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Research report

Sequence of the voltage-gated sodium channel β_1-subunit in wild-type and in *quivering* mice

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Abstract

SCN1B, the human gene encoding the β_1-subunit of the voltage-gated sodium channel has previously been cloned and mapped to Chr 19q13.1. The sequence of the homologous mouse gene, *Scn1b*, has now been determined from cDNA. The mouse gene is highly conserved, encoding a predicted protein with 99%, 98% and 96% amino acid identity to the rat, rabbit, and human homologs, respectively. DNA sequence conservation is also striking in the 3′ untranslated region which shows 67% and 98% to human and rat, respectively. Unlike the human and rat homologs, high expression of mRNA from the mouse gene is confined to adult skeletal muscle and brain, and is not observed in heart. As *Scn1b* maps to Chr 7, in close genetic proximity to the *quivering* gene (*qv*), the coding region of *Scn1b* was also cloned from a qv^J/qv^J homozygous mouse and assessed as a candidate for the site of this genetic defect. Comparison of *qv* and wild-type cDNAs showed no changes in the predicted amino acid sequence that could cause the *qv* phenotype. However, three silent polymorphisms in the DNA coding region indicate that *Scn1b* is close to *qv*, and is within a region of genetic identity with DBA/2J, the inbred background on which the qv^J allele arose.

Keywords: Voltage-gated sodium channel; β_1-Subunit; Mouse; *Quivering*; Neuromuscular disease; Chromosome 7

1. Introduction

The voltage-gated sodium channel is comprised of a channel-forming α-subunit that can associate with either no, one, or two additional β-subunits [5]. Although the α-subunit has development- and tissue-specific isoforms encoded by separate loci, there is only a single gene for the β_1-subunit [12,16]. The β_1-subunit is a small (36 kDa) heavily glycosylated protein that has a single putative transmembrane domain [11]. It is expressed at high levels in adult brain and skeletal muscle where it associates with and modulates specific isoforms of the α-subunit [13,14]. The α-subunit expressed alone in *Xenopus* oocytes has an abnormally long inactivation time compared to heteromeric channels in mammalian cells. The inactivation time is more normal when the α-subunit is co-expressed with the β_1-subunit [3,11]. In humans and horses, mutations in the skeletal muscle α-subunit cause myotonias and periodic paralyses, apparently by altering channel inactivation [2,4]. Thus, the β_1-subunit is potential target for mutations that could cause neuromuscular disease.

The human gene encoding the β_1-subunit of the sodium channel, *SCN1B*, maps to Chr 19q13.1, while the mouse homolog, *Scn1b*, lies in a region of synteny conservation on mouse Chr 7 [13,16]. This segment of Chr 7 contains one mutant, *quivering* (*qv*) that displays a central nervous system lesion. *Quivering* is an autosomal recessive neurologic disorder characterized by locomotor instability and pronounced quivering as early as 14 days of age [15,19]. The *qv* mice also suffer from deafness that, unlike most other deafness mutants, is of central origin [7]. In this report we have cloned the mouse homolog of *SCN1B*, determined its cDNA sequence, and assessed this locus as a potential site for the *qv* defect.

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2. Materials and methods

2.1. Mice

All mice and DNA were purchased from the Jackson Laboratory, except for BALB/c DNA, which was isolated from a mouse purchased from Charles River Laboratories. The *quivering* mice used in these experiments have the qv^J allele.

2.2. Scn1b cDNA

Total RNA was isolated from C57BL/6J, C3HeB/FeJ, and qv^J/qv^J brain and skeletal muscle [6]. cDNA was reverse transcribed in reactions that contained 5–10 μg total RNA, 0.5 mM each dNTP, 28 U RNAsin (Promega), 100 U M-MLV Reverse Transcriptase (Gibco), 1 × supplied First Strand Buffer, 0.5 μg oligo$(dT)_{12-18}$ primer (Gibco) in 20 μl volume. Reactions were run at 37°C for 2 h. The cDNA was amplified by PCR in a total volume of 50 μl with 0.5 μl of the reverse transcription reaction, 100 ng each primer, 0.2 mM of each dNTP, 0.5 U *Taq* DNA polymerase (Fisher), and 1 × supplied buffer. The coding region of *Scn1b* was amplified using the primers KM6742 (5′-ATCTCCTGTCGCCGCGCT-3′) and KM6566 (5′-CGTAGGGGCTGGCTCTTCC-3′) (741 bp product). The thermal cycles were as follows: 1 @ 94°C 5 min; 35 @ 60°C 1 min, 72°C 1 min, 94°C 1 min; 1 @ 60°C 2 min, 72°C 5 min. PCR products were cloned into pCRII (Invitrogen) according to standard protocols, and at least three separate clones were sequenced (USB) to eliminate potential PCR artifacts. Similarly, the coding region and a significant portion of the 3′-UTR were amplified by RT-PCR from C57BL/6J brain using primers KM6742 and 3215 (5′-CCCAGGTCCAGCCGGAGGAA-3′) (1170 bp product) and thermal cycles were as follows: 1 @ 94°C 5 min; 35 @ 58°C 1 min, 72°C 1 min, 94°C 1 min; 1 @ 58°C 2 min, 72°C 5 min.

2.3. SSCP analysis

Polymorphism A/G579 is located next to a small (~ 400 bp) intron. The intron and flanking exon sequence was amplified by PCR and directly sequenced using standard procedures. A new primer was designed from intronic sequence, and used to PCR the polymorphism. The primers used were as follows: C/T78, KM7715 (5′-TATCCT-CAGCCTGGGGGG-3′) and KM7716 (5′-GTCCACTC-CGTGAAGGTTTC-3′) (141 bp product); A/G156, KM7717 (5′-CCGATACCGAGGCTGTGTAT-3′) and KM7718 (5′-TGACAAATTCCTCTGTCCCC-3′) (129 bp product); A/G579 + intron, KM7719 (5′-AGAT-TGCTGCTGCTACGGAG-3′) and KM7720 (5′-CG-GATGTAATGGCCAGGTAT-3′) (~ 460 bp product); A/G579, KM7719 and CLSG7 (5′-CCTCCTGTTCTCTG-GGTTTC-3′) (163 bp product). All reactions were as follows: 50 ng genomic DNA, 40 ng each primer, 0.2 mM each dNTP, 0.5 U r*Taq* DNA polymerase (Oncor), 50 mM KCl, 15 mM $MgCl_2$, 20 mM Tris (pH 8.6), 0.1 μg BSA, and 1 μCi [α-^{32}P]dCTP (NEN); total volume was 10 μl; thermal cycles were as follows: 1 @ 94°C 5 min; 25 @ 55°C 1 min, 72°C 1 min, 94°C 1 min; 1 @ 55°C 2 min, 72°C 5 min. Products were diluted 1:10 in 85% formamide; C/T78 and A/G156 were run on an 8% polyacrylamide and 10% glycerol non-denaturing gel at 6 W. A/G579 was run on an 6% polyacrylamide, no glycerol non-denaturing gel at 25 W.

2.4. Northern analysis

All Northern blots were purchased from Clontech. Probes used are 5B1-4, a 1.4 kb human *SCN1B* cDNA, and a 2.0 kb human β-actin cDNA. Probes were labeled by random priming [9]. Blots were pre-hybridized overnight at 42°C in 50% formamide, 5 × Denhardt's Solution, 0.5% SDS, 5 × SSC, and 100 μg/ml herring testes DNA. Blots were hybridized in the same solution overnight at 42°C, then washed in 0.5 × SSC and 0.5% SDS twice at 42°C for 30 min. The human fetal blot was carried along in the same reactions as the rat adult blot, therefore providing a positive control for the technique.

3. Results

We have cloned a 1125 bp cDNA for *Scn1b* by RT-PCR of C57BL/6 mRNA. This clone includes the entire 657 bp coding region and a significant portion (450 bp) of the 3′-untranslated region (3′-UTR) (Fig. 1). An examination of GenBank with the corresponding rat cDNA sequence identified a mouse brain expressed sequence tag (EST) generated by priming with a poly$(T)_n$ primer [1]. The 5′ end of the EST overlapped the 3′ end of our clone by 46 bp, thereby providing a 164 bp extension that reached the polyA tail site, completing the 3′-UTR sequence. DNA sequence homology between species is very high across both the coding region and the 3′-UTR. The mouse coding region displays DNA sequence identity of 97%, 90% and 89% with rat, rabbit and human, respectively, reflecting an even higher degree of protein conservation of 99%, 98% and 96% amino acid identity. Surprisingly, the 3′-UTR is also extremely conserved, suggesting a functional importance that constrains its divergence. The 3′-UTR of the mouse cDNA is 98% and 67% identical to that of rat and human, respectively.

We have also compared tissue-specific expression of *Scn1b* between species, using Northern blot analysis (Fig. 2). In mouse, rat, and human, *Scn1b* is highly expressed in skeletal muscle, and is also expressed in brain. Although it appears that expression is higher in skeletal muscle than in brain in all three species, the amount of RNA loaded in each lane, as reflected by the actin control, indicates that

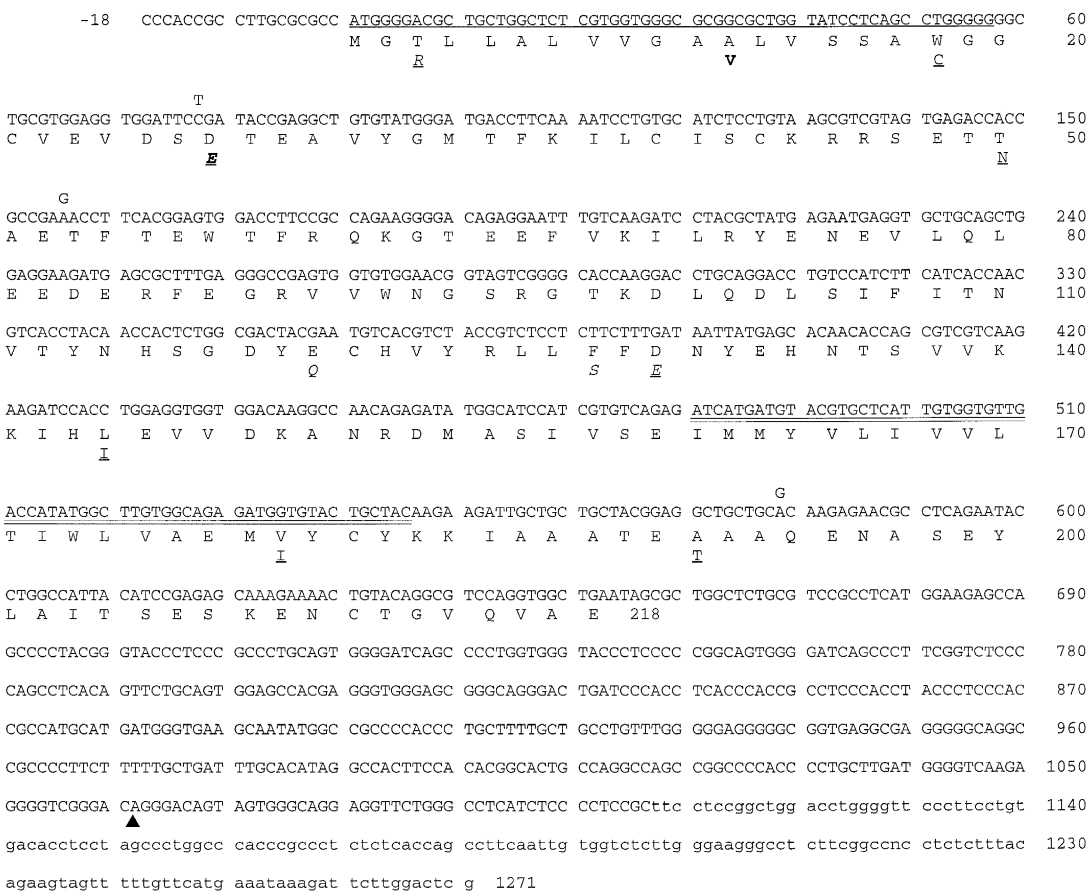

Fig. 1. Mouse *Scn1b* sequences. The sequence of *Scn1b* was amplified by RT-PCR from C57BL/6J brain. DNA sequence in lower case is supplied by the EST. The triangle marks the extent of overlap between the RT-PCR clone and the EST. The predicted amino acid structure is shown underneath. The single line indicates the transmembrane signal sequence; the double line indicates the putative transmembrane domain. Bold letters below the amino acid sequence indicate differences in the rat β_1-subunit; italic letters indicate differences in the rabbit β_1-subunit; underlined letters indicate differences in the human β_1-subunit. Letters above the DNA sequence indicate the positions of the polymorphisms and the alleles associated with qv^J.

expression in the mouse and human brain may be closer to the respective skeletal muscle. *Scn1b* is also expressed in rat and human heart. Indeed, in rat heart, *Scn1b* appears to be expressed at a higher level than in rat brain. Interestingly, there is no detectable *Scn1b* in mouse heart, although previous reports have shown that it is possible to amplify *Scn1b* cDNA from mouse heart RNA by RT-PCR [16]. An examination of the mouse actin control indicates

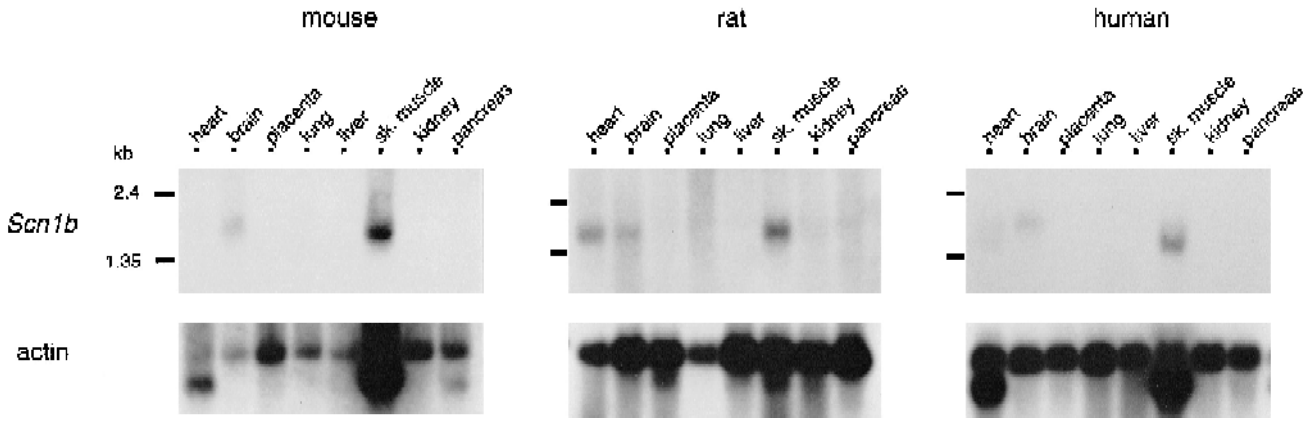

Fig. 2. Northern analysis of tissue-specific expression of *Scn1b* RNA. Species is indicated above the blots. The top row of blots were probed with human *SCN1B* cDNA. The blots were reprobed with actin cDNA for control and are shown immediately below the corresponding *Scn1b* blot.

Table 1
Scn1b polymorphism alleles associated with various mouse strains

Strain	Polymorphisms		
	C/T96	A/G174	A/G597
qv^J/qv^J	T	G	G
C57Bl/6J	C	A	A
C3HeB/FeJ	C	A	A
Sv129/J	C	G	A
BALB/c	C	G	A
vb/*vb*	C	A	A
DBA/2J	T	G	G

that, if anything, more RNA is loaded in the heart lane than in the brain lane. Therefore, if there was significant *Scn1b* expression in heart, this experiment would have been able to detect it. The different *SCN1B* transcript sizes between human tissues has been noted previously, however the coding region does not seem to be altered [12]. Mouse and rat do not have this tissue-specific size difference. *Scn1b* expression was not observed in placenta, lung, liver, kidney, or pancreas in any species. *SCN1B* was not expressed at detectable levels in human fetal tissues (data not shown).

To assess *Scn1b* as a potential site of the *qv* defect, we have also cloned the coding sequence by RT-PCR from qv^J/qv^J mice. Sequence analysis indicated that there are no differences in predicted protein sequence between the wild-type and qv^J associated *Scn1b*, arguing against any causative involvement of the β_1-subunit in generating the *quivering* phenotype. However, we detected three silent third-base polymorphisms in the coding region (Fig. 1), designated C/T78, A/G156, and A/G579, that distinguished the qv^J-associated allele from those of the C57BL/6 and C3HeB/FeJ strain background on which the mutation is carried. We therefore examined various inbred strains of mice for these polymorphisms. The qv^J associated allele for polymorphism A/G174 occurs in several of the inbred strains that we tested (see Table 1), whereas the qv^J associated alleles for polymorphisms C/T78 and A/G579 only occur in qv^J/qv^J and DBA/2J. As the qv^J allele originally arose in the *vibrator* (*vb*) strain which has some DBA/2J contribution [15], these changes are evidently polymorphisms and not mutations.

4. Discussion

Determination of the sequence of the mouse version of the sodium channel β_1-subunit has revealed an extremely highly conserved amino acid sequence, reinforcing the already noted strong homology between rat, rabbit and human homologs [13]. Bases 457–800 of the sequence match the partial genomic sequence recently published by Dib-Hajj and Waxman [8], which indicates the presence of introns after bases 590 and 662. Interestingly, analysis of the 3′-UTR sequence has also confirmed the homology previously noted for the rat and human genes and is consistent with the proposal that the 3′-UTR may be crucial for appropriate regulation of *Scn1b* expression [13]. However, despite these strong similarities, the mouse β_1-subunit mRNA differs in one crucial aspect from its human and rat counterparts. Although all three species express the β_1-subunit at high levels in adult brain and skeletal muscle, the mouse does not exhibit significant *Scn1b* mRNA in heart. The α-subunits of adult brain and adult skeletal muscle are known to associate with the β_1-subunit which is thought to be critical to ensure appropriate kinetics of inactivation and reactivation [18]. The human adult cardiac α-subunit is thought to be allelic with the fetal muscle α-subunit isoform [10], whose expression greatly predates the β_1-subunit which first appears in rat approximately 5 days after birth [18]. Thus, the reason for high β_1-subunit expression in the heart of the human and especially the rat is puzzling, and comparison of these tissues with the mouse heart may prove revealing.

The absence of any amino acid changes in the predicted sequence of the β_1-subunit in qv^J/qv^J mice does not support the notion that a defect in this protein is the cause of this neuromuscular disorder. Moreover, concentric needle electromyographic recordings from hind limb and paraspinous muscles in two qv^J/qv^J mice did not show evidence of myotonia or other forms of enhanced electrical activity that would implicate a specific channel abnormality (Cannon, unpublished results). While it did not identify a defect, the genetic analysis does reinforce the view that *Scn1b* and *qv* are in close genetic proximity. In 1961, the vibrator mutation emerged in a DBA/2J mouse and was rescued by a single cross to C57BL/6J and subsequent brother-sister mating [17]. The qv^J allele arose at F40 in this *vb* strain in 1974 and has been maintained on a C3FeB6-$A/A^{w\text{-}J}$ (C3HeB/FeJ × C57BL/6J-$A^{w\text{-}J}$) hybrid background by the cross (qv^J/qv^J female × C3FeB6-$A/A^{w\text{-}J}$ male) intercross ($+/qv^J$ brother × $+/qv^J$ sister) system of mating (Hope Sweet, personal communication). The mice analyzed here represent the products of 30 backcross generations in which most of the genome would be expected to match either C3HeB/FeJ or C57BL/6J. Since the *Scn1b* alleles for all three polymorphisms are those characteristic of DBA/2J, the *Scn1b* must lie in a portion of chromosome contributed by the *vb* strain and maintained by close proximity to *qv*.

There are no other mutants known to map to this vicinity of Chr 7 that are likely to be due to mutations at *Scn1b*. Similarly, no human disorders have shown to be due to defects in *SCN1B* on Chr 19, although it remains a prime candidate in disorders with altered sodium channel kinetics. However, our studies have provided the reagents necessary for detailed in vitro and in vivo comparison of the biochemical and physiological function of mouse and human β_1-subunits in model systems.

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