

Identification and Functional Analysis of Sulfonylurea Receptor 1 Variants in Japanese Patients With NIDDM

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The sulfonylurea receptor 1 (SUR1) is an essential regulatory subunit of the β -cell ATP-sensitive K^+ channel (K_{ATP}). The possible role of SUR1 gene mutation(s) in the development of NIDDM remains controversial as both a positive association and negative linkage results have been reported. Therefore, we examined the SUR1 gene at the single nucleotide level with single strand conformation polymorphism analysis in 100 Japanese NIDDM patients. We identified a total of five amino acid substitutions and 17 silent mutations by examining all 39 exons of this gene. Two rare novel mutations, D⁸¹¹N in exon 20 and R⁸³⁵C in exon 21, were identified in the first nucleotide-binding fold (NBF), a functionally important region of SUR1, in one patient each, both heterozygotes. To analyze possible functional alterations, we reconstituted the mutant K_{ATP} by coexpressing β -cell inward rectifier (BIR) (Kir 6.2), a channel subunit of K_{ATP} , and mutant SUR1 in HEK293T and COS-7 cells. As demonstrated by the patch clamp technique and rubidium (Rb^+) efflux studies, neither mutation alters the properties of channel activities. Two other rare missense mutations, R²⁷⁵Q in exon 6 and V⁵⁶⁰M in exon 12, were also identified. The R²⁷⁵Q substitution was not found in 67 control subjects, and V⁵⁶⁰M was present in three control subjects. Neither of these substitutions appeared to cosegregate with NIDDM in the probands' families. A previously reported S¹³⁷⁰A substitution located in the second NBF was also common in the Japanese subjects (allelic frequency 0.37), and was found at an equal frequency in nondiabetic control subjects. In conclusion, SUR1 mutations impairing K_{ATP} function do not appear to be major determinants of NIDDM susceptibility in Japanese. *Diabetes* 47:476–481, 1998

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BIR, β -cell inward rectifier; DMEM, Dulbecco's modified Eagle's medium; IRI, immunoreactive insulin; K_{ATP} , ATP-sensitive potassium channel; NBF, nucleotide-binding fold; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single-strand conformation polymorphism; SUR1, sulfonylurea receptor 1.

Insufficient glucose-induced insulin secretion is one of the key pathophysiological features observed in NIDDM patients. Although the defect is, at least in part, genetically determined, the underlying molecular mechanisms are largely unknown except in a few rare forms of NIDDM (1–7).

The β -cell ATP-sensitive potassium channel (K_{ATP}) plays a central role in glucose-induced insulin secretion by linking signals derived from glucose metabolism to membrane depolarization and insulin secretion (8–11). Recently, the K_{ATP} was shown to be a heteromultimeric structure composed of two essential subunits, sulfonylurea receptor 1 (SUR1) (12) and β -cell inward rectifier (BIR) (Kir 6.2), a member of the weak inward rectifier family (13). SUR1 is a member of the ATP-binding cassette superfamily containing two highly conserved nucleotide-binding folds (NBFs) 1 and 2 and serves as an ATP/ADP regulatory subunit of K_{ATP} (14–16). Mutations of the SUR1 gene were identified in patients with persistent hyperinsulinemic hypoglycemia of infancy, underscoring the importance of SUR1 in the physiological regulation of insulin release (16–19).

Given the central role of SUR1 in regulated insulin secretion, it is also possible that sequence variations in the gene play a role in other conditions characterized by abnormal insulin release, such as NIDDM. Recently, associations were reported between two polymorphisms of the SUR1 gene and NIDDM in Caucasians (20,21), suggesting the presence of a common mutation(s) determining susceptibility at or near this locus. However, linkage studies failed to demonstrate linkage of the disease to the SUR1 locus in the same racial group (20–22), and the nature of the mutation(s) is still unknown.

Results from previous studies involving Japanese subjects have suggested that primary β -cell defects play important roles in the development of NIDDM (23), such that the K_{ATP} genes are good candidates for the susceptibility determinant. A previous linkage analysis in Japanese NIDDM sibships found no evidence of linkage (24). However, the study sample size was relatively small, and linkage analysis would detect only major gene defects in this sample size, making the results inconclusive. Analysis of the entire SUR1 gene at the single nucleotide level has not yet been reported in any population. We conducted a single nucleotide level screening of the entire coding region of the SUR1 gene, and found several nucleotide changes resulting in amino acid substitutions. We further evaluated the variants by means of expression experiments.

RESEARCH DESIGN AND METHODS

Subjects. NIDDM patients ($n = 100$) and nondiabetic control subjects ($n = 67$) were chosen at random from among subjects recruited for our previous studies (25–27). All subjects were from the outpatient clinics at Yamaguchi University Hospital and affiliated hospitals. They were unrelated Japanese over age 40 years. Individuals with NIDDM met the diagnostic criteria of the World Health Organization (WHO). Nondiabetic subjects had a random plasma glucose level of <6.7 mmol/l and had no personal or family history of diabetes within second-degree relatives. At the time of recruitment, informed consent was obtained from each individual. The study protocol was approved by the Human Studies Committee of Yamaguchi University.

In the current study, control subjects were younger than diabetic patients (50.0 ± 9.5 vs. 58.0 ± 10.1 years, means \pm SD; $P < 0.01$). There were no differences between the NIDDM and control group in terms of sex (50 vs. 40% male) or BMI (22.9 ± 3.7 vs. 22.3 ± 3.6). Genomic DNA was extracted from peripheral blood as reported previously (28).

A 75-g oral glucose tolerance test was performed by the standard method, using WHO diagnostic criteria. The ratio of the change in immunoreactive insulin to that in plasma glucose at 30 min on the glucose tolerance test— Δ (immunoreactive insulin [IRI] 30 min)/ Δ (plasma glucose 30 min), the insulinogenic index—was calculated as a parameter of the glucose-induced insulin response (23).

SSCP analysis of the human SUR1 gene. We synthesized 39 sets of oligonucleotide primers flanking the coding regions of each of the 39 exons of the human SUR1 gene (19,20). Polymerase chain reaction (PCR) was carried out as described previously (19). Single-strand conformation polymorphism (SSCP) analysis was performed essentially as described by Orita et al. (29). Then 10 μ l of the PCR products were mixed with 30 μ l of 95% formamide, 20 mmol/l EDTA, 0.05% bromophenol blue, and xylene cyanol. Immediately before electrophoresis, samples were heat denatured at 95°C for 5 min. Aliquots of the samples (1.5 μ l) were electrophoresed under two different conditions: 1) 5% polyacrylamide (49:1, acrylamide:*N,N'*-methylene-bis-acrylamide) gel in 1 \times TBE [90 mmol/l Tris-borate (pH 8.3), 2 mmol/l EDTA] at 10 W for 5–7 h and 2) 5% polyacrylamide gel with 10% glycerol in 1 \times TBE at 10 W for 7–12 h, both at room temperature. After electrophoresis, the gels were dried and exposed to X-ray film with an intensifying screen for 16–30 h at -80°C .

Nucleotide changes corresponding to each SSCP were determined by direct sequencing of the PCR products using a model 373A automated sequencer and dye terminator sequencing kit (Applied Biosystems, Norwalk, CT).

Genotyping of the SUR1 gene. Genotypic and allelic frequencies of the missense mutations identified in the NIDDM patients were also determined in nondiabetic control subjects by either the PCR-SSCP or PCR-restriction fragment length polymorphism (RFLP) method. The Ser³⁷⁰ Ala (S³⁷⁰A) substitution created a *Mvu*I restriction site. After PCR-amplification of the 258 base pair (bp) DNA fragment containing exon 33, the products were digested with a 10-fold excess of the restriction enzyme for 16 h and electrophoresed on 2% agarose gel. The RFLP was detected by ethidium bromide staining. The Asp⁸¹¹ Asn (D⁸¹¹N) mutation in exon 20 and Arg⁸³⁵ Cys (R⁸³⁵C) mutation in exon 21 eliminated *Taq*I and *Hinc*II restriction sites, respectively. The mutations were detected by PCR-RFLP as described above.

Statistical analysis. The statistical significance of differences between groups in quantitative variables was analyzed by unpaired (two-tailed) *t* tests. Associations between the polymorphic markers in the human SUR1 gene and NIDDM were evaluated using Fisher's exact test or the χ^2 test.

Family studies. The proband with the R²⁷⁵Q mutation, a 54-year-old man, had been diagnosed as having NIDDM at age 40 years and was being treated with sulfonylureas. He had a 64-year-old sister with NIDDM. She had been diagnosed as having NIDDM and Basedow's disease at age 54 years, and her NIDDM persisted after her thyroid function had been normalized. She did not have the mutation.

The proband with the D⁸¹¹N mutation, a 54-year-old man, had been diagnosed as having NIDDM at age 44 years and was being treated with insulin. His family had a high prevalence of NIDDM, with four of his siblings plus himself having NIDDM. Two other brothers had already died, and their disease status was unknown. The proband had simple retinopathy, neuropathy, and proteinuria with normal serum creatinine. His sister had advanced retinopathy, neuropathy, and nephropathy, requiring hemodialysis. None of the family members had hearing disturbance, a neuromuscular disorder, or other systemic manifestations suggesting mitochondrial disease. One of the proband's sisters and her two daughters, who were all diabetic, were available for the mutation analysis. The sister and one of her daughters had the D⁸¹¹N mutation, but the other diabetic daughter did not.

The proband with the R⁸³⁵C mutation was a 71-year-old man who had been diabetic for 25 years without developing complications. He was managed with a sulfonylurea and an α -glucosidase inhibitor. He had four sisters and one brother. Two of the siblings plus the proband were overtly diabetic. Unfortunately, none of the proband's family members consented to participate in the study.

Another unrelated individual, who had normal glucose tolerance, was identified during the extended screening for the R⁸³⁵C mutation in nondiabetic controls.

The proband, a 51-year-old woman, and her 25-year-old daughter were available for the study. Both were apparently healthy and had the R⁸³⁵C mutation. There was no family history of NIDDM. The proband and her daughter had 75-g oral glucose tolerance tests. Both showed normal glucose tolerance. In the proband, the peak insulin level during the test was relatively low (282 pmol/l), as well as delayed (60 min), and her Δ (IRI 30 min)/ Δ (plasma glucose 30 min) was decreased (36.8; >43.2 in the normal Japanese population). The insulin response profile of her daughter appeared to be normal with an Δ (IRI 30 min)/ Δ (plasma glucose 30 min) of 526.4.

Functional analysis of mutant K_{ATP}. A full-length mouse SUR1 cDNA (mSUR1a; Y.T., unpublished observation) was isolated by reverse transcription (RT)-PCR from total RNA of the mouse insulinoma cell line MIN6 using oligonucleotide primers synthesized based on the reported rat and hamster SUR1 sequences (12). Mouse BIR (Kir6.2) cDNA was also isolated by RT-PCR from MIN6 RNA based on the reported sequence (13). Both SUR1 and BIR cDNA were inserted into the pcDNA3 vector (Invitrogen, San Diego, CA) to make pcDNA-mSUR1a and pcDNA-mBIR for expression in mammalian cells. Site-directed mutagenesis was performed using PCR according to a standard protocol (30). After the sequence confirmation, plasmids for the expression of mutant SUR1 (pcDNA-mSUR1a^{S11}, pcDNA-mSUR1a^{C85}) were constructed.

Wild-type and mutant K_{ATP} were reconstituted in human embryonic kidney (HEK) 293T cells by co-transfection of pcDNA-mBIR and pcDNA-mSUR1a (or pcDNA-mSUR1a^{S11} or pcDNA-mSUR1a^{C85}). Transfection was performed using lipofectamine reagent (GIBCO-BRL, Gaithersburg, MD) in Opti-MEM1 (GIBCO-BRL), as recommended by the supplier. After transfection, cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum for 24–48 h before electrophysiological assay. Single-channel recordings were made at room temperature in the inside-out configuration of the patch clamp technique. The pipette solution contained 140 mmol/l KCl, 1 mmol/l CaCl₂, 1 mmol/l MgCl₂, and 5 mmol/l HEPES-KOH (pH 7.4). The intracellular surface of the patch membranes was perfused with an internal solution containing 140 mmol/l KCl, 5 mmol/l EGTA, and 5 mmol/l HEPES (pH 7.3) with the free Mg²⁺ concentration adjusted to 1.4 mmol/l with MgCl₂, or with an internal solution containing no MgCl₂ but EDTA (5 mmol/l) instead of EGTA. ATP was dissolved in the Mg²⁺-containing or Mg²⁺-free internal solution and applied to the internal side of the patch membrane. When ATP was added to the internal solution containing Mg²⁺, the free-Mg²⁺ concentration was always adjusted to 1.4 mmol/l by supplementing MgCl₂ with reference to the stability constant of a Mg-ATP complex.

For the Rb⁺ efflux study, 1.3×10^5 COS-7 cells were seeded onto a 35-mm-diameter culture dish and incubated in DMEM supplemented with 4,500 mg/l glucose and 10% fetal calf serum (DMEM-HG-10FC) under 5% CO₂. On the following day, COS-7 cells were co-transfected with 1.5 μ g each of pcDNA-mSUR1a (or its mutant), pcDNA-mBIR, and pAdvantage vector (Promega, Madison, WI) using TransIT-LT1 (PanVera, Madison, WI) and Opti-MEM1 according to the supplier's protocol. Media were replaced with fresh DMEM-HG-10FC 6 h after the transfection. After additional incubation for 10–18 h, media were changed to fresh DMEM-HG-10FC containing ⁸⁶RbCl (Amersham, Buckinghamshire, U.K.) at 1 μ Ci/ml, and incubation was continued for an additional 24 h. Rb⁺ efflux was measured essentially as described previously (13). Oligomycin (Sigma, St. Louis, MO), 2-deoxyglucose (Sigma), and glibenclamide were used at the concentrations of 2.5 μ g/ml, 1 mmol/l, and 1 μ mol/l, respectively.

RESULTS

Human SUR1 gene variants. PCR-SSCP analysis of each of the 39 exons of the human SUR1 gene identified a total of 22 sequence variants in 100 Japanese NIDDM patients (Table 1). Of these, 5 were missense mutations, 10 were silent mutations of the codons, and 7 were mutations in the introns. None of the intron mutations were in the known consensus sequences for splicing, and thus did not appear to be functionally significant.

A missense mutation, TCC GCC (Ser¹³⁷⁰ Ala [S¹³⁷⁰A]) in exon 33 was located in NBF-2. The mutation was common, being found in 56 NIDDM patients, 38 of whom were heterozygous and 18 of whom were homozygous. The frequency of the S¹³⁷⁰A mutation was also determined in 67 control subjects by PCR-RFLP. When analyzed in NIDDM patients and control subjects, neither allelic (Table 1) nor genotypic (data not shown) frequencies differed between the two groups. Other missense mutations, CGG CAG (Arg²⁷⁵ Gln [R²⁷⁵Q]) in exon 6, GTG ATG (Val⁵⁶⁰ Met [V⁵⁶⁰M]) in exon 12,

TABLE 1
Sequence variations of SUR1 gene

Position	Common	Variant	Allelic frequency	
			NIDDM	Control
Exon 2	CCT (Pro ⁶⁹)	CCC (Pro ⁶⁹)	71/200	ND
Exon 5	ATC (Ile ²⁵⁰)	ATT (Ile ²⁵⁰)	1/200	ND
Exon 6	CGG (Arg ²⁷⁵)	CAG (Gln ²⁷⁵)	1/200	0/134
Exon 10	ACC (Thr ⁵⁴⁰)	ACT (Thr ⁵⁴⁰)	1/200	ND
Exon 12	GTG (Val ⁵⁶⁰)	ATG (Met ⁵⁶⁰)	1/200	3/134
Exon 12	CAT (His ⁵⁶²)	CAC (His ⁵⁶²)	137/200	95/134*
Intron 12	C [†]	T	1/200	ND
Exon 14	AAG (Lys ⁶⁴⁹)	AAA (Lys ⁶⁴⁹)	27/200	ND
Intron 15	C [‡]	T	109/200	67/134 [§]
Intron 19	C	A	1/200	ND
Exon 20	GAC (Asp ⁸¹¹)	AAC (Asn ⁸¹¹)	1/200	0/134
Exon 21	CTG (Leu ⁸³⁰)	TTG (Leu ⁸³⁰)	11/200	ND
Exon 21	CGC (Arg ⁸³⁵)	TGC (Cys ⁸³⁵)	1/200	1/134
Exon 25	TCG (Ser ¹⁰¹⁴)	TCA (Ser ¹⁰¹⁴)	1/200	ND
Exon 27	ACG (Thr ¹¹¹⁶)	ACT (Thr ¹¹¹⁶)	1/200	ND
Exon 29	GCC (Ala ¹²⁰⁵)	GCT (Ala ¹²⁰⁵)	1/200	ND
Exon 31	AGG (Arg ¹²⁷⁴)	AGA (Arg ¹²⁷⁴)	20/200	ND
Intron 32	C [¶]	A	2/200	ND
Exon 33	TCC (Ser ¹³⁷⁰)	GCC (Ala ¹³⁷⁰)	74/200	51/134 [#]
Intron 33	T ^{**}	C	24/200	ND
Intron 33	C ^{††}	T	3/200	ND
Intron 38	T ^{‡‡}	A	1/200	ND

ND, not determined. * $P = 0.2818$ (by two-sided Fisher's exact test); [†]seven base pairs upstream from exon 13 (c/tgagcag CGT-exon 13); [‡]three base pairs upstream from exon 16 (c/tag GCC-exon 16); [§] $P = 0.5188$ (by two-sided Fisher's exact test); ^{||}22 base pairs upstream from exon 20 (c/acctgectctaccacgcccag GTA-exon 20); [¶]71 base pairs upstream from exon 33; [#] $P = 1.00$ (by two-sided Fisher's exact test); ^{**}25 base pairs upstream from exon 34 (t/cggccggtgccttctcttccag ATC-exon 34); ^{††}16 base pairs upstream from exon 34 (c/tcttctcttccag ATC-exon 34); ^{‡‡}15 base pairs upstream from exon 39 (t/acctgcctgcccag CAT-exon 39).

GAC AAC (Asp⁸¹¹ Asn [D⁸¹¹N]) in exon 20, and CGC TGC (Arg⁸³⁵ Cys [R⁸³⁵C]) in exon 21, were rare. These mutations were found in one diabetic patient each, all heterozygous. The R²⁷⁵Q mutation in exon 6 (in the second extracellular loop) was not identified in the 67 control subjects. The V⁵⁶⁰M mutation in exon 12 (in the 8th transmembrane domain) was identified in three control subjects, all heterozygous for the mutation (Table 1).

D⁸¹¹N and R⁸³⁵C mutations are potentially important, since they are in the first nucleotide binding domain, a functionally important domain of SUR1 (19,31). After the initial screening in 100 NIDDM patients and 67 nondiabetic control subjects, the search for the D⁸¹¹N mutation was extended to a total of 201 NIDDM subjects and 114 control subjects, using PCR-RFLP with *Taq* I. No other individuals with the mutation were found. The missense mutation in exon 21 was examined in a total of 192 NIDDM subjects and 108 control subjects by PCR-RFLP with *Hinc*II. The mutation was detected in one control subject in addition to the NIDDM subject found during the initial screening.

In Caucasians, strong associations were found between two sequence variations of the SUR1 gene and NIDDM (20). One of the variants, a C T transition located three base pairs upstream from exon 16 (previously called exon 24), was also common in Japanese individuals. Neither the allelic nor the genotypic frequencies, however, differed between NIDDM patients and control subjects (Table 2). Another variant, ACC ACT (T⁷⁶¹T), was not present in any of the 199 Japanese NIDDM subjects screened.

Family studies. To evaluate the possible contribution of the R²⁷⁵Q, D⁸¹¹N, and R⁸³⁵C substitutions to the development of NIDDM, we studied the probands' families. The results of segregation analyses were not consistent with these substitutions playing a role in the pathogenesis of NIDDM in these families.

Functional study of mutant SUR1. Possible functional alterations of SUR1 due to the D⁸¹¹N and R⁸³⁵C mutations in the NBF-1 were assessed by the patch clamp technique and Rb⁺ efflux studies of K_{ATP} reconstituted in cultured cells.

The patch clamp studies revealed no significant difference in single-channel conductance or kinetics among the wild-type SUR1a/BIR, SUR1a^{N811}/BIR, and SUR1a^{C835}/BIR channels. As shown in Fig. 1, all of the spontaneously opening K_{ATP} channels formed with these three combinations of SUR1 and BIR were inhibited by 100 μmol/l ATP to a similar extent. ATP had a stronger effect on all three in the absence rather than in the presence of intracellular Mg²⁺. The channel activities in the presence of 100 μmol/l ATP normalized to the value obtained in the absence of ATP were 0.080 ± 0.007, 0.019 ± 0.017, and 0.13 ± 0.13 for the wild-type SUR1a/BIR, SUR1a^{N811}/BIR, and SUR1a^{C835}/BIR channels in the absence of Mg²⁺, respectively, whereas the corresponding values in the presence of Mg²⁺ were 0.43 ± 0.26, 0.39 ± 0.20, and 0.48 ± 0.24. Figure 2 shows the concentration-response relationship of the ATP-induced inhibition of the activities of the wild-type SUR1a/BIR, SUR1a^{N811}/BIR, and SUR1a^{C835}/BIR channels. The data were fitted with the Hill equation, the parameters of which are listed in Table 3. In the

TABLE 2
Genotypic and allelic frequencies of the intron 15 variants

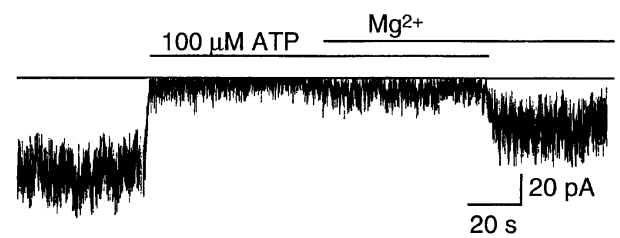
	NIDDM	Control
Genotype*		
c/c	21 (0.21)	16 (0.23)
c/t	49 (0.49)	35 (0.52)
t/t	30 (0.30)	16 (0.23)
Total	100 (1.00)	67 (1.00)
Allele†		
t	109 (0.55)	67 (0.50)
c	91 (0.46)	67 (0.50)
Total	200 (1.00)	134 (1.00)

Data are *n* (frequency). * $P = 0.7358$ ($\chi^2 = 0.5653$); † $P = 0.5188$ (by two-sided Fisher's exact test).

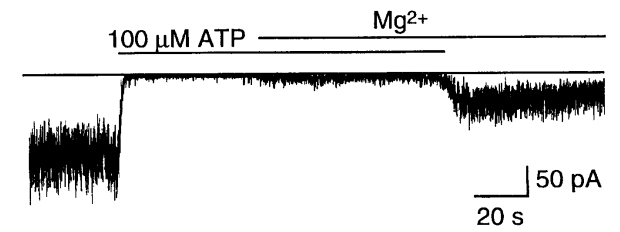
absence of Mg^{2+} , it is obvious that there was no significant difference in ATP sensitivity among these channels. In the presence of Mg^{2+} , however, the SUR1a^{N811}/BIR channel appeared to be less sensitive to ATP than the others. Thus we assessed whether the significant differences in ATP sensitivity in the presence of Mg^{2+} among the three types of the channels were statistically significant by using the analysis of variance statistical method. There were, however, no significant differences ($P = 0.800$).

We also assessed the functions of the SUR1a^{N811}/BIR and SUR1a^{C835}/BIR K_{ATP} channels by means of Rb^+ efflux studies. For certain mutations, Rb^+ efflux studies may be more sensitive as a screening test (16). When wild-type K_{ATP} (wild type mSUR1a/BIR) was expressed in COS-7 cells, and intracellular ATP was depleted in response to metabolic inhibition by incubation with 2-deoxyglucose (1 mmol/l) and oligomycin (2.5 μ g/ml), significant Rb^+ efflux was observed as compared with the control cells, which expressed bacterial β -galactosidase (*lacZ*) and mBIR (Fig 3A). Comparable amounts of Rb^+ efflux were observed from the cells expressing variant K_{ATP} (SUR1a^{N811}/BIR and SUR1a^{C835}/BIR) (Fig 3A). Efflux was inhibited by 1 μ mol/l glibenclamide to the same extent among the cells expressing wild-type and variant K_{ATP} channels (Fig. 3B).

SUR1a/BIR (wild)



SUR1a^{C835}/BIR



SUR1a^{N811}/BIR

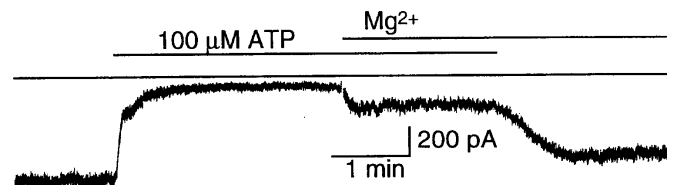


FIG. 1. Inhibition of spontaneously opening K_{ATP} channels by intracellular ATP in the presence and absence of 1.4 mmol/l free Mg^{2+} in inside-out patches. The membrane potential was -60 mV. These patches were exposed to ATP (100 μ mol/l) for a period of time, as indicated by the bars. Lines indicate the zero current level.

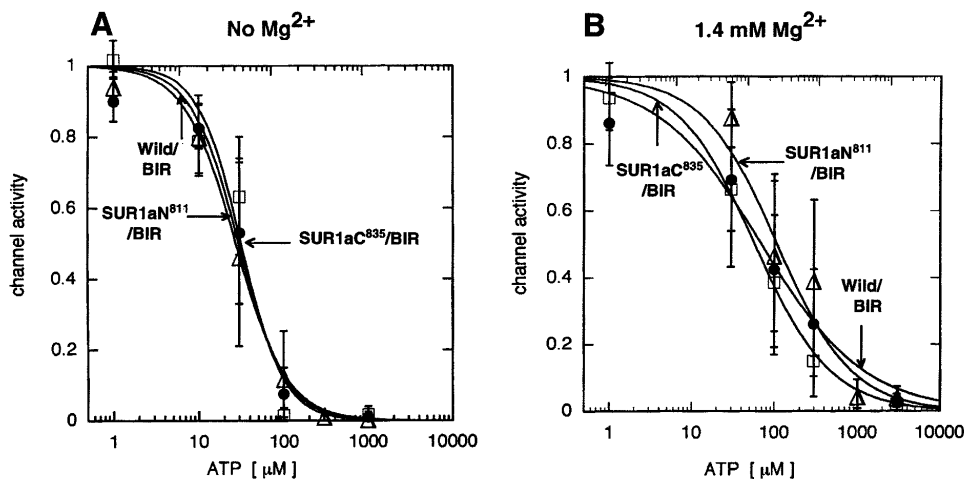


FIG. 2. The concentration-response relationships of ATP-induced inhibition of the wild-type SUR1a/BIR (●), SUR1a^{N811}/BIR (△), and SUR1a^{C835}/BIR (□) channels in the absence (A) and the presence (B) of Mg^{2+} . The channel activity in the presence of the indicated concentrations of ATP was normalized to the value obtained in the absence of ATP. Symbols and bars represent means \pm SD, and the curves represent the fitting of the data with the Hill equation. Each point represents at least five observations.

TABLE 3

Values of parameters obtained by fitting the data shown in Fig. 2 with the Hill equation

	No Mg ²⁺		1.4 mmol/l free Mg ²⁺	
	K _i (μmol/l)	H	K _i (μmol/l)	H
SUR1a/BIR (wild)	29	1.6	70	0.70
SUR1aN ⁸¹¹ /BIR	27	1.4	108	0.96
SUR1aC ⁸³⁵ /BIR	31	1.7	57	0.94

K_i, apparent dissociation constant of ATP; H, Hill coefficient.

DISCUSSION

We scanned all 39 exons of the human SUR1 gene in 100 Japanese NIDDM patients by SSCP analysis and found five, including four novel, amino acid substitutions. A missense variant in NBF-2 of exon 33 (S¹³⁷⁰A) was found in more than 50% of Japanese NIDDM patients, 18% of whom were homozygous for the substitution. The allelic frequency of A¹³⁷⁰ was 0.37 in NIDDM patients and 0.38 in nondiabetic subjects (Table 1). It was recently reported that NBF-2 of SUR1 mediates the regulatory effect of intracellular MgADP, which binds to the NBF-2, thereby antagonizing the inhibitory effect of ATP on K_{ATP} (16). Although the mutation in this region is likely to impair the function of K_{ATP}, it is unlikely that this variant constitutes a significant risk factor for NIDDM, given the lack of any difference between the frequencies observed in patients and control Japanese subjects, in accordance with a report on Caucasian subjects (20).

The other four missense mutations—R²⁷⁵Q, V⁵⁶⁰M, D⁸¹¹N, and R⁸³⁵C—have not been reported previously (Table 1). Amino acid sequences of human, rat, hamster, and mouse SUR1 are conserved at these positions.

The D⁸¹¹N and R⁸³⁵C mutations are particularly interesting because these are in NBF-1. We evaluated the possible role of these amino acid substitutions in the development of NIDDM. Analysis of the families with these mutations did not provide evidence supporting a causal relation to NIDDM; namely, in the family with the D⁸¹¹N substitution, there was one NIDDM patient without the mutation. The nondiabetic individual with

the R⁸³⁵C had a decreased early-phase insulin response to glucose loading evaluated by Δ(IRI 30 min)/Δ(plasma glucose 30 min), possibly suggesting involvement of the mutation in impaired glucose-induced insulin secretion. However, her 25-year-old daughter had a normal insulin response.

We next directly evaluated the channel function of the mutants using patch clamp and Rb⁺ efflux analyses. Both SUR1aN⁸¹¹ and SUR1aC⁸³⁵ constituted functional K_{ATP} with BIR/Kir 6.2, and their channel activities were inhibited by 1 μmol/l glibenclamide (Figs. 1–3). Although the SUR1aN⁸¹¹/BIR channel appeared to be less sensitive to intracellular ATP in the presence of Mg²⁺, the difference was not statistically significant (Fig. 2, Table 3). Neither of the mutations had a major effect on response to metabolic inhibition (Fig. 3), the effect of which seems to be through a (relative) increase in intracellular ADP concentration (16), suggesting that the response to ADP of these mutant channels was not impaired. These lines of evidence suggest that both the D⁸¹¹N and the R⁸³⁵C substitution are functionally silent. We introduced human mutations into mouse SUR1 cDNA, which were then coexpressed with mouse BIR in heterologous cell lines (COS-7 and HEK293T cells) but not in β-cell lines, to evaluate the functional consequences of these mutations. When a protein product has a highly specialized role in a specific cell type, and if there is species-specific interaction between SUR1 and BIR, the possibility arises that our system may not detect the real functional impact of amino acid changes. However, this is very unlikely.

The functional consequences of the R²⁷⁵Q and V⁵⁶⁰M substitutions are less certain. R²⁷⁵ lies in the extracellular junctional region between the 4th transmembrane domain and the 5th transmembrane domain. V⁵⁶⁰ lies in the 8th transmembrane domain. The R²⁷⁵Q mutation was found in only 1 NIDDM subject, and in none of the 67 nondiabetic control subjects. The patient with the R²⁷⁵Q mutation had a sister with NIDDM. However, she did not have the mutation (data not shown). V⁵⁶⁰M, which was found in 1 out of 100 NIDDM patients, was also present in three of 67 nondiabetic subjects (Table 1). These findings do not support the involvement of these two mutations in the development of NIDDM. Conclusions cannot, however, be drawn until functional analyses of the SUR1 mutants are completed, given the conservation of the amino acids at these positions among species.

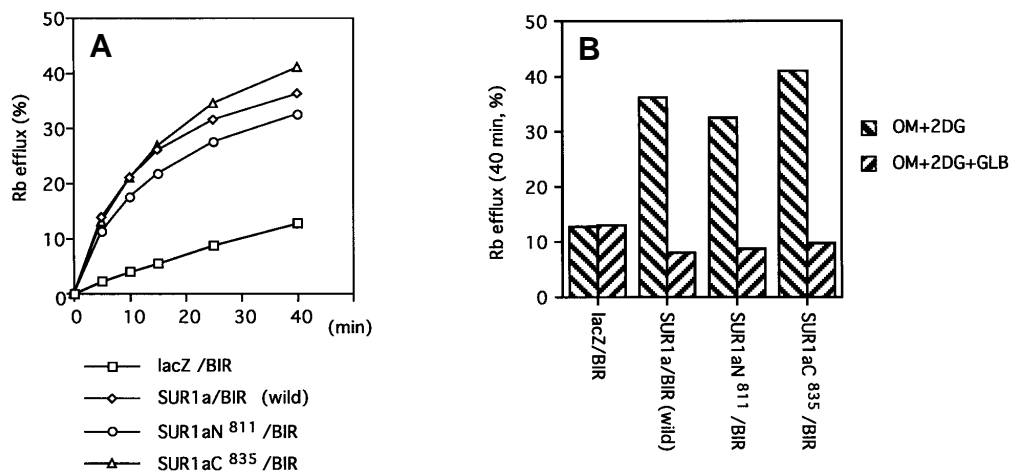


FIG. 3. Rb⁺ efflux studies. *A*: Time courses of Rb⁺ efflux from COS-7 cells expressing wild-type or mutant K_{ATP} in the presence of oligomycin (OM; 2.5 μg/ml) and 2-deoxyglucose (2DG; 1 mmol/l). *B*: Efflux of Rb⁺ during a 40-min incubation in the presence or absence of glibenclamide (GLB; 1 μmol/l) under metabolic inhibition. Means of duplicate measurements in the representative experiment are plotted. The experiments were repeated three times with similar results. As a control, Rb⁺ efflux was measured from COS-7 cells expressing bacterial β-galactosidase (lacZ) and BIR.

Recently, two sequence polymorphisms of the SUR1 gene were found to be strongly associated with NIDDM in Caucasian subjects (20). This result was replicated in French patients (21), suggesting that genetic defects at this locus may account for a significant component of the inherited basis of NIDDM in this racial group. In the Japanese subjects, however, we did not observe the association between NIDDM and one of the polymorphisms examined in the Caucasian studies (Table 2). The other polymorphism was not found in the present group of Japanese patients. These results, along with our SSCP analysis, indicate that mutation of the SUR1 gene is not a major determinant of susceptibility to NIDDM in Japanese. This may imply racial differences in the genetic basis of the disease, although there is still a possibility that the previous population studies (20,21) could reflect genetic mixture in the Caucasian population studied.

In conclusion, we analyzed the entire coding region of the SUR1 gene at the single nucleotide level in Japanese NIDDM patients. Although we identified several missense mutations, none appeared to determine susceptibility to NIDDM. Thus defects in SUR1 do not appear to account for the impaired glucose-induced insulin secretion commonly observed in Japanese NIDDM patients.

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