Identification of Microsatellite Markers Near the Human Genes Encoding the β-Cell ATP-Sensitive K^+ Channel and Linkage Studies with NIDDM in Japanese

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ATP-sensitive K^+ (K_{ATP}) channels play a key role in stimulus-secretion coupling in pancreatic β-cells. Recent studies have shown that the β-cell K_{ATP} channel comprises two subunits: a novel member of the inwardly rectifying K^+ channel family, designated BIR and expressed at highest levels in pancreatic islets, and the sulfonylurea receptor (SUR). Moreover, the genes encoding these two proteins are adjacent to one another on human chromosome 11. Genetic factors contribute to the development of NIDDM, and it seems likely that mutations in genes encoding proteins involved in insulin secretion or action may contribute to NIDDM susceptibility. The present study examined the contribution of the linked BIR and SUR genes to the development of NIDDM. These genes were localized to the same yeast artificial chromosome as two microsatellite DNA polymorphisms, D11S902 and D11S921. These microsatellite DNA polymorphisms were typed in 140 Japanese NIDDM-affected sib pairs. There was no evidence for linkage between these markers and NIDDM, suggesting that genetic variation in the BIR and SUR genes does not play a major role in susceptibility to NIDDM in Japanese. *Diabetes* 45: 267–269, 1996

Pancreatic β-cells secrete insulin in response to a rise in blood glucose concentration (1). The increase in glucose levels leads to an increase in glucose metabolism and a concentration-dependent inhibition of the ATP-sensitive K^+ (K_{ATP}) channel, the ion channel responsible for maintaining the cell's negative resting potential. The resulting depolarization of the plasma membrane opens voltage-dependent Ca^{2+} channels, and the influx of calcium triggers insulin release. The sulfonylureas also stimulate insulin secretion and do so by blocking ATP-sensitive potassium currents (I_{KATP}). Thus, the K_{ATP} channel plays a key role in determining the secretory response of the β-cell to glucose, the main physiological regulator of insulin secretion, and sulfonylureas (2), the major class of pharmacological agents used clinically to stimulate insulin secretion.

Recent studies have shown that the β-cell K_{ATP} channel is composed of two different subunits: a novel member of the inwardly rectifying K^+ channel family, termed BIR, that is expressed at highest levels in pancreatic islets and at lower levels in heart, skeletal muscle, and brain (3) and the sulfonylurea receptor (SUR) (4). (The two subunits of the K_{ATP} channel, BIR and SUR, are named K_{ATP}-α [K_{ATP} channel α-subunit] and K_{ATP}-β [K_{ATP} channel β-subunit], respectively [3].) Interestingly, the genes encoding these two proteins are adjacent to one another, with the BIR gene being 4.5 kb downstream of the gene encoding the SUR. Loss-of-function mutations in the SUR gene cause familial persistent hyperinsulinemic hypoglycemia of infancy, a rare autosomal recessive disorder characterized by unregulated secretion of insulin and profound hypoglycemia (5–8). The key roles played by these proteins in the regulation of insulin secretion suggest that mutations in the BIR and SUR genes may contribute to the impaired β-cell function that characterizes NIDDM. We have examined this hypothesis by testing for linkage between two microsatellite DNA polymorphisms located in the region of the BIR and SUR genes and NIDDM in a group of 140 affected sib pairs of Japanese origin.

RESEARCH DESIGN AND METHODS

Patient selection and clinical characterization. Subjects with NIDDM diagnosed according to the criteria of the World Health Organization (9) were recruited from the Diabetes Center of Tokyo Women's Medical College. The study group consisted of 213 subjects from 97 families for a total of 140 affected sib pairs (Table 1). At the time of recruitment, informed consent was obtained from each subject and a blood sample was taken for DNA isolation and determination of insulin, glucose, and HbA_{1c} levels. Fasting plasma insulin and glucose levels were determined for 85 patients who were not being treated with insulin at the time of study. Plasma insulin levels were measured using the EIA kit Test Insulin 2 (Boehringer Mannheim–Yamanouchi, Tokyo, Japan). Plasma glucose levels were measured by the glucose oxidase method (Glucose AUTO & STAT, Kyoto Dai-ichi Kagaku, Kyoto, Japan), and HbA_{1c} was measured by a high-performance liquid chromatography (HPLC) method (Hi-Auto A1c HA-8121, Kyoto Dai-ichi Kagaku).

Identification of yeast artificial chromosomes (YACs) by polymerase chain reaction (PCR)-based screening. Pooled DNA samples from the Centre d'Etude du Polymorphisme Humain (CEPH) YAC 'B' library were purchased from Research Genetics (Huntsville, AL). PCR screening of the YAC pools was carried out using primers from the human SUR (SUR16, 5'-CACGCTCAGGTTCTGGAT-3'; and SUR34AL, 5'-ACAAGGAGCCTGGGGAT-3') (4) and BIR genes (IKATP-P3, 5'-TC-

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K_{ATP}, ATP-sensitive K^+; BIR, a novel member of the inwardly rectifying K^+ channel family; CEPH, Centre d'Etude du Polymorphisme Humain; IBS, identity by state; PCR, polymerase chain reaction; PIC, polymorphism information content; STS, sequence-tagged site; SUR, sulfonylurea receptor; YAC, yeast artificial chromosome.

TABLE 1
Structure of Japanese sibships

Number in sibship	Number of such sibships	Number of subjects	Possible pairs per sibship	Total possible pairs
2	82	164	1	82
3	12	36	3	36
4	2	8	6	12
5	1	5	10	10
Total	97	213	—	140

CACTCCTTCTCGTCTGCCTTC-3′; and IKATP-P4, 5′-ATGCTTGCTGAAGATGAGGGTCT-3′) in a volume of 10 μl, containing 100 ng (1 μl) of YAC pool DNA, 4 pmol of each primer (^{32}P-labeled SUR16 or IKATP-P3 and unlabeled SUR34AL or IKATP-P4), 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.4), 1.0 mmol/l $MgCl_2$, 200 μmol/l of each dNTP, and 1 U *Taq* DNA polymerase. A GeneAmp 9600 PCR system was used for 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The PCR products were separated by electrophoresis on a 5% sequencing gel and visualized by autoradiography. The presence of a sequence-tagged site (STS) in a YAC was confirmed using DNA prepared from recombinant yeast clones (Research Genetics). Information about CEPH YACs was obtained from the Human Physical Mapping Project at the Whitehead Institute/Massachusetts Institute of Technology (MIT) Center for Genome Research (Data Release 7, May 1995) over the World-Wide Web.

DNA studies. DNA was isolated from peripheral blood lymphocytes. PCR-based genotyping of markers D11S902 and D11S921 (the sequences are available from the Genome Database) was described previously (10,11). PCR products were separated by electrophoresis on a 5% sequencing gel and visualized by autoradiography. For D11S902, the PCR consisted of 30 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 1 min. For D11S921, the PCR consisted of 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. The $MgCl_2$ concentration was 1.5 mmol/l.

Linkage analysis. Evidence for linkage between D11S902 and D11S921 and NIDDM was assessed using the nonparametric affected–sib pair methods of Bishop and Williamson (12) and Holmans (13). The identity-by-state (IBS) method of analysis described by Bishop and Williamson (12) compares the observed numbers of sib pairs sharing zero, one, or two alleles IBS with the expected values under no linkage. The significance of deviations from the null hypothesis was evaluated by a χ^2 test with 2 df. Marker allele frequencies and polymorphism information content (PIC) values were estimated from the sib-pair data as described by Holmans (13) using the computer program SPLINK, as were the estimated identity-by-descent (IBD) distribution and maximum likelihood of odds value.

RESULTS

Clinical characteristics of the subjects. The clinical features of the subjects are summarized in Table 2. The average age of the subjects at the time of study was 56.6 ± 10.3 years (mean ± SD), and at diagnosis of NIDDM it was 43.0 ± 11.7 years. The BMI at the time of study and maximum BMI were 23.1 ± 3.3 and 26.5 ± 4.1 kg/m^2, respectively. Thus, this group was not particularly obese, implying that obesity and associated insulin resistance plays a less important role in the development of NIDDM in Japanese than in other groups (14). The majority of the subjects were treated with either diet (28.0%) or oral hypoglycemic agents (38.7%), with only 33.3% being treated with insulin.

Isolation of a YAC containing BIR and SUR genes. The BIR and SUR genes have been localized by fluorescence in situ hybridization to human chromosome band 11p15.1, and physical mapping studies have shown that the BIR gene is located about 4.5 kb downstream of the SUR gene (3). Since no high-information simple-sequence repeat DNA polymorphisms suitable for linkage studies had been described in these genes, we used a strategy to identify such polymorphisms whereby BIR and SUR genes were physically mapped in the CEPH megabase-insert YAC database and the YAC identification number was used to gain information on polymorphic STSs that had been mapped to the same or an overlapping YAC. STSs for the BIR and SUR genes mapped to the YAC 966_E_8. The information in the database of the Human Physical Mapping Project at the Whitehead Institute/MIT Center for Genome Research indicated that this YAC also contained the polymorphic STSs D11S902 and D11S921 and two expressed sequence tags: the human testis cDNA clone g05006 and the infant brain cDNA clone c-12h08. The colocalization of the BIR and SUR genes and the markers D11S902 and D11S921 to YAC 966_E_8 was confirmed by direct analysis of the isolated yeast clone.

TABLE 2
Clinical characteristics of subjects

n	213
Sex (M/F)	103/110
Age at time of study (years)	56.6 ± 10.4
Age at diagnosis (years)	43.0 ± 11.7
Duration (years)	13.8 ± 9.3
Fasting plasma glucose levels at time of study (mmol/l)	8.7 ± 2.8
Fasting plasma insulin at time of study (pmol/l)	48.0 ± 26.4
HbA_{1c} at time of study (%)	7.6 ± 1.8
BMI at time of study (kg/m^2)	23.2 ± 3.3
BMI at maximum body weight (kg/m^2)	26.5 ± 4.1
Current treatment (diet/OHAs/insulin)	58/80/69 (28.0/38.7/33.3)

Data are means ± SD or *n* (%). Information on current treatment was available for 207 subjects. Fasting plasma glucose and insulin levels were obtained from 85 subjects who were being treated with diet or oral hypoglycemic agents. OHAs, oral hypoglycemic agents.

Linkage analysis. The affected sib pairs were typed with the markers D11S902 and D11S921. There were 12 and 4 alleles, respectively, at these loci in Japanese subjects. There was no evidence for linkage of either marker with NIDDM in Japanese (Table 3), indicating that mutations in the BIR and/or SUR genes are not a major cause of NIDDM in this population.

DISCUSSION

Genetic factors play an important role in the development of NIDDM (15). Although there has been considerable progress in unraveling the genetics of maturity-onset diabetes of the

TABLE 3
Allele sharing IBS in affected sib pairs for markers near the BIR and SUR genes

Marker	Alleles IBS			χ^2	*P*	MLOD
	0	1	2			
D11S902 (PIC = 0.82)						
Pairs observed	19	76	45	0.22	0.89	0.00
Pairs expected	18	74	48			
D11S921 (PIC = 0.28)						
Pairs observed	1	31	99	0.89	0.64	0.08
Pairs expected	2	35	94			

The maximum likelihood of odds (MLOD) was derived from estimated identity-by-descent (IBD) as described by Holmans (13). MLOD values of 0.742, 1.377, and 2.324 correspond to *P* values of 0.05, 0.01, and 0.001, respectively.

young, a form of NIDDM with early onset and autosomal dominant inheritance (16), linkage studies of the more common late-onset NIDDM have not led to the confirmed identification of genes responsible for this form of diabetes. NIDDM is characterized by insulin resistance and impaired β-cell function (17), and it is likely that the genes responsible for NIDDM susceptibility will affect insulin action and secretion. The I_{KATP} plays a key role in integrating metabolism and insulin secretion in the pancreatic β-cell, and the identification and functional characterization of the protein(s) that comprise this current have been an area of intense investigation. Previously, we and others speculated that an inwardly rectifying K^+-channel–like protein encoded by a gene on chromosome 21 and expressed at high levels in insulinoma cells might be the β-cell K_{ATP} channel itself or a component of this channel (10,18,19). However, the results of a recent study (3) suggest that the previous studies were mistaken in this regard and that the K_{ATP} channel is a complex of a novel member of the inwardly rectifying K^+ channel family (BIR) expressed at highest levels in pancreatic islets and the sulfonylurea receptor. Moreover, the latter study showed that the genes encoding these two proteins are adjacent to one another on chromosome band 11p15.1. We localized the BIR and SUR genes in the emerging physical map of the human genome (20) and identified two microsatellite DNA polymorphisms located near them. Linkage studies in a group of 140 Japanese sib pairs with NIDDM indicate that genetic variation in the BIR and SUR genes is not a major factor contributing to NIDDM susceptibility in Japanese. However, these studies do not exclude a causative role for these loci in other populations or in a subgroup of NIDDM patients.

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