Biochemical and Biophysical Research Communications **274,** 302–309 (2000)
doi:10.1006/bbrc.2000.3136, available online at http://www.idealibrary.com on IDEAL®

Genomic Structure and Expression of Human *KCNJ9 (Kir3.3/GIRK3)*

Jeannette Vaughn, Johanna K. Wolford, Michal Prochazka, and Paskasari A. Permana[1]
Clinical Diabetes and Nutrition Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona 85016

Received June 21, 2000

The human KCNJ9 (Kir 3.3, GIRK3) is a member of the G-protein-activated inwardly rectifying potassium (GIRK) channel family. Here we describe the genomic organization of the *KCNJ9* locus on chromosome 1q21-23 as a candidate gene for Type II diabetes mellitus in the Pima Indian population. The gene spans ~7.6 kb and contains one noncoding and two coding exons separated by ~2.2 and ~2.6 kb introns, respectively. We identified 14 single nucleotide polymorphisms (SNPs), including one that predicts a Val366Ala substitution, and an 8 base-pair (bp) insertion/deletion. Our expression studies revealed the presence of the transcript in various human tissues including pancreas, and two major insulin-responsive tissues: fat and skeletal muscle. The characterization of the *KCNJ9* gene should facilitate further studies on the function of the KCNJ9 protein and allow evaluation of the potential role of the locus in Type II diabetes.

Inwardly rectifying K^+ (Kir) channels play a major role in controlling resting membrane potential and initiating action potentials. G-protein-activated K^+ (GIRK) channels constitute a subfamily activated by pertussis toxin-sensitive G proteins (1). These GIRK channels play an important role in regulating heart beat and neuronal firing rate. At present the GIRK subfamily consists of four different proteins encoded by mammalian genes *GIRK1/Kir3.1/KCNJ3* (according to the most recent human locus nomenclature, see http://www.ncbi.nlm.nih.gov; (2, 3), *GIRK2/Kir 3.2/BIR1/KATP2/KCNJ6* (4), *GIRK3/Kir3.3/KCNJ9* (4), and *GIRK4/Kir3.4/CIR/KATP1/KCNJ5* (5, 6). An additional member of this subfamily isolated from *Xenopus* oocyte is called XIR (7).

Only some genes encoding GIRK proteins have fully characterized genomic structures. Human *KCNJ3* contains 3 exons spanning an ~45-kb region (8) that has been mapped to chromosome 2q24.1 (9). *GIRK2* in the mouse contains 7 exons and produces differentially spliced transcript isoforms (10–12). Human *KCNJ6* is on chromosome 21q22.1-22.2 and consists of at least 2 exons (Ref. 13; Schoots, O., and Van Tol, H. H. M., unpublished, GenBank Accession No. NM_002240). *KCNJ5* has been localized to chromosome 11q24. The mouse homolog, *GIRK4*, contains 2 coding exons and possibly noncoding exons encompassing the 5′ and 3′-*UTR*s (14). To date, only the *KCNJ9* mRNA sequence has been determined (Schoots, O., unpublished; GenBank Accession No. NM_004983/U52152).

The human KCNJ9 protein is encoded by the *KNCJ9* locus that has been localized to chromosome 1q21-23 (15). We have previously found that this chromosomal region is linked to Type II diabetes mellitus in Pima Indians of Arizona with a LOD score of 2.6 (16), a population with the highest reported prevalence of the disease (17). The *KCNJ9* locus is also a functional candidate gene, since the ability of the encoded protein to regulate membrane potential resembles the role of K_{ATP} channels in glucose-induced insulin secretion in pancreatic beta cells. The K_{ATP} channels consist of inwardly rectifying potassium channel subunits and sulfonylurea receptors (18). Sulfonylureas, as treatment of Type II diabetes mellitus, stimulate insulin secretion by closing the K_{ATP} channels (19). The position and function of *KCNJ9* as a candidate gene for this disease in the Pima Indians led us to characterize its genomic structure as well as its expression in different human tissues.

MATERIALS AND METHODS

YAC and BAC library screening. We identified a YAC clone containing the *KCNJ9* gene, clone 891b7, by screening the Human CEPH B YAC Library (Research Genetics, Huntsville, AL) according

[1] To whom correspondence should be addressed at Clinical Diabetes and Nutrition Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 4212 N. 16th Street, Phoenix, AZ 85016. Fax: (602)-200-5335. E-mail: ppermana@mail.nih.gov.

0006-291X/00 $35.00

to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was performed with the forward primer 5′-CTG-TCTAATGACACGGTAGG-3′ and the reverse primer 5′-CTGAAC-TTGCGGACTAAGG-3′. This YAC clone was used to determine exon/intron boundaries of *KCNJ9*. To avoid potential chimerism that is common among YAC clones, we used BAC clones to sequence upstream of the gene. BAC clones containing the *KCNJ9* gene were identified in the CITB Human BAC library, Release IV (Research Genetics) using the above primers. This library screening identified 5 positive BAC clones: 261F14, 567J5, 338E13, 440N13, and 317N23. The first two clones were used for further study.

Determination of exon/intron boundaries. Primers designed from the cDNA sequence (GenBank Accession No. U52152 or NM_004983) were utilized with Expand Long Template PCR System (Boehringer Mannheim, Indianapolis, IN) to determine the exon–intron boundaries and intron sizes of *KCNJ9* on YAC 891b7. The PCR and sequencing primers are listed in Table 1. Amplification conditions for fragments larger than ~1 kb (except for exon 3) consisted of a denaturing step for 1 min at 96°C, followed by 30 cycles of 20 s at 96°C, 30 s at 60°C, and 18 min with 10 s increments per cycle at 68°C, and a final extension for 10 min at 68°C. The genomic region was sequenced using the Big Dye Terminator Cycle Sequencing kit and an ABI 377 sequencer (Applied Biosystems Division of Perkin Elmer, Foster City, CA). Cycling parameters for sequencing the fragments were as recommended by the manufacturer, except for introns 1 and 2 that required a primer annealing temperature of 60°C.

Sequencing the putative promoter region. The region upstream of the first *KCNJ9* exon was sequenced directly using DNA from BAC clones 261F14 and 567J5. Subsequent primers were designed from the new sequences to cover a ~1.7 kb segment containing the putative promoter region. The BAC DNA was denatured for 5 min at 95°C, followed by 99 cycles of 30 s at 95°C, 20 s at 53°C, and 4 min at 60°C. The sequencing primers are listed in Table 1. Unless otherwise mentioned, the nucleotide numbering used throughout this paper is based on the assignment of nucleotide A of the first translation start codon (ATG) as 1, and the preceding nucleotide as −1 (20).

Identification of KCNJ9 polymorphisms in genomic DNA from Pima Indian subjects. The *KCNJ9* gene was screened for variants by sequencing genomic DNA from 8 diabetic and 8 nondiabetic, full-blooded Pima Indians who were not first degree relatives. The subjects are members of the Gila River Indian community who have been participating in a longitudinal study of the development of Type II diabetes. The genomic DNA samples were prepared from transformed lymphocyte cultures or peripheral white blood cells (21). The putative promoter region was amplified using the Expand Long Template PCR System (Boehringer Mannheim) with a denaturing step for 1 min at 96°C, followed by 35 cycles of 20 s at 96°C, 30 s at 57°C, and 18 min with 10 s increments per cycle at 68°C, and a final extension for 10 min at 68°C. The fragment was sequenced on an ABI 377 sequencer (Applied Biosystems Division of Perkin Elmer) with internal primers (Table 1) according to the manufacturer's recommendation with a primer annealing temperature of 57°C. The exonic, intronic, and 3′-*UTR* regions were amplified and sequenced as described above. All primers used are described in Table 1.

Expression analysis. *KCNJ9* expression in a variety of human tissue-specific cDNA (Clontech, Palo Alto, CA) was determined using RT-PCR. Primers Kir 3FA and Kir 2R located in exons 2 and 3 respectively (Table 1) were utilized to minimize the possibility of amplifying genomic fragments. These primers were used with a cycling condition of a 3-min denaturing step at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C with a final extension step for 5 min at 72°C. The expected 282-bp amplicon products from cDNA were analyzed on a 1.2% agarose gel stained with ethidium bromide (Fig. 4).

RESULTS

To analyze the genomic structure of the *KCNJ9* gene, we screened a human YAC library with primers in the 3′*UTR*. A clone containing the gene, YAC 891b7, was used as a sequencing template to determine the exon/intron boundaries. Comparison of the cDNA and the genomic sequence showed that the gene consists of one noncoding exon, two coding exons, and two introns, encompassing an approximately 7.6 kb region (Fig. 1). The 5′*UTR* includes the short noncoding exon 1 (128 bp) and part of exon 2. The protein coding sequence (1182 bp) consists of parts of exons 2 and 3. The exon–intron splice junctions adhere to the GT-AG consensus sequence of splice donor and acceptor sites in eukaryotic genes (22).

To determine the sequence of the putative promoter of *KCNJ9*, we identified BAC clones 261F14 and 567J5 that contain the gene. As we sequenced ~1.7 kb of the 5′ upstream region of exon 1 using these BAC clones, we noted the absence of the first 16 nucleotides (nt) of the published human cDNA (GenBank Accession No. NM_004983/U52152). We also observed that the immediate genomic sequence preceding exon 1 did not contain a consensus splice acceptor site similar to those found in introns 1 and 2. To determine if there was indeed an intron preceding exon 1, we performed Long Distance PCR using forward primers ending with the 16 nt sequence and various reverse primers in the exon 1 and putative promoter regions; these attempts did not amplify any PCR product. To establish if these 16 nt are conserved in other species, the human cDNA sequence was aligned with sequences from mouse and rat (Fig. 2). Interestingly, the 5′ sequence homology among the 3 species starts beyond the first 16 nt of the human cDNA sequence. This 16 nt sequence is also absent in known genomic sequences of other human G-protein-coupled inward rectifiers. The final genomic sequence that we obtained has been submitted to GenBank with Accession No. AF193615.

The putative promoter region of the *KCNJ9* locus was analyzed for the presence of transcription factor consensus binding sites using MatInspector program v2.2 (23). The sequence directly upstream of the 5′*UTR* lacks the canonical TATA and CAAT boxes that typically characterize mammalian RNA polymerase II promoters, but contains a common enhancer element in eukaryotic genes generally found near the transcriptional start site of many TATA-less promoters that binds the transcription factor PEA3 (24). We also identified several other consensus sequences for transcription factors in this region (Fig. 3), including a beta-cell-specific transcription factor PDX-1 that regulates expression of the insulin gene (25), and transcription activators of muscle specific genes: RSRFC4 (26) and MEF-2 (27).

TABLE 1

Primers Used in Sequencing the *KCNJ9* Gene

Primer	Forward (F)/reverse (R)	Sequence 5′–3′[b]	Primer location	Size (bp)
Amplicon primers[a]				
Prom F1	F	CAT GGC CAA ATC TTG AAA GC	promoter	1935
K-flank 3R	R	AAA ACT CCC CCA AGA ACC AG	exon 1	
Kir-1F	F	AGA AAC AGC GGT GTC TGC G	exon 1	2578
Kir-1R	R	GTA TGT CTC GCG CAC GTT G	exon 2	
KFM1	F	GCG CTA CGT GGA GAA GGA TG	exon 2	687
KRM1	R	ATC TCG TGG CTG ATA ACC AGC	exon 2	
Kir-3FA	F	GCT TCG ACA CGG GAG ACG	exon 2	2888
Kir-2R	R	TCT CGT GAA AGC TGG CAT AG	exon 3	
KFO1	F	CAC GTC AGT GCT GAC TCT GG	exon 3	435
KRO1	R	GTT TCT AGA CTT GAG GTT CTG G	3′ UTR	
IRP-1A	F	GAA CTG CAT ATC GGA GGT G	exon 3	1486
IRP-1R	R	CAT TGA TGG TTG AGT TTC TGG	3′ UTR	
Sequencing primers				
Prom F1	F	CAT GGC CAA ATC TTG AAA GC	promoter	
Prom F2	F	AGT TGT TGC CAG GGG TTA GG	promoter	
Prom F3	F	AGT TTG GCT CCT GTC TCT GC	promoter	
Prom F4	F	ACA TTT GGG TTG TTT CTA GG	promoter	
Prom F6	F	AGA CCC ATG TTG AAG GT	promoter	
PMT F1	R	CCT CCT GGA CAA CTC AGA GC	promoter	
K-flank 4R	R	TGC CTG CTT CAC AGA GTC AG	promoter	
K-flank 6R	R	ACA CAT GCC TCA TGT CAT CC	promoter	
PMT F2	R	TTC CAC GGC AAC CAG TCT GG	promoter	
K-flank 3R	R	AAA ACT CCC CCA AGA ACC AG	exon 1	
Kir-1F	F	AGA AAC AGC GGT GTC TGC G	exon 1	
KFA1	F	GTC CTT GAT CTT GAG ATA GAG G	intron 1	
KFA2	F	AGG AAA AGG AAG CCC ATT GT	intron 1	
KFA4	F	TCG TTT TCT GCA TGA CCT GG	intron 1	
KFAP	F	GAT CTG GGC CTG GGT CTT	intron 1	
KFA3	F	CAT ACC CAG GCA GTT TCT CC	intron 1	
KRA1	R	GGG TCA AAT GGA ATG ATG TAC C	intron 1	
KRA2	R	CCT CCT TGG ACC TCA CTC AC	intron 1	
KRA3	R	CTG CCA TAT GCC CCA AAG	intron 1	
Kir-1R	R	GTA TGT CTC GCG CAC GTT G	exon 2	
KFM1	F	GCG CTA CGT GGA GAA GGA TG	exon 2	
KRM1	R	ATC TCG TGG CTG ATA ACC AGC	exon 2	
Kir-3FA	F	GCT TCG ACA CGG GAG ACG	exon 2	
KFB1	F	GGA ATG TGT CAC TTG GAA TAG G	intron 2	
KFB2	F	TAG TGG TCC CGA GTT GGA AG	intron 2	
KFB3	F	ACA AGC TGG TGC CTT CAG AT	intron 2	
KRB1	R	TGT CAT TTG GTG ATG CCA ACC	intron 2	
KRB2	R	ACC CTG GGA TTT CCA ACT CT	intron 2	
KRB3	R	CTG CTC CGT GCA TAA GAG AG	intron 2	
Kir-2R	R	TCT CGT GAA AGC TGG CAT AG	exon 3	
KFO1	F	CAC GTC AGT GCT GAC TCT GG	exon 3	
IRP-1A	F	GAA CTG CAT ATC GGA GGT G	3′ UTR	
IRP-20	F	GGT AGG CCA AGC TGA AGT GA	3′ UTR	
IRP-63	F	AAC CTG GTT CCT GTC CTC AA	3′ UTR	
IRP-1R	R	CAT TGA TGG TTG AGT TTC TGG	3′ UTR	
IRP-1RA	R	CTG CAC AGG TTG TCA GTT TG	3′ UTR	
IRP-2	R	CTG AAC TTG CGG ACT AAG G	3′ UTR	
KRO1	R	GTT TCT AGA CTT GAG GTT CTG G	3′ UTR	

[a] Amplicon primers were also used as sequencing primers.
[b] Annealing temperatures ranged from 56 to 60°C.

We screened the genomic region of *KCNJ9* for potential polymorphisms by comparative sequencing using genomic DNA from 8 diabetic and 8 nondiabetic Pima Indians and identified 14 single nucleotide polymorphisms (SNPs) summarized in Table 2. One SNP changes the 366th amino acid residue in the C terminus from Val to Ala. In a preliminary typing of additional 100 people, we also detected an 8-bp insertion/

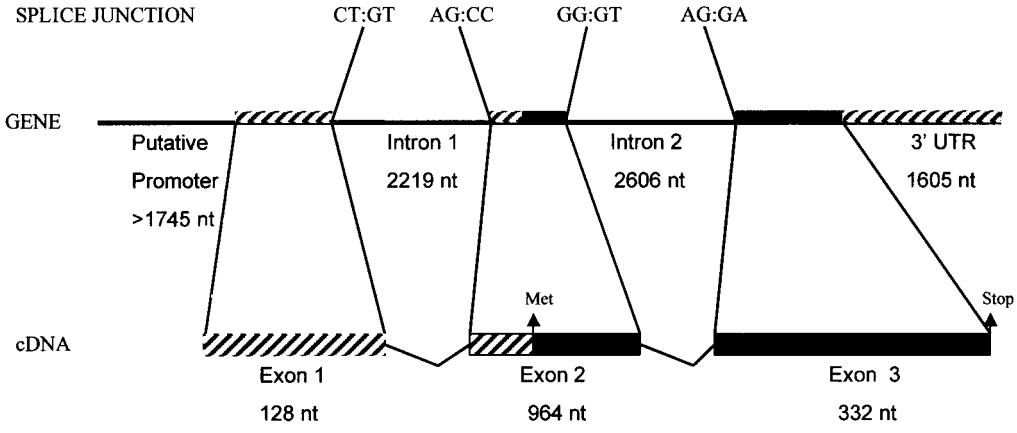

FIG. 1. A schematic diagram showing the genomic structure and splice junction donor and acceptor sequences of the human *KCNJ9* gene. The length of each region is expressed in nucleotides (nt). Solid and hatched boxes represent coding and noncoding regions of the cDNA, respectively. The thin line at the genomic level represents the putative promoter and intronic regions.

deletion polymorphism in the 3′*UTR* of 4 subjects; this mutation does not appear to disrupt any known regulatory motif.

To determine the tissue distribution of the *KCNJ9* transcript, we performed RT-PCR in cDNA samples from various human tissues (Fig. 4). The expected 282 bp fragment was seen in pituitary, small intestine, testis, adult brain, fetal brain, fat, kidney, skeletal muscle, smooth muscle, and pancreas. There was no detectable expression in ovary, placenta, spleen, stomach, thyroid gland, adrenal gland, heart, liver, and mammary gland. Reaction using water as the negative control template did not produce any detectable band (data not shown).

DISCUSSION

The gene encoding the third human subfamily of G-protein-activated K^+ channels (*Kir3.3/GIRK3/KCNJ9*) resides on chromosome 1q21-23, where there is a suggestive linkage to diabetes mellitus in the Pima Indian population. Our genomic characterization of this candidate gene revealed that the gene spans ~7.6 kb region and consists of one noncoding exon and 2 coding exons separated by ~2.2 and ~2.6 kb introns, respectively. The position of intron 1 is unique, whereas that of intron 2 is exactly the same as in other characterized human G-protein-coupled inward rectifiers (GIRK1, 2, and 4; 6, 8, 13). The size of *KCNJ9* intron 2 (~2.6 kb) is smaller than those of other members of this family that have a range of 5.5 kb to over 20 kb. The putative promoter region of *KCNJ9* lacks both the CAAT and TATA boxes that are usually present in mammalian promoters utilizing RNA polymerase II (28). This characteristic is shared by the 5′ flanking sequence of the human *KCNJ3* gene (8), the only other member of the human GIRK family whose promoter region has been analyzed.

Since the *KCNJ9* gene is a good candidate gene for Type II diabetes in the Pima Indians, we have screened this gene for potential polymorphisms in selected groups of diabetic and nondiabetic subjects. In the ~10 kb region that we screened, we found 14 SNPs. These polymorphisms are currently being typed in a larger group of subjects.

```
NM_004983     1 GCATCAAGACCATCAGACATTTAGGAGAAACAGCGGTGTCTGCGGCTCCCACCCTTCGGG
AF130860      1 ------------------------------------------------------------
U11860        1 ---CTG..CTGCCGTT.....C.............A.....G..........AT..CA.A.
L77929        1 ----------------------------GG..CGA.-..............T..CA.A.

NM_004983  1324 CGGCTGGATGAGAAGGTGGAGGAGGAGGGGGTGGGGGAGGGGG-CGGGTGGGGAAGCTGG
AF130860   1217 A.....................A..A....CT..........-.A....C..G..A...
U11860     1373 A.....................A..A....CT..........G.A....C..G..A...
L77929     1317 A.....................A..A....CT..........-.A..C.CA.G..A...
```

FIG. 2. Homology alignment of human *KCNJ9* cDNA (GenBank Accession No. NM_004983) with cDNA from other mammalian species, mouse (AF130860 and U11860) and rat (L77929), at the beginning of the transcript and surrounding the Val366Ala substitution. Dots in the bottom 3 rows represent nucleotide homology to human *KCNJ9* cDNA. Dashes indicate absent nucleotides. The cDNA numbering system used is taken from the GenBank files. The single nucleotide polymorphism that results in Val to Ala substitution in human sequence is bold and underlined (position c.1356).

FIG. 3. Potential transcription factor binding sites in the putative promoter region. Shaded areas may contain more than one transcription factor binding site. The different transcription factors are represented by lowercase letters placed above the first nucleotide of their consensus binding sites. A minus sign (−) after the transcription factor indicates that the consensus binding site is complimentary to the shaded sense strand displayed in this figure. The bold and underlined nucleotides are SNPs found within this region. The **R** corresponds to an A/G transition. The numbering corresponds with the nucleotide A, in the first ATG codon, as 1 and the previous nucleotide as −1, which is not shown.

Position	Transcription factor	Core Binding Site Sequence 5′-3′
a	GATA1 (-)	AAATAGATAAATAA
b	NFY-1	AGAAGCCAATTTGAAA
c	BRN2 (-)	GCCAATTTGAAATGGC
d	TCF11 (-)	CCAACGACATGAC
e	OCT1 (-)	GACATTCTG
f	NFAT	TTCTGGAAAAGG
g	MZF1	AAGGGGGA
h	AP1,AP1FJ	ATTGACTAGGC
i	CEBPB	TTTTACAGCAATGA
j	PDX-1	TAAT
k	E47 (-)	TAAGACAGGTGTTATT
l	LMO2 (-)	AGACAGGTGTTA
m	E-box	CACCTG
n	PEA3	AGGAAG
o	MEF2	ACTTTGGCTAAAAATAGCTATG
p	RSRFC4(-)	TAGCTATTTTTAGCCA
q	G-BOX	GGGAGGGG

FIG. 3—*Continued*

The SNP that causes an amino acid substitution V366A lies in the highly variable cytoplasmic C terminus. Even though this domain is reportedly not involved in heteromultimerization with other GIRK subunits, the high variability content of this domain is postulated to determine the specific function of each subunit, such as the ability of GIRK1 to bind $G_{\beta\gamma}$ protein (29). The amino acid V366 is not conserved in other human GIRKs (30); in addition, the mouse and rat homologs of *KCNJ9* have an Ala in this position (Fig. 2). These observations, coupled with the fact that both Val and Ala are hydrophobic amino acids, suggest that the substitution may not significantly alter the function of the protein.

TABLE 2

Polymorphisms in the *KCNJ9* Gene

Region	Position	Surrounding sequences
Promoter	g.-3429	AGACC(A/G)TGATG
Promoter	g.-3370	TCCTG(G/A)GTTAA
Promoter	g.-2908	TATCC(A/G)ATGCT
Intron 1	g.-1956	TTTAC(C/T)TAGAT
Intron 1	g.-1933	GGAGA(G/A)TGAGA
Intron 1	g.-1725	CTCTT(C/T)CTGAG
Intron 1	g.-1702	TCAGG(G/A)AAGGA
Intron 2	g.1285	AAGGA(T/A)GTTGT
Intron 2	g.2112	GTGTC(T/C)CTTAT
Exon 3	g.3703[a]	GGGGG(T/C)GGGGG
3′UTR	g.4145	GACAA(T/C)TGGTC
3′UTR	g.4181	GAACA(C/T)CCCTG
3′UTR	g.4433	AAGGC(A/G)TGCAA
3′UTR	g.4728-35**	TTCACA(TCACTGAT)TGGGTG
3′UTR	g.4793	AGGGG(T/C)ACCTG

[a] Polymorphism at position g.3703 causes an amino acid substitution from VAL-ALA.

[b] 8 bp insertion/deletion.

The four SNPs in the 3′*UTR* do not reside in any obvious consensus regions for mRNA stability, such as an AU-rich element (31). The four SNPs in intron 1 are not located in any consensus sequences for human transcription factors in the Transfac database (http://transfac.gbf-braunschweig.de/cgi-bin/matSearch/matsearch.pl). Of the two SNPs in intron 2, only one (position g.1285; sequence: 5′-AACA/TTCCTTAATTTCT-3′) resides in a consensus binding site of a human transcription factor, namely N-Oct-3 or Brn-2, a nervous-system specific factor that binds to the octamer DNA motif (32). The actual binding of Brn-2 to this site and its potential effects remain to be determined. The three SNPs in the promoter region do not reside in any consensus binding site for human transcription factors listed in the database.

In addition to identifying polymorphisms in the *KCNJ9* gene, we also investigated the tissue distribution of its transcript. The *KCNJ9* transcript appears to be quite abundant in the brain tissue of both fetal and adult humans. This is in accordance with several studies reporting that the mouse and rat GIRK3 may be the most abundant subtype of the GIRK subfamily at least in the brain (33–35). Other human GIRK family members are also present in the brain, apparently at a lower level than GIRK3 (30). GIRK subunits typically function as heterotetramers and have the potential to regulate neuronal excitability in the central nervous system, consistent with their expression in the brain (30).

We also found *KCNJ9* expression in pituitary, testis, small intestine, kidney, and smooth muscle. *KCNJ9* transcript was identified in major insulin-responsive sites involved in the development of diabetes: fat and skeletal muscle, as well as pancreas, where the insulin-

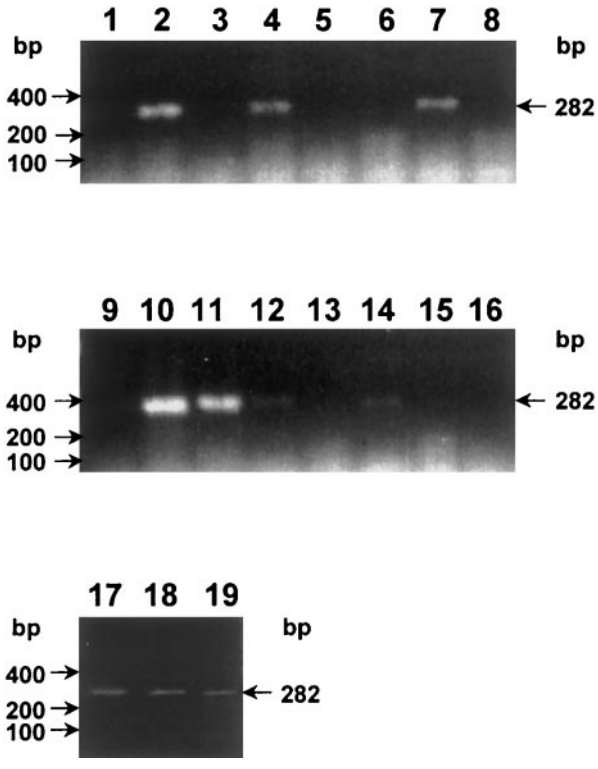

FIG. 4. Human tissue distribution of the *KCNJ9* transcript. *KCNJ9*-specific primers were utilized to amplify a fragment of 282 bp from various tissue-specific cDNA. The PCR products were analyzed on an agarose gel stained with ethidium bromide. The arrows on the left-hand side of the figure indicate positions of DNA marker sizes. *KCNJ9* expression is shown in lanes 2, 4, 7, 10–12, 14,and 17–19 which correspond to pituitary, small intestine, testis, adult brain, fetal brain, fat, kidney, skeletal muscle, smooth muscle, and pancreas, respectively. There is no detectable expression in lanes 1, 3, 5, 6, 8, 9, 13, 15, and 16 corresponding to ovary, placenta, spleen, stomach, thyroid gland, adrenal gland, heart, liver, and mammary gland, respectively.

producing beta cells reside. Interestingly, GIRK2 has been found to be expressed in the pancreas (36), and at least one function of GIRK3 has been proposed to be a negative regulator of GIRK2 when both subunits are coexpressed in *Xenopus* oocytes (37). Nevertheless, the specific functions of KCNJ9 in the different cell types remain to be elucidated.

In conclusion, the *KCNJ9* gene spans an ~7.6 kb region with one noncoding and 2 coding exons. We also sequenced ~1.7 kb region upstream of the gene into the putative promoter region. The 14 SNPs and one insertion/deletion found in this gene should facilitate further association studies in different subject groups.

ACKNOWLEDGMENTS

We thank the members of the Gila River Indian Community for their helpful cooperation and participation, without whom this study would not have been possible. We also thank Linda Phillips for genomic DNA preparation and Dr. Jeff Bunkelman for technical advice. We are grateful for the assistance and patient care provided by Michael Milner, PA, Carol Massengill, RN, the nurses of the Clinical Research Ward, and Dr. Arline Salbe as well as the metabolic kitchen staff.

REFERENCES

1. Isomoto, S., Kondo, C., and Kurachi, Y. (1997) *Jpn. J. Physiol.* **47,** 11–39.
2. Kubo, Y., Reuveny, E., Slesinger, P. A., Jan, Y. N., and Jan, L. Y. (1993) *Nature* **364,** 802–806.
3. Dascal, N., Lim, N. F., Schreibmayer, W., Wang, W., Davidson, N., and Lester, H. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6596–6600.
4. Lesage, F., Duprat, F., Fink, M., Guillemare, E., Coppola, T., Lazdunski, M., and Hugnot, J. P. (1994) *FEBS Lett.* **353,** 37–42.
5. Krapivinsky, G., Gordon, E. A., Wickman, K., Velimirovic, B., Krapivinsky, L., and Clapham, D. E. (1995) *Nature* **374,** 135–141.
6. Spauschus, A., Lentes, K. U., Wischmeyer, E., Dissmann, E., Karschin, C., and Karschin, A. (1996) *J. Neurosci.* **16,** 930–938.
7. Hedin, K. E., Lim, N. F., and Clapham, D. E. (1996) *Neuron* **16,** 423–429.
8. Schoots, O., Voskoglou, T., and Van Tol, H. H. (1997) *Genomics* **39,** 279–288.
9. Stoffel, M., Espinosa, R., 3rd, Powell, K. L., Philipson, L. H., Le Beau, M. M., and Bell, G. I. (1994) *Genomics* **21,** 254–256.
10. Lesage, F., Guillemare, E., Fink, M., Duprat, F., Heurteaux, C., Fosset, M., Romey, G., Barhanin, J., and Lazdunski, M. (1995) *J. Biol. Chem.* **270,** 28660–28667.
11. Isomoto, S., Kondo, C., Takahashi, N., Matsumoto, S., Yamada, M., Takumi, T., Horio, Y., and Kurachi, Y. (1996) *Biochem. Biophys. Res. Commun.* **218,** 286–291.
12. Wei, J., Hodes, M. E., Piva, R., Feng, Y., Wang, Y., Ghetti, B., and Dlouhy, S. R. (1998) *Genomics* **51,** 379–390.
13. Sakura, H., Bond, C., Warren-Perry, M., Horsley, S., Kearney, L., Tucker, S., Adelman, J., Turner, R., and Ashcroft, F. M. (1995) *FEBS Lett.* **367,** 193–197.
14. Wickman, K., Seldin, M. F., Gendler, S. J., and Clapham, D. E. (1997) *Genomics* **40,** 395–401.
15. Lesage, F., Fink, M., Barhanin, J., Lazdunski, M., and Mattei, M. G. (1995) *Genomics* **29,** 808–809.
16. Hanson, R. L., Ehm, M. G., Pettitt, D. J., Prochazka, M., Thompson, D. B., Timberlake, D., Foroud, T., Kobes, S., Baier, L., Burns, D. K., Almasy, L., Blangero, J., Garvey, W. T., Bennett, P. H., and Knowler, W. C. (1998) *Am. J. Hum. Genet.* **63,** 1130–1138.
17. Knowler, W. C., Bennett, P. H., Hamman, R. F., and Miller, M. (1978) *Am. J. Epidemiol.* **108,** 497–505.
18. Seino, S. (1999) *Annu. Rev. Physiol.* **61,** 337–362.
19. Schmid-Antomarchi, H., De Weille, J., Fosset, M., and Lazdunski, M. (1987) *J. Biol. Chem.* **262,** 15840–15844.
20. Antonarakis, S. E. (1998) *Hum. Mutat.* **11,** 1–3.
21. Prochazka, M., Lillioja, S., Tait, J. F., Knowler, W. C., Mott, D. M., Spraul, M., Bennett, P. H., and Bogardus, C. (1993) *Diabetes* **42,** 514–519.
22. Shapiro, M. B., and Senapathy, P. (1987) *Nucleic Acids Res.* **15,** 7155–7174.
23. Quandt, K., Frech, K., Karas, H., Wingender, E., and Werner, T. (1995) *Nucleic Acids Res.* **23,** 4878–4884.
24. Yu, M., Yang, X. Y., Schmidt, T., Chinenov, Y., Wang, R., and Martin, M. E. (1997) *J. Biol. Chem.* **272,** 29060–29067.
25. Sander, M., and German, M. S. (1997) *J. Mol. Med.* **75,** 327–340.

26. Pollock, R., and Treisman, R. (1991) *Genes Dev.* **5,** 2327–2341.
27. Andres, V., Cervera, M., and Mahdavi, V. (1995) *J. Biol. Chem.* **270,** 23246–23249.
28. Bucher, P. (1990) *J. Mol. Biol.* **212,** 563–578.
29. Chan, K. W., Sui, J. L., Vivaudou, M., and Logothetis, D. E. (1997) *J. Biol. Chem.* **272,** 6548–6555.
30. Dascal, N. (1997) *Cell. Signal.* **9,** 551–573.
31. Ross, J. (1995) *Microbiol. Rev.* **59,** 423–450.
32. Schreiber, E., Tobler, A., Malipiero, U., Schaffner, W., and Fontana, A. (1993) *Nucleic Acids Res.* **21,** 253–258.
33. Kobayashi, T., Ikeda, K., Ichikawa, T., Abe, S., Togashi, S., and Kumanishi, T. (1995) *Biochem. Biophys. Res. Commun.* **208,** 1166–1173.
34. Chen, S. C., Ehrhard, P., Goldowitz, D., and Smeyne, R. J. (1997) *Brain Res.* **778,** 251–264.
35. Karschin, C., and Karschin, A. (1997) *Mol. Cell. Neurosci.* **10,** 131–148.
36. Ferrer, J., Nichols, C. G., Makhina, E. N., Salkoff, L., Bernstein, J., Gerhard, D., Wasson, J., Ramanadham, S., and Permutt, A. (1995) *J. Biol. Chem.* **270,** 26086–26091.
37. Kofuji, P., Davidson, N., and Lester, H. A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6542–6546.