Mammalian Genome 11, 831–835 (2000).
DOI: 10.1007/s003350010178

Mammalian Genome
Incorporating Mouse Genome

A new spontaneous mouse mutation in the *Kcne1* gene

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Received: 20 April 2000 / Accepted: 2 June 2000

Abstract. A new mouse mutant, punk rocker (allele symbol *Kcne1*^{pkr}), arose spontaneously on a C57BL/10J inbred strain background and is characterized by a distinctive head-tossing, circling, and ataxic phenotype. It is also profoundly and bilaterally deaf. The mutation resides in the *Kcne1* gene on Chromosome (Chr) 16 and has been identified as a single base change within the coding region of the third exon. The C to T nucleotide substitution causes an arginine to be altered to a termination codon at amino acid position 67, and predictably this will result in a significantly truncated protein product. The *Kcne1*^{pkr} mutant represents the first spontaneous mouse model for the human disorder, Jervell and Lange-Nielsen syndrome, associated with mutations in the homologous KCNE1 gene on human Chr 21.

Introduction

The recessive human disorder Jervell and Lange-Nielsen syndrome (JLNS) is one of several disorders characterized by long QT (LQT) cardiac arrhythmias. The clinical symptoms of JLNS include a prolonged QT interval in surface ECG recordings, recurrent syncopes, ventricular tachyarrhythmias, very high mortality, and profound deafness (Jervell and Lange-Nielsen 1957). The JLNS condition is rare, with an incidence of about 0.01% in the human population.

Interestingly, JLNS patients have been identified with homozygous mutations in the KCNQ1 gene on human Chr 11 or the KCNE1 gene on human Chr 21. The autosomal dominant LQT Romano-Ward syndrome is also associated with mutations in the KCNQ1 gene. JLNS is observed only when both alleles of the KCNQ1 or the KCNE1 genes are mutated, indicating that, although the LQT syndrome requires the presence of only one affected allele in the KCNQ1 gene, the mutation must be in the homozygous state to observe the profound deafness characteristic of JLNS. The analysis of the KCNE1 DNA sequence from JLNS patients has revealed a common mutation resulting in an aspartate to asparagine substitution at position 76 of the 130 amino acid protein (Schulze-Bahr et al. 1997; Splawski et al. 1997; Duggal et al. 1998). This amino acid change occurs soon after the single transmembrane spanning domain of the protein within the carboxy terminus. Other mutations giving rise to JLNS include two amino acid substitutions, threonine–leucine to proline–proline substitution at positions 59 and 60 within the hydrophobic domain (Tyson et al. 1997), and a compound heterozygous mutation with the asparagine substitution described above and a threonine to isoleucine change within the amino terminus (Schulze-Bahr et al. 1997). Two studies of JLNS patients also revealed frameshift rearrangements in the KCNQ1 gene (Neyroud et al. 1997; Tyson et al. 1997).

Many in vitro studies of the KCNE1 and KCNQ1 gene products have been undertaken to examine the functional interaction of these two subunits (Barhanin et al. 1996; Sanguinetti et al. 1996; Takumi et al. 1991; Wang and Goldstein 1995). The KCNE1 gene encodes the minimal potassium (minK) protein that has been identified as the beta subunit associating with the alpha channel subunit (KvLQT1) encoded by KCNQ1 gene. These two proteins form the slowly activating, delayed rectifier potassium I_{SK} channel; thus, it is not so surprising that mutations in either gene can give rise to the same disorder. The KvLQT1 protein is the major structural component of the channel and has the typical six transmembrane domains associated with the voltage-gated potassium channel superfamily. The minK subunit cannot form a functional channel, but acts to regulate the slow activation kinetics and amplitude of the I_{SK} channel, and is predicted to prevent the premature activation of further action potentials.

Animal models with mutations in the homologous KCNQ1 and KCNE1 genes provide a further opportunity to study the behavioral interaction between these gene products. Presently, the *Kcne1* gene has been knocked out in the isk-null and minK (−/−) mice, and these knockouts were engineered to delete all expression of the protein (Vetter et al. 1996; Kupershmidt et al. 1999). Interestingly, the isk-null mouse shows symptoms similar to those of the human JLNS, including hearing and inner ear defects in addition to cardiac arrhythmias (Vetter et al. 1996; Drici et al. 1998). More subtle DNA rearrangements may give us further insight into this I_{SK} channel, and we describe here the characterization of a novel spontaneous mouse mutation in the *Kcne1* gene.

Materials and methods

Mice. The *Kcne1*^{pkr} mutation arose as a spontaneous mutation at The Jackson Laboratory and, together with the parental C57BL/10J and control mice (DBA/2J, CAST/Ei and CBA/J), continues to be maintained at The Jackson Laboratory, Bar Harbor, Maine. All animal procedures were approved by the Animal Care and Use Committee (ACUC).

DNA preparation. Mouse genomic DNA was prepared as described (Taylor et al. 1993). DNA (5 μg/ml) was PCR amplified in a M/J Research PTC-100 machine (1 min 94°C, 2 min 55°C (62°C for 3′ end primer), 2 min 72°C, for a total of 25–35 cycles). DNA clones were sequenced with the Amplicycle Sequencing kit (Perkin Elmer).

Inner ear preparations. Inner ears were dissected from 3-month-old affected and control mice, and whole mounts were fixed overnight in 10% formalin. Following graded dehydration through ethanol from 70% to 100%, the ears were cleared for 24 h in methyl salicylate. For sections, ears were fixed in 2% paraformaldehyde, 2% glutaraldehyde in 0.1 M sodium phosphate pH 7.3. Following alcohol dehydration and decalcification, the cochleas were embedded in plastic and dissected across the middle turns.

Auditory brainstem recording (ABR). Four- to 6-week old mice were anesthetized with avertin (tribromoethanol in tertiary amyl hydrate) at a dose of 5 mg/10 g body weight. Fine platinum electrodes were placed

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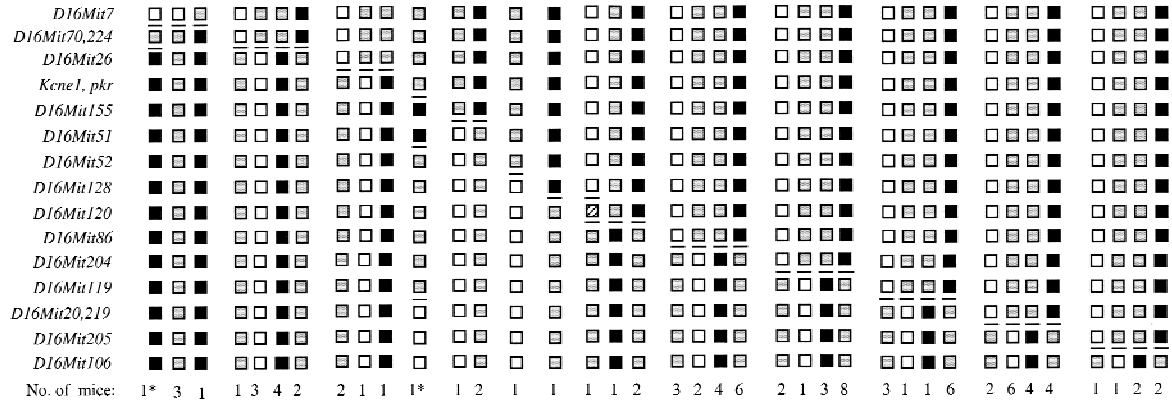

Fig. 1. Haplotype analysis of 90 F_2 mice with crossovers around the *Kcne1* and *pkr* loci. Open boxes represent homozygosity for the C57BL/10J (B10) allele; shaded boxes, heterozygosity for one B10 and one CAST allele; and filled boxes, homozygosity for the CAST allele. The diagonal striped box represents one locus that could not be resolved between the homozygous B10 and the heterozygous haplotype. The asterisk represents mice with two close crossover events.

subdermally at three positions on the head to record brain responses to auditory stimuli. The results were recorded from tests with broad-band click stimuli and 8, 16, and 32 kHz pure tone pips at sound level intensities from 10 to 99 decibels (Zheng et al. 1999).

Results

Mutant mouse phenotype. Two mutant mice were observed in the progeny from a mating pair in the C57BL/10J (B10) inbred strain colony at The Jackson Laboratory. The mutation arose spontaneously, and neither parent was affected. The mutants were originally noticed by their rapid bidirectional circling movements and distinctively jerky head-tossing activity. The mutant mice were unable to swim and did not respond to tapping or clicking sounds, unlike their normal littermates, indicating that they also had hearing loss. Initially the mutant mouse was named punk rocker (*pkr*) because of its jerky movements, reminiscent of the shaker and waltzer mice.

Genetic mapping. Crosses between mutant and inbred strains DBA/2J, CBA/J, and CAST/Ei confirmed that the defect was an autosomal recessive mutation. Initially, the mutation was localized to mouse Chromosome (Chr) 16 by REVEAL PCR from genotyping the DBA/2J F_2 intercross (Kaushik and Stoye 1994). Fine-mapping studies continued with DNA from the F_2 generation of the intersubspecific cross between the mutant and *M. mus castaneus* (CAST/Ei) mice to refine the position of the mutation to the distal region of Chr 16, illustrated by the haplotype analysis in Fig. 1. In total, 400 mice were genotyped, and 90 mice had crossovers in the critical interval. The *Kcne1* gene also maps to the same region as the mutation, *pkr* (Figs. 1 and 2), but there is only one haplotype with two close crossover events indicating that the *Kcne1* is proximal to *D16Mit155*, and future studies of BAC clones covering this interval will verify the actual DNA sequence. The map, including many distal *D16Mit* markers, in Fig. 2 is generally in good agreement with the mapping data previously described by Cabin and Reeves (1999).

Kcne1 gene as a candidate for this mutation. The phenotype of this new spontaneous mutation mirrored the phenotype studies of the targeted *Kcne1* knockout mouse, the "isk-null" mouse, with a deletion of the *Kcne1* gene (Vetter et al. 1996), and, coupled with the genetic mapping of the new mutation to the region of *Kcne1*, the *Kcne1* gene appeared to be an excellent candidate for this

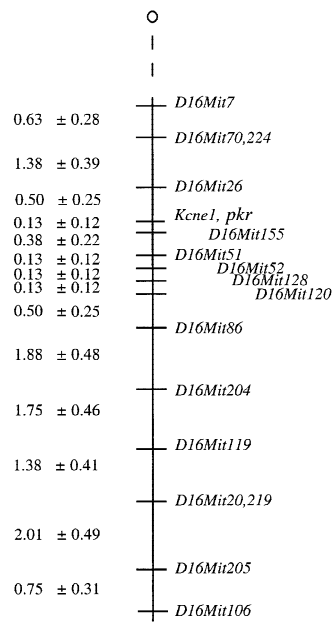

Fig. 2. Distal map of mouse Chr 16 showing the *Kcne1* gene located between *D16Mit26* and *D16Mit155.*

mutation. Northern analysis of the mutant, heterozygous, and B10 control RNAs revealed no quantitative or size differences in the *Kcne1* transcripts from several tissues, including heart, lung, and kidney, and this was confirmed by RT-PCR analysis (results not shown). However, from genomic DNA sequence analysis, it was discovered that the mutant had a C to T base change at position 199 of the open reading frame (Fig. 3A and B), changing the CGA codon for arginine to a stop codon, TGA, at the 67th amino acid (Fig. 3C).

Hearing and inner ear analysis. Auditory brainstem recordings at 5 weeks of age confirmed that the mutants from the CBA/J backcross and the CAST/Ei intercross were profoundly and bilaterally deaf. They were unable to hear even at the upper limit of the test at 99 decibels at 4 weeks of age, while the heterozygous control mice at the same age retained normal hearing levels.

Dissections of the inner ear revealed abnormalities in both the hearing and vestibular apparatus. There were significant changes within the cochlea of the mutant mouse, including the collapse of the Reissner's membrane against the stria vascularis, the tectorial

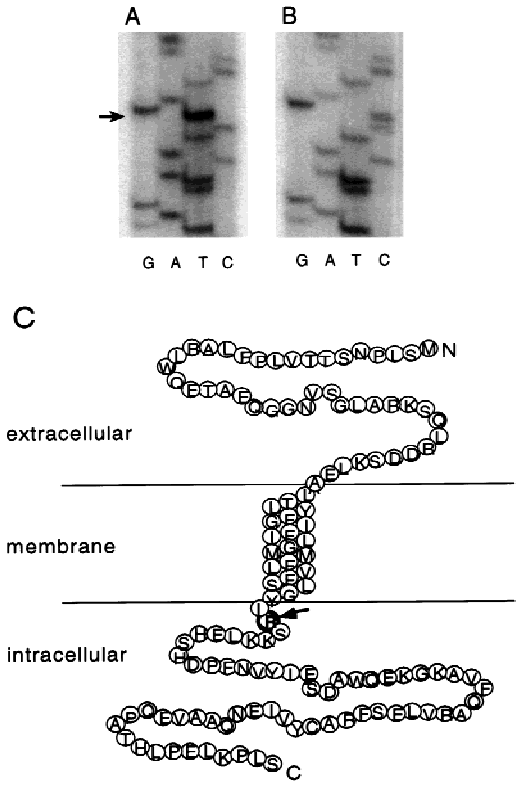

Fig. 3. Autoradiogram of the DNA sequencing gel with an arrow indicating the T in Panel **A,** *Kcne1*^{pkr}/*Kcne1*^{pkr} mutant; and the C residue at the same position in **(B)** control C57BL/10J. Panel **C** illustrates the two-dimensional protein structure of the mouse minK protein. The arrow indicates the arginine residue at position 67 that is changed to a stop codon in the *Kcne1*^{pkr}/*Kcne1*^{pkr} mutant.

membrane, and the Organ of Corti, causing the scala media to become very reduced in size (Fig. 4). The tectorial membrane was displaced and no inner and outer hair cells were observed, including the stereocilia normally found arrayed on the outer hair cells (Figure 4). In the vestibular region, the otoliths of the saccule and utricle also appeared to be displaced in a 3-month cleared-inner ear preparation (Fig. 5). In addition, the *Kcne1*^{pkr}/*Kcne1*^{pkr} mutant had abnormally thin semicircular canals (Fig. 5), although the supporting crus commune structure did not appear to be significantly reduced in size.

Discussion

We report the identification of a new spontaneous mouse mutation in the *Kcne1* gene, initially called punk rocker and now referred to as *Kcne1*^{pkr}. This mutation is an autosomal recessive mutation mapping to the distal region of mouse Chr 16. The mouse *Kcne1* gene has three exons, and the entire open reading frame coding for a protein of 129 amino acids is contained within the final exon (Lesage et al. 1992). The single base change at amino acid position 67, observed in the *Kcne1*^{pkr} mutation, converts an arginine to a stop codon and presumably gives rise to a minK protein that includes the amino terminus and single transmembrane domain but lacks the entire carboxy tail. It is believed that the minK protein is oriented with the carboxy terminus projecting into the cytosol, and as potassium ions flow out of the cell, the carboxy terminus would be expected to play an important role in initiating and directing this process.

The *Kcne1*^{pkr}/*Kcne1*^{pkr} mouse mutant has the profound bilateral deafness found with JLNS and the isk-null mouse, and common defects in the inner ear include the collapse of Reissner's membrane and loss of hair cells within the cochlea (Friedmann et al. 1996; Vetter et al. 1996). The I_{SK} channels, composed of the KCNQ1 and KCNE1 gene products, are located at the apical membrane of the marginal cells of the stria vascularis and maintain a high potassium ion concentration within the endolymph, the fluid filling the scala media of the cochlea (Neyroud et al. 1997). In the isk-null mouse, the cochlea appears to be normal at birth, but the collapse of the Reissner's membrane occurs before P3 (Vetter et al. 1996). This is probably owing to the endolymph draining away from the scale media because of its ionic depletion, although it could also be attributable to a defect in endolymph production following birth. Not only is this fluid normally rich in potassium ions, but potassium also plays an essential role in the stimulation of the hair cells to mediate hearing. The mechanical vibrations passing from the outer and middle chambers to the inner ear are converted by the inner hair cells into electrical impulses. The stimulated inner hair cells become permeable to potassium ions, triggering the synaptic discharges from these cells to the auditory nerve. The collapse of Reissner's membrane and the degeneration of the hair cells in the *Kcne1*^{pkr} mutation would appear to be the primary causes of deafness in this and the isk-null mice.

Interestingly, the collapse of the Reissner's membrane is also observed in the *shaker* (*sy*) and *shaker with no syndactylism* (*sy*^{ns}) mutants that have defects in the Na-K-Cl cotransporter, SLC12A2, located in the basolateral membrane of strial marginal cells (Dixon et al. 1998; Delpire et al. 1998). This gene product is responsible for potassium ion transport into the marginal cells, and the KLQT1 and KCNE1 proteins then allow the potassium ions to flow out through the apical membrane into the endolymph. Both *sy* and *sy*^{ns} have thin semicircular canals, and we observed a similar phenotype in the *Kcne1*^{pkr} mutation. Since the canals are also filled with endolymph, the thinning of the canals is probably due to the reduced volume of endolymph in the inner ear, and the thin canal walls are subsequently preserved by condensation of the supporting mesenchymal cells.

From the in vitro site-directed mutagenesis of the minK protein and the identification of mutations in the KCNE1 gene of humans with JLNS, it is evident that key amino acids in the carboxy terminus close to the transmembrane domain play a critical role in forming a functional human I_{SK} channel, both within the inner ear and in the heart (Schulze-Bahr et al. 1997) (Splawski et al. 1997; Duggal et al. 1998). However a truncated protein retaining only the first 63 amino acids, including most of the transmembrane domain, also appeared to be sufficient to maintain a functional channel in *Xenopus* oocyte preparations (Takumi et al. 1991). We have yet to confirm that any minK protein is actually made in the *Kcne1*^{pkr}/*Kcne1*^{pkr} mutant, although we have evidence that this gene appears to be transcribed normally. Future experiments will address whether the truncated 66 amino acid minK product is indeed present in the *Kcne1*^{pkr}/*Kcne1*^{pkr} mutants and, if so, whether it is localized correctly at the membrane with the KCNQ1 gene product.

The isk-null and the *Kcne1*^{pkr} mutations are both good mouse models for the deafness associated with JLNS. Do they also exhibit the cardiac arrhythmias found in JLNS patients? Interestingly, the KCNE1 gene product localizes to the same regions that support specialized slow conduction in both mouse and human hearts (Kupershmidt et al. 1999), and initially it was also reported that the isk-null mutation showed evidence of cardiac dysfunction (Drici et al. 1998), including longer QT intervals at slow heart rates and, conversely, shorter QT intervals at fast heart rates. However a second study of this knockout mouse failed to find any difference in ventricular action potentials by in vitro analysis (Charpentier et al. 1998). Further analysis of another knockout, the minK (−/−) mouse, also found that minK modulates both I_{SK} and ERG activity, associated with the rapidly activated rectifier potassium channel I_{RK} (Kupershmidt et al. 1999). Both the I_{SK} and I_{RK} currents play key roles in cardiac potassium repolarization before birth in mice, but I_{SK} is down-regulated postnatally (Davies et al. 1996;

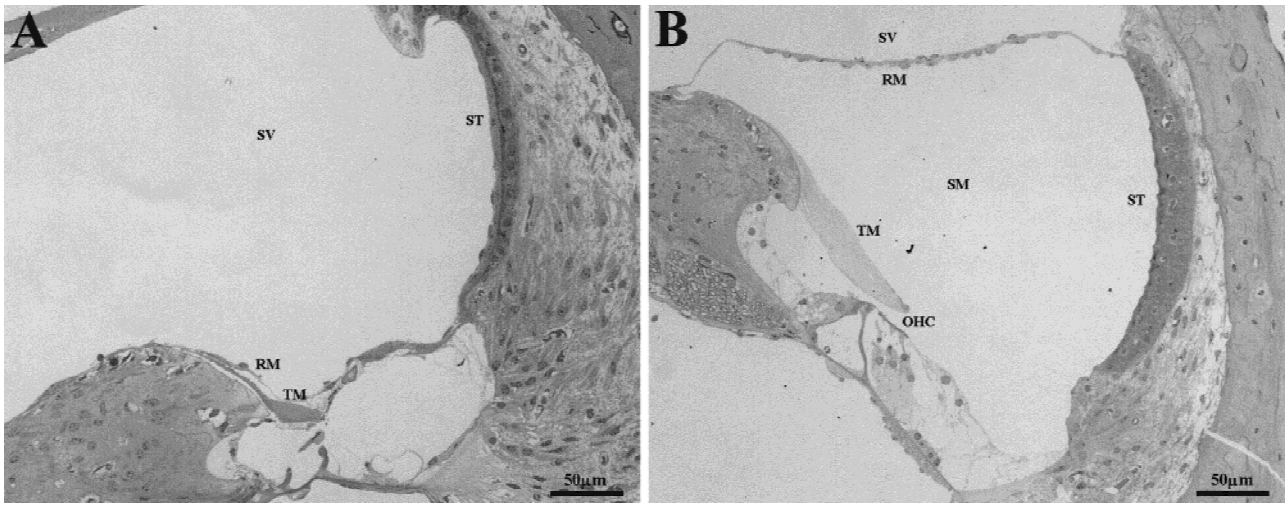

Fig. 4. Cross-section of the cochlea from the inner ear of the **(A)** *Kcne1*^{pkr}/*Kcne1*^{pkr} mutant and **(B)** control heterozygote at 3 months. The Reissner's membrane in the mutant has collapsed against the stria vascularis and tectorial membrane. SV, scale vestibule; ST, stria vascularis; SM, scala media; RM, Reissner's membrane; TM, tectorial membrane; OHC, outer hair cells.

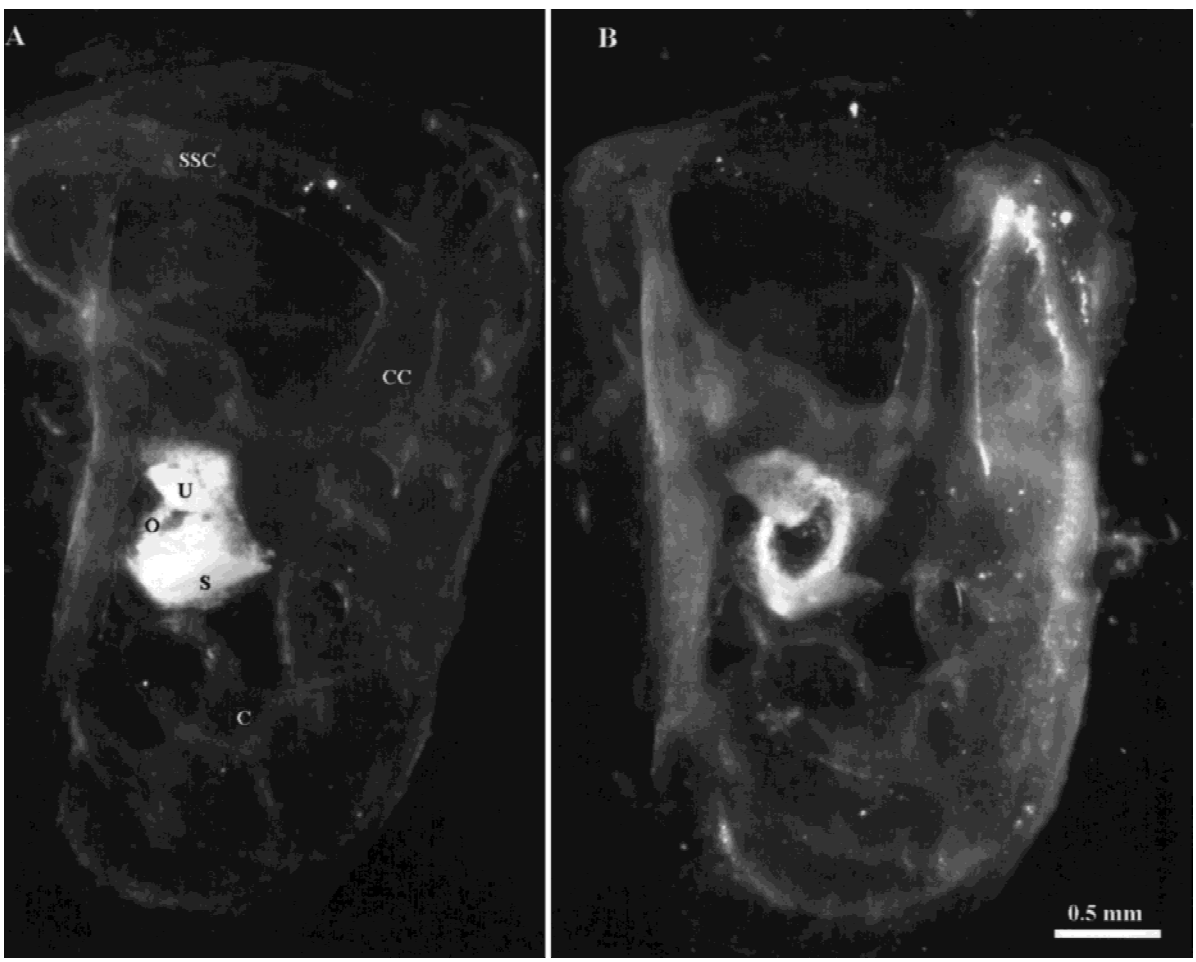

Fig. 5. Whole-mount inner ear preparations comparing the thin superior semicircular canal and the displacement of the white otoliths in the saccule and utricle in the **(A)** control and **(B)** mutant at 3 months. SSC, superior semicircular canal; CC, crus commune; U, utricle; S, saccule; O, oval window; C, cochlea.

Drici et al. 1998; Kupershmidt et al. 1999). Cardiac function has not yet been tested in the *Kcne1*^{pkr}/*Kcne1*^{pkr} mutant, and perhaps these studies will help to clarify the role of the *Kcne1* gene in the adult mouse heart.

Acknowledgments. The authors are grateful for the assistance of two summer students, Sarah Hipkens and Christopher Berry. We also thank the histology and microchemistry services (supported by Cancer core grant CA34196). We greatly appreciate the helpful discussions with Drs. Wayne

Giles, Karen Steel, and Bruce Tempel. The authors were supported by the National Institute of Health grants NS32801 to V.A. Letts; DC62108 to K.R. Johnson and Q.Y. Zheng; and NS31348 and a Klingenstein Fellowship to W.N. Frankel.

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