SHORT COMMUNICATION

SCNN1, an Epithelial Cell Sodium Channel Gene in the Conserved Linkage Group on Mouse Chromosome 6 and Human Chromosome 12

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***SCNN1*, a gene encoding a nonvoltage-gated sodium channel, was detected using a rat colon cDNA probe with homology to *Caenorhabditis elegans* degenerin genes. Human *SCNN1* was assigned to chromosome 12 using the NIGMS hybrid mapping panel 2. Mouse *SCNN1* was mapped to a conserved linkage group on distal chromosome 6. The observed order of mouse genes was centromere–*Raf1*–(2.1 ± 2.1)–*Scnn1, Vwf*–(1.9 ± 1.9)–*Ntf3*, with 0/101 recombinants between *Scnn1* and *Vwf*. No rearrangements of genomic DNA were detected in the linked mouse mutations deaf waddler (*dfw*) and opisthotonus (*opt*).** © 1994 Academic Press, Inc.

cDNAs encoding an epithelial, nonvoltage-gated, amiloride-sensitive sodium channel were recently isolated from rat colon and human lung cDNA libraries (2, 6, 10). Expression of the rat cDNA clone αrENaC in *Xenopus* oocytes resulted in generation of an amiloride-sensitive sodium current (2). The rat cDNA shares significant sequence homology with two degenerin genes from *Caenorhabditis elegans, mec-4* and *deg-1*. Mutations in *mec-4* and *deg-1* result in degeneration of specific neurons. To determine the potential relationship of this cation channel gene to known mouse mutants, we have mapped the mouse homolog.

To expedite localization of the mouse gene, a human/rodent somatic cell hybrid panel was first analyzed. The 2.1-kb *Sac*I fragment of the rat αrENaC cDNA (2) was used to probe genomic DNA from the NIGMS human/rodent somatic cell hybrid mapping panel 2 (Coriell Institute for Medical Research, Camden, NJ). Species-specific *Hin*dIII restriction fragments of 18, 5.5, and 5.1 kb were detected in human genomic DNA (Fig. 1A). These fragments were also detected in cell line GM 10862, which contains human chromosome 12 as the only human chromosome. All of the other hybrids in this panel were negative, indicating that the human locus, designated *SCNN1* (sodium channel, nonvoltage-gated 1; gene symbol reserved), is located on chromosome 12. No additional cross-hybridizing human fragments were detected when the temperature was reduced to 55°C, suggesting that there may be no other closely related genes in the mammalian genome. The assignment to chromosome 12 is consistent with the results of *in situ* hybridization reported while the manuscript was in preparation (10).

Homologs of loci from human chromosome 12 are present in three large linkage groups on mouse chromosomes 6, 10, and 15 (3). To determine whether the mouse *Scnn1* gene is part of the linkage group on chromosome 6, we analyzed two mapping panels that were previously typed for markers on this chromosome (1, 5). Restriction fragment length polymorphisms were identified by hybridization of the αrENaC cDNA to blots containing genomic DNA from strains C57BL/6J, SPRET/Ei, and CAST/Ei that was digested with eight restriction endonucleases. In addition to several invariant bands, we observed a 1.3-kb *Pst*I fragment specific to strain CAST/Ei and a 17-kb *Bgl*II fragment specific to strain SPRET/Ei (data not shown). These fragments were analyzed in DNA from the two chromosome 6 mapping panels. The results were consistent and demonstrated close linkage (0/101 recombinants) between *Scnn1* and *Vwf* (Fig. 1B). The indicated gene order from the CAST F_2 is centromere–*D6Mit8*–(12.5 ± 4.8)–*Raf1, D6Mit11*–(2.1 ± 2.1)–*Scnn1, Vwf, D6Mit12*–(10.4 ± 4.4)–*D6Mit14*. The distance between *Scnn1* and neurotrophin 3 (*Ntf3*), also found on human 12p13, was determined on the BSB backcross with the following results: centromere–*D6Mit8*–(7.5 ± 3.6)–*mi*–(9.4 ± 4.0)–*Scnn1, Vwf, D6Mit12*–(1.9 ± 1.9)–*Ntf3*. The gene order indicated by the two crosses is combined in Fig. 1C.

Scnn1 mapped to the same region of chromosome 6 as two mutants with neurological disorders, deaf waddler (*dfw*) and opisthotonus (*opt*) (4, 8). To determine whether *Scnn1* is rearranged in either mutant, genomic DNA samples were obtained from The Jackson Laboratory (Bar Harbor, ME). *dfw* was compared with the strain of origin, C3H/HeJ, and *opt* was compared

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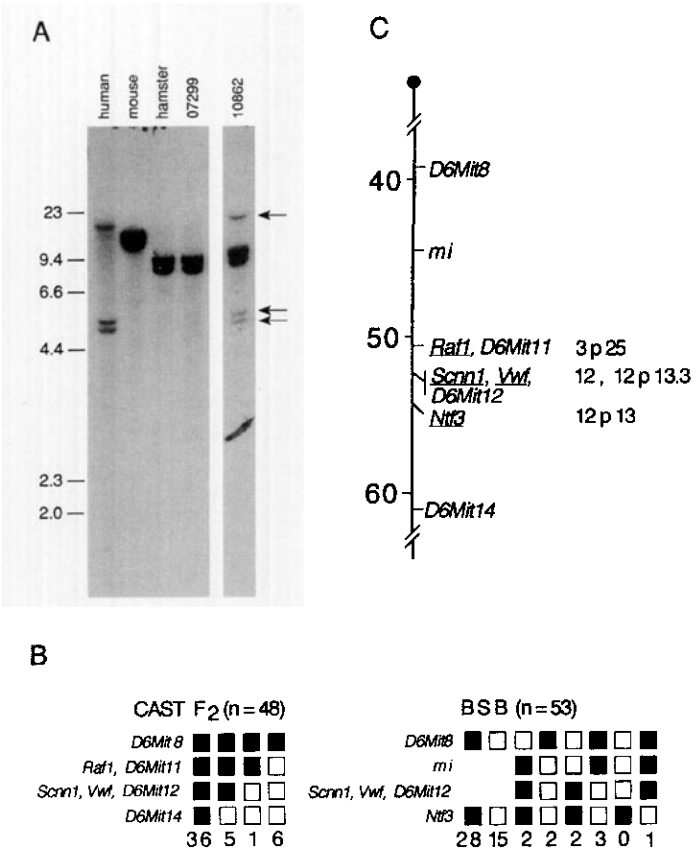

FIG. 1. Mapping of the human and mouse sodium channel genes. (**A**) Human mapping panel. Genomic DNA from NIGMS human/rodent somatic cell hybrid panel 2 was digested with *Hin*dIII, blotted, and hybridized at 65°C with radiolabeled αrENaC cDNA (Canessa *et al.*, 1993) as described previously (1). In addition to the hamster chromosomes, line GM 07299 contains human chromosome 1 only, and line GM 10862 contains human chromosome 12 only. Arrows, human-specific restriction fragments. Molecular weight markers in kilobasepairs are shown to the left. (**B**) Haplotype data for mouse chromosome 6. *Scnn1* genotypes were determined using the strain-specific RFLPs described in the text. RFLPs were analyzed on recombinant animals from two crosses that were previously typed for the other markers on chromosome 6 (1, 5). Each column represents an observed haplotype; the number of mice with each haplotype is indicated at the bottom of each column. CAST F_2, (C57BL/6J-*mnd2* × CAST/Ei)F_2 progeny homozygous for *mnd2* (5); BSB, (C57BL/6J-tg9257 × SPRET/EI)F_1 × C57BL/6J interspecific backcross (1). Filled symbols, C57BL/6J homozygotes; open symbols, heterozygotes. (**C**) Position of *Scnn1* in a conserved linkage group on mouse chromosome 6. The positions of human homologs of the underlined genes are shown to the right (7, 9).

with the background strains C57BL/KsJ and STOCK Mi. Genomic DNA was digested with *Taq*I, *Sac*I, and *Pst*I, and Southern blots were hybridized with the sodium channel cDNA. No rearrangement or deletion was detected in either mutant (data not shown). However, the possibility of a more subtle mutation has not been eliminated.

In addition to *SCNN1, VWF,* and *NTF3,* 20 other genes have been assigned to the conserved linkage group on human chromosome 12 and distal mouse chromosome 6 (3, 7). Human *VWF* encoding von Willebrand factor is the most distal gene mapped to chromosome 12p13.3. The close linkage of *Scnn1* and *Vwf* in the mouse indicates that human *SCNN1* is likely to be located at the distal terminus of the chromosome arm. This is consistent with the recently reported *in situ* hybridization of the human lung cDNA to chromosome 12p13 (10).

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