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Initial isolation and analysis of the human *Kv1.7* (*KCNA7*) gene, a member of the voltage-gated potassium channel gene family

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Abstract

A novel human potassium channel gene was identified and isolated. The maximal open reading frame encodes a protein of 456 amino acids. The predicted product exhibits 91% amino acid identity to the murine voltage-gated potassium channel protein Kv1.7 (Kcna7), which plays an important role in the repolarization of cell membranes. Based on the high similarity, the human gene has been classified as the ortholog of the mouse *Kcna7* and given the name *Kv1.7 (KCNA7)*. A structural prediction identified a pore region characteristic of potassium channels and six membrane-spanning domains. Northern expression analysis revealed the gene is expressed preferentially in skeletal muscle, heart and kidney. However, it is expressed at lower level in other tissues, including liver. A single mRNA isoform was observed, with a size of approximately 4.5 kb. Using fluorescence in situ hybridization, the gene was mapped to chromosomal band 19q13.4 (269.13 cR_{3000}). A genomic sequence was identified in the database from this region, and the *KCNA7* gene structure determined. Computational analysis of the genomic sequence reveals the location of a putative promoter and a likely muscle-specific regulatory region. Initial comparison to the published murine *Kcna7* cDNA suggested a different N-terminal sequence for the human protein, however, further analysis suggests that the original mouse sequence contained an error or an unusual polymorphism.

Keywords: *Not*I-linking clone; Gene structure; Gene mapping

1. Introduction

As *Not*I sites are preferentially located in CpG islands (Bird, 1987), the sequences flanking *Not*I sites can aid in the identification of the edges of genes. This localization to CpG islands adjoining and within genes has resulted in the discovery of a number of new genes from the *Not*I clone sequencing efforts (Kashuba et al., 1999; Zabarovsky et al., 2000).

Here we report the cloning of a novel, putative member of the human voltage-gated potassium channel protein family, the potassium channel gene *Kv1.7* (*KCNA7*), originally identified from a partial exon sequence in the NotI human genomic sequence collection.

Potassium channels, the largest sub-family of the ion channel superfamily, play important roles in a wide variety of cells. Members of this channel family play critical roles in cellular signalling processes regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume regulation. K^+ channels are membrane–spanning proteins that selectively conduct K^+ ions across the cell membrane. Membrane depolarization activates voltage-gated potassium channels that, once opened, conduct potassium ions along the concentration gradient against the electric field. This outward current leads to repolarization of the membrane. To accomplish this, K^+ channels are endowed with a set of features: (1) a water-filled permeation pathway (pore) that allows K^+ ions to flow across the cell membrane; (2) a selectivity filter that specifies K^+ ions as permeate ion species; (3) a gating mechan-

Abbreviations: BAC, bacterial artificial chromosome; EST, expressed sequence tag; FISH, fluorescence in situ hybridization; ORF, open reading frame; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends

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ism that serves to switch between open and closed channel conformations (Shier et al., 2000).

Three groups of potassium channels with six, two and four putative transmembrane segments are recognized: (1) voltage-gated potassium channels (Shaker-like) containing six transmembrane regions (S1-S6) with a single pore; (2) inward rectifiers K^+ channels containing only two transmembrane regions and a single pore; (3) two-pore K^+ channels containing four transmembrane regions.

Mutations in potassium channel genes underlie diseases as diverse as persistent hyperinsulinemia of infancy, cardiac long QT syndrome, cerebellar degeneration, and certain ataxias (Dukes and Philipson, 1996).

Potassium channels in mammalian cells are encoded by extended family genes. The largest subfamily, *Kv1*, is related to the fly Shaker gene and contains at least seven members, *Kv1.1-Kv1.7*. The murine *Kv1.7* gene (Kalman et al., 1998) has been linked to diabetes via its expression in pancreatic beta cells and the localization of the human ortholog to 19q13.3-q13.4, which has been linked to diabetes susceptibility (Elbein et al., 1994; Mein et al., 1998).

In this work we present the cloning of the *KCNA7*, the human ortholog of murine Kv1.7, the tissue distribution of its expression, and the analysis of the genomic sequence encoding the gene. A significant difference between the human gene and the published mouse sequence suggests a different N-terminal sequence for the protein than previously proposed.

2. Materials and methods

2.1. General molecular biology methods

Construction of *Not*I linking libraries was described previously (Zabarovsky et al., 1994).

Isolation of the DNA, RNA and all other molecular biology and microbiology procedures were performed according to standard methods (Sambrook et al., 1989; Ausubel et al., 1990).

cDNA library from heart (Stratagene, La Jolla, CA, USA) in λ ZAP II was used for the screening and isolation of cDNA clones. Marathon-Ready™ cDNA from skeletal muscle (Clontech, Palo Alto, CA, USA) was used for 5′-RACE PCR according to the instruction with PCR primers:

RACE1: 5′ CCCACCGGACTGGTAGTAGTAGAGCAC 3′ (584-558nt).

RACE2: 5′ CGGCGTCGAAGCTGGGCCGGTC 3′ (558-537nt).

To confirm the long 3′- untranslated region of human *Kv1.7* gene we designated forward primer Left 4 and reverse primer Right 3, situated on the 3′-UTR of human *Kv1.7* gene, and amplified a 2.0 kb cDNA fragment from skeletal muscle Marathon-Ready cDNA:

Left 1: 5′ GCGAATGGCAATGGGTTGTGTT 3′ (1924-1945 nt).

Right 1: 5′ TGAGCCTCAGTTTCCCCGTCTG 3′ (4223-4202 nt).

PCR was performed in 30 μl of a solution containing 67 mM Tris–HCl, pH 9.1, 16.6 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 0.1% Tween 20, 200 μM dNTPs, 0.05 μg of the genomic DNA, 400 nM of each primer and 5U of Taq polymerase. The PCR cycling conditions were 95°C for 1.5 min, followed by 35 cycles of 95°C for 0.5 min, 68°C for 3 min and a final extension at 68°C for 7 min. The PCR product was gel-purified and sequenced.

To confirm the *Kv1.7* transcription start site we used the following PCR primers:

Left 2: 5′ AAACTTGGAGAGACGCAGGACAG 3′ (1-23 nt).

Left 3: 5′ AAAGAAAAGAGGCGACTGCAACTG 3′ (-56 to -33 nt).

Right 2: 5′ TACACAGCGTCTCCACCACGAAGA 3′ (1005-982 nt).

PCR was performed as described above but with addition of 5% DMSO.

For the generation of murine *Kcna7* gene fragment using mouse liver DNA, the following PCR primers were used:

Left 4: 5′ AAAGCTCAAGAGATCCACGGAAAAGCG 3′ (274-300 nt, NM_010596).

Right 3: 5′ CGACTGGTAGTAGTAGAGCACCGCATC 3′ (717-691 nt, NM_010596).

PCR was done as described for the obtaining of *Kv1.7* 3′-UTR.

All primers were designed using *Primer3* program: http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi.

The hybridization with Human Multiple Tissue #7765-1 and #7780-1 Northern blots (Clontech, Palo Alto, CA, USA) was done according to the manufacturer's protocols. Human *Kv1.7*cDNA fragment (1-1005 bp) was used as a probe.

Sequencing was performed with ABI377 (Applied Biosystems) sequencers according to standard procedures.

The FISH of *Not*I linking clone NR1-253, insert size 4.7 kb, with normal metaphase chromosomes was done as described previously (Protopopov et al., 1996). Sixty metaphase spreads with specific signal were analyzed.

The KCNA7 nucleotide sequence has been submitted to the EMBL/GeneBank data banks with Accession # AJ310479.

2.2. Bioinformatics

Homology searches were performed using *blastx* and *blastn* programs (Altschul et al., 1990; Gish and States, 1993).

Search of significant matches against the PROSITE-library of protein profiles was done at the ISREC server (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html).

A prediction of membrane-spanning regions and their orientation was made by the *TMpred* (http://www.ch.embnet.org/software/TMPRED_form.html) and the *HMMTOP* (http://www.enzim.hu/hmmtop) programs.

Exon positions in the genomic sequence were determined with *est_genome* (Mott, 1997). Promoter prediction was performed with *PromoterInspector* (Scherf et al., 2000). The positions of putative transcription regulatory regions for muscle-specific expression were determined with a logistic regression model (Wasserman and Fickett, 1998).

3. Results and discussion

3.1. The structure of the human KCNA7 gene

The *Not*I linking clone NR1-253 (flanking sequence length is approximately 900 bp, GenBank Accession No. AQ939522) displays 85% identity over 658 nucleotides with the murine voltage-gated potassium channel *Kv1.7* gene (*Kcna7*, Accession No. AF032099) (Kalman et al., 1998). A set of overlapping human cDNA sequences was obtained via a combination of cDNA library screening and RACE-PCR. The homologous human EST clones were found in public databases. All of them were outside a putative open reading frame (ORF): BF325690 in the middle of the gene (1943–2174 nt), F21796 (3922–4284 nt) and AA193007 (3994–4372 nt) at the 3′ end. AA193007 was used to obtain 3′ UTR of the gene. The final human sequence for *KCNA7* gene contained 4372 bp, encoding a predicted protein of 456 amino acids (Fig. 1). Screening the genome sequence database revealed the *KCNA7* gene in the draft sequence of the human BAC clone CTB-60B18 (Accession No. AC008687). Like the murine ortholog (and unlike most/all other human *Kv1* channels genes), the *KCNA7* gene is split into two exons of length 911 and 3461 bp, respectively (Fig. 2A). The human gene intron is 1153 bp in length, compared to the reported murine intron of length 1.9 kb (Kalman et al., 1998).

Analysis of the human genomic sequence suggested the location of some regulatory control regions. The *Promoter-Inspector* algorithm suggests the presence of a single promoter adjacent to the identified first exon. A unique algorithm (Wasserman and Fickett, 1998; Wasserman et al., 2000) for the identification of transcriptional regulatory regions directing skeletal muscle-specific transcription was applied to the *KCNA7* genomic sequence. A putative regulatory region was identified at approximately −1100 relative to the 5′ end of the first exon (Fig. 2B). Potential binding sites for both Mef-2 and Sp-1 transcription factors were identified within this region.

To investigate 5′ flanking region of human *Kv1.7* gene we performed 5′-RACE using Marathon-Ready™ cDNA from skeletal muscle (Clontech, Palo Alto, CA, USA). One specific 5′-RACE product of 600 bp was generated with the gene-specific primers RACE1 and RACE2 and a Marathon adapter primer AP1 and AP2, respectively. This PCR product was gel-purified and cloned. 15 clones were sequenced. These sequences demonstrated that the 5′ end extends by 356 bp upstream of the ATG initiation codon.

To confirm the *Kv1.7* transcription start site we performed PCR with two forward primers Left 2 (the 5′ end of primer corresponds to transcription start site, defined by 5′ RACE) and Left 3 (56 bp upstream from primer Left 2) and one reverse primer Right 2. To ensure that the cDNA product could be clearly distinguished from genomic products, the reverse primer Right 2 was designated within exon 2. Using forward primer Left 2 and reverse primer Right 2 a part of human *Kv1.7* gene was amplified from both leukocytes genomic DNA (about 2.0 kb) and skeletal muscle Marathon-Ready cDNA (about 1.0 kb). A cDNA product could not be amplified using forward primer Left 3 and reverse primer Right 2. However we have amplified *Kv1.7* gene from genomic DNA using these primers. All PCR products were gel-purified and sequenced. Thus, the 5′ end of the human *Kv1.7* mRNA is located between Left 2 and Left 3 primers (–56 to 1 nt). RNA protection experiments (Multi-NPA RNA protection assay kit # 1428, Ambion Inc., Austin, Texas, USA) and RNA-Ligase-Mediated RACE kit (# 1700, Ambion Inc.) did not define transcription initiation site more precisely.

The human KCNA7 protein displays a high level of amino acid identity to the mouse Kcna7 (90% in 451 aa overlap, score 809 bits) and less identity to a variety of human potassium channel proteins (70% to Kv1.4 in 405 aa overlap). In fact, in many extended regions the human and mouse proteins are identical (Fig. 3). Based on the observed similarity, we have named the human gene *KCNA7* and suggest that these genes are true orthologs. Nucleic acid similarity is less profound, in the best cases reaching 82–86%, which is consistent with the variability between orthologous human and rodent genes (Makalowski and Boguski, 1998).

3.2. The N-terminal sequence of the mouse Kv1.7 protein

The human and murine cDNAs suggest different N-terminal protein sequences in the putative ORFs. The murine ORF encodes 532 aa, which is 76 aa longer than the human. Additionally, there is no similarity between the first 10 aa of the human protein and the first 88 aa of the mouse protein (Fig. 3A). While potassium channels can vary considerably at the N-terminal ends, the high similarity of these orthologous genes suggests that an alternative explanation is more probable. A frameshift within the murine cDNA sequence is likely to have produced an inaccurate N-terminal sequence. Consistent with this hypothesis, the human cDNA sequence contains CGGC at the positions 382–385, which corresponds to CGC in the murine sequence positions 523–525 (Accession No. NM_010596.1). In support of this difference being a frameshift sequencing error, the murine ESTs for *Kcna7* (Accession Nos. AI322534.1 and AI324179) contain the sequence CGGC. Additionally, a murine genomic sequence (Accession No. AC073711) for the *Kcna7* gene, or possibly for a recently created paralog or pseudogene, contains the same CGGC sequence present in the human cDNA. If the

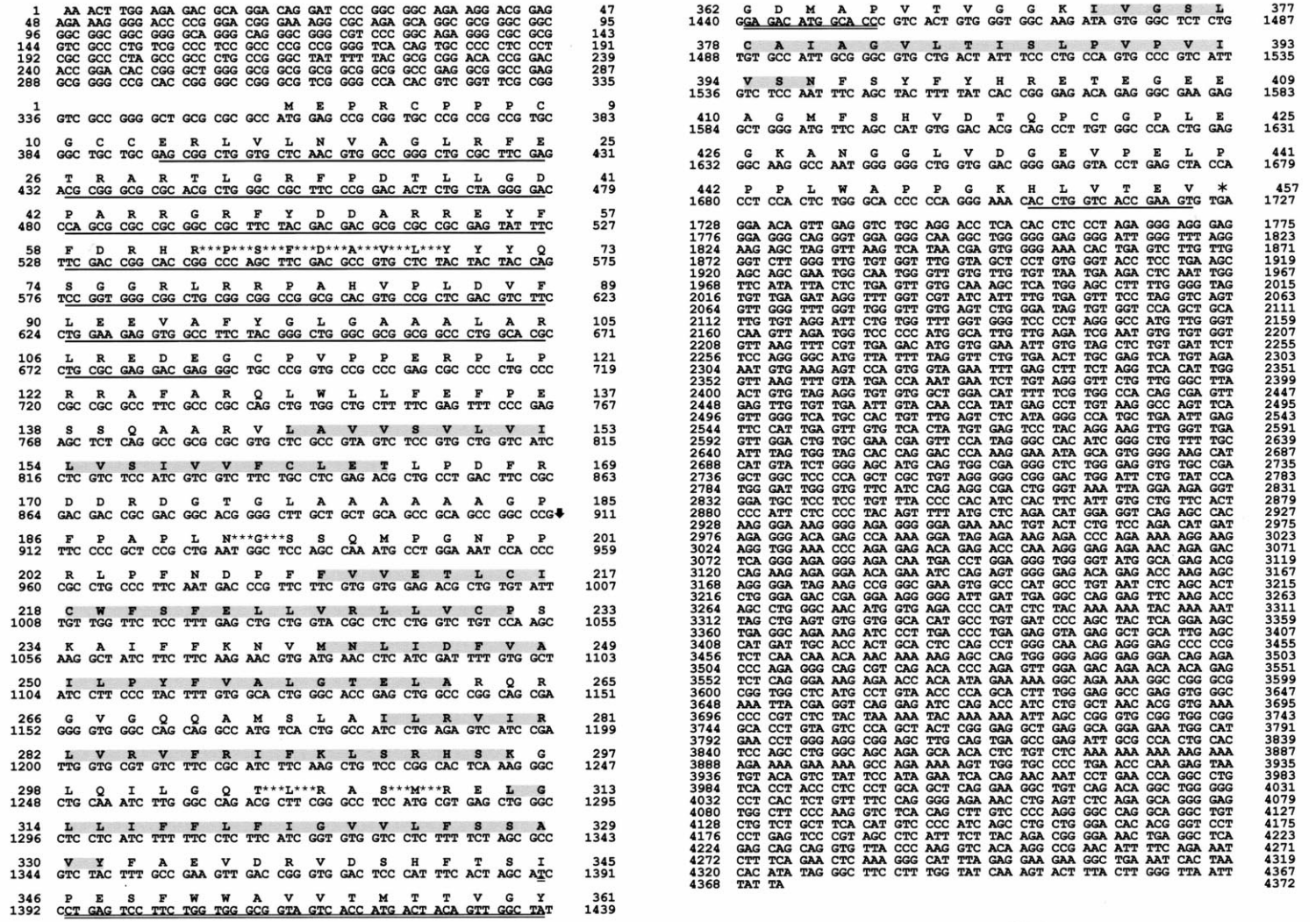

Fig. 1. Nucleotide and translated amino acid sequences of the *KCNA7* cDNA. Six transmembrane domains are shown in shadow. N-terminal T1 domain and C-terminal PDZ stretch are underlined and pore region of potassium channels is double underlined. Putative tyrosine kinase phosphorylation (RPSFDAVLY), glycosylation (NGS) and protein kinase C (TLR and SMR) sites are designated by stars. The arrow indicates exons splice site.

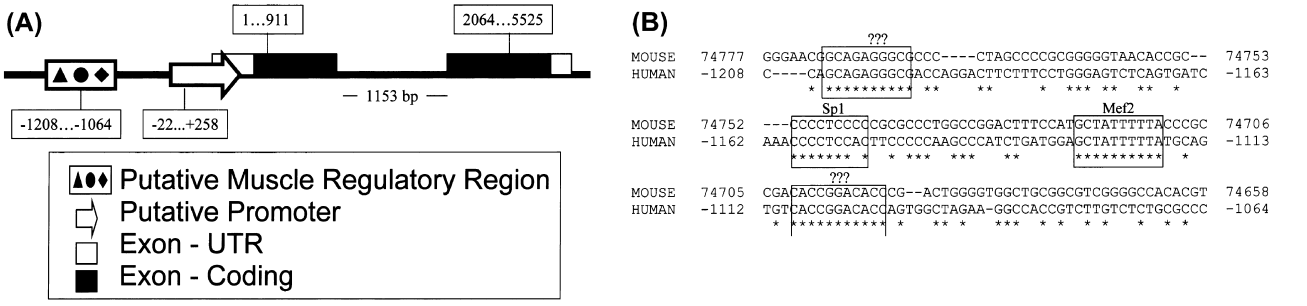

Fig. 2. Structure of the human *KCNA7* gene. (A) Genomic structure of *KCNA7*, including a putative promoter and the exon positions. (B) Computational detection of potential skeletal muscle regulatory regions (boxes marked by question marks) in human *KCNA7* gene and putative mouse genomic sequence (Accession No. AC073711). Mef2 and Sp1 binding sites are also shown.

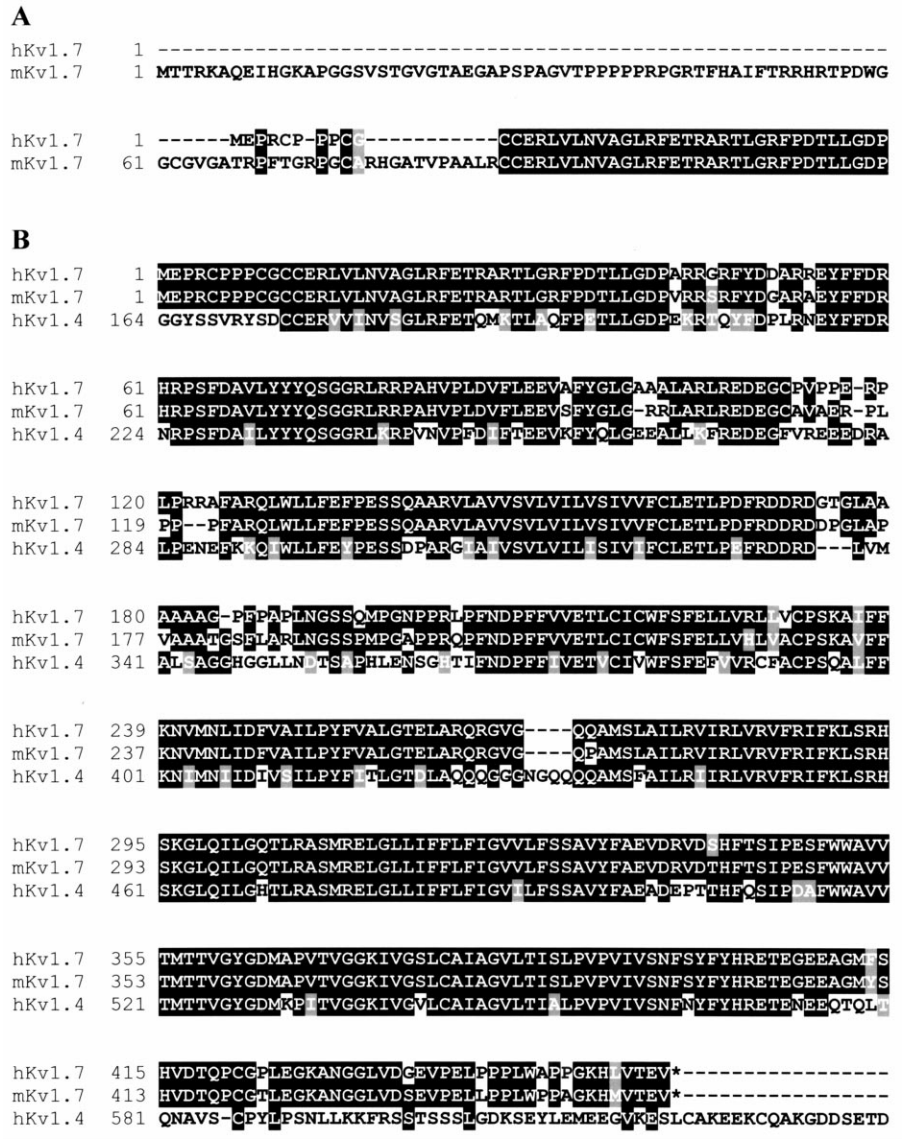

Fig. 3. Alignment of the deduced amino acid sequence of KCNA7 with murine Kcna7 and human Kv1.4. Black and grey boxes indicate identical and similar amino acid residues, respectively. (A) Alignment of N-termini of the human KCNA7 and murine Kcna7 (Accession Nos. AF032099 and NM_010596.1). (B) Alignment of the human KCNA7, murine Kcna7 and human Kv1.4 proteins suggested after the introduction of an additional nucleotide G in the position 524 of the *Kcna7* sequence (Accession No. NM_010596.1).

CGGC sequence is correct, the murine ORF would be altered at the N-terminal such that the first 88 amino acids of the published murine sequence would be replaced by 10 amino acids identical to the human N-terminal sequence. With this correction to the murine sequence, the sequence alignment gives 91% identity over 457 aa (score 834 bits, Fig. 3B).

To directly check the sequence of murine *Kcna7* gene we performed PCR with mouse genomic DNA and primers described in Materials and Methods. Sequencing of the PCR product confirmed that the mouse gene contain the CGGC sequence (Accession No. AJ 409348) and therefore its N-terminal protein sequence is identical to the human.

3.3. Domain organization of the human Kv1.7 protein

Human *KCNA7* gene is predicted to encode a protein, hKv1.7, of 50.5 kdal molecular weight (Fig. 1). This protein has all the standard hallmarks of a voltage-gated potassium channel, which include six membrane-spanning domains S1-S6, the S4 being positively charged (Arg-X-X-Arg-X-X-Arg-X-X-Arg-X-X-Lys-X-X-Arg-X-X-Lys). The amino- and carboxyl-terminal ends are proposed to be intracellular. The search of protein profiles revealed the N-terminal K^+ channel tetramerization domain (T1, Pfam: PF02214) and potential C-terminal stretch of PDZ domain (Songyang et al., 1994) needed for protein-protein interaction. Single potential N-linked glycosylation site (Asn-X-Ser, PROSITE: PS00001) is identified at position 191, which resides in the region between the first two

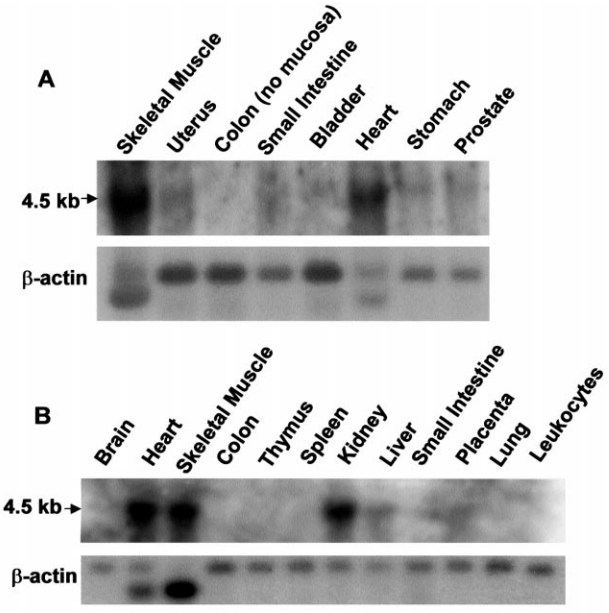

Fig. 4. Hybridization of the *KCNA7*to a Human Multiple Tissue Northern blots (Clontech) #7765-1 (A) and #7780-1 (B).

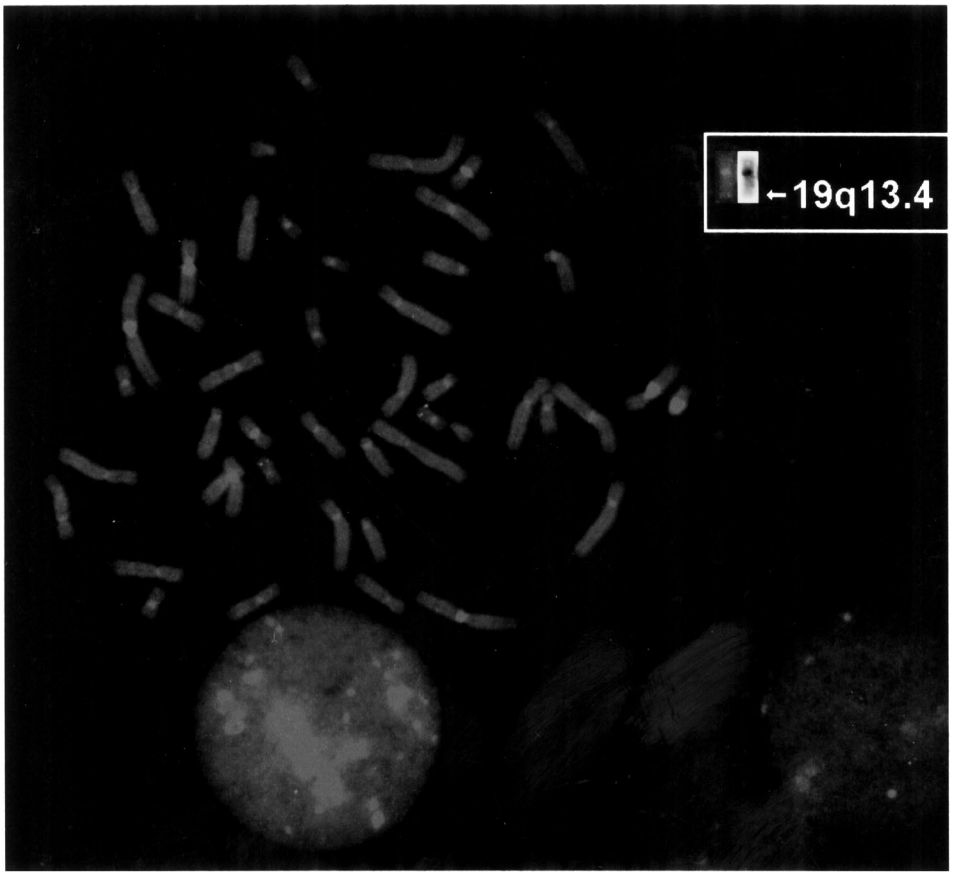

Fig. 5. FISH-based assignment of *KCNA7* to human chromosome band 19q13.4.

membrane-spanning domains and is predicted to be extracellular. The segment between 237–448 is similar to the PF00914 (transmembrane region of the cyclic nucleotide gated channel) and contains a pore region (340–397; PS50265). As do all other Shaker-related channels, hKv1.7 has a potential tyrosine kinase phosphorylation site (RPSFDAVLY) in its N-terminal region. Two protein kinase C consensus sites (Ser/Thr-X-Arg) are present in the cytoplasmic loop; at least one of these sites is present in all members of the Kv1 family (Chandy and Gutman, 1995). In general, the mouse Kcna7 and human KCNA7 have very similar predicted structures.

3.4. Expression pattern of the human KCNA7 gene

As potassium channels are often preferentially expressed in muscle tissues, we performed a Northern hybridization using a filter containing RNA from a variety of muscle tissues. Northern blot expression analysis revealed that the highest expression of *KCNA7* is in skeletal muscle and heart, agreeing with the computational detection of a putative muscle regulatory region. A single band of approximately 4.5 kb was observed. Expression in smooth muscles was detected at a lower level (Fig. 4A), consistent with the expression of *Kcna7*. At the same time *KCNA7* was expressed in other tissues (Fig. 4B) like kidney or liver. Several mouse ESTs (AI324179, AA021711, AI322534) were isolated from placenta. However, no significant expression is evident in human placenta (Fig. 4B).

3.5. Chromosomal assignment of the human KCNA7 gene

Using FISH we assigned the *Not*I linking clone NR1-253, to which human voltage-gated potassium channel gene *Kv1.7* corresponded, to chromosomal band 19q13.4 (Fig. 5). The *Kv1.7* gene shares the same BAC clone CTB-60B18 with *CGB*, *SNRP70*, *NTF5* genes and few STSs, which are assigned around position 269.13 cR_{3000} by RH mapping. As previously observed by Kalman et al. (1998), this chromosomal location is consistent with a putative diabetes susceptibility gene that has been suggested to be present at 19q13 (Mein et al., 1998). This suggestion is especially strong for Finnish families with associated hypertension and difficulties in insulin-stimulated glucose storage (Elbein et al., 1994). For the *Kcna7* gene, expression in mouse pancreatic islet cells was demonstrated (Kalman et al., 1998). Therefore, *KCNA7* is potentially linked to the pathogenesis of type I and II diabetes mellitus in some humans. On the other hand, as *KCNA1* gene is the site of the mutations in type 1 episodic ataxia (Browne et al., 1994), and type 2 episodic ataxia (EA2) is linked to chromosome 19, *KCNA7* might be involved in EA2.

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