Mitochondrial FAD-Glycerophosphate Dehydrogenase and G-Protein–Coupled Inwardly Rectifying K^+ Channel

No Evidence for Linkage in Maturity-Onset Diabetes of the Young or NIDDM

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Two genes that have potentially important regulatory roles in insulin secretion are both located on chromosome 2q24.1. G-protein–coupled muscarinic potassium channel (GIRK1) is an inwardly rectifying K^+ channel that helps to maintain the resting potential and excitability of cells. Mitochondrial FAD-linked glycerophoshate dehydrogenase (m-GDH) catalyzes a rate-limiting step of the glycerol phosphate shuttle in pancreatic islets. Reduced m-GDH activity has been demonstrated in islets isolated from diabetic subjects compared with islets from nondiabetic control subjects and from the diabetic GK rat. To study the relationship between these candidate genes and NIDDM, we have examined a simple tandem-repeat polymorphism (STRP) close to both the KCN J3 (GIRK1) locus and the m-GDH locus. In a linkage study of three maturity-onset diabetes of the young (MODY) pedigrees, not linked to MODY1, MODY2, or MODY3, a cumulative score of −9.6 at a recombination fraction of $\theta = 0$ excluded linkage. In a population-association study, no linkage disequilibrium for the STRP was found between 190 unselected NIDDM patients and 60 geographically and age-matched white nondiabetic subjects ($\chi^2 = 1.51$ on 3 df, $P = 0.68$). Thus, mutations involving the genes for GIRK1 or FAD-glycerophosphate dehydrogenase are unlikely to cause MODY, and a common mutation in either gene is unlikely to contribute to NIDDM in whites. These data do not exclude mutations in some families or other ethnic groups. *Diabetes* 45:639–641, 1996

Two genes that have potentially important regulatory roles in insulin secretion are both located on chromosome 2q24.1. Human G-protein–coupled inwardly rectifying potassium channel, designated GIRK1, is a member of a new superfamily of K^+ channels (1). The membrane topology, amino acid sequence, and electrophysiological properties of these channels are distinct from those of voltage-gated K^+ channels. Inwardly rectifying K^+ channels are important in regulating the resting potential of the cell and controlling excitability. GIRK1 was initially characterized from rat heart, where it is abundant in the atrial myocardium, and it is also expressed in the brain (1) and in the rat insulinoma cell line RINm5F (2). Recent evidence suggests that K^+ channels may form heteromultimers comprising a combination of inwardly rectifying subunits with different properties, possibly with the sulfonylurea receptor as a regulator (3). K^+ channel genes expressed in pancreatic β-cells are potentially important candidate genes for NIDDM because they may be involved in the regulation of insulin secretion. However, no evidence for linkage has been demonstrated for GIRK2 (gene symbol KCN J7), an inwardly rectifying potassium channel that belongs to the same superfamily as GIRK1 (4,5). GIRK1 (designated KCN J3) has recently been cloned and localized to chromosome 2q24.1 (2).

A second candidate gene in this region that may be involved in the genetic susceptibility for NIDDM is the mitochondrial enzyme FAD-linked glycerophosphate dehydrogenase (m-GDH). This enzyme is an essential component in the glycerol phosphate shuttle in pancreatic islets. The m-GDH gene has been characterized in rats (6) and localized to chromosome 2q24.1 in humans (7). Reduced activity of m-GDH has been reported in islet homogenates from streptozotocin-injected rats (8), GK rats (9), and *db/db* mice (10). Decreased activity of m-GDH was recently observed in T-cells (11) and islets (12) isolated from NIDDM patients compared with islets from nondiabetic control subjects. The m-GDH deficiency in islets from NIDDM patients coincided with a decreased rate of both glycolysis and glucose oxidation and a low rate of insulin secretion from islets in response to D-glucose compared with islets from nondiabetic control subjects (12). It has been suggested that an islet

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CEPH, Centre d'Etude du Polymorphisme Humain; GIRK, G-protein–coupled inwardly rectifying potassium channel; LOD, logarithm of odds; m-GDH, mitochondrial FAD-linked glycerophosphate dehydrogenase; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction; STRP, simple tandem-repeat polymorphism; STS, sequence tagged site; YAC, yeast artificial chromosome.

TABLE 1
Linkage analysis of simple tandem repeated DNA polymorphism in KCN J3 and m-GDH with MODY

	Recombination fraction (θ)						
Pedigree no.	0.00	0.01	0.05	0.1	0.2	0.3	0.4
1	−1.07	−1.00	−0.74	−0.49	−0.21	−0.08	−0.02
2	−3.60	−1.40	−0.72	−0.44	−0.19	−0.08	−0.02
3	−4.90	−2.86	−1.72	−1.11	−0.48	−0.16	−0.02
Cumulative LOD score	−9.57	−5.26	−3.18	−2.04	−0.88	−0.32	−0.06

LOD scores are shown at different recombination fractions.

m-GDH defect may represent a contributing factor in the pathogenesis of NIDDM (11).

A simple tandem-repeat polymorphism (STRP) close to both the GIRK1 and m-GDH gene loci on chromosome 2q24.1 has been identified (2). The present report describes a linkage study of three large maturity-onset diabetes of the young (MODY) pedigrees with the STRP for GIRK1 and m-GDH and a population-association study of this marker in white NIDDM patients and geographically and age-matched nondiabetic subjects.

RESEARCH DESIGN AND METHODS

Yeast artificial chromosome (YAC) libraries and sequence tagged site (STS) screening. The YAC libraries constructed at the Centre d'Etude du Polymorphisme Humain (CEPH) (Research Genetics, Huntsville, AL) were used in this study. STS primers for m-GPD (F: 5′-GTGCCTGCCTAACGCAGCTT-′3 and R: 5′-GACAATTCACATACCTCTGC-3′) amplified a fragment of 177 bp. The primary screening of the library was performed by polymerase chain reaction (PCR) analysis of DNA pools. PCR was performed in 30 μl in 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 400 μmol /l of each dNTP, 100 pmol of each primer, and 2.5 U *Taq* polymerase. For amplification, ~10 ng YAC DNA were denatured for 5 min at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C, and elongation at 72°C for 1 min 30 s. Amplification products were detected after electrophoresis on 2% SeaKem agarose gels.

MODY pedigrees. Three white MODY families that are not linked with glucokinase, the adenosine deaminase locus on chromosome 20 (13), or the MODY3 locus on chromosome 12q (14) were studied. They live in London, Norwich, and Leicester. Pedigree 1 is the original MODY family described as "M" by Tattersall (15).

Diabetic patients and nondiabetic subjects. A total of 190 white NIDDM patients recruited into the U.K. Prospective Diabetes Study (16) and 60 nondiabetic white control subjects from the same geographical area and of similar age were studied. The patients with newly diagnosed NIDDM had a mean age of 52 years, BMI of 29 kg/m^2, mean fasting plasma glucose level of 12.5 mmol/l, and geometric mean fasting plasma insulin level of 14 mU/l.

Methods. Genomic DNA was extracted from peripheral blood using either the standard phenol/chloroform method (17) or a Nucleon II DNA extraction kit (Scotlab, Coatbridge, U.K.). Subjects were genotyped at the microsatellite polymorphism for GIRK1 and m-GDH using PCR. The PCR products generated using the primers 5′-GGACATATATGGTCTTTCAGACC-3′ and 5′-GGTAATAGAGTACATTCCTTTCC-3′ were separated by electrophoresis on an 8% nondenaturing polyacrylamide gel, and the alleles were detected by silver staining (Bio-Rad, Richmond, CA).

Linkage analysis. Linkage analysis was performed with previously described parameters (18) using the software LINKAGE program (version 5.1).

Statistical analysis. Comparison of the allele frequencies at the STRP was by χ^2 analysis after combining alleles 1, 2, and 6 because of the small numbers of these alleles.

RESULTS

Physical integration of KCN J3 and m-GPD into the chromosome 2 YAC contig map. STS oligonucleotide primers of m-GPD were used to screen the CEPH-B human YAC library. Five YAC clones (813H2, 874A12, 883F11, 908B4, and 952C6) were isolated. These YACs are located in contig WC-744 of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research database. The contig spans ≤ 2 cM and contains marker D2S141. We previously showed significant linkage between D2S141 and the STRP in KCN J3 (recombination fraction [$\theta_m = \theta_f$] of 0.023, Z_{max} 20.85) (2). Thus, the genes for KCN J3 and m-GPD are linked to the same genetic locus.

TABLE 2
Frequencies of alleles of STRP in NIDDM and nondiabetic subjects

Allele	Size (bp)	NIDDM subjects	Nondiabetic control subjects
n		380	120
1	235	0.02 (8)	0.00 (0)
2	233	0.02 (8)	0.03 (3)
3	231	0.15 (58)	0.16 (19)
4	229	0.38 (143)	0.41 (49)
5	227	0.40 (152)	0.39 (47)
6	225	0.03 (11)	0.02 (2)

n refers to the number of alleles. Observed numbers are in parentheses.

Linkage analysis. Table 1 shows the logarithm of odds (LOD) scores for each of the three MODY pedigrees studied. The cumulative LOD score for this marker at a recombination fraction of θ = 0.00 was −9.57, excluding linkage in these families. The maximum possible positive LOD scores that could be achieved in these three pedigrees are 4.0, 1.4, and 3.3, respectively, with a cumulative maximum of 8.7.

Population-association study. No linkage disequilibrium was found between the STRP for GIRK1 and m-GDH and NIDDM in the 190 diabetic and 60 nondiabetic subjects studied (χ^2 = 1.51 on 3 df, P = 0.68). Six alleles were observed with a size range of 225–235 bp and a heterozygosity of 60%. The frequencies of the alleles in the NIDDM patients and nondiabetic control subjects are shown in Table 2. The proportion of alleles in the diabetic subjects was similar to that in nondiabetic control subjects, and the frequencies observed are similar to those noted for this polymorphism in whites (2).

DISCUSSION

These data indicate that inherited defects in the GIRK1 or m-GDH are unlikely to be major causes of MODY or NIDDM in whites. The linkage study excluded mutations in GIRK1 or m-GDH loci as causing dominant inheritance in each of the MODY pedigrees. The population association study gave no indication that there is a single common mutation in either GIRK1 or m-GDH in NIDDM patients. The results of these family and population-association studies, however, do not exclude the possibility that mutations in either of these two genes may contribute to diabetes in some pedigrees or in other ethnic groups.

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