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Genomic organization and promoter analysis of human *KCNN3* gene

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Abstract *KCNN3* is a member of the gene family, *KCNN1–4*, encoding the small and intermediate conductance calcium-activated potassium channels. Long CAG-repeat alleles of this gene have been found to be over-represented in patients with schizophrenia in a number of population-based association studies, and this gene maps to human chromosome 1q21, a region recently implicated in schizophrenia by linkage. To set the stage for a further functional evaluation of *KCNN3*, we defined the nature of the genomic locus in the size, structure, and sequence of its introns and exons and the function of potential upstream regulatory regions. We isolated P1-derived artificial chromosome (PAC) clones from a genomic library and identified an overlapping available bacterial artificial chromosome (BAC) clone. Cosmids subcloned from the PAC and BAC clones were then sequenced and merged with the sequence in the public database. The *KCNN3* gene spans over 163.1 kb and is composed of eight exons and seven introns. All of the exon-intron junctions conform closely to consensus splice sites. The proximal 2.5 kb of the 5′-flanking sequence was obtained and analyzed for potential transcription factor binding sites. In the proximal 2.5 kb upstream region, potential sites for the Ikaros factor (IK2), homeodomain factor Nkx-2.5/Csx (NKX25), nuclear factor of activated T-cells (NFAT), upstream stimulating factor (USF), c-AMP responsive element binding protein (CREB), POU factor Brn2 (BRN-2), myeloid zinc finger protein (MZF1), vitellogenin binding protein (VBP), HNF3 forkhead homologue 2 (HFH2), and transcription initiation were identified, as well as several potential AP-1 and AP-4 sites. Finally, a 2261-bp fragment of this upstream region was cloned into a promoterless pGL3-luciferase vector, where it produced orientation-dependent expression of the reporter gene in transiently transfected PC12 cells, cells which natively express functional KCNN3 channels, suggesting that this cloned fragment includes competent promoter elements of this gene.

Key words hSKCa3 · Calcium-activated potassium channel · Ion channel gene · Schizophrenia · Trinucleotide repeat · PC12

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Introduction

We had previously cloned and sequenced the cDNA of *KCNN3* (also known as *hSKCa3*), an apamin- and scyllatoxin-sensitive small conductance calcium-activated potassium channel gene (Chandy et al. 1998). It is a member of a family of four genes, *KCNN1–4* (also known as *KCa1–4*) (Kohler et al. 1996; Vergara et al. 1998), that encode channel subunits that function as homo- and hetero-tetramer channels and play a critical role in controlling the firing pattern of neurons via the generation of slow after-hyperpolarizations (Vergara et al. 1998). Northern blot studies showed *KCNN3* to be expressed in a narrow subset of tissues, and in situ hybridization studies showed the gene to be most abundantly expressed in dopaminergic regions of the human brain, particularly in the substantia nigra and the ventral tegmental area, sites giving rise to the two major central nervous system (CNS) tracts expressing the D_2 subtype of the dopamine receptor (Dror et al. 1999), an implied target of antipsychotic medications (Pickar 1998).

The *KCNN3* cDNA encodes an intrinsic membrane protein of 736 amino acids with six putative transmembrane segments, S1-S6, a characteristic P-loop linking S5 and S6 (the potassium channel pore region that forms the ion conduction pathway), and cytosolic N- and C-termini (Kohler et al. 1996; Chandy et al. 1998; Doyle et al. 1998). *hSKCa3/*

KCNN3 was found to contain two CAG-repeats that encode two N-terminal polymorphic polyglutamine repeats, which were subsequently found to be an evolutionarily conserved feature of only this family member (Chandy et al. 1998). In humans, the second CAG-repeat is longer and highly polymorphic, ranging from 4 to 30 repeats in length, with a modal value of 19 repeats. Many hereditary late-onset human neurodegenerative diseases are caused by expanded trinucleotide repeats (Cummings and Zoghbi 2000) and several suggestions of such expansions have been reported in patients with schizophrenia (Morris et al. 1995; O'Donovan et al. 1996), a common, heterogeneous, polygenic neuropsychiatric disease (Gargus et al. 1998). Initially, a number of case-control studies showed a consistent significant over-representation of longer CAG-repeat alleles in European, Welsh, and North American Caucasians, as well as in Israeli Ashkenazi, patients with schizophrenia (Bowen et al. 1998; Chandy et al. 1998; Cardno et al. 1999; Dror et al. 1999; Wittekindt et al. 1998). However, a number of subsequent case-control, family-based association and linkage studies in diverse populations have failed to support the initial observation (O'Donovan and Owen 1999; Vincent et al. 2000), clouding interpretation of the findings.

KCNN3 had been localized to chromosome 1q21 by fluorescence in situ hybridization (FISH) (Dror et al. 1999; Wittekindt et al. 1998), a region undetected in genome-wide scans for schizophrenia-susceptibility genes at the time (Vincent et al. 2000). Last year, however, a genome-wide scan for schizophrenia-susceptibility loci in 22 extended families with a high rate of schizophrenia provided highly significant evidence of linkage to 1q21 with a lod score of 6.50 (Brzustowicz et al. 2000), again raising the disease-candidate status of *KCNN3*. To set the stage for a further functional evaluation of *KCNN3*, and particularly to facilitate screening for additional polymorphic or mutant alleles, in this study we defined the intron-exon sequence and structure and functional promoter elements of this gene.

Materials and methods

Materials

The pGEM4Z, pGL3-basic, and pRL-CMV plasmid vectors, TransFast Transfection Reagent, and restriction enzymes were purchased from Promega (Madison, WI, USA). Luciferase activities were measured using the Dual-Luciferase Reporter Assay System of Promega. The TaqPlus Long PCR System, StrataPrep Total RNA Miniprep kit, ProSTAR Ultra HF RT-PCR System, β-actin primers, and the SuperCos 1 cosmid vector were obtained from Stratagene (La Jolla, CA, USA) and the bacterial artificial chromosome (BAC) genomic clone RP11-809K21 was obtained from Research Genetics (Huntsville, AL, USA). Plasmid Maxi kits, were purchased from Qiagen (Valencia, CA, USA).

Isolation and characterization of P1-derived artificial chromosome (PAC) clone

A human genomic PAC library (RPCI from Dr. Pieter de Jong at Roswell Park Memorial Institute) (Church et al. 1997) was screened by PCR with primers selected to flank the 5′-end of the *KCNN3* cDNA sequence. The primers were 5′-tctttcacccctcttctttctcc-3′ (forward) and 5′-tctttggcttgcttcggttctctg-3′ (reverse). The annealing temperature was 55°C and the reaction mix contained dNTPs at 200 μM, KCl at 75 mM, and MgCl at 1.5 mM. After several rounds of selection, clone PAC no. 14A26 was isolated.

Defining the genomic sequence and intron-exon boundaries

The PAC no. 14A26 and BAC RP11-809K21 clones were subcloned into SuperCos 1 cosmid vector. A contig of overlapping cosmid clones was assembled and, based upon the cDNA sequence and sequence in the public database, we designed primer pairs to attempt to define the intron/exon junctions of *KCNN3*. We performed automated sequence analysis with an ABI 377 system (Applied Biosystems Foster City, CA, USA), using the cloned cosmid DNAs as template. Size estimates for three of the introns (introns 4, 6, and 7) could be directly obtained by PCR with the TaqPlus Long PCR System and cosmid DNA, using primer pairs IN4F/IN4R, IN6F/IN6R, and IN7F/IN7R, respectively (Table 1). PCR products were confirmed by sequencing with the same primers. Introns that could not be spanned with PCR were obtained by performing end sequencing of the isolated cosmids, using T7 and T3 primers, executing a search of the derived sequence against the public database (http://www.ncbi.nlm.nih.gov/blastnd) and repeated rounds of PCR and sequencing with sequence-derived primers to close the gaps.

Sequence and analysis of the upstream region

We sequenced the cosmid containing the 5′-untranslated region (UTR) of *KCNN3* (cosmid 1 in Fig. 1), obtaining

Table 1. Primers used for characterization of exon-intron boundaries

Primer name	Primer sequence (5′-3′)	Primer position
IN1F	aggagggccccgtttgaa	Exon1
IN1R	acaggatgcgctcgtaggtc	Exon3
IN2F	gccttatcagtctgtccaccatca	Exon2
IN2R	agaagaacttgtactcgccaggaa	Exon3
IN3F	cctggcactgtgctgctcgtgtt	Exon3
IN3R	gggcaccatgtccccataaccaat	Exon4
IN4F	gggtgccatgtggctcatctccat	Exon4
IN4R	gccaccacggccaccacaag	Exon5
IN5F	gtggcccgaaagctggaactcac	Exon5
IN5R	cctcactttggcatggtcaatctt	Exon6
IN6F	acacaaagctgctaaagaagattg	Exon6
IN6R	ccagagtgttggcttggtca	Exon7
IN7F	aggagcgtcaagatggaacagagg	Exon7
IN7R	aggtcttcgctccggtcattgagt	Exon8

F, Forward; R, reverse

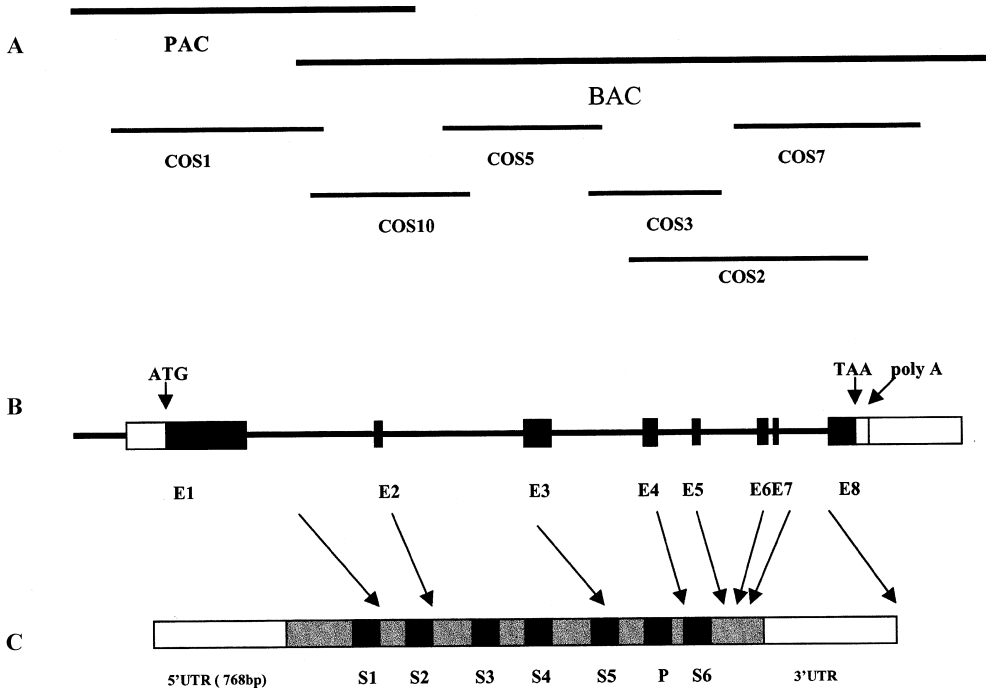

Fig. 1A–C. Organization of *KCNN3*. **A** The overlapping P1-derived artificial chromosome (*PAC*) and bacterial artificial chromosome (*BAC*) genomic clones are indicated by *thick continuous lines*. Beneath, derived cosmid clones are shown by *thinner continuous lines*. **B** The gene structure is shown below the clones, with the exon numbering indicated. **C** The structure of the transcript is shown, with the translated sequences shown as *filled boxes* and untranslated sequences as *open boxes*. The transmembrane segments (*S1–S6*) and pore (*P*) in the coding region are indicated as black boxes and labeled *below the diagram*. The 3′-end of the corresponding exon is indicated with an *arrow above the diagram*. *UTR*, Untranslated region

2.5 kb of sequence upstream from the first codon. The suite of analysis programs at http://www.hgsc.bcm.tmc.edu/searchlauncher was utilized to detect potential transcription initiation sites (score, >0.85) and potential transcription factor binding sites in this putative promoter region (solution parameters: core similarity = 1; matrix similarity ≥ 0.9). This program also was used to identify potential poly-A^+ addition sites in the region downstream of the stop codon in the genomic sequence.

pGL3-Luc expression vector construction

Two *Pst*I restriction sites naturally occur at −2283 bp and −22 bp upstream from the first codon of the *KCNN3* gene (Fig. 2). Therefore, the 2261 bp fragment between these two sites was cloned into the *Pst*I site of the pGEM4Z vector in both the sense and antisense orientation. Oriented subcloning was used to transfer this fragment into pGL3-basic to form the pGL3-Luc expression constructs. Their structure was confirmed by sequencing.

Cell culture and luciferase assay

PC12 cells were plated in 35-mm six-well plates in α-MEM medium supplemented with 10% fetal calf serum, and incubated for 24 h in an atmosphere containing 5% CO_2 to obtain 70%–80% confluence. pGL3-Luc expression vectors were transfected using cationic liposomes. The transfection mixture was freshly prepared by adding 2.5 μg of the desired pGL3-derived construct, 20 ng of pRL-CMV vector, and 15.75 μl of TransFast Reagent (Promega) into 1 ml of serum-free α-MEM medium. The pGL3-derived constructs were always co-transfected with the pRL-CMV vector to allow correction for variation in transfection efficiency. The luciferase assay was carried out according to the manufacturer's protocol. Briefly, cultured cells are washed and lysed, and the lysate is transferred into a microfuge tube. Then 20 μl of supernatant is mixed with 100 μl of Luciferase assay reagent II, provided by the manufacturer, and the mixture is assayed for firefly luciferase activity in a luminometer (Monolight 2010; Analytical Luminescence Laboratory, San Diego, CA, USA). Next, 100 μl of the provided 1× Stop and Glo Reagent is added before the sample is returned to the luminometer to measure the Renilla luciferase activity. At least two independent experiments were performed for each condition, and the mean and SD values are presented.

Patch electrode recording of native *KCNN3* channels

Experiments were carried out in the whole cell configuration of the patch clamp technique with a holding potential of −80 mV and an internal solution containing 145 mM K aspartate, 10 mM K_2 ethyleneglycoltetraacetic acid (EGTA), 2.08 mM $MgCl_2$, and 8.55 mM $CaCl_2$, resulting in a calculated free calcium concentration of 1 μM. External solutions used were either Na aspartate (155 mM Na aspartate, 4.5 mM K aspartate, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM hydroxyethylpiperazine ethanesulfonic acid (HEPES) at pH 7.4), or K aspartate (with identical ingredients except that all Na aspartate was substituted by K aspartate). All membrane currents were recorded at room temperature (22°C–26°C) with an EPC-9 patch clamp amplifier (Heka, Lambrecht, Germany). SKCa/KCNN currents were elicited by 200-ms voltage ramps, from −120 to 40 mV applied every 5 s.

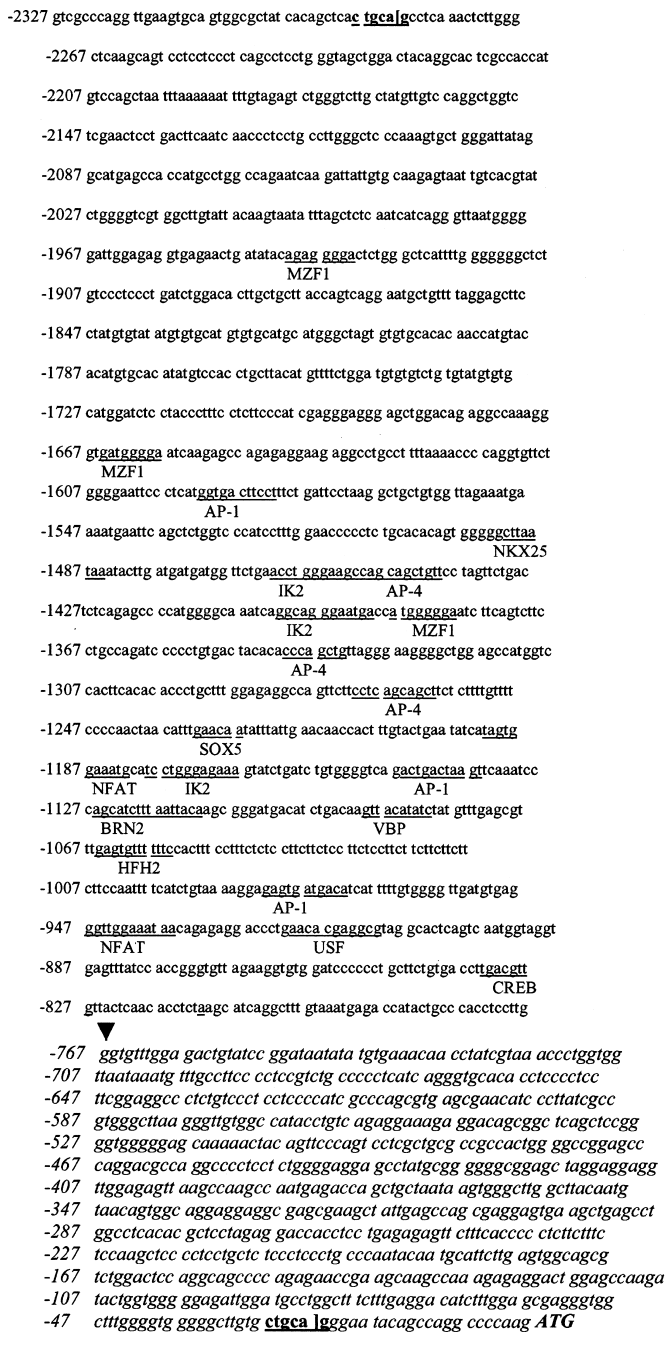

Fig. 2. The primary sequence of the 5′-flanking untranslated and promoter region of *KCNN3*. This region was used to construct the pGL3-Luc expression vectors. Potential mammalian regulatory sites identified by computer analysis are *underlined*. The *arrowhead* indicates the consensus transcription start site. The *italic sequence* shows the untranslated region, and the upper case *bold sequence* shows the coding region. The sequence flanked by *square brackets* indicates the region subcloned into the luciferase reporter vector, with the *PstI* sites in bold type. *MZF1*, *NKX25*, *IK2*, *NFAT*, *VBP*, *HFH2*, *USF*, and *CREB*: see text for definitions

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from PC12 cells cultured in 75-cm^2 flasks, using the StrataPrep Total RNA Miniprep kit (Stratagene). Then 500 ng total RNA of PC12 cells, and 1 µl of RNase-free water as control, were reverse transcribed to single-strand DNA (cDNA) using Oligo-dT primer and StrataScript reverse transcriptase provided with the ProSTAR Ultra HF RT-PCR System (Stratagene). Then 1 µl of the transcription mixture was amplified, using primer pair IN1F/IN1R (spanning intron 1 and intron 2 of the genomic locus; Table1), primer pair IN3F/IN3R (spanning intron 3; Table 1), and β-actin primers. PCR was performed using a GenAmp PCR System 9600 (Applied Biosystems, Foster City, CA, USA) in a volume of 50 µl containing: cDNA, 10 mM dNTP, 1× PfuTUrbo DNA polymerase buffer, 2.5 unit of PfuTUrbo DNA polymerase, and 50 pmol of each primer. PCR conditions were 95°C for 1 min, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s, and 68°C for 1.5 min, with a final extension at 72°C for 10 min. Ten-µl aliquots of PCR products for a given RNA with each primer set were mixed, separated on a 2% agarose gel, and visualized by ethidium bromide staining.

Results and discussion

Structural organization of *KCNN3*

In order to isolate the complete *KCNN3* gene, we screened a human PAC genomic DNA library with primers designed from the cDNA sequence. The PAC no. 14A26 clone, containing the 5′-upstream region and the first two exons of *KCNN3*, was obtained in this fashion. One overlapping BAC clone was identified in the public database (accession number, AC027645), which additionally contained the 3′-end of the cDNA sequence. Both of the clones were subcloned into SuperCos I vector, and screened for exon sequence. A contig of six overlapping cosmid clones from the PAC and BAC clones spanned the gene (Fig. 1). The intron/exon junction sequences were defined by automated sequencing of these cosmids with primers designed from the cDNA sequence (Table 1). Consensus GT-AG donor and acceptor splice sites are found at all junctions. Three introns (introns 4, 6, and 7) could be spanned by long PCR reactions and direct sequencing of the PCR products. End-sequencing of the cosmids in the contig (with T7 and T3 primers) and alignment of the sequence with the sequence in the public database, followed by sequential long PCR reactions to close gapped regions not present in the database, allowed us to obtain the sizes and full sequence of introns 1, 2, 3, and 5. The overall structure of this gene and the clones used in this work are shown in Fig. 1. The *KCNN3* gene is divided into eight exons that vary in length from 70 bp to 1716 bp, and its overall length spans approximately 163.1 kb. The size of the introns ranges from 676 bp to 49.71 kb (Table 2). This annotated sequence was deposited with GenBank and given accession number AF336797.

Functional analysis of the upstream region

We obtained 2.5 kb of the 5′-flanking untranslated region by sequencing cosmid 1. After the analysis of this sequence

Table 2. Sequences at the exon-intron boundaries and the sizes of exons and introns

Exon no.	Exon size[a]	Splicing acceptor	5′-Exon junction	3′-Exon junction	Splice donor	Intron size[a]
1	1716		TSS	-GTACTCAAAG	**gt**agg ggctg-	46.75
2	96	-gttcctgc**ag**	GACTCCATGT-	-TGAAGTCCAG	**gt**agtgcccc-	49.71
3	419	-ctcccacc**ag**	CTCTTCGTGA-	-TCTGTGAAAG	**gt**gaaaggta-	39.33
4	142	-ccattggc**ag**	GTACCATGAC-	-TGGCATCATG	**gt**gagtaccc-	7.00
5	111	-ccccccgc**ag**	GGTGCAGGCT-	-CACCAAGCGG	**gt**aagatgcc-	10.91
6	128	-tcttcctt**ag**	ATCAAGAATG-	-CTATCCACCA	**gt**gagtatgc-	0.68
7	70	-ttccccac**ag**	GTTGAGGAGC-	-CCTTTCCAAG	**gt**gagtggca-	5.19
8	324	-ctccacac**ag**	ATGCAGAATG-	poly(A)		

Intron sequence is shown in lower case and exon sequence in uppercase. The highly conserved gt-ag motif is shown in boldface
TSS, Transcription start site
[a] Size of exons is in base pairs and size of introns is in kilobase pairs

(see "Methods"; "sequence and analysis of the upstream region"), three potential transcription start sites (TSS) were identified, at 768 bp, 1596 bp, and 1811 bp upstream of the initiation codon. Thus, exon 1 includes at least 768 bp of the 5′-untranslated sequence and 948 bp of the coding sequence. After inspecting the upstream sequence, we identified, between 1543 and 768 bp 5′ to the initiation codon, consensus sequence binding sites for, the Ikaros factor (IK2), homeo domain factor Nkx-2.5/Csx (NKX25), nuclear factor of activated T-cells (NFAT), upstream stimulating factor (USF), POU factor Brn2 (BRN2), c-AMP-responsive element binding protein (CREB), myeloid zinc finger protein (MZF1), HNF3 forkhead homologue 2 (HFH2), and vitellogenin binding protein (VBP), as well as several AP-1 and AP-4 sites. Similar to findings for the *KCNN4* gene (Ghanshani et al. 2000), no TATA elements were found (Fig. 2).

To test the hypothesis that this 5′-flanking region contains *KCNN3* promoter elements, the 2261-bp *Pst*I restriction fragment upstream of the initiation codon was inserted in both a sense and antisense orientation in front of the promoterless luciferase gene of the pGL3-basic vector to generate promoter-reporter constructs for transfection into PC12 cells. Like the brain regions that natively express *KCNN3*, PC12 cells are known to have dopaminergic properties, as they possess functional pathways for dopamine biosynthesis and release (Lamensdorf et al. 2000). They are also known to natively express apamin-sensitive small conductance calcium-activated potassium channels (Schmid-Antomarchi et al. 1986; Wadsworth et al. 1997; Terstappen 1999). Figure 3A shows the PC12 cell calcium-activated current to be K^+-selective, as the current had a reversal potential at −80 mV in the Na aspartate solution and 0 mV in the K aspartate solution, corresponding to the Nernst potential for potassium in these respective extracellular solutions. Figure 3B shows this current to be blocked almost completely with 100 nM apamin. Apamin, at 10 nM, which would have completely blocked KCNN2 channels, blocked only 20% of the current (data not shown). Figure 3C shows this current to be partially blocked by 10 nM scyllatoxin. This is a toxin profile that is characteristic of only KCNN3 channels, demonstrating that PC12 cells natively express *KCNN3*. The RT-PCR results (Fig. 3D) confirmed the presence of *KCNN3* transcripts in PC12 cells.

The normalized luciferase activity in PC12 cells transfected with the sense vector construct was 17-fold higher than the activity in PC12 cells transfected with the promoterless control or with the antisense construct ($P < 0.001$) (Fig. 4), demonstrating an orientation-dependence of the promoter activity. These results suggest that the 2261-bp upstream flanking fragment of *KCNN3* cloned into the reporter constructs contains functional promoter elements.

Functional analysis of the exon structure

As would be expected, the *KCNN3* exons primarily define easily recognized functional domains of the channel protein; however, some of the exon-encoded domains are somewhat surprising. Particularly intriguing is the finding that exon 1 encodes the two N-terminal polymorphic polyglutamine-repeat tracts, in addition to the first transmembrane alpha-helical domain, S1. Because, in this gene family, only the *KCNN3*-encoded channel has a long cytoplasmic N-terminal domain (283 amino acids vs a range of 27 to 133 amino acids in the others) and specifically only it has the polyglutamine repeats, one might imagine this cytoplasmic region to represent a new motif, added as an exon, to the core channel structure, rather than this cytoplasmic region being an intrinsic component of the exon encoding an S1 domain that is quite similar to that of all the other family members. On the other hand, exon 2 encodes the S2 transmembrane region and exon 3 encodes the S3, S4, and S5 transmembrane spans. Exon 4 encodes the critical P region that creates the key permeability pathway in the extended family of potassium channels, as well as the outer-third of the S6 transmembrane segment (7 of 21 amino acids). Exon 5 encodes the cytoplasmic two-thirds of the S6 segment, the region that forms the cytoplasmic part of the ion conduction pathway, as well as the channel "gate" (Doyle et al. 1998). Exon 5 also encodes the highly conserved initial 24 residues in the C-terminus that forms part of the critical calmodulin-binding domain in this class of channels (Xia et al. 1998; Fanger et al. 1999). The unexpected division of this final membrane-spanning domain between two exons is a feature shared with *KCNN1* (Litt et al. 1999) and *KCNN4* (Ghanshani et al. 2000), as is the

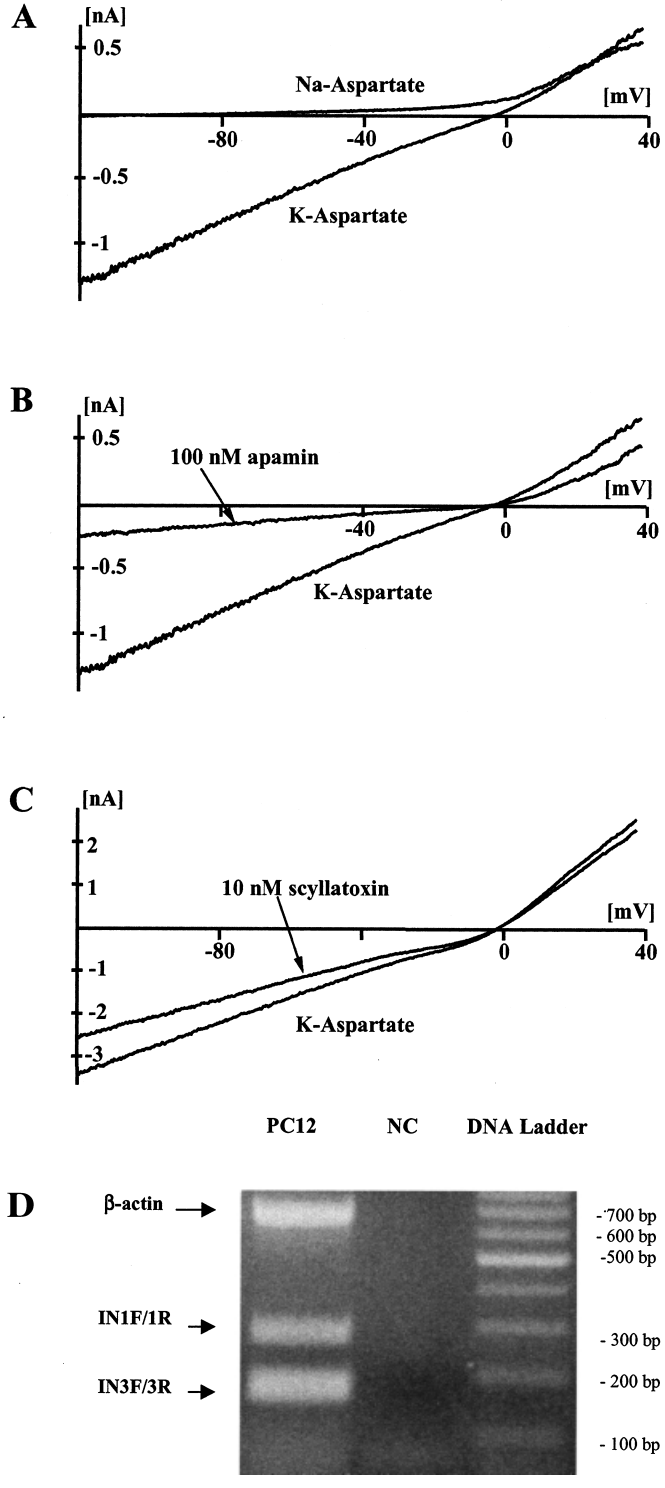

Fig. 3A–D. Native KCNN3 channel expression in PC 12 cells. **A** Calcium-activated current recorded with 1 μM free intracellular calcium, a physiological intracellular potassium concentration, and the cell in an extracellular bath solution of either Na or K aspartate. **B** Inhibitory effect of 100 nM apamin on the calcium-activated potassium current. **C** Inhibitory effect of 10 nM scyllatoxin on the calcium-activated potassium current. **D** Reverse transcription-polymerase chain reaction (RT-PCR) products from PC12 cells and control (*NC*), separated on a 2% agarose gel. The sizes of the products were 661 bp, 277 bp, and 170 bp for the β-actin primers, the IN1F/IN1R primers, and the IN3F/IN3R primers, respectively

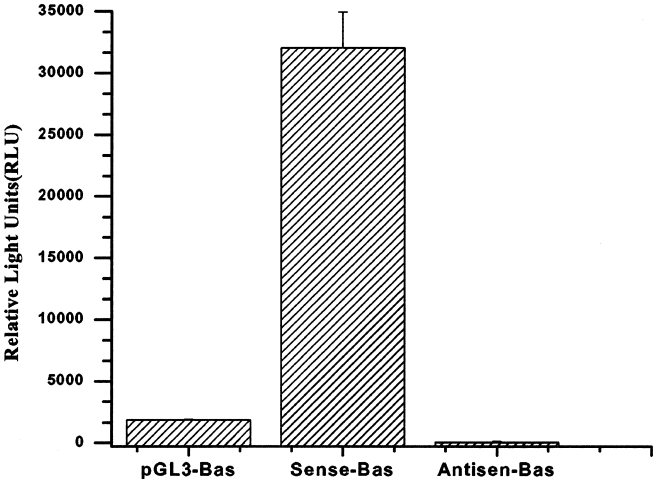

Fig. 4. Promoter activity of the 5′-flanking region of *KCNN3*. Relative reporter activity of the empty pGL3-basic vector (*pGL3-Bas*), the antisense promoter construct (*Antisen-Bas*), and the sense promoter construct (*Sense-Bas*) transfected into PC12 cells is shown. The luciferase activity of transient transformants with each construct is presented as relative light units (*RLU*). RLU represents the ratio of the firefly luciferase activity to the renilla luciferase activity (multiplied by a factor of 10^4 in order to bring the renilla luciferase values into the range of the original firefly luciferase values). Two to three independent experiments were conducted, and the mean values are expressed. The *line* extending above the *bar* represents the SD

exonic distribution of all other transmembrane spans. The remainder of the calmodulin-binding segment of *KCNN3* is encoded by exons 6–8. The stop codon is found 297 bp from the start of exon 8.

Interestingly, two forms of the *KCNN3* transcript have been reported, with differing lengths in the 3′ non-coding sequence. The first of these contains a poly-A^+ signal (AATAAA) that overlaps the stop codon and that is conserved in both humans (Genbank accession number, AF031815) and rats (AF292389). This transcript ends in a poly-A^+ tail 27 bp downstream from the signal. Several expressed sequence tags (ESTs) in the database correspond to this form of the transcript, including human ESTs (AW968762, AI669070, AA767647, AA741463, AI201553, AI671578, AA731772, AA285078, AA491238, AW136136, and AW977583) and rat EST (AI044511). The second type of transcript extends further 3′, and is represented by human cDNA (AJ251016), and by human ESTs (AU118918, AU145368, AW575885, AI769660, AI813448, AA255937, AW074697, AI611826, AI242020, and AW411312) and mouse ESTs (AI121990, BB203858, and BB149689). The AJ251016 cDNA contains a poly-A stretch, but no authentic upstream poly-A^+ signal (Fig. 5), suggesting that the transcript might actually extend further 3′. Consistent with this notion, the poly-A sequence in AJ251016 is present in our genomic sequence, indicating that it is not an authentic poly-A^+ tail. Three additional poly-A^+ signals are present further downstream in the genomic sequence, the first only 11 bp downstream from the genomic poly-A tract, 562 bp downstream of the stop codon. Thus, exon 8 contains 297 bp of coding sequence, at least 562 bp of 3′-untranslated

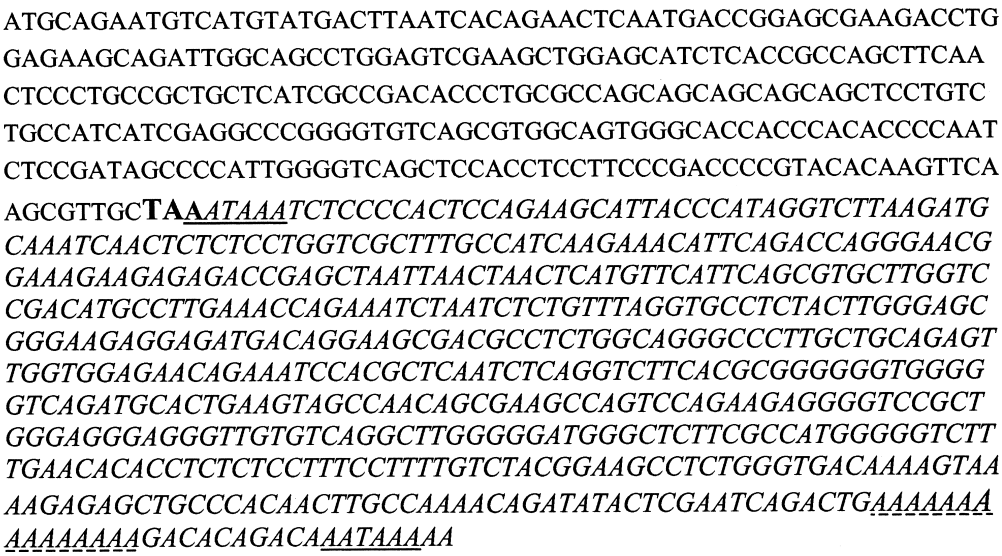

Fig. 5. Poly-A$^+$ addition signals in the 3′-UTR of *KCNN3*. The exon 8 sequence is shown, with the TAA stop codon *in bold*. The AATAAA Poly-A$^+$ addition signals are *underlined*. The genomic poly-A tract is shown with a *broken underline*. The 3′-UTR is shown in *italics*

sequence, and at least two utilized alternative poly-A$^+$ addition signals.

We have previously shown that Northern blot analysis of human brain and lymphoid tissues reveals the presence of several *KCNN3*-specific transcripts, the smallest of which is ~2.5 kb (Dror et al. 1999). The transcript that begins at the most proximal TSS and terminates at the first poly-A$^+$ signal (e.g., AF031815, AF292389) is 3006 bp in length and contains the 768-bp 5′-UTR, 2211-bp coding region, and 27-bp 3′-UTR. This is too large to account for the smallest transcript seen, but it would account for the second from the smallest transcript seen on Northern blotting. The transcript that ends at the second poly-A$^+$ signal (e.g., AJ251016) would be 566 bp longer and would account for the third from the smallest transcript. The presence of several other transcripts suggests that *KCNN3* might be alternatively spliced, utilizing exons that remain to be identified.

Analysis of the introns

The initial three introns are consistently large (49–39 kb), whereas the final four introns are only modest in size. The most interesting and potentially useful feature identified within the introns is an AC-dinucleotide found to be repeated 21 times in intron 6. Such dinucleotide repeats are often highly polymorphic; therefore, this site promises to prove a valuable marker for association and linkage studies with the *KCNN3* gene.

Conclusions

1. This article reports the genomic organization of the *hSKCa3/KCNN3* gene. This gene spans 163.1 kb and has eight exons, all with splice junctions consistent with standard GT-AG rules.
2. The 2261-bp region upstream from the first codon has several recognized promoter elements and conveys in vitro promoter activity.
3. The final exon contains at least two alternatively utilized poly-A$^+$ addition signals.

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