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SCIENCE DIRECT

Genomics 86 (2005) 581 – 593

GENOMICS

www.elsevier.com/locate/ygeno

Structure, alternative splicing, and expression of the human and mouse KCNIP gene family☆

Priit Pruunsild, Tõnis Timmusk*

Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, Tallinn 19086, Estonia
National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia

Received 14 April 2005; accepted 5 July 2005
Available online 19 August 2005

Abstract

Potassium channel-interacting proteins (KCNIPs, also named KChIPs) modulate A-type potassium channels and favor their surface expression. In addition, KCNIPs have been shown to interact with presenilins and also to function as transcriptional repressors. Here we describe the structures and alternative splicing of the human and mouse *KCNIP* genes, including novel splice variants for *KCNIP1, KCNIP3,* and *KCNIP4,* and show the expression of different *KCNIP* mRNAs in various mouse and human tissues and brain regions by RT-PCR. Furthermore, we describe the expression of *KCNIP1, KCNIP2, KCNIP3,* and *KCNIP4* mRNAs in the adult mouse brain with in situ hybridization and show that all *KCNIP* mRNAs were expressed in the neurons of the mouse brain with specific patterns for each *KCNIP.* Our results show that alternatively spliced *KCNIP* mRNAs are expressed differentially and could contribute to the diversity of functions of the KCNIP proteins.

Keywords: DREAM; Calsenilin; KChIP; Alternative splicing; Potassium channels; Transcriptional repressor; Presenilin; Nervous system

KCNIPs, potassium channel-interacting proteins, are a family of calcium binding proteins with four EF-hand motifs, which have been implicated in the modulation of A-type potassium channels [1]. They have been shown to modulate and favor the surface expression of neuronal and cardiac A-type Kv-4 channels [2,3]. Interestingly, two members of the family, KCNIP3 and KCNIP4, have been shown to interact with presenilins and to regulate the levels of presenilin fragments and have also been named calsenilin [4] and CALP [5], respectively. This knowledge of the dual activity of KCNIPs has expanded further, because KCNIP3/calsenilin was found to be also a Ca^{2+}-regulated transcriptional repressor and was named DREAM (downstream regulatory element antagonist modulator) [6]. It was shown to bind to a regulatory element downstream of the transcription start site of the prodynorphin gene (DRE) and repress the expression of prodynorphin in the absence of calcium. Putative DREs are present in many genes [7,8] and in addition to DREAM, other KCNIP family proteins have the ability to bind DRE sites in vitro and to repress transcription in transient overexpression assays [9].

The KCNIPs differ from other proteins in the family of neuronal calcium binding proteins because they contain a variable N-terminal sequence that shares no homology with other calcium-binding protein domains (reviewed in [10]). Interestingly, the N-termini among the KCNIPs themselves are also unique and share no homology [1]. It has also been found that one *KCNIP* gene can encode more than one

☆ Sequence data from this article have been deposited in the GenBank Data Libraries under Accession Nos. DQ148476, DQ148477, DQ148478, DQ148479 (Human KCNIP1 isoforms); DQ148480, DQ148481, DQ148482, DQ148483, DQ148484 (Human KCNIP2 isoforms); DQ148485, DQ148486 (Human KCNIP3 isoforms); DQ148487, DQ148488, DQ148489, DQ148490, DQ148491, DQ148492 (Human KCNIP4 isoforms); DQ148493, DQ148494, DQ148495 (Mouse KCNIP1 isoforms); DQ148496, DQ148497, DQ148498, DQ148505 (Mouse KCNIP2 isoforms); DQ148499, DQ148500 (Mouse KCNIP3 isoforms); DQ148501, DQ148502, DQ148503, DQ148504 (Mouse KCNIP4 isoforms).

* Corresponding author. Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, Tallinn 19086, Estonia. Fax: +372 620 4401.

E-mail address: tonist@kbfi.ee (T. Timmusk).

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doi:10.1016/j.ygeno.2005.07.001

unique N-terminal sequence due to alternative splicing [11] or alternative usage of transcription start sites [12], which in turn could have importance in the diverse roles of the KCNIP proteins. However, the only evidence that the unique N-termini have functional significance in determining the role for KCNIP has been shown in the case of KCNIP4, which has different N-termini that cause distinct effects upon Kv4 channel modulation [12].

In addition to studies showing that KCNIPs have diverse roles in different compartments of cells, some studies have presented opposite results about the function of KCNIPs in vivo. In fact, three different *KCNIP* knockout mice have been developed so far, two of them being *KCNIP3* knockouts, which show different phenotypes. The first study on *KCNIP3* knockout mice showed that these mice had elevated levels of prodynorphin mRNA and dynorphin A peptides in the spinal cord [13]. KCNIP3 was presumed to be a transcriptional repressor modulating pain processing by repressing prodynorphin expression in vivo [13]. These mice were shown to have no impairment of Kv4 channels in the heart [13], which was expected due to KCNIP3 involvement in Kv4 channel modulation [1]. In addition, these mice did not have detectable differences in the presenilin-1 and presenilin-2 expression levels or presenilin processing compared to wild-type mice, which was expected because KCNIP3 is implicated in the proteolytic processing of presenilins [4]. In contrast, the other study for which the *KCNIP3*knockout mice were developed showed that there were no changes in dynorphin expression in the knockout mice and additionally these mice were shown to have altered long-term potentiation and presenilin processing, which indicate a role for KCNIP3 in modulating the Kv4 channels and γ-secretase function, respectively [14]. The third *KCNIP* knockout developed was the *KCNIP2* knockout in which there was no transient outward potassium current in the ventricular myocytes, which is well in accordance with *KCNIP2* expression data in the heart and proposed involvement of KCNIP2 in Kv4 channel modulation [15]. As KCNIP2 was not implicated in presenilin processing and gene transcription regulation at the time the study was conducted, there is no information about dynorphin expression or presenilin processing in *KCNIP2* knockout mice. However, recent data have shown that KCNIP2 could also be in involved in gene regulation [9].

Therefore, considering the data about the functions of the KCNIP proteins obtained from the *KCNIP3* knockouts and the finding that KCNIPs have unique N-termini that could have functional significance, it is very important to study the expression pattern of different KCNIP isoforms. Accordingly, in this study we have described the structures of the human and mouse *KCNIP1, KCNIP2, KCNIP3,* and *KCNIP4* genes. We have also analyzed alternative splicing of the *KCNIP* genes and coding potentials of the alternative transcripts. Furthermore, we have analyzed the expression of different *KCNIP1, KCNIP2, KCNIP3,* and *KCNIP4* mRNA splice variants in various mouse and human tissues and brain regions by RT-PCR and describe the expression of *KCNIP1, KCNIP2, KCNIP3*, and*KCNIP4* mRNA in the adult mouse brain with in situ hybridization.

Results

The structure of human KCNIP genes

Recent studies on *KCNIPs* have identified several alternative transcripts for each *KCNIP* [1,5,11,16], but with the exception of *KCNIP2* [17], none of the studies has thoroughly analyzed the structures and alternative splicing of the *KCNIP* genes. In this study, we characterized the exon/intron structure of human *KCNIP* genes and analyzed the alternative splicing pattern of each *KCNIP* gene in both human and mouse using bioinformatics and RT-PCR. For each *KCNIP* a search for mRNA sequences and expressed sequence tags (ESTs) was performed. Furthermore, the identified cDNAs were cloned and verified by sequencing. RT-PCR analyses were carried out for characterization of the expression pattern of the alternative transcripts of the *KCNIP* family in human and mouse. Expression of human *KCNIP* alternative transcripts was analyzed in a panel of 23 adult tissues and in 30 adult brain regions. Expression of mouse *KCNIP* transcripts was studied in 8 adult nonneural tissues, 10 adult brain regions, and 6 different developmental stages of brain.

The lengths of the four human *KCNIP* genes vary from approximately 17 kb for *KCNIP2* up to approximately 1.2 Mb for *KCNIP4* (Fig. 1A). All *KCNIP* genes have multiple unique 5′ exons and share homology in the 3′ part of the genes: the last seven exons are highly homologous and have identical lengths. The identity among the most conserved last seven exons of the *KCNIP* genes is 81% on the

Fig. 1. (A) Structures and alternative transcripts of human *KCNIP* genes. The structural organization of human *KCNIP1, KCNIP2, KCNIP3*, and *KCNIP4* exons and introns was determined by analyzing genomic and mRNA sequence data using bioinformatics and RT-PCR. Exons are shown as boxes and introns are shown as lines. Filled boxes indicate the translated regions of the exons, white boxes indicate the untranslated regions of the exons. The numbers above the exons and below the introns indicate their sizes. For the 5′ and 3′ exons the sizes of the protein coding parts are shown. Vertical dashed lines indicate putative transcription start and stop sites. Green vertical lines indicate translation start codon positions and red vertical lines indicate translation stop codon positions. Exon numbers are shown in roman characters. Only the major transcript variants for each *KCNIP* gene are shown. Asterisks mark very rarely used exons. (B) N-terminal sequences of the human KCNIP proteins. For each KCNIP exon III the first 10 amino acids encoded are shown in colored letters. The color code results from an alignment of all human full-length KCNIPs in ClustalW and the colors indicate as follows: red, identity of amino acids; green, strong similarity of amino acids; and blue, weak similarity of amino acids. The homologous N-terminal sequences of human KCNIP1 and KCNIP4 are marked with a red asterisk.