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Cloning, structure and assignment to Chromosome 19q13 of the human Kir2.4 inwardly rectifying potassium channel gene (*KCNJ14*)

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Inwardly rectifying K^+ (Kir) channels are assembled from four subunits, each consisting of two transmembrane segments flanking a pore loop region. According to sequence similarity and functional properties, 15 differentially distributed subunits are now grouped into six subfamilies (Kir1, Kir2, Kir3, Kir5, Kir6, and Kir7). Kir channels have evolved to serve diverse cellular functions such as K^+ homeostasis, setting the membrane resting potential, modulation of neuronal firing rates, controlling pacemaker activity in the heart, as well as tuning metabolite-dependent insulin/hormone secretion (Doupnik et al. 1995; Isomoto et al. 1997). Their crucial cellular function is illustrated by mutated genes associated with inheritable diseases; for example, familial persistent hyperinsulinemic hypoglycemia (Thomas et al. 1996) or hyperprostaglandin E syndrome (Simon et al. 1996).

Constitutively active Kir channels with strong rectification are probably formed by homomeric assembly of Kir2.1, Kir2.2, Kir2.3, and Kir2.4 subunits, which are present in the mammalian brain, heart, skeletal muscle, endothelial cells, and cellular components of the immune system (Kurachi et al. 1999). Kir2.4 is unique in its expression pattern in the rat brain. It is restricted to the large motoneurons in the spinal cord and all motor nuclei associated with cranial nerves 3–7 and 9–12, thus being the first example of an ion channel associated with a functional system (Töpert et al. 1998). Its distinct distribution pattern renders Kir2.4 a potential candidate to be involved in the pathology of motoneuron diseases such as amyotrophic lateral sclerosis (Brown 1997) or spinal muscular atrophy (Scharf et al. 1999). To characterize the human Kir2.4 ortholog (hKir2.4), we screened the GenBank expressed sequence tag- (EST-) database with the rat Kir2.4 (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). All identified EST clones (GenBank Accession No. W25800 and I.M.A.G.E. clones 839696, 1837089, 1523087; kindly supplied by the RessourcenZentrum/PrimärDatenbank, Berlin) lacked the majority of the 5′ ORF. EST clones W25800 and 839696 were isolated from human retina cDNA libraries, suggesting that Kir2.4 subunits are also expressed in retinal neurons.

For determination of the entire ORF, as well as the gene structure of hKir2.4, a human genomic λ-phage library (DASH; Stratagene, La Jolla, Calif., USA) was homology screened with a specific 565-bp ^{32}P-labeled probe that covered the COOH-terminus of Kir2.4 and was PCR-amplified with sequence information from the EST clone AA504857. Three positive phage clones containing inserts between 16.5 kb and 18.5 kb were plaque purified, propagated for phage-DNA preparations, and were directly sequenced by PCR cycle sequencing. The deduced ORF of the complete hKir2.4 codes for a polypeptide of 436 amino acids. It differs from the rat Kir2.4 subunit in 22 residues mostly located in the variable NH_2- and COOH-terminal regions (Fig. 1A; Töpert et al. 1998) and shares similar sequence identity with the guinea pig ortholog of Kir2.4 (Genbank accession number AF187876; Fig. 1A).

Structural analysis of the human Kir2.4 gene (*KCNJ14*) revealed the existence of an intron that disrupts the ORF at the codon for glutamine 238. The intron is 1742 bp in length and is flanked by adequate splice donor and acceptor consensus sites (Fig. 1B). Thus, the first 238 amino acids of hKir2.4, which include transmembrane segments M1, M2, and the pore-forming H5 region, are coded by a single exon (Exon 1), whereas the complete cytosolic COOH-terminus is coded by a second exon (Exon 2). Comparison of the genomic sequence with the 3′ untranslated regions (UTR) of the EST clones 1837089 and 839696 (1238 bp) revealed no further intronic disruption downstream of Exon 2. On the basis of the analysis of the closely related guinea pig *Kir2.4* gene (Genbank accession number AF187875), in which a 238-amino-acid long first exon is followed by intronic sequences, it is assumed that the gene structure in both species is identical.

Genomic analysis shows that intronic sequences may be variably distributed both in the ORFs and UTRs of Kir subunit genes. In human Kir3.4 (*KCNJ5*) and Kir3.1 (*KCNJ3*), the ORF is disrupted by one and two introns, respectively, whereas the 5′ and 3′ UTRs are intronless (Spauschus et al. 1996; Schoots et al. 1997). In contrast, the rat *Kir2.1* gene (*KCNJ2*) contains an intron only in the 5′ UTR (Redell and Tempel 1998). As a common feature, however, the coding sequences for the transmembrane segments and the pore-forming loop are located on the same exon (Shuck et al. 1997; Derst et al. 1998; Erginel-Unaltuna et al. 1998; Redell and Tempel 1998; Schoots et al. 1997; Wei et al. 1998).

To identify disease phenotypes potentially associated with mutations in *KCNJ14*, we determined its physical map position by PCR analysis on two panels of human/rodent somatic cell hybrids. In a first step, a 304-bp fragment of the human *KCNJ14* was amplified from the NIGMS Human Genetic Mutant Cell Repository monochromosomal human/rodent somatic cell hybrid panel 2/version 2 (Drwinga et al. 1993) only from DNA of clone NA10449 with primer pair Kir2.4-FOR and -REV (sequence shown in legend of Table 1), thus unambiguously assigning *KCNJ14* to human Chr 19. In a second step, the regional localization was analyzed with the GeneBridge 4 radiation hybrid panel (Gyapay et al. 1996) and the same pair of primers. Two-point maximum-likelihood analysis of the data vector indicated a linkage of *KCNJ14* to the genetic framework markers D19S420 (centromeric) and D19S218 (telomeric; Table 1). The closest flanking markers for *KCNJ14* were WI-9028 and NIB1805, at cytogenetic band 19q13 (5.76 cR from WI-9028). In addition to *KCNJ14*, five other potassium channel genes, *KCNA7*/Kv1.7, *KCNC2*/Kv3.2, *KCNC3*/Kv3.3, *KCNN4* (hSK4), and *KCNK6*/TWIK-2, have also been mapped to chromosomal locus 19q13.

In general, K^+ channel genes are good candidates for epilepsies, and indeed region 19q13 contains one gene for benign famil-

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                                                                  M1
hKir2.4  MGLARALRRLSGALDSGDSRAG-----DEEEAGPGLCRNGWAPAPVQSPVGRRRGRFVKK  55
rKir2.4  ·············EP·N·············A·········G····AGN·········  53
gKir2.4  ·············E··N··E·NSRPD···A···········QG···GA·········  58

                                                                  M1
hKir2.4  DGHCNVRFVNLGGQGARYLSDLFTTCVDVRWRWMCLLFSCSFLASWLLFGLAFWLIASLH 115
rKir2.4  ····························································· 113
gKir2.4  ····························································· 118

                                        H5                    M2
hKir2.4  GDLAAPPPPAPCFSHVASFLAAFLFALETQTSIGYGVRSVTEECPAAVAAVVLQCIAGCV 175
rKir2.4  ·············Q····························································· 173
gKir2.4  ····························································· 178

hKir2.4  LDAFVVGAVMAKMAKPKKRNETLVFSENAVVALRDHRLCLMWRVGNLRRSHLVEAHVRAQ 235
rKir2.4  ·····································R····················· 233
gKir2.4  ·····································R····················· 238
         ▽ Intron
hKir2.4  LLQPRVTPEGEYIPLDHQDVDVGFDGGTDRIFLVSPITIVHEIDSASPLYELGRAELARA 295
rKir2.4  ····························································· 293
gKir2.4  ····························································· 298

hKir2.4  DFELVVILEGMVEVTAMTTQCRSSYLPGELLWGHRFEPVLFQRGSQYEVDYRHFHRTYEV 355
rKir2.4  ············A·········································· 353
gKir2.4  ············A·········································· 358

hKir2.4  PGTPVCSAKELDERAEQASHSLKSSFPGSLTAFCYENELALSCCQEEDEDDETEEGNGVE 415
rKir2.4  ····························A···················EEDTK··TSA· 413
gKir2.4  ····························A···················EE·TK·ET·A· 418

hKir2.4  TEDGAASPRVLTPTLALTLPP 436
rKir2.4  ·P·R····QA·········· 434
gKir2.4  ··········I·····S·L· 438
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B

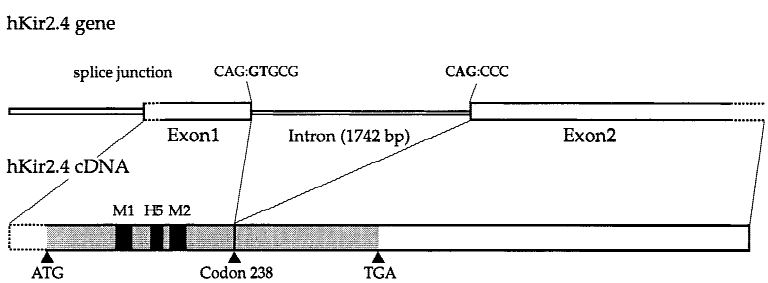

Fig. 1. Protein sequence and partial gene structure of the human Kir2.4 subunit. **(A)** The predicted 436 amino acids of hKir2.4 (GenBank accession No. AF181988) are aligned with the rat (rKir2.4) and guinea pig (gKir2.4) orthologs. Transmembrane segments M1 and M2 and the pore-forming loop H5 are boxed in grey. Residues in rKir2.4 and gKir2.4 that are identical to hKir2.4 are depicted as dots. The arrowhead after the glutamine at position 238 indicates the position of the intronic interruption. **(B)** Genomic structure of the human Kir2.4 gene (*KCNJ14*). The diagram in the upper panel shows a 5780-bp Kir2.4 λ phage clone with two exons separated by an intron of 1742 bp in length (consensus sequences of splice junctions are given). The lower diagram is a schematic representation of the hKir2.4 cDNA with the ORF shown in grey, and subunit core domains marked as black bars. Start and stop codon and the position of the intron are indicated by arrowheads.

Table 1. GeneBridge 4 panel radiation hybrid mapping data.

Gene/ locus	Primer pair (product size)	Data vector[a]	lod score[b]	Map location	Flanking markers[c]
KCNJ14	Kir2.4/FOR[d] Kir2.4/REV[d] (304 bp)	00000010010000000101010 00001001001000000111110 11000110000000001010110 01101001000001000101110 00001	>3	19q13	D19S420 WI-7903 WI-9028 *KCNJ14* NIB1805 WI-5264 WI-5423 D19S218

[a] The data vector is based on two independent PCR analyses of the entire panel, with data arranged in the order specified for the Whitehead/MIT on-line Radiation Hybrid Mapper Program, June 1998 (http://www.genome.wi. mit.edu). 0 = negative, 1 = positive, 2 = uncertain data.
[b] The lod score quoted is the highest for which this linkage is supported.
[c] Centromeric flanking markers are shown above, telomeric markers below *KCNJ14*.
[d] For chromosomal assignment, two oligonucleotide primers amplifying a 304-bp fragment of the 3′-nontranslated part of *KCNJ14* (FOR-Primer: 5′-ctgtgctccctc-ctgagaac-3′; REV-Primer: 5′-ccttccctccttaggtggtc-3′).

ial infantile convulsions (BFIC; Guipponi et al. 1997). More specifically, however, Kir2.4 channels provide the dominant K^+ conductance of many cranial motoneurons at rest. Inhibition of Kir2.4 channels in rat hypoglossus motoneurons dramatically enhances tonic activity and firing rates in response to suprathreshold stimuli (Töpert et al. 1998). In addition, in situ hybridizations in rat and PCR analysis in human tissues demonstrated Kir2.4 expression in spinal cord, suggesting similar Kir2.4 functions in ventral horn α-motoneurons. Thus, mutations in the *Kir2.4* gene may possibly result in dysfunctional coordination or impaired development of skeletal muscles as it is known from other muscular diseases (Brown 1997; Scharf et al. 1999). In view of the distinct expression in cranial motoneurons, dysfunctions affecting head muscle control, for example, facial expression or eye positioning, would be of particular interest. In a first screen of 232 entries associated with Chr 19q13 in the OMIM database (http://www.ncbi.nlm.nih.gov/Omim/searchomim.html), no disorder with the predicted phenotype was identified except dystrophia myotonica. This type of skeletal muscle disorder, however, is probably correlated with a variable number of trinucleotide insertions in a protein kinase gene (Fu et al. 1993). Due to the Kir2.4 expression in the retina, disease phenotypes associated with impaired visual processing may also derive from mutations in *KCNJ14*. Two candidate disorders have been mapped to locus 19q13: (i) Cone rod dystrophy 2 is linked to mutations in the homeodomain transcription factor gene CRX (Freund et al. 1997). (ii) For optic atrophy 3, no gene correlate has been identified so far (Nystuen et al. 1997); thus, it would be worthwhile to examine affected individuals for mutations in *KCNJ14*.

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