Y, Bennet PH, Hamman RF, Knowler WC: Diabetes mellitus and its vascular complications in Japanese migrants on the island of Hawaii. *Diabetes Care* 2:161–70, 1979
6. Takashina S, Tomaru H, Komatsu K, Utsumi N, Nakashima S, Yamada S, Nagashima M, Murata T, Koyama W: Prevalence of diabetes mellitus in a rural area of Japan: by 75g OGTT. *Tohoku J Exp Med* 141 (Suppl.):229–34, 1983
7. Fujimoto WY, Leonetti DL, Kinoun JL, Newell-Morris L, Shuman WP, Stolov WC, Wahl PW: Prevalence of diabetes mellitus and impaired glucose tolerance among second-generation Japanese-American men. *Diabetes* 36:721–29, 1987
8. Pyke DA: Diabetes: the genetic connections. *Diabetologia* 17:333–43, 1979
9. Permutt MA: Use of DNA polymorphisms for genetic analysis of non-insulin-dependent diabetes mellitus. In *Genetics of Diabetes Part II*. Harrison L, Tait B, Eds. London, Baillere Tindall/Saunders, 1991, p. 495–526
10. Owerbach D, Nerup J: Restriction fragment-length polymorphism of

the insulin gene in diabetes mellitus. *Diabetes* 31:275–77, 1982
11. Permutt MA, Rotwein P, Andreone T, Ward WK, Porte D Jr: Islet β-cell function and polymorphism in the 5′-flanking region of the human insulin gene. *Diabetes* 34:311–14, 1985
12. Elbein SC, CorsettiL, 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
13. McClain DA, Henry RR, Ullrich A, Olefsky JM: Restriction fragment-length polymorphism in insulin receptor gene and insulin resistance in NIDDM. *Diabetes* 37:1071–75, 1988
14. Li SR, Baroni MG, Oelbaum RS, Stock J, Galton DJ: Association of genetic variant of the glucose trasporter with non-insulin-dependent diabetes mellitus. *Lancet* 2:368–70, 1988
15. Cox NJ, Xiang KS, Bell GI, Karam JH: Glucose transporter gene and non-insulin-dependent diabetes mellitus. *Lancet* 2:793–94, 1988
16. Kaku K, Matsutani A, Mueckler M, Permutt MA: Polymorphisms of HepG2/erythrocyte glucose transporter gene: linkage relationships and implications for genetic analysis of NIDDM. *Diabetes* 39:49–56, 1990
17. Matsutani A, Koranyi L, Cox N, Permutt MA: Polymorphisms of GLUT2 and GLUT4 genes: use in evaluation of genetic susceptibility to NIDDM in Blacks. *Diabetes* 39:1534–42, 1990
18. Buracynska M, Hanzlik J, Grzywa M: Apolipoprotein A-1 gene polymorphism and susceptibility of non-insulin-dependent diabetes mellitus. *Am J Hum Genet* 37:1129–37, 1985
19. Xiang KS, Cox NJ, Sanz N, Huang P, Karam JH, Bell GI: Insulin-receptor gene and apolipoprotein genes contribute to the development of NIDDM in Chinese American. *Diabetes* 38:17–23, 1989
20. Colowick SP: The hexokinases. In *The Enzymes*. Vol. 9. Boyer PD, Ed. New York, Academic, 1973, p. 1–48
21. Magnuson MA: Glucokinase gene structure: functional implications of molecular studies. *Diabetes* 39:523–27, 1990
22. Matschinsky FM: Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. *Diabetes* 39:647–52, 1990
23. Iynedjian PB, Mobius G, Seitz HJ, Wollheim CB, Renold AE: Tissue-specific expression of glucokinase: identification of the gene product in liver and pancreatic islets. *Proc Natl Acad Sci USA* 83:1998–2001, 1986
24. Tanizawa Y, Koranyi LI, Welling C, Permutt MA: Human glucokinase gene: cloning and sequence determination of two alternatively spliced CDNAS. *Proc Natl Acad Sci USA* 88:7294–97, 1991
25. Koranyi LI, Tanizawa Y, Welling CM, Rabin DU, Permutt MA: Human islet glucokinase gene: isolation and sequence analysis of a full-length cDNA. *Diabetes* 41:807–11, 1982
26. Matsutani A, Janssen R, Donis-Keller H, Permutt MA: A polymorphic (CA)n repeat element maps the human glucokinase gene (GCK) to chromosome 7p. *Genomics* 12:319–25, 1992
27. Tanizawa Y, Matsutani A, Chiu KC, Permutt MA: Human glucokinase gene: Isolation, structural characterization, and identification of a microsatellite repeat polymorphism. *Mol Endocrinol* 6:1070–81, 1992
28. Froguel Ph, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, Lesage S, Vionnet N, Clement K, Fougerousse F, Tanizawa Y, Weissenbach J, Beckmann JS, Lathrop GM, Passa Ph, Permutt MA, Cohen D: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. *Nature (Lond)* 356: 162–64, 1992
29. Hattersley AT, Turner RC, Permutt MA, Patel P, Tanizawa Y, CHiu KC, O'Rahilly S, Watkins PJ, Waisoct JS: Linkage of type II diabetes to the glucokinase gene. *Lancet* 339:1307–10, 1992
30. Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, Lesage S, Velho G, Iris F, Passa Ph, Frougel Ph, Cohen D: Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature (Lond)* 356:721–22, 1992
31. Stoffel M, Froguel Ph, Takeda J, Zouali H, Vionnet N, Nishi S, Weber IT, Harrison RW, Pilkis SJ, Lesage S, Vaxillaire M, Velho G, Sun F, Iris F, Passa Ph, Cohen D, Bell GI: Human glucokinase gene: isolation, characterization, and identification of two missense mutations linked to early-onset non-insulin-dependent (type II) diabetes melllitus. *Proc Natl Acad Sci USA* 89:7698–702, 1992
32. Stoffel M, Patel P, Lo Y-MD, Hattersley AT, Lucassen AM, Page R, Bell JI, Bell GI, Turner RC, Wainscoat JS: Missense mutation in maturity-onset diabetes of the young and mutation screening in late-onset diabetes. *Nature Genet* 2:153–56, 1992
33. Froguel Ph, Zouali H, Vionnet N, Velho G, Vaxillaire M, Sun F, Lesage S, Butel MOB, Stoffel M, Takeda J, Robert JJ, Passa Ph, Permutt MA, Beckmann JS, Bell GI, Cohen D: Familial hyperglycemia due to mutations in glucokinase: definition of a new subtype of non-insulin-dependent (type II) diabetes mellitus. *N Engl J Med* 328:697–702, 1993
34. Chiu KC, Province MA, Permutt MA: The glucokinase gene is a genetic marker for non-insulin-dependent diabetes mellitus in American Blacks. *Diabetes* 41:843–49, 1992
35. Chiu KC, Province MA, Dowse GK, Zimmet PZ, Serjeantson S, Permutt MA: A genetic marker at the glucokinase gene locus for non-insulin-dependent diabetes mellitus in Mauritian Creoles. *Diabetologia* 35:632–38, 1992
36. Weir BS: *Genetic Data Analysis*. Sunderland, MA, Ed. Sinauer Associates, 1990
37. MacLean CJ, Morton NE: Estimation of myriad haplotype frequencies. *Genet Epidemiol* 2:263–72, 1985
38. Otto J: *Analysis of Human Genetic Linkage*. Baltimore, MD, Johns Hopkins University Press, 1991
39. Rothman KJ: No adjustments are needed for multiple comparisons. *Epidemiology* 1:43–46, 1990
40. Weber JL: Informativeness of human (dC-dA):(dG-dT)n polymorphisms. *Genomics* 7:524–30, 1990
41. Cox NJ, Bell GI: Disease associations: chance, artifact, or susceptability genes? *Diabetes* 38:947–50, 1989
42. O'Rahilly S, Wainscoat JS, Turner RC: type II (non-insulin-dependent diabetes mellitus: new genetics for old nightmares. *Diabetes* 31:407–14, 1988
43. Lander ES, Bostein D: Strategies of studying heterogeneous genetic traits in human by using a linkage map of restriction fragment-length polymorphism. *Proc Natl Acad Sci USA* 83:7353–57, 1986
44. Cox NJ, Xiang K-S, Fajans SS, Bell GI: Mapping diabetes-susceptability genes: lessons learned from search for DNA marker for maturity-onset diabetes of the young. *Diabetes* 41:401–407, 1992
45. Bodmer WF: Human genetics: the molecular challege. In *Cold Spring Harbor Symposia on Quantitative Biology. Vol L1. Molecular Biology of Homo Sapiens*. Cold Spring Harbor, NY, Cold Spring Harbor, 1986, p. 1–13
46. Velho G, Froguel PH, Clement K, Pueyo M, Rakotoambinina B, Zouali H, Passa PH, Cohen D, Robert JJ: Primary pancreatic β-cell secretory defect caused by mutations in glucokinase gene in kindreds of maturity-onset diabetes of the young. *Lancet* 340:444–48, 1992
47. Katagiri H, Asano T, Ishihara H, Inukai K, Anai M, Miyazaki J, Tsukuda K, Kikuchi M, Yazaki Y, Oka Y: Nonsense mutation of glucokinase gene in late-onset non-insulin-dependent diabetes mellitus. *Lancet* 340:1316–17, 1992
48. Tattersall RB, Fajans SS: A difference between the inheritance of classical juvenile-onset and maturity-onset diabetes of young people. *Diabetes* 24:44–53, 1975
49. Cook JTE, Hattersley AT, Christopher P, Bown E, Barrow B, Patel P, Shaw JAG, Cookson WOCM, Permutt MA, Turner RC: Linkage analysis of gluockinase gene with NIDDM in Caucasian pedigrees. *Diabetes* 41:1496–500, 1992
50. Elbein SC, Hoffman M, Chiu L, Tanizawa Y, Permutt MA: Linkage analysis of the glucokinase locus in familial type II (non-insulin-dependent) diabetic pedigrees. *Diabetologia* 36:141–45, 1993
51. Tanizawa Y, Chiu KC, Province MA, Morgan R, Ownes DR, Rees A, Permutt MA: Two microsatellite repeat polymorphisms flanking opposite ends of the human glucokinase gene: use in haplotype analysis of Welsh Caucasians with type II (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:409–13, 1993
52. Kadowaki T, Miyake Y, Hagura R, Akanuma Y, Kajinuma H, Kuzuya N, Takaku F, Kosaka K: Risk factors for worsening to diabetes in subjects with impaired glucose tolerance. *Diabetologia* 26:44–49, 1984
53. Yoneda H, Cha T, Ikegami H, Kawaguchi Y, Yamamoto Y, Tahara Y, Yamato E, Ogihara T: Analysis of early-phase insulin responses in nonobese subjects with mild glucose intolerance. *Diabetes Care* 15:1517–21, 1992
54. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaced syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173–94, 1991
55. Reaven GM: Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension: parallels between human disease and rodent models. *Diabetes Care* 14:195–202, 1991
56. Nankervis A, Proietto J, Aitken P, Harewood M, Alford F: Differential effects of insulin therapy on hepatic and peripheral insulin sensitivity in type II (non-insulin-dependent) diabetes. *Diabetologia* 23:320–25, 1982
57. Hidaka H, Nagulesparan M, Klimes I, Clark R, Sasaki H, Aronoff SL, Vasques B, Rubenstein AH, Unger RH: Improvement of insulin secretion but not insulin resistance after short-term control of plasma glucose in obese type II diabetes. *J Clin Endocrinol Metab* 54:217–22, 1982
58. Warram JH, Martin BC, Gleason RE, Soeldner JS: Slow glucose removal rate but not insulin secretion predicts development of NIDDM in offspring of two NIDDM patients. *Diabetes* 36(Suppl. 1):14A, 1987