Novel Susceptibility Gene for Late-Onset NIDDM Is Localized to Human Chromosome 12q

Joanne T.E. Shaw, Paul K. Lovelock, Janine B. Kesting, John Cardinal, David Duffy, Brandon Wainwright, and Donald P. Cameron

NIDDM has a substantial genetic component, but the nature of the genetic susceptibility is largely unknown. Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous monogenic form of NIDDM characterized by an early age of onset and autosomal dominant inheritance, and linkage studies have identified genes that are mutated in different MODY pedigrees on chromosome 20 (*MODY1* locus, hepatocyte nuclear factor-4α [HNF-4α] gene), chromosome 7 (*MODY2* locus, glucokinase gene), and chromosome 12 (*MODY3* locus, HNF-1α gene). We studied an extended pedigree in which multiple members are affected by late-onset NIDDM associated with insulin resistance and performed linkage analysis with four microsatellite markers in the *MODY3* region of chromosome 12q. We found significant evidence for linkage between NIDDM and the *MODY3* locus (logarithm of odds score 3.65 at θ = 0.008 telomeric to marker D12S321), but sequencing of the 10 exons and promoter of HNF-1α did not identify any causative mutation in this gene. Our results indicate that the region of chromosome 12q close to *MODY3* harbors a novel susceptibility gene or genes for NIDDM. *Diabetes* 47:1793–1796, 1998

NIDDM is characterized by hyperglycemia caused by impaired insulin secretion, insulin resistance in muscle, and elevated hepatic glucose production (1). The causes of NIDDM are poorly understood, but its familial clustering and high rate of concordance in monozygotic twins implicate genetic factors (2). It is a genetically and clinically heterogeneous disorder and can be divided into early- and late-onset forms. The early-onset form includes maturity-onset diabetes of the young (MODY), a genetically heterogeneous monogenic form of NIDDM characterized by early age of onset (usually <25 years) and autosomal dominant inheritance (3). Linkage studies have identified genes that are mutated in different MODY pedigrees on chromosomes 20 (*MODY1* locus, hepatocyte nuclear factor-4α [HNF-4α] gene), 7 (*MODY2* locus, glucokinase gene), and 12 (*MODY3* locus, HNF-1α gene) (4–6), and clinical studies indicate that mutations in these genes are associated with abnormal patterns of glucose-stimulated insulin secretion (7–10).

Although there has been considerable success in identifying the genes for MODY, there has been relatively little progress in identifying the genes responsible for the more common forms of late-onset NIDDM; they do not appear to have a simple Mendelian basis and are thought to result from the joint action of genetic and environmental factors. This lack of progress can be attributed to a number of factors, including the paucity of extended pedigrees because of the late age of onset of the disorder, the prevalence of bilineal inheritance, and the genetic heterogeneity that occurs within and between pedigrees (2). To optimize the likelihood of finding novel NIDDM susceptibility genes, we have chosen to study an extended pedigree in which late-onset NIDDM segregates in a pattern consistent with an autosomal dominant disorder. Our strategy was to undertake linkage analysis with the three loci that are known to cause MODY followed by a systematic search for linkage if those results were negative. We report positive linkage between markers at the *MODY3* region and NIDDM in this pedigree.

RESEARCH DESIGN AND METHODS

The protocol was approved by the Princess Alexandra Hospital Research Ethics Committee, and informed consent was obtained from all participants. The proband and relatives were clinically characterized by a diabetes physician or a clinical nurse, with the majority of the assessments performed in the homes of the study participants. The protocol included a standardized interview that recorded age of onset of diabetes and history of its treatment and complications; measurement of height, weight, BMI, and blood pressure; and collection of a fasting blood sample for measurement of glucose, insulin, and C-peptide levels. For deceased study subjects, information about diabetes (age of onset and type of treatment) was obtained from spouse or offspring and, where possible, confirmed by medical record review.

Plasma glucose was measured by a hexokinase method using the Hitachi 747–100 analyzer (Boehringer Mannheim, Mannheim, Germany) with CV <2%. Plasma C-peptide was measured by radioimmunoassay (RIA; Diagnostic Systems Laboratories, Webster, TX). Cross-reactivity with proinsulin species was negligible. The fasting plasma glucose and C-peptide concentrations were interpreted by homeostasis model assessment of β-cell function and insulin sensitivity (11). This method uses a mathematical model of the body's glucose and insulin interactions as a frame of reference. The major feedback loops are stimulation of insulin secretion by glucose, reduction of hepatic glucose output, and increase in uptake of glucose into muscles by insulin. Different degrees of insulin resistance and impaired β-cell function can be introduced into the model, and for each combination the homeostatic fasting plasma glucose and C-peptide results achieved by the feedback loops are calculated. Each patient's fasting plasma glucose and C-peptide measurements can be interpreted by the model to predict the β-cell function and insulin sensitivity that are likely to have given those meas-

From the Department of Diabetes and Endocrinology (J.T.E.S., P.K.L., J.B.K., J.C., D.P.C.), Princess Alexandra Hospital; the Centre for Molecular and Cellular Biology (P.K.L., B.W.), University of Queensland; and the Epidemiology Unit (D.D.), Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

Address correspondence and reprint requests to Dr. Joanne T.E. Shaw, Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia. E-mail: amdgenet@gil.com.au.

Received for publication 14 May 1998 and accepted in revised form 6 August 1998.

FPG, fasting plasma glucose; HNF, hepatocyte nuclear factor; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction; TBE, tris-borate-EDTA.

urements. β-Cell function and insulin sensitivity are expressed as centile groups defined relative to a nondiabetic population <35 years old and <115% of ideal weight. β-cell function and insulin sensitivity measured by homeostatic model assessment have been shown to correlate with measures obtained by hyperglycemic and euglycemic clamp (11,12).

The following subjects were considered affected: *1*) those with diabetes diagnosed according to World Health Organization criteria and treated with oral hypoglycemic agents, insulin, or a specific diet and *2*) those with impaired fasting glucose as defined by the American Diabetes Association (fasting plasma glucose [FPG] =6.1 mmol/l) (13). Normoglycemia was defined as FPG =5.5 mmol/l.

DNA was extracted from blood samples using the salting-out method (14). Paternity was established by the use of highly polymorphic markers in a protocol based on polymerase chain reaction (PCR) and the incorporation of [^{32}P]dCTP during amplification. Based on the Cooperative Human Linkage Centre database and the physical map, we selected four markers that span the expanded *MODY3* region: D12S86, D12S321, D12S807, and D12S342. PCR reactions were performed in 96-well plates in 10-µl volumes containing 50 ng genomic DNA, 1× PCR buffer (Perkin Elmer Cetus, Norwalk, CT), 0.5 pmol of each primer, 0.5 U of AmpliTaq Gold (Perkin Elmer Cetus), 2.5 mmol/l $MgCl_2$, 1 µCi [^{32}P]dCTP (Bresatec, Adelaide, South Australia), 0.025 mmol/l dCTP, and 0.25 mmol/l each of dATP, dGTP, and dTTP (Pharmacia Biotech, Uppsala, Sweden). After a 10-min initial denaturing step at 94°C in a 96-well thermal cycler (Corbett Research, Sydney, Australia), samples were subjected to 40 cycles of 15 s at 55°C for annealing, 72°C for 30 s, and 94°C for 15 s for denaturing; a final 5-min incubation took place at 72°C. After amplification, the products were denatured by the addition of 9 µl sequencing stop solution followed by heating at 95°C for 5 min. Next, 2 µl of each sample was loaded onto a 6% denaturing polyacrylamide gel in 1× TBE (tris-borate-EDTA) buffer and resolved electrophoretically at 100–120 W. Gels were fixed, dried, and exposed to autoradiographic film. After film development, microsatellite genotypes were scored without knowledge of the clinical or pedigree data. Consistency within and between gels was maintained by using size standards.

Linkage analysis was performed using the FASTLINK 2.0 versions of MLINK and LINKMAP (15–17), which performed multipoint analyses at each location along chromosome 12q spanned by the region D12S86, D12S321, D12S807, and D12S342. Four liability classes were defined on the basis of age: class 1, >60 years; class 2, 40–60 years; class 3, 20–40 years; and class 4, <20 years. To accommodate for potential heterogeneity, phenocopy rates were used for each liability class (0.01, 0.001, 0.0001, and 0.0001, respectively) (18). The penetrance factors used for the heterozygous dominant genotype were 0.90, 0.75, 0.45, and 0.15, and for the homozygous dominant genotype, 0.95, 0.90, 0.90, and 0.80. The frequency of the disease allele was estimated to be 5%. The allele frequencies were calculated from the pedigree data.

Using primers described previously (19), the 10 exons, the flanking intronic sequence, and the promoter region were amplified by PCR. Ten members of the pedigree were included in the sequencing studies, and both DNA strands were sequenced. Another pair of primers, BGF (5′-AGC CAG CAC TGT TCT TGG CAC-3′) and BGR (5′-GAC TTC AGC CCT GCA AAG TGC AGG-3′), was designed to generate a 430-bp fragment further upstream of, and partially overlapping, the most 5′ region amplified by Kaisaki et al. (19). The PCR products were sequenced directly using an ABI Prism Big Dye automated sequencing kit and an ABI Prism 377 sequencer. Sequence data was compared with that previously presented (19).

RESULTS

The pedigree structure and *MODY3* haplotypes at markers D12S86, D12S321, D12S807, and D12S342 are shown in Fig. 1, along with inferred haplotypes for the deceased founders. The haplotype assignments were confirmed with the GENEHUNTER program (20). The haplotype 5654 was found to segregate with diabetes in the pedigree. Individual IV-6 is an obligate recombinant, placing the NIDDM susceptibility locus telomeric of marker D12S321. Subjects II-7 and III-20 represent possible phenocopies. Subjects IV-1 and IV-3 have inherited the at-risk haplotype but are nonpenetrant at this stage. Because of the strong family history of NIDDM, these two subjects adopted a low-fat diet and a physical activity program; their BMIs are 26 and 20 kg/m^2. Subject IV-5 was diagnosed with insulin-requiring diabetes at age 8 years and had a clinical history consistent with IDDM.

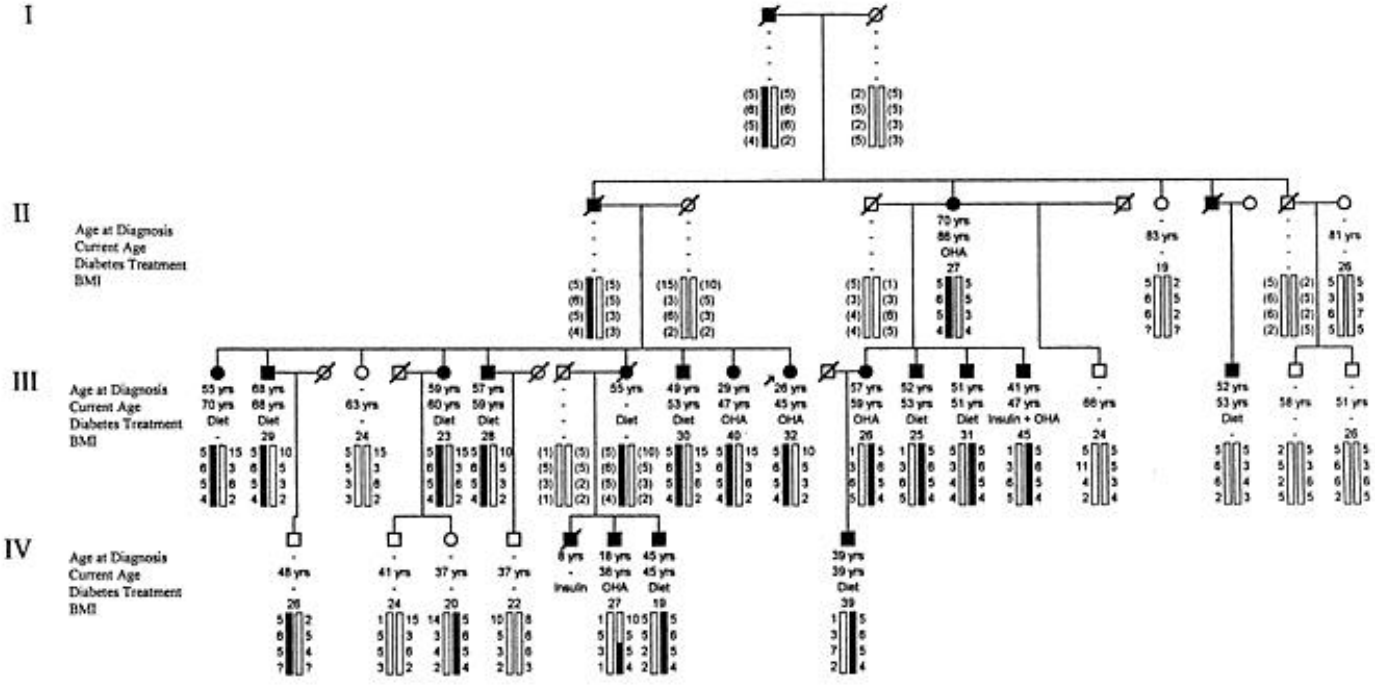

FIG. 1. NIDDM pedigree (● ■, affected family members; ○ □, normoglycemic subjects; ↗, proband; ⁄, deceased subjects). The clinical features of the subjects are noted below each symbol, including age at diagnosis, present age, diabetes treatment (diet, oral hypoglycemic agent [OHA], and insulin therapy), and BMI expressed as weight in kilograms divided by height in meters squared. The genotypes at markers D12S86, D12S321, D12S807, and D12S342 are shown, and the haplotypes based on GENEHUNTER estimates are indicated. The haplotypes for deceased founders and other unavailable individuals are inferred and indicated within parentheses. The at-risk haplotype is noted by shading. Individual IV-6 is an obligate recombinant, placing the NIDDM locus telomeric of marker D12S321. Subjects II-7 and III-20 represent possible phenocopies. Subjects IV-1 and IV-3 have inherited the at-risk haplotype but are nonpenetrant at this stage.

Age at diagnosis of NIDDM for those affected members of the pedigree available for testing was 48 ± 14 years (mean ± SD), and BMI was 30 ± 7 kg/m^2. Eleven subjects required treatment with diet alone, five were on oral hypoglycemic therapy, and one was on nocturnal long-acting insulin in combination with sulfonylurea. Eight of the diabetic subjects had clinical evidence of macrovascular complications (ischemic heart disease or cerebrovascular disease). Subject IV-6, who had had NIDDM for 20 years, had diabetic nephropathy and proliferative retinopathy. The affected subjects had heterogeneous β-cell function (median 92%, interquartile range 47–140%) and substantial insulin resistance (median 35%, interquartile range 16%-42%) relative to a normal reference value of 100%.

Two-point linkage analysis logarithm of odds (LOD) scores for each of the *MODY3* markers with NIDDM in the pedigree are presented in Table 1. A graphic representation of the results of parametric multipoint linkage analysis of the markers at the *MODY3* locus are shown in Fig. 2. The maximal LOD score was 3.65 at θ = 0.008 telomeric to marker D12S321.

For 10 pedigree members, we sequenced the 10 exons and the promoter of the HNF-1α gene in both directions and found no evidence of a mutation coinherited with NIDDM in the pedigree, although several previously described polymorphisms were found in affected and unaffected individuals (19). No mutations or polymorphisms were found in the minimal promoter of the HNF-1α gene, a region containing the binding sites for HNF-4α and HNF-3, both of which have been implicated in the regulation of HNF-1α expression (19). No variants were identified in exons 2, 3, 4, 5, 6, 8, 9, or 10 of HNF-1α. Three previously described polymorphisms in exon 1 were identified in affected and unaffected pedigree members: a C-G Leu/Leu polymorphism in codon 17, an A-C Ile/Leu polymorphism in codon 27, and a C-T Ala/Val polymorphism in codon 98. Two previously described polymorphisms were identified in affected and unaffected pedigree members in exon 7: a C-T Leu/Leu polymorphism in codon 459 and a G-A Ser/Asn polymorphism in codon 487 (19).

DISCUSSION

This report of a large pedigree with late-onset NIDDM associated with insulin resistance presents evidence of linkage between late-onset NIDDM and markers in the *MODY3* region of chromosome 12q. There was no evidence of mutation in the HNF-1α gene or its promoter, and we hypothesize that another gene or genes in this region of chromosome 12q contribute to diabetes susceptibility in the pedigree.

Our negative mutation data for HNF-1α is not surprising given the phenotype of affected subjects in our pedigree. Subjects with mutations in HNF-1α are described as having diabetes characterized by an early age at diagnosis, predominant insulin deficiency, lean body mass, early microvascular complications, and an early therapeutic requirement for insulin (10,21). In contrast, the affected subjects in our pedigree were obese, had a relatively late age at diagnosis, were often treated with diet, had a low prevalence of microvascular complications but a high prevalence of macrovascular disease, and pathophysiologically had predominant insulin resistance associated with heterogeneous levels of β-cell function.

TABLE 1
Two-point linkage analysis LOD scores for the *MODY3* markers versus affection status in the pedigree

	Recombination fraction (θ)					
	0	0.01	0.05	0.1	0.2	0.3
Markers						
D12S86	2.37	2.47	2.52	2.34	1.73	1.01
D12S321	0.02	0.05	0.14	0.19	0.16	0.07
D12S807	1.47	1.64	1.83	1.79	1.43	0.91
D12S342	0.68	0.72	0.79	0.80	0.67	0.45

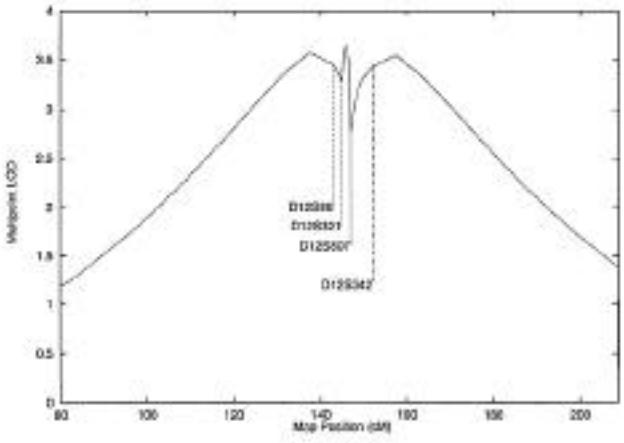

FIG. 2. Multipoint location score curve for *MODY3* with the near-dominant model and markers D12S86, D12S321, D12S807, and D12S342.

That there is genetic heterogeneity in diabetes susceptibility between different ethnic groups would appear evident from other reports concerning the role of the *MODY3* locus in NIDDM. There is no evidence of linkage of NIDDM with this locus in Mexican-American (22) or Pima Indian sib pairs (23,24). Lesage et al. (25) reported no evidence for linkage of NIDDM with *MODY3* markers in a large collection of French NIDDM families. Mahtani et al. (26) described linkage of *MODY3*-linked markers with NIDDM in a subset of families from an isolated population in western Finland, the linkage being present in the group with the lowest insulin secretion. The authors inferred the presence of a gene in this region ("*NIDDM2*") that affects susceptibility to adult-onset diabetes associated with low insulin secretion. The Finnish families in the low-insulin quartile had a later age of onset (mean 58 years) than that described for families with HNF-1α mutations. In the current study, the pathophysiological defect in the affected pedigree members is predominantly insulin resistance with heterogeneous levels of β-cell function. Nevertheless, it is possible that the locus identified by Mahtani et al. (26) corresponds with the one identified in the present study, as impaired β-cell compensation in the context of insulin resistance is an important factor in the development of hyperglycemia (1). Bowden et al. (27) documented evidence for linkage of NIDDM with markers in the *MODY3* region in Caucasian sib pairs with a history of adult-onset nephropathy but did not demonstrate linkage in African-American sib pairs. Ours is the first report of late-onset NIDDM associated with severe insulin resistance showing linkage to the *MODY3* region. The ethnic background of the pedigree is relevant because the founder was a Pacific Islander, and NIDDM in

Oceania is commonly associated with severe insulin resistance. There are no other reports of linkage or mutation analysis with the *MODY3* locus in this population.

NIDDM is characterized by defects in both insulin secretion and insulin action. Hyperinsulinemia compensates for insulin resistance in the early prediabetic state, and the subsequent development of hyperglycemia results from the failure of β-cells to secrete enough insulin for effective compensation (1). The genetic susceptibility to NIDDM in the pedigree may affect β-cell function, insulin resistance, or both. Secondary defects due to hyperglycemia may be relevant to the combination of pathophysiologies in the affected family members. Alternatively, a single molecular abnormality may underlie both insulin resistance and the failure of effective β-cell compensation, as occurs with the disruption of IRS2 in mice (28).

In summary, the results of our study indicate that the region of chromosome 12q close to *MODY3* harbors a novel susceptibility gene for late-onset NIDDM. Positional cloning will be undertaken to further refine its localization and define its nature in the pedigree.

ACKNOWLEDGMENTS

The study was supported by a National Health and Medical Research Council Project Grant, Canberra; a grant from the Australian government's Cooperative Research Centre for the Discovery of Genes for Common Human Diseases; the Princess Alexandra Hospital Research and Development Foundation, Brisbane, Australia; and Novo Nordisk Regional Diabetes Support Scheme. J.T.E.S. is a Viertel Senior Research Fellow in Medical Science.

We are grateful to the family in this study for their assistance.

REFERENCES

1. DeFronzo RA: Lilly Lecture 1987: The triumvirate: B-cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* 37:667–687, 1988
2. Turner RC, Hattersley AT, Shaw JTE, Levy JC: Type II diabetes: clinical aspects of molecular biological studies. *Diabetes* 44:1–10, 1995
3. Fajans SS: Maturity-onset diabetes of the young (MODY). *Diabetes Metab Rev* 5:579–606, 1989
4. Yamagata K, Furuta H, Oda N, Kaisaki JK, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4 alpha gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
5. Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, Lesage S, Velho G, Iris F, Passa P, Froguel P, Cohen D: Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:721–722, 1992
6. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, LeBeau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Turner RC, Velho G, Chevre J-C, Froguel P, Gell GI: Mutations in the hepatocyte nuclear factor-1 alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458, 1996
7. Herman WH, Fajans SS, Ortiz FJ, Smith MJ, Sturis J, Bell GI, Polonsky KS, Halter JB: Abnormal insulin secretion, not insulin resistance is the genetic or primary defect of MODY in the RW pedigree. *Diabetes* 43:40–46, 1994; erratum *Diabetes* 43:1171, 1994
8. Froguel P, Zouali H, Vionnet N, Velho G, Vaxillaire M, Sun F, Lesage S, Stoffel M, Takeda J, Passa P, Permutt A, Beckmann JS, Bell GI, Cohen D: Familial hyperglycemia due to mutations in glucokinase: definition of a subtype of diabetes mellitus. *N Engl J Med* 328:697–702, 1993
9. Byrne MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Dronsfield MJ, Bain SC, Hattersley AT, Velho G, Froguel P, Bell GI, Polonsky KS: Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. *Diabetes* 45:1503–1510, 1996
10. Lehto M, Tuomi T, Mahtani MM, Widen E, Forsblom C, Sarelin L, Gullstrom M, Isomaa B, Lehtovirta M, Hyrkko A, Kanninen T, Orho M, Manley S, Turner RC, Brettin T, Kirby A, Thomas J, Duyk G, Lander E, Taskinen M-R, Groop L: Characterization of the MODY3 phenotype: early-onset diabetes caused by an insulin secretion defect. *J Clin Invest* 99:582–591, 1997
11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
12. Levy JC, Rudenski A, Burnett M, Knight R, Matthews DR, Turner RC: Simple empirical assessment of beta-cell function by a constant infusion of glucose test in normal and type 2 (non-insulin-dependent) diabetic subjects. *Diabetologia* 34:488–489, 1991
13. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 21 (Suppl. 1):S5-S19, 1998
14. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215, 1988
15. Cottingham RW Jr, Idury RM, Schaffer AA: Faster sequential genetic linkage computations. *Am J Hum Genet* 53:252–263, 1993
16. Schaffer AA, Gupta SK, Shriram K, Cottingham RW Jr: Avoiding recomputation in linkage analysis. *Hum Hered* 44:225–237, 1994
17. Lathrop GM, Lalouel J-M, White RL: Construction of human genetic linkage maps: likelihood calculations for multilocus analysis. *Genet Epidemiol* 3:39–52, 1986
18. Cook JTE, Hattersley AT, Christopher P, Bown E, Barrow B, Patel P, Shaw JAG, Cookson WOCM, Permutt MA, Turner RC: Linkage analysis of glucokinase gene with NIDDM in Caucasian pedigrees. *Diabetes* 41:1496–1500, 1992
19. Kaisaki PJ, Menzel S, Lindner T, Oda N, Fjasanowski I, Sahn J, Meincke G, Schulze J, Schmechel H, Petzold C, Ledermann HM, Sachse G, Boriraj VV, Menzel R, Kerner W, Turner RC, Yamagata K, Bell GI: Mutations in the hepatocyte nuclear factor-1α gene in MODY and early-onset NIDDM: evidence for mutational hotspot in exon 4. *Diabetes* 46:528–535, 1997
20. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES: Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347–1363, 1996
21. Velho G, Charpentier G, Vaxillaire M, Froguel P, Boccio V: Diabetes complications in NIDDM kindreds linked to the MODY3 locus on chromosome 12q. *Diabetes Care* 19:915–919, 1996
22. Stern MP, Duggirala R, Mitchell BD, Reinhart LJ, Shivakumar S, Shippman PA, Uresandi OC, Benavides E, Blangero J, O'Connell P: Evidence for linkage of regions on chromosomes 6 and 11 to plasma glucose concentrations in Mexican Americans. *Genome Res* 6:724–734, 1996
23. Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wapelhorst B, Spielman RS, Gogolin-Ewens KJ, Shephard JM, Williams SR, Risch N, Hinds D, Iwasaki N, Ogata M, Omori Y, Petzold C, Rietzsch H, Schroder HE, Schulze J, Cox NJ, Menzel S, Boriraj VV, Chen X, Lim LR, Lindner T, Mereu LE, Wang YQ, Xiang K, Yamagata K, Yang Y, Bell GI: A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nat Genet* 13:161–166, 1996
24. Knowler WC, Hanson RL, the Pima Diabetes Genes Group: Genomic scan for genetic markers linked to diabetes in Pima Indians (Abstract). *Diabetologia* 40 (Suppl. 1):A8, 1997
25. Lesage S, Hani EH, Philippi A, Vaxillaire M, Hager J, Passa P, Demenais F, Froguel P, Vionnet N: Linkage analyses of the MODY3 locus on chromosome 12q with late-onset NIDDM. *Diabetes* 44:1213–1217, 1995
26. Mahtani MM, Widen E, Lehto M, Thomas J, McCarthy M, Brayer J, Bryant B, Chan G, Daly M, Forsblom C, Kanninen T, Kirby A, Kruglyak L, Munnelly K, Parkkonen M, Reeve-Daly MP, Weaver A, Brettin T, Duyk G, Lander ES, Groop LC: Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. *Nat Genet* 14:90–94, 1996
27. Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, Rothschild CB, Akots G, Rich SS, Freedman BI: Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. *Diabetes* 46:882–886, 1997
28. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren J-M, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF: Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391:900–904, 1998

Author Queries (please see Q in margin and underlined text)

Q1:Author: The names of human genes should be italicized. Please ensure that all names referring to specific genes are italic.

Q1a: Please provide locations for the 5 manufacturers underlined in Methods.

Q2: Au: please define TBE.

Q2a: In Figure 1, would you like to provide an explanation of symbols with slashes through them?

Q3: Au: To ensure that the last phrase is placed correctly in the sentence, please confirm that Kaisaki et al. amplified the region (as opposed to designed the pair of primers).

Q4: Please expand “NHMRC” and provide locations for NHMRC, the Cooperative Research Centre, and the Princess Alexandra Hospital Research and Development Foundation.

Ref. 5: Please provide all authors for this reference.

Ref 24: Au: Please provide page numbers for ref. 24.