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Assignment¹ of the 2P domain, acid-sensitive potassium channel OAT1 gene KCN3 to human chromosome bands 2p24.1 → p23.3 and murine 5B by in situ hybridization

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¹ This is a more precise localization of the human gene previously mapped to 2p by Lesage and Lazdunski (1998) and to our knowledge this is the first time the murine gene has been mapped.

Rationale and significance

Potassium channels are a diverse group of proteins with wide cellular and tissue distribution that serve a broad spectrum of physiologic functions and participate in a variety of cellular processes (Jan and Jan, 1997). They exhibit marked genetic, biochemical, structural, and functional diversity and defects in these channels have been associated with various genetic disorders. Potassium channels share a common pore-forming P domain that determines channel conductance, selectivity, and sensitivity to open-channel blockade. Recently, a novel family of potassium channels differing in cellular, biochemical, structural, and functional characteristics has been identified that contains two P domains in tandem (Goldstein et al., 1998). Members of this family, OAT channels, so named for their Open, Acid-sensitive Two P domain characteristics, have been identified in mouse, rat, and man (Kim et al., 1998; Lopes et al., 1998; Duprat et al 1997; Leonoudakis et al., 1998). Localization and functional characterization of OAT1 suggest that it may participate in cardiac action potential amplitude and duration, and may play a role in either inherited or acquired disorders of cardiac conduction (Lopes et al., 1998).

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Materials and methods

Isolation of genomic DNA probes

A human genomic DNA library in PACs was screened with primers corresponding to the middle of the coding region of KCN3 cDNA as described (Ioannou et al., 1994). These primers, 5'-GCGAGCGCATCAACACCTTGGTGAGG-3' and 5'-GCGGCTGCGTCTGCAGGGCCTGGTC-3', amplify a 265-bp fragment from human genomic DNA. Three PCR-positive clones verified to be KCN3-gene specific were identified. A murine genomic DNA library in bacteriophage P1 was screened with primers corresponding to the 3' end of the coding region of murine *Kcnk3* cDNA as described (Pierce et al., 1992). These primers, 5'-GCAGACGCAGCCGCA-GTATG-3' and 5'-GCCTGGCCGTTGTGCGTGAGCAGGG-3', amplify a 168-bp fragment from murine genomic DNA. Three PCR-positive clones verified to be KCN3-gene specific were identified.

In situ hybridization

Preparation of metaphase chromosomes, labeling of genomic clones with digoxigenin-11-dUTP, hybridization conditions, post hybridization washes, and probe detection were performed as previously described (Ried et al., 1992). The digoxigenin-labeled clones were co-hybridized with a biotin-labeled Alu oligonucleotide (MG-009) to generate an R-banded karyotype (Matera et al., 1992). Probes were detected with FITC-avidin and rhodamine-antidigoxigenin antibodies as described (Matera et al., 1992). Images were captured using a computer-controlled Zeiss Axioskop epifluorescence microscope coupled to a CCD camera. FITC and rhodamine signals were recorded separately as gray scale images, enhanced, pseudo-colored and merged.

Human KCN3:

Probe names: 18531, 18532, 18533

Probe type: human genomic DNA

Insert sizes: ~ 180 kb

Vector: pADsacBII

Proof of authenticity: restriction enzyme analysis, Southern blotting, and limited DNA sequencing

Gene reference: AF006823

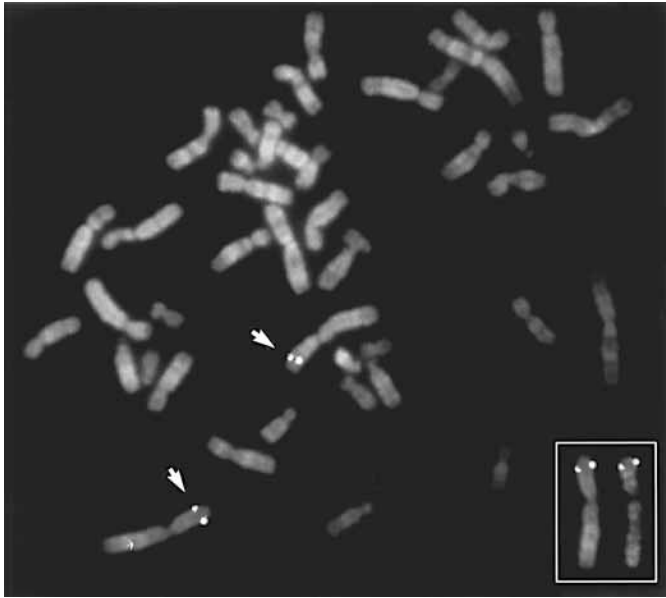


Fig. 1. Chromosome mapping of human *KCNK3* by fluorescence in situ hybridization. Specific labeling was observed at 2p24.1 → p23.1 (arrows). Inset. The chromosome on the left is DAPI stained and the chromosome on the right is Alu banded.

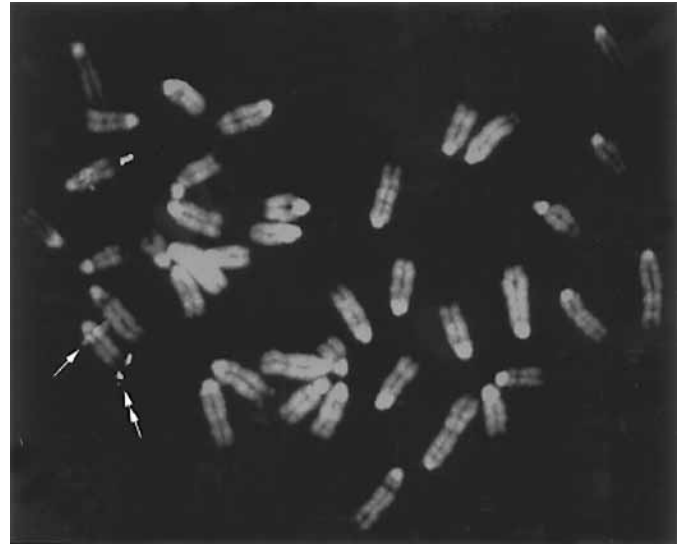


Fig. 2. Chromosome mapping of murine *Kcnk3* by fluorescence in situ hybridization. Specific labeling was observed at 5B (arrow). Localization to chromosome 5 was confirmed by co-hybridization with a murine chromosome 5 probe ATCC# 63383 (double arrow) (Mongelard et al., 1996).

Murine *Kcnk3*:

Probe names: 15456, 15457, 15458

Probe type: murine genomic DNA

Insert sizes: ~110 kb

Vector: pAd10-SacBII

Proof of authenticity: restriction enzyme analysis, Southern blotting, and limited DNA sequencing

Gene reference: AB008537

Results

Mapping results human *KCNK3*

Location: 2p24.1 → p23.1

Number of cells examined: 24

Number of cells with specific signal: 24

Most precise assignment: 2p24.1 → p23.1 (FLpter values 0.10–0.12)

Location of background signals (sites with >2 signals): 0

Mapping results murine *Kcnk3*

Location: 5B

Number of cells examined: 22

Number of cells with specific signal: 22

Most precise assignment: 5B

Location of background signals (sites with >2 signals): 0

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