Assignment of K_{ATP}-1, the Cardiac ATP-Sensitive Potassium Channel Gene (*KCNJ5*), to Human Chromosome 11q24

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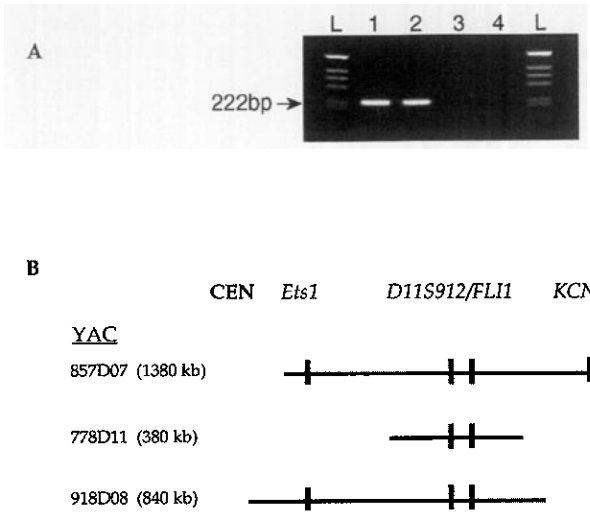

FIG. 1. (**A**) Amplification of *KCNJ5* from chromosome 11. This figure shows the 222-bp product amplified from (**1**) human genomic DNA, (**2**) the chromosome 11 hybrid, (**3**) mouse/hamster genomic DNA control, and (**4**) no DNA control (**L**, size markers). PCR amplification conditions were 35 cycles at 94°C for 20 s, 65°C for 30 s, and 72°C for 20 s. (**B**) Relative position of the *KCNJ5* gene on YAC 857D07. The three YACs known to contain the *FLI1* gene are shown. Only YAC 857D07 was found to contain the *KCNJ5* gene. The relative positions of the *Ets1*, *FLI1*, and *KCNJ5* genes and the microsatellite D11S912 are indicated. The orientation of the YACs is shown by CEN (centromeric) and TEL (telomeric).

Potassium channels inhibited by cytosolic ATP (K_{ATP}) are found in a wide variety of tissues. In the pancreatic β-cell, they play a critical role in the regulation of insulin secretion, and in smooth muscle they are responsible for hypoxic vasodilation (1). Moreover, these channels are the targets for several important classes of therapeutic drugs, including the anti-diabetic sulfonamides and the anti-hypertensive potassium channel openers (1). In the heart, as in other tissues, K_{ATP} channels are thought to couple the membrane potential to the metabolic status of the cell (1), and there is substantial evidence that these normally quiescent channels are activated during transient ischemic and hypoxic periods when they contribute to shortening of the cardiac action potential duration (6). It has been proposed that such activation of cardiac K_{ATP} channels may also precondition and protect the myocardium against subsequent infarction (6). The fact that these channels are involved in such physiologically significant events suggests that they may be candidate genes for certain forms of diabetes, hypertension, and cardiac disorders (1, 8).

Heterogeneity in the properties of K_{ATP} channels among different tissues suggests that they may have distinct molecular identities (1). The recent cloning of a cDNA encoding a K_{ATP} channel from rat heart (K_{ATP}-1) (2) has enabled the human homologue of this cardiac channel to be isolated (2) and a similar but distinct gene (βIR-1) to be isolated from the pancreatic β-cell (J.P.A., unpublished observation). The primary structures of K_{ATP}-1 and βIR-1 place them in the family of inwardly rectifying potassium channels (3). The cardiac K_{ATP} channel and its role in the duration of the ischemic cardiac action potential (6) proposes K_{ATP}-1 as a candidate gene for the inherited form of ventricular arrhythmias, the long QT (LQT) syndrome, in which patients exhibit a longer interval between the Q and the T waves of their electrocardiographs (11).

In this study we have determined the chromosomal localization of K_{ATP}-1 (gene symbol *KCNJ5;* GenBank Accession No. X83582) with high resolution by PCR (polymerase chain reaction) screening of somatic cell hybrids, radiation hybrids, and yeast artificial chromosomes (YACs). By using two PCR primers based upon sequences within the open reading frame of the human K_{ATP}-1 gene (flanking nucleotides 191–413; sense, 5′-CGGCAACGTCCAGGAGA-3′, and antisense, 5′-GGACACGAAGCCACTGAG-3′), we developed a highly stringent and species-specific PCR capable of amplifying the predicted 222-bp product from human genomic DNA but not from rat, hamster, or mouse genomic DNA (Fig. 1A). Restriction analysis was used to confirm the presence of an internal *Nco*I site (at nucleotide position 281) and thus the identity of the amplified fragment. Screening of the NIGMS human/rodent somatic cell hybrid mapping panel 2 unambiguously assigned this product to chromosome 11. A single 222-bp product was amplified only from the hybrid containing chromosome 11 and the human genomic DNA control; no product was amplified from any of the other hybrids or rodent genomic DNA control (see Fig. 1A, where for clarity only the positive result from the chromosome 11 hybrid is shown).

The identification of *KCNJ5* on chromosome 11 is of extreme interest. Linkage analysis in several different LQT families has implicated three different chromosomes as carrying the LQT locus (13). The locus on chromosome 3 has recently been identified as mutations in a sodium channel, *SCN5A* (14), and the locus on chromosome 7 as mutations in a putative potassium channel, *HERG* (5); however, the LQT locus on chromosome 11 has yet to be identified. The chromosome 11 LQT locus has been mapped to the short arm, 11p (8), and significant linkage to the *H-ras* proto-oncogene at

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11p15.5 initially implicated this gene (9). However, recent studies have discounted *H-ras* and suggest that the defect is more centromeric (10). Another cardiac disorder, familial hypertrophic cardiomyopathy (FHC), has also been mapped to chromosome 11p13–q13 (4). Although the pathophysiology of this disease is less indicative of a defective ion channel, it still suggested that the precise localization of *KCNJ5* should be determined to assess possible linkage to LQT or FHC.

To localize *KCNJ5* further, a panel of 78 radiation hybrids (RH) each containing different fragments of human chromosome 11 in a hamster background was used to map *KCNJ5* with high resolution. Scoring of the presence or absence of *KCNJ5* by PCR in the 78 RH was performed exactly as previously described (7). The first step in data treatment was two-point analysis with all markers on the existing chromosome 11 RH map. The highest lod score of *KCNJ5* with another marker was 10 with *FLI1* (Friend leukemia virus integration 1), and subsequent inspection of results showed near-identity of scores with this gene. Based on this result, three YACs from the CEPH YAC library previously shown to contain *FLI1* (M.R.J., unpublished observations) were screened for the presence of *KCNJ5* by PCR as before. Only one YAC, 857D07, was found to contain *KCNJ5,* and this same YAC, in addition to containing *FLI1,* also contains the *Ets1* gene and the microsatellite D11S912 (AFM 157xh6). These results are summarized in Fig. 1B and indicate that *KCNJ5* is at 11q24 within approximately 500 kb on the telomeric side of *FLI1.*

The mapping of *KCNJ5* to the distal end of the long arm indicates no probable linkage of this gene to the LQT or FHC loci associated with chromosome 11. However, the localization of this gene close to the *FLI1* gene may be of interest since this locus is disrupted by certain translocations (12). Thus, the high-resolution mapping of *KCNJ5* to 11q24 not only excludes it as a candidate gene for LQT and FHC but also assigns another gene to a region currently sparse in identified transcripts.

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