View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Neuron, Vol. 31, 537–544, August 30, 2001, Copyright ©2001 by Cell Press

A Novel Gene Causing a Mendelian Audiogenic Mouse Epilepsy

Shana L. Skradski,[1] Anna M. Clark,[2] Hiamio Jiang,[3] H. Steve White,[1,4] Ying-Hui Fu,[5] and Louis J. Ptáček[2,3,6,7,8]
[1] Department of Pharmacology and Toxicology
[2] Howard Hughes Medical Institute
[3] Department of Human Genetics
[4] Anticonvulsant Screening Project
[5] Department of Neurobiology and Anatomy
[6] Department of Neurology
[7] Program in Neuroscience
University of Utah
Salt Lake City, Utah 84112

Summary

***Frings* mice are a model of generalized epilepsy and have seizures in response to loud noises. This phenotype is due to the autosomal recessive inheritance of a single gene on mouse chromosome 13. Here we report the fine genetic and physical mapping of the locus. Sequencing of the region led to identification of a novel gene; mutant mice are homozygous for a single base pair deletion that leads to premature termination of the encoded protein. Interestingly, the mRNA levels of this gene in various tissues are so low that the cDNA has eluded detection by standard library screening approaches. Study of the MASS1 protein will lead to new insights into regulation of neuronal excitability and a new pathway through which dysfunction can lead to epilepsy.**

Introduction

The *Frings* mouse represents one of many strains of mice and rats that are sensitive to audiogenic seizures (AGS) and arose as a spontaneous mutation on the Swiss Albino background (Frings et al., 1951). AGS-susceptible rodents represent models of generalized reflex epilepsy and include the well-studied DBA/2 mouse and GEPR-9 rat. The *Frings* mouse seizure phenotype is similar to other described AGS and is characterized by wild running, loss of righting reflex, tonic flexion, and tonic extension in response to high intensity sound stimulation (Schreiber et al., 1980). The *Frings* mouse seizure phenotype is due to the autosomal recessive transmission of a single gene, *mass1* (*m*onogenic *a*udiogenic *s*eizure-*s*usceptible), located on chromosome 13 (Skradski et al., 1998). Thus, while audiogenic seizures have been observed in polygenic rodent models, such as the DBA/2 mouse (Collins, 1970; Seyfried and Glaser, 1981; Neumann and Collins, 1991) and GEPR-9 rat (Ribak et al., 1988), the monogenic Frings model provided a unique opportunity for cloning and characterization of an AGS gene.

Human epilepsies can be separated into symptomatic and nonsymptomatic forms. Symptomatic epilepsy is a seizure disorder related to a known cause such as metabolic disease, infection, ischemia, brain malformations, or brain tumors. In these cases, seizures presumably occur because of a very abnormal focus (or foci) in the brain. Genetic models of symptomatic epilepsy include the weaver mouse (*wv*), where a mutation of the G protein-gated inwardly rectifying potassium channel GIRK2 results in neurodevelopmental abnormalities and seizures (Signorini et al., 1997). Fragile X-associated protein knockout mice have a neurodevelopmental syndrome with lowered thresholds to AGS (Musumeci et al., 2000). Interestingly, AGS can also be induced in seizure-resistant mice such as C57BL/6 by repetitive sound stimulation (Henry, 1967), suggesting that seizure susceptibility is influenced not only by multiple genetic, but also environmental factors.

Nonsymptomatic epilepsies are defined when no structural or metabolic lesions are recognized and other neurological abnormalities are not present. This latter group of patients is more likely to have primary neuronal hyperexcitability. Molecular characterization of electrical hyperexcitability in human muscle diseases led to the hypothesis that such disorders might be the result of mutations in neuronal ion channels, the primary determinants of neuronal membrane excitability (Ptáček et al., 1991).

All nonsymptomatic human epilepsy syndromes and genetic mouse seizure models that have been characterized at a molecular level are caused by mutations in ion channels (Ptáček, 1999; Jen and Ptáček, 2000; Noebels, 2000). Some patients with febrile seizures have been recognized to have mutations in sodium channel α and β subunits (Wallace et al., 1998; Escayg et al., 2000a) while some patients with epilepsy and episodic ataxia were shown to have calcium channel mutations (Escayg et al., 2000b). The voltage-gated potassium channel genes *KCNQ2* and *KCNQ3*, when mutated, result in benign familial neonatal convulsions (Biervert et al., 1998; Charlier et al., 1998; Singh et al., 1998). Ligand-gated channels can also result in epilepsy as demonstrated by mutations in the α4 subunit of the neuronal nicotinic acetylcholine receptor that result in autosomal dominant nocturnal frontal lobe epilepsy (Steinlein et al., 1995). In mice, the α, β, and γ subunits of the voltage-sensitive calcium channel have been associated with the tottering (*tg*), lethargic (*lh*), and stargazer (*stg*) models of absence seizures (Fletcher et al., 1996; Burgess et al., 1997; Letts et al., 1998). Finally, AGS susceptibility has been characterized in a mouse knockout model of the 5-HT_{2C} receptor (Tecott et al., 1995; Brennan et al., 1997); homozygous mice have AGS and altered feeding behavior.

Here we report the fine mapping of the *mass1* locus and the identification of a novel gene that is mutated in these epileptic mice. The *mass1* message is a very rare transcript and encodes a protein that shares no homology with any known ion channels or other proteins. Two motifs in the predicted protein hint at possible functions of MASS1. Therefore, the *Frings* mice are an important naturally occurring monogenic model of a nonsymptomatic epilepsy and will provide information on a novel

[8] Correspondenc: ptacek@genetics.utah.edu

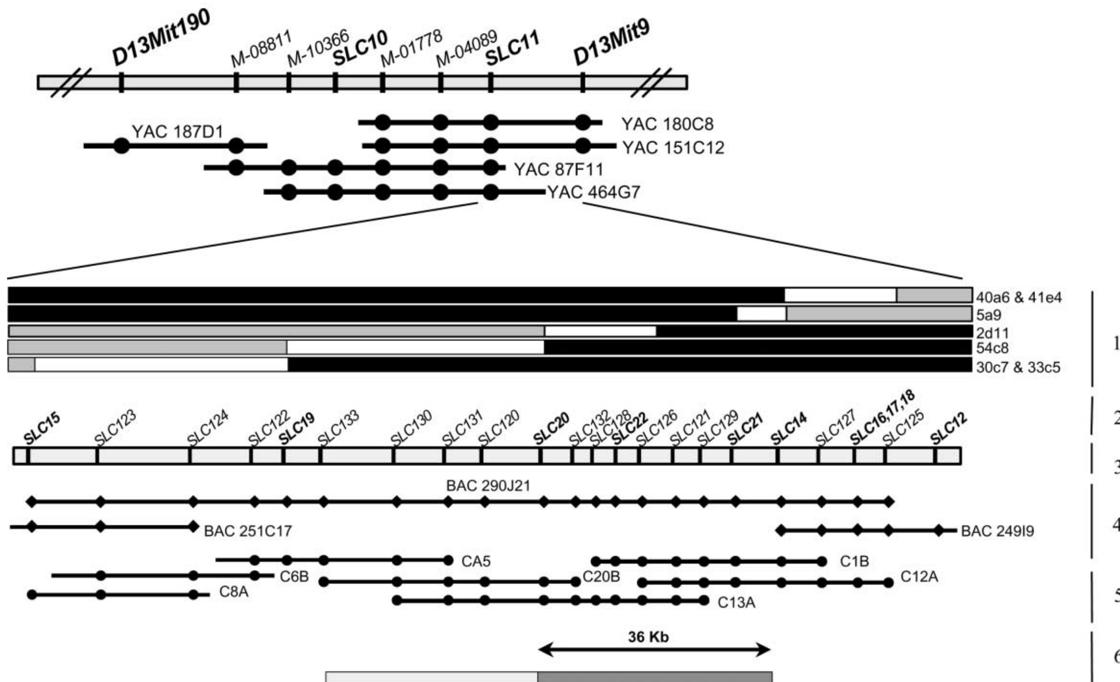

Figure 1. Detailed Summary of Genetic Mapping of *mass1*

The map at the top represents the large-scale physical map of the *mass1* interval spanned by YACs. *SLC10* and *SLC11* are novel SSLP markers, the remaining labels are STS markers. The critical region defined by markers SLC11 and D13Mit9 is expanded to show fine-scale physical mapping defined by BACs (4) and cosmids (5). *SLC*- numbers between 10 and 100 (in bold) are novel SSLP markers, and *SLC*- numbers 100 to 200 are STS markers (2) on chromosome 13 (3). The bars (1) represent the genotypes of the nearest recombinant mice. The gray bars represent regions where the mice are recombinant, black filled bars are regions where the mice are nonrecombinant, and white filled bars are regions where the markers were not informative. Names of recombinant mice are shown at the right of the bars. The final *mass1* interval was spanned by cosmids C13A and C1B, and the complete genomic sequence was generated between the markers *SLC20* and *SLC14*. The location of the *mass1* gene is shown by the bar at the bottom (6). The darker area represents completely sequenced genomic DNA and the lighter has been partially sequenced.

mechanism of seizure susceptibility as well as CNS excitability in general.

Results

Fine Mapping and Physical Mapping

Approximately 1200 (*Frings* X C57BL/6J)F1 intercross mice were genotyped with microsatellite markers spanning the *mass1* interval (Skradski et al., 1998). Genotyping of the border-defining recombinant mice with the two additional markers, *D13Mit9* and *D13Mit190* (identified from the Chromosome 13 Committee map), narrowed the critical interval (Figure 1). Of the 1200 F2 mice, three were recombinant at *D13Mit9* and ten mice were recombinant at *D13Mit190*. No other known simple sequence length polymorphisms (SSLPs) markers were mapped within this interval.

This distance between the markers *D13Mit9* and *D13Mit190* was covered by three overlapping YACs, 151C12, 87F11, and 187D1 (Figure 1). Four known sequence-tagged sites (STSs) were used to identify BACs from the library (Figure 1). Using small-insert pUC19 subclone libraries of the BACs, we screened for new single nucleotide polymorphisms (SNPs) by sequencing. Two polymorphic markers, *SLC10* and *SLC11*, were identified and further narrowed the distal border and defined the *mass1* interval to the distance spanned by a single YAC, 151C12, between markers *SLC11* and *D13Mit9* (Figure 1).

A physical map of the region was constructed by using end sequences of BAC clones to develop new STSs to rescreen the library for overlapping BACs. Simultaneous with the physical mapping, identification of SSLPs from the new BACs continued to narrow the interval. Seven overlapping BACs were required to cover the distance between *SLC11* and *D13Mit9*. SSLPs from each end of the insert of BAC 290J21, *SLC14* and *SLC15*, were recombinant and localized the *mass1* gene to this small region which is less than 150 kb based on the insert size of the BAC (Figure 1).

This BAC insert was subcloned into both a cosmid vector and pUC19. Sequences from randomly selected pUC19 clones were used to develop STSs across the BAC, and these markers were then used to align cosmids into a complete contiguous map of BAC 290J21 (Figure 1). SSLP screening of the pUC19 library detected five new repeat markers within BAC 290J21 (*SLC16–20*). Two of these, *SLC19* and *SLC20,* were mapped within the *mass1* interval. Analysis of recombinants at these markers showed a recombination with *SLC20* that re-

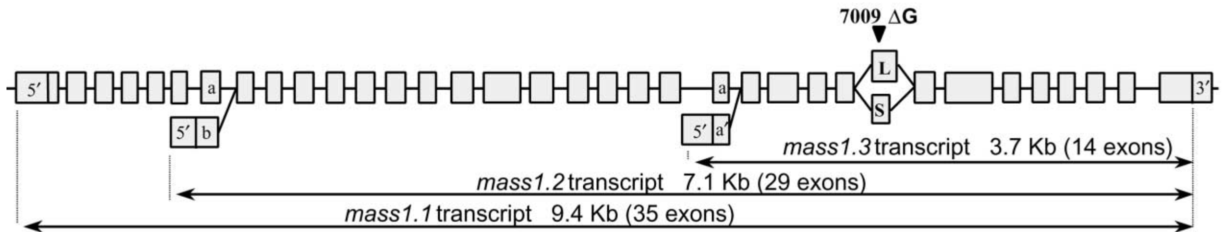

Figure 2. The Diagram of the *mass1* Gene which Spans 35 Exons

Three putative transcripts and the exons that are included in each transcript are also shown. The short transcript, *mass1.3*, has putative 5′ untranslated sequence leading into exon 22 (a′). The medium transcript, *mass1.2*, has a putative 5′ untranslated sequence leading into exon 7b. Exons 7a and 7b represent two alternate exons that have been identified in mouse brain cDNA. The longest transcript, *mass1.1* (35 exons), has only been shown to contain exon 7a. Long and short splice variants were identified in exon 27 (L and S). The 27S variant removes 83 bp and changes the reading frame. The 7009G deletion is located in exon 27.

fined the interval to two overlapping cosmids, C1B and C13A, between the markers *SLC14* and *SLC20* each with a single recombinant mouse (5a9 and 2d11; Figure 1).

Candidate Gene Identification

To directly identify candidate genes from the two cosmids, C1B and C13A, we screened mouse brain cDNA libraries by hybridization using cosmid DNA as probe, but were unsuccessful at identifying any candidate cDNAs from the region. We also began sequencing cosmids C1B and C20B and produced complete genomic sequence from marker *SLC14* to *SLC20*. The complete nonrecombinant *mass1* interval is approximately 36 kb. Analysis of the sequence by Genefinder predicted one multiple exon gene spanning the *mass1* interval oriented from the distal to proximal end. Reverse transcription-PCR (RT-PCR) with primers spanning putative introns amplified products of the appropriate sizes from *Frings* and C57BL/6J brain RNA. Sequence analysis of these bands confirmed that they matched the genomic sequence within the exons and identified the first intron-exon boundries.

Cloning and Analysis of *mass1* cDNA

RT-PCR experiments produced 1 kb of open reading frame that could be amplified from mouse brain RNA. Subsequently, rapid amplification of cDNA ends (RACE) defined the 3′ end of the gene which contained 330 base pairs (bp) of untranslated sequence from the first stop codon to the poly(A) tail. Multiple 5′ RACE reactions produced the complete cDNA sequence of *mass1* and identified three putative alternate transcripts each containing a unique 5′ untranslated sequence. When the cDNA sequence was aligned with 36 kb of complete genomic sequence from cosmid C1B, 15 exons were noted to correspond to the 3′ end of the cDNA sequence; primers were designed from the remaining 5′ cDNA sequence and used to sequence cosmid C20B. Analysis of this genomic sequence revealed an additional 20 exons (Figure 2).

The longest transcript is approximately 9.4 kb, the second 7.1 kb, and the shortest 3.7 kb. Northern blot analyses of mouse RNA failed to produce conclusive data to confirm these transcript sizes, and further suggested that the transcript levels were very low. However, several autoradiograms with very long exposure times (3–4 weeks) suggested that the 9.4 and 7.1 kb transcripts are expressed in mouse brain (data not shown). In situ hybridizations using a 1 kb product of the cDNA (exons 24–28) to probe mouse brain did not reveal any signal above background (data not shown).

Each putative transcript contains a unique 5′ untranslated region leading into the rest of the gene sequence. All three transcripts contain a possible splice variant in exon 27 where 83 bp of sequence are either included (27L) or removed (27S) from the transcript (Figure 2).

Analysis of the expression of *mass1* in mouse tissues by RT-PCR only yielded a product from brain, lung, and kidney RNAs (Figure 3a). Further analysis of the adult mouse brain showed *mass1* by RT-PCR of RNA from all regions tested (Figure 3b) and from mouse RNA isolated from cultured astrocytes or aspirated from cultured cortical neurons (data not shown).

Mapping of the *hMass1* Gene

A human genomic clone (RP11-535M5) containing the human homolog of the *mass1* gene was identified by screening a BAC library by PCR with primers from the mouse *mass1* gene under lower stringency. This clone was used in flourescent in situ hybridization experiments and mapped to human chromosome 5q14.

Identification of a *mass1* Mutation in DNA from *Frings* Mice

Using single strand conformation polymorphism analysis (SSCP, see Experimental Procedures), 17 SNPs were identified between *Frings* and C57BL/6J mice within the nonrecombinant coding region. Of these, six alter protein sequence and could, theoretically, be the genetic basis of *Frings* audiogenic seizure susceptibility. To determine which of the ORF-changing SNPs (G5941C, A6847G, 7009ΔG, G7450A, A8176G, and G8326A) corresponds to the mass1 mutation, twelve different strains of Swiss-Albino mice were tested. The six regions harboring the polymorphisms were amplified by PCR and sequenced. Ten strains resembled C57BL/6J at all six positions, whereas two, BUB/BnJ and SWR/Bm, were similar to Frings. SWR/Bm had four (G5941C, A6847G, G7450A, and A8176G) changes, but did not carry the G7009 deletion. BUB/BnJ, on the other hand, had all six Frings polymorphisms, including the deletion. The G7009 deletion results in a frame shift of the open read-

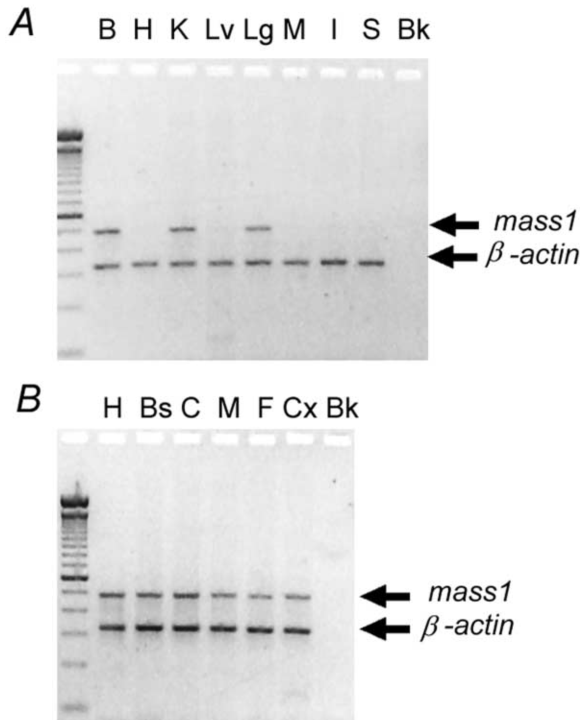

Figure 3. Expression Analysis of the *mass1* Gene by RT-PCR in Different Tissue RNA Samples Using Primers from Exons 22 and 23

(A) Analysis of *mass1* in multiple tissue RNA samples of a CF1 mouse shows that expression is primarily in the brain (B), kidney (K), and lung (L), and not in the other tissues: heart (H), liver (Lv), skeletal muscle (M), small intestine (I), or spleen (S). Bk represents a blank. (B) Further analysis of brain RNA detected *mass1* expression in all regions tested: hippocampus (H), brain stem (Bs), cerebellum (C), midbrain (M), forebrain (F), and cortex (Cx). The *mass1*-specific primers span exons 22–23, and the expected product size was 487 bp. The β-actin primers also spanned two exons and the expected product size was 327 bp. The DNA ladder in the first lane is in 100 bp increments.

ing frame changing the valine to a stop codon; this change is expected to produce a truncated MASS1 protein in *Frings* mice. The deletion is located in exon 27 before the long and short splice variants. The only other allele unique to the Frings and BUB/BnJ strains (G8326A) is downstream of the deletion and would not be translated because of premature truncation of the protein.

This finding demonstrated that the BUB/BnJ strain is closely related to the Frings strain and that it could also be susceptible to audiogenic seizures. To test this, a small colony of BUB/BnJ mice was established and subjected to qualitative seizure phenotyping (Skradski et al., 1998). A total of 29 animals were tested. There were five time points starting at postnatal day 19. At 19 days of age, three out of three mice exhibited full tonic extension seizures resulting in one death. Nine out of thirteen animals seized when tested at ages 22–24 days and two of these died. Two additional animals exhibited a partial phenotype (wild running and loss of righting reflex without tonic-clonic seizure) and the remaining two did not seize. There were no seizures in mice beyond day 25 days of age. The results correlate with a progressive hearing loss in this strain of mice, which exhibit hearing impairment at 3–4 weeks (K. Johnson, personal communication) and become completely deaf by the age of two to three months (Zheng et al., 1999; Johnson et al., 2000). In contrast, SWR/Bm mice did not exhibit audiogenic seizure susceptibility. These data demonstrate that the G7009 deletion is the mutation causing the Frings phenotype.

Analysis of the MASS1 Protein Sequence

The *mass1* gene produces three putative transcripts. The long transcript (*mass1.1*) contains 9327 nucleotides (nt) and is expected to produce an approximately 337 kDa protein. The medium transcript (*mass1.2*) contains 6714 nt and the predicted protein size is 244 kDa. The short transcript (*mass1.3*) is 2865 nt and the predicted protein size is 103 kDa. These transcripts and isoforms are based on incorporation of the longer splice form of exon 27 (27L). Further putative variants are possible as a result of the 27S alternate splicing event. Using the 27S exon theoretically shortens all the transcripts by 83 nt and each of the isoforms by 645 amino acids (69 kDa). The conceptual translation of the amino acid sequence for the *mass1.1*(27L) transcript is shown in Figure 4. The MASS1 protein is strongly acidic and has a −192 charge at pH 7.0. The hydropathy plot indicated numerous hydrophobic domains that are candidates for transmembrane segments.

Database searches using the *mass1.1* sequence identified no ESTs that were identical and no homologous genes. However, a small repetitive motif from MASS1 shared homology with numerous Na^+/Ca^{2+} exchangers. This homology was to the β1 and β2 repeats in the third cytosolic loop of the exchanger that contains the Ca^{2+} regulatory binding domain (Nicoll et al., 1996). This motif occurs 18 times within the sequence. Alignment of these sequences shows several highly conserved amino acids within this motif (Figure 5), including a Proline-Glutamate-X-X-Glutamate (PEXXE) sequence preceded by one to three acidic residues (D or E). The proline and first glutamate are completely conserved in all 18 related motifs, and the second glutamate is conserved in 16 of the motifs. The PEXXE motif occurs twice more within the MASS1 sequence; however, these repeats (repeats 19 and 20) have a lower degree of identity (Figure 4).

Three aspartic acid residues (DDD) are found in the Na^+/Ca^{2+} exchanger β1 segment and in the segment of the very large G protein-coupled receptor-1 directly preceding the PEXXE motif (Nikkila et al., 2000). In MASS1, this DDD motif is identical only in repeat number 3; conservative substitutions of the DDD motif occur in repeats 1, 9, and 18. The 18 repeats are distributed across the MASS1 protein and repeats 14 to 18 would be missing from the truncated MASS1 protein (Figure 4).

Analysis of the MASS1 sequence by Pattern Match identified a multicopper oxidase (MCOI) consensus site in the carboxy-terminal region of MASS1 in a region encoded by exon 29 (Figure 4) that would be eliminated by the *Frings* 7009ΔG mutation. Other motifs found within MASS1 include three tyrosine kinase phosphorylation motifs, two cAMP/cGMP-dependent phosphorylation motifs, and one glycosaminoglycan attachment motif. Further analysis of the MASS1 protein will be required

1	MVTVTFDVSGGPNPPEEDLNPVR GNITFPPGRATVIYNVTVLDDEVPENDELFLIQLR SVEGGAEINASRSSVEIIVKKNDSPVNFMQSVYVVPEDDHVL
101	TIPVLRGKDSDGNLIGSDETQVSIRYKVMTWDSTAHAQQNVDFIDLQPD TTLVFPPFVHESHLKFQIIDDLIPEIAESFHIMLL KNTLQGDAVLMGPSTV
201	QVTIKPNDKPYGVLSFNSILFERPVIIDEDTASSSRFEE IAVVRNGGTHGNVSVSWVLTRNSSDPSPVTADITPAS GTLQFAQGQMLAPISLVVFDDDLP
301	EEAEAYLLTIL PHTIQGGAEVSEPAQLLFYIQDSDNVYGEIAFFPGESQKIESSPSERSLSLSLARRGGSKGDVRVIYSALYIPAGAMDPLRAKDGILNT
401	SRRSSLLFPEQNQQVSIKLPIRNDAFLQNGAHFLVQLEAVVLVNIFPPIPPVSPRFGEIRNISLLVTPAIANGEIGFLSNLPI ILHEPKDSSAEVVSIPL
501	HRDGTDGQATVYWSLRPSGFNSKAVTLDDAGPFN GSVVFLSGQNETSINITVKGDDIPELNETVTLSLD RVSVDSDVLKSGYTSRDLIILENDDPGGIFE
601	FSYDSRGPYVIKEGDAVELRITRSRGSLVKQFLRFHVEPRESNEFYGNM GVLEFTPGEREVVITLLTRLDGTPELDEHFWAILS SHGERESKLGRATLVN
701	ITILKNDYPHGIIEFVSDGLS ASIKESKGEDIYHAVYGVIRTRGNFGAVNVSWMVSPDFTQDVFPVQ GTVCFGDQEFFKNITVYSLVDEIPEEMEEFTII
801	LL NATGGAQTGIRTTASLRILRNDDPVYFAEPCVLRVQEGETANFTVLRNGSVDGACTVQYATVDGKASGEEGDFAPVEKG ETLVFEVGSREQSISVHVK
901	DDGIPETDEPFYIVLF NSTGDTVVYEYGVATVIIEANDDPNGVFSLEPIDKAVEEGKTNAFWILRHRGHFGNVSVAWQLFQNASLQPGQEFYETS GTVNF
1001	TDGEETKPVILRAFPDRIPEFNEFYILRLV NISGPGGQLAETNFQVTVMIPFNDDPFGIFILDPECLEREVAEDVLSEDDMSYITSFTILRQQGVFGDVR
1101	VGWKVLSREFTAGLPPMIDFILLGSFPSTVPLQPHMRRHHSGTDVLYFSGLEGAFGTVDPKYQPFRNNTIANFTFSAWVMPNANTNGFLIAKDDSHGSIY
1201	YGVKIQTNETHVTLSLHYKTFGSNVTYIAKSTVMKYLEEGVWLHVLIILDDGIIEFYLDGKAMPRGIKSLKGEAITDGPGILRIGAGMDGGARFTGWMQD
1301	VRTYERKLTPEEIYELHAVPARTDLHPISEYLEFRQGESNKSFIVAARDDSEEEGEELFLLKLVSVDGGAQISKENTTARLRIQKSDNANGLFGFTGACI
1401	PEMTEEGSTVSC VVERTRGALGYVHVFYTISQIESEGINYLVDDFANAS GTITFLPWQRSEVLNLYVLDEDMPELNEYFRVTLV SAVPGDGKLGSTPISG
1501	ASIDPEKETTGITVKASDHPYGLMQFSTGLPPQPEDSMSLPASSVPHITVQEEDGEIRLLVIRAQGLLGRVTVGFRTVSLTAFSPEDYQSTA GTLEFQSG
1601	ERYKYIFVNITDNSIPELEKSFKVELL NLVGGVSDLFRVDGSGSGEADTDFFLPPVLPHASLGVASQILVTIAASDHAHGVFEFSPESLFVSGTEPEDGY
1701	STVVLNVTRTRGALSAVTLQWKVDSDLDGDLAITS GNITFETGQRIASITVEILPDEEPELDKALTVSIL NVSSGSMGVLTNATLTILASDDPYGVFIFP
1801	NKTRPLSVEEATQNVTLSIIRLKGLMGEVAVSYATIDDMEKPPYFPPNLARATQGGDYISAS GLALFRANQTEATITISILDDAEPERSESVFIELF NSS
1901	LVDKVQNRPIPHSPRLGPKVETVAHLVIV ANDDAFGTVQLSATSVHVAENHVGPIINVTRTGGTFADVSVKFKAVPITAAGEDYSIAS SDVVLLEGETT
2001	KAVPIYIINDIYPELEETFLVQLL NETTGGATLGPLREAVITIEASDDPYGLFGFQNTKFIVEEPEFNSVR *V SAPIIRNSGTLGNVTVQWVAIINGQFAT
2101	GDLRVVS GNVTFAPGETIQTLLLEVLADDVPEIEEVVQVQLA AASGGGTIGLDRVANIVIPANDNPYGSVAFVQSVFRVQEPLERSSYANITVRRSGGHF
2201	GRLLLCYGTSDIDVVARAVEEGEDVLSYYESPTQGVPDPLWRTWVNVSAVEETQYTCATLCLKERACSAFSVVSGAEGPRCFWMTSWVSGTVNSSDFQTY
2301	KKNMTRVASLFSGQAVAGSDYEPVTR QWAVILEGDEFANLTVSVLPDDAPEMDESFLISLL EVHLMNISDSFKNQPTIGHPNTSAVVIGLNGDAF **GVFII**
2401	**YSVSPNTSEDGLCVEV** QEQPQTSVELVIYRTGGSLGQVMVEWRVVGGTATEGLDFMGAG DILTFAEGETKKMAILTILDDSEPEDNESILVRLV ATEGGS
2501	RILPSSDTVTVNILANDNVAGIVSFQTASRSVIGHEGEMLQFHVVRTPPGRGNVTVNWKVVGQNLEVNFANFT GQLFFSEGTLNKTIFVHLLDDNIPEEK
2601	EVYQVVLY DVKTQGVSPAGVALLDAQGYAAVLTVEASDEPHGVLNFALSSRFVVLQEANVTIQLFVNREFGSLGAINVTYATVPGIVSLKNNTEGNLAEP
2701	ESDFIPVV GSLVLEEGETTAAISITVLEDDIPELKEYFLVNLT HVDLIMAPLTSSPPRLGMGLSFMNLLTNCESQRTSLF

Figure 4. The Conceptual Amino Acid Translation of the *mass1.1* Transcript

The 18 MASS1 repetitive motifs are boxed with a solid line. The two less conserved possible repeats encompass amino acids 1041–1075 and 1378–1412. The putative MCO I domain is underlined. The valine → stop mutation in the *Frings* MASS1 protein is located at amino acid number 2072 marked with the "*".

to determine if any of these consensus sites are functional.

Discussion

The *Frings* mouse is a naturally occurring single gene model of audiogenic generalized seizures without associated neurological or behavioral phenotypes caused by mutations in the novel *mass1* gene. The low abundance of this message could be due to low expression of the *mass1* mRNA, or to the message being unstable and quickly degraded.

Convincing data suggest that *mass1* is the gene causing AGS in the *Frings* mice. This is the only gene that we have found in the small nonrecombinant *mass1* interval. We can amplify the cDNA from both mouse and human Marathon cDNA libraries (Clontech, Palo Alto, CA), and for genomic structure of *hMass1* thus far obtained, intron-exon boundaries are conserved (not shown). The alternate transcript of mouse *mass1* exon 27 is also found in *hMass1*. The *mass1* transcripts contain long open reading frames which are disrupted by a single base-pair deletion in the *Frings* mouse.

The deletion is located in the nonrecombinant coding region containing exons 21 through 35 and leads to an immediate stop codon. Polymorphisms detected in Frings were found only in two out of twelve mouse strains tested, SWR/Bm (four out of six, not including the deletion) and BUB/BnJ (all six changes). The fact that BUB/BnJ carried the 7009G mutation suggested that this strain, like *Frings*, was prone to audiogenic seizures. Seizure tests of the BUB/BnJ mice confirmed that there was a strong correlation between the presence of the 7009G deletion and susceptibility to audiogenic seizures. This argument was further strengthened by the absence of seizures in the SWR/Bm mice strain, which carried four of the upstream SNPs but not the 7009G deletion.

The homology of the MASS1 protein sequence repetitive motifs to the Na^+/Ca^{2+} exchanger β1 and β2 repeat domains may provide an important clue toward identifying the function of this novel protein. Although the identity between these proteins is limited to a short segment of the cytosolic loop of the exchanger, it is likely to be functionally significant in MASS1 because this motif is repeated 18 times within the protein sequence (Figures 4 and 5). The Na^+/Ca^{2+} exchanger is a membrane-associated protein that cotransports three sodium ions into and one calcium ion out of the cell using the sodium electrochemical gradient (Nicoll et al., 1996). The Na^+/Ca^{2+} exchanger can be regulated by

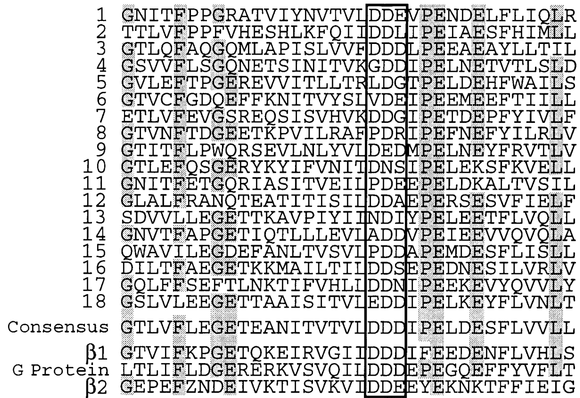

Figure 5. Amino Acid Sequence Alignment of the MASS1 Repeats

The first 18 lines represent the well-conserved amino acid repeat motif found in MASS1. Positions of highly conserved amino acids are shaded gray. The next line shows the consensus sequence for the MASS1 repeat, and below it are the sequences of the Na^+/Ca^{2+} exchanger (β1 and β2) segments that share homology with the MASS1 repeat. Also shown is a homologous region of the very large G protein-coupled receptor-1 (accession number AF055084). The boxed segment outlines the DDD motif that has been shown to be a Ca^{2+} binding site in the Na^+/Ca^{2+} exchanger β1 segment.

intracellular calcium at a Ca^{2+} binding site on the third cytosolic loop that is distinct from the Ca^{2+} transport site. This site contains three aspartate residues (DDD) (Figure 5). When Ca^{2+} is bound at this site, the transporter is activated (Matsuoka et al., 1995). One of the MASS1 repeats contains the DDD motif, and three others have conservative D to E substitutions, suggesting that these domains may be involved in Ca^{2+} binding.

The MCO I consensus sequence identified within the MASS1 amino acid sequence is also an interesting putative functional domain. The MCOs represent a family of proteins that oxidize substrates while reducing molecular O_2 to H_2O. The oxidation of multiple substrate molecules occurs serially while storing electrons in the copper atom (presumably to prevent the formation of reactive species) until a molecule of O_2 is reduced. Two known MCOs, Fet3p in yeast (Askwith et al., 1994) and ceruloplasmin in humans (Harris et al., 1995), have been shown to oxidize and transport iron. And a third, hephaestin, has been suggested to be a feroxidase (Vulpe et al., 1999). Other known MCO substrates include Mn^{2+} (Brouwers et al., 1999), serotonin, epinephrine, dopamine, and (+)-lysergic acid diethylamide (LSD) (Zaitsev et al., 1999). Therefore, loss of this putative functional domain could possibly result in problems with the metabolism of iron or other metals, neurotransmitter processing, and/or oxidative stress. Detailed biochemical analysis of the protein will be required to determine which of the consensus motifs are functional domains.

The human ortholog of the *mass1* gene resides on chromosome 5q. Interestingly, a gene causing a human epilepsy has also been mapped to this region of chromosome 5. This locus, FEB4, was mapped in families with a phenotype of febrile convulsions (Nakayama et al., 2000). While this temperature-sensitive phenotype is different than AGS, *hMass1* will be an important candidate to test in the FEB4-linked families.

To date, all genes that have been shown to cause nonsymptomatic epilepsies have encoded ion channels (voltage- or ligand-gated and exchangers) (Jen and Ptáček, 2000; Noebels, 2000). The *mass1* gene therefore represents the first non-ion channel gene shown to cause a nonsymptomatic epilepsy. The seizures in the *Frings* mice are different from those recognized to be caused by ion channels. The phenotype is a reflex epilepsy with seizures in response to loud auditory stimuli. This suggests that the genesis of episodes may be in brainstem rather than being due to hyperexcitability of cortical neurons. There is a growing appreciation of the role that deep brain structures and brainstem play in the integration and modulation of cortical discharges. Perhaps some of the reflex epilepsies in humans are not the result of primary cortical hyperexcitability, but rather of abnormal function of circuits critical for integration and modulation of cortical activity. Much work will be required to test this hypothesis, but some fascinating episodic CNS disorders have clinical and electrical manifestation that may be consistent with this idea (Fouad et al., 1996; Ptáček, 1998; Plaster et al., 1999; Swoboda et al., 2000).

Identification and characterization of the *mass1* gene reveals it to be a novel transcript. Further research to determine the function of MASS1 will lead to an understanding of how a defect in this protein results in seizures in these audiogenic seizure-susceptible mice. From the mouse *mass1* cDNA, a human *mass1* ortholog has been identified; the genomic structure is conserved. Through mapping and characterization of the human ortholog, it may be possible to find an association of *mass1* with a human epilepsy disorder. Together, the studies of the mouse and human MASS1 will provide information about the function of this novel protein and are likely to lead to new insights into normal neuronal excitability and dysfunction of membrane excitability that can lead to seizures and epilepsy.

Experimental Procedures

Mouse Breeding, Seizure Testing, and DNA Collection

BUB/BnJ and SWR/Bm mice were purchased from Jackson Laboratory (Bar Harbor, ME). Small colonies of each of these strains were established. A total of five litters were produced resulting in 29 BUB/BnJ and 11 SWR/Bm offspring. *Frings* mice were crossed to the seizure-resistant strain C57BL/6J to produce F1 animals, which were intercrossed to generate F2 mice. All mice were phenotyped at postnatal day 21 as described previously (Skradski et al., 1998). Tail sections were cut for DNA preparation. Potential recombinant mice within the region were tested again to confirm the seizure phenotype, a second tail section was cut, and the mice were euthanized by CO_2 and bilateral thoracotomy.

Fine Mapping and Physical Mapping

All known MIT microsatellite markers between *cD13Mit200* and *D13Mit126* were identified from the Chromosome 13 Committee map. Primer sequences and information for the markers was obtained from the Whitehead Institute Database site Genetic and Physical Maps of the Mouse Genome. Primer synthesis and SSLP analysis were performed as previously described (Skradski et al., 1998).

YAC maps spanning the region were obtained from the Physical Maps of the Mouse Genome. All STSs shown to be associated with each YAC clone from the map were tested to confirm that the clones were correct and aligned with overlapping YAC clones. Standard PCR conditions for physical mapping analyses were 10 mM Tris-HCl, 50 mM NaCl, 1.5 mM $MgCl_2$, 30 μM dNTPs, 0.5 μM of forward

and reverse primers, and 50 ng of DNA in a 25 μl reaction volume. PCR thermocycles were 94°C for 2 min, followed by 35–40 cycles of 94°C for 10 s, 54°C for 30 s, and 72°C for 30 s with a 5 min final extension at 72°C.

BACs were identified and isolated from the mouse BAC library available from Research Genetics using all known STSs and SSLPs found in the region, and BAC DNA was prepared using purification columns (Magnum columns, Genome Systems, Inc). Individual BAC insert sizes were determined by complete digestion of the BAC DNA with NotI and separating the fragments on a 1.0% agarose gel in 0.5X TBE circulating buffer by field inversion gel electrophoresis (FIGE).

SSLP Identification

BAC DNA was partially digested with Sau3A1 into fragments ranging from 1 to 3 kb and subcloned into the BamHI site of pUC18 with the Ready-To-Go cloning kit (Amersham Pharmacia Biotech). New repeats were identified by plating the subclone library, lifting duplicate Hybond-N membranes (Amersham Pharmacia Biotech), and hybridizing with $(CA)_{20}$ and $(AT)_{20}$ oligonucleotide end-labeled with γ^{32}P-ATP. Membranes were exposed to film overnight. Positive clones were sequenced and primer pairs were designed to amplify new repeat sequences. New SSLP markers were tested with control and recombinant mice to fine-map the interval.

Cosmid Subcloning

BAC 290J21 was partially digested with Sau3A1 into 30–40 kb fragments which were subcloned into SuperCos 1 according to the manufacturer's instructions (Stratagene) and packaged with Gigapack III Gold Packaging Extract (Stratagene) using XL1-Blue mrf′ competent cells. Cosmids were then aligned by amplification with all STSs across the region. Cosmid sequencing was performed by standard techniques using 1200 ng of cosmid DNA and 3.2 pmol of gene-specific *mass1* oligos ranging from 18 to 24 nt in length.

Identification of the *mass1* Gene

The *mass1* cDNA was identified by RT-PCR using primers developed from a sequence of exons predicted by Genfinder. Total RNA was prepared from mouse brain using Trizol (Molecular Research Center, Inc.). The standard reverse transcription reaction was carried out using the Superscript II cDNA synthesis system (Gibco BRL). First strand cDNAs were amplified using *pfx* DNA polymerase (Gibco BRL) and multiple reactions were sequenced for each. Since the entire gene was not contained within the genomic sequence generated, 5′- and 3′-RACE were used to identify the remaining cDNA sequences.

Reverse Transcription-PCR

The RT reactions to determine tissue specificity of *mass1* expression were performed as described in the previous section on RNAs from CF1 (Charles Rivers, Wilmington, MA), C57BL/6J, or *Frings* mice. The tissue panel samples were isolated from a single C57BL/6J mouse. The neuronal cDNA was produced from the pooled cellular extracts of 4–6 CF1 mouse cultured cortical neurons, and the astrocyte cDNA from CF1 astrocyte culture RNA. PCR conditions to amplify the cDNAs were as described above with 1 μl of the cDNA in a 25 μl reaction volume. The *mass1* primers spanned from exon 22 to exon 23, and were 5′ CAG AGG ATG GAT ACA GTA C 3′ and 5′ GTA ATC TCC TCC TTG AGT TG 3′ with an expected product size of 487 bp. The β-*actin* primers also spanned an intron and were 5′ GCA GTG TGT TGG CAT AGA G 3′ and 5′ AGA TCC TGA CCG AGC GTG 3′ with an expected product of 327 bp. PCR products for each tissue were mixed and separated by electrophoresis on 2% agarose gels in 1X TAE buffer.

Polymorphism and Mutation Identification

SSCP was carried out using standard procedures (Ptáček et al., 1991). The PCR forward primer sequence was 5′ TTT ATT GTA GAG GAA CCT GAG 3′ and the reverse primer sequence was 5′ GCC AGT AGC AAA CTG TCC 3′, and the expected product size was 126 bp. Exon 27 PCR products were sequenced to determine that the aberrant band was due to a single G deletion in the *Frings* mouse *mass1* gene. In addition to C57/BL6 and Frings, twelve Swiss-Albino strains were analyzed. Genomic DNA samples of BUB/BnJ, EL/Suz, FVB/NJ, NOD/LtJ, NON/LtJ, RF/J, SJL/Bm, and SWR/Bm were purchased from Jackson Laboratory (Bar Harbor, ME). DNAs of CD-1, NFS/N, STS/A, and Swiss-Webster were kindly provided by Dr W. Frankel. Six different regions of the gene carrying single nt changes resulting in amino acid substitutions or premature stop [(G5941C, A6847G, 7009DG, G7450A, A8176G, and G8326A) or (A1716 P, T2018 P, V2072Stop, V2219I, I2461V, and V2511I)] were PCR amplified and sequenced.

MASS1 Amino Acid Sequence Analysis

The amino acid sequence of MASS1 was deduced from the nt sequence of the cloned *mass1* cDNA by DNA Star. The amino acid sequence was compared to known proteins by BLAST sequence similarity searching. Identification of functional domains utilized PSORT II Prediction [http://psort.nibb.ac.jp/form2.html], Sequence Motif Search [http://www.motif.genome.ad.jp/], Global and Domain Similarity Search [http://www-nbrf.georgetown.edu/pirwww/search/dmsim.html], and Pattern Match [http://www-nbrf.georgetown.edu/pirwww/search/patmatch.html].

Identification and Mapping of the *hMass1* Gene

Several primer sets corresponding to different exons of mouse *mass1* were used to amplify human fetal brain cDNA with PCR conditions as above except at an annealing temperature of 47°C. These primers were used to identify a human genomic BAC clone containing a part of the *hMass1* gene from the CITB human BAC library. This BAC clone was localized using standard FISH mapping.

Acknowledgments

The authors wish to thank Nicole Harris for technical assistance and Drs. J. Kaplan, T. Constantino, and G. Goellner for helpful discussions and comments on this manuscript, and Dr. T. Seyfried, K. Johnson, and W. Frankel for mouse reagents and helpful discussions. Finally, we thank the many families who participated in this work. This investigation was supported by NIH grant NS32711 (L.J.P. and Y.-H.F.) and Public Health Service research grant M01-RR000064 from the National Center for Research Resources. L.J.P. is an Investigator of the Howard Hughes Medical Institute.

Received January 10, 2001; revised June 27, 2001.

References

Askwith, C., Eide, D., Van Ho, A., Bernard, P.S., Li, L., Davis-Kaplan, S., Sipe, D.M., and Kaplan, J. (1994). The FET3 gene of S. cerevisiae encodes a multicopper oxidase required for ferrous iron uptake. Cell *76*, 403–410.

Biervert, C., Schroeder, B.C., Kubisch, C., Berkovic, S.F., Propping, P., Jentsch, T.J., and Steinlein, O.K. (1998). A potassium channel mutation in neonatal human epilepsy. Science *279*, 403–406.

Brennan, T.J., Seeley, W.W., Kilgard, M., Schreiner, C.E., and Tecott, L.H. (1997). Sound-induced seizures in serotonin 5-HT2c receptor mutant mice. Nat. Genet. *16*, 387–390.

Brouwers, G.J., de Vrind, J.P., Corstjens, P.L., Cornelis, P., Baysse, C., and de Vrind-de Jong, E.W. (1999). cumA, a gene encoding a multicopper oxidase, is involved in Mn2+ oxidation in Pseudomonas putida Gb-1. Appl. Environ. Microbiol. *65*, 1762–1768.

Burgess, D.L., Jones, J.M., Meisler, M.H., and Noebels, J.L. (1997). Mutation of the Ca2+ channel beta subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. Cell *88*, 385–392.

Charlier, C., Singh, N.A., Ryan, S.G., Lewis, T.B., Reus, B.E., Leach, R.J., and Leppert, M. (1998). A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat. Genet. *18*, 53–55.

Collins, R.L. (1970). A new genetic locus mapped from behavioral variation in mice: audiogenic seizure prone (ASP). Behav. Genet. *1*, 99–109.

Escayg, A., MacDonald, B.T., Meisler, M.H., Baulac, S., Huberfeld, G., An-Gourfinkel, I., Brice, A., LeGuern, E., Moulard, B., Chaigne,

D., et al. (2000a). Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nat. Genet. *24*, 343–345.

Escayg, A., De Waard, M., Lee, D.D., Bichet, D., Wolf, P., Mayer, T., Johnston, J., Baloh, R., Sander, T., and Meisler, M.H. (2000b). Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. Am. J. Hum. Genet. *66*, 1531–1539.

Fletcher, C.F., Lutz, C.M., O'Sullivan, T.N., Shaughnessy, J.D., Jr., Hawkes, R., Frankel, W.N., Copeland, N.G., and Jenkins, N.A. (1996). Absence epilepsy in tottering mutant mice is associated with calcium channel defects. Cell *87*, 607–617.

Fouad, G.T., Servidei, S., Durcan, S., Bertini, E., and Ptáček, L.J. (1996). A gene for familial paroxysmal dyskinesia (FPD1) maps to chromosome 2q. Am. J. Hum. Genet. *59*, 135–139.

Frings, H., Frings, M., and Kivert, A. (1951). Behavior patterns of the laboratory mouse under auditory stress. J. Mammal. *32*, 60–76.

Harris, Z.L., Takahashi, Y., Miyajima, H., Serizawa, M., MacGillivray, R.T., and Gitlin, J.D. (1995). Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. Proc. Natl. Acad. Sci. USA *92*, 2539–2543.

Henry, K.R. (1967). Audiogenic seizure susceptibility induced in C57BL-6J mice by prior auditory exposure. Science *158*, 938–940.

Jen, J., and Ptáček, L.J. (2000). Channelopathies: episodic disorders of the nervous system. Metabolic and Molecular Bases of Inherited Disease, C.R. Schriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds. (New York: McGraw-Hill), pp. 5223–5238.

Johnson, K.R., Zheng, Q.Y., and Erway, L.C. (2000). A major gene affecting age-related hearing loss is common to at least ten inbred strains of mice. Genomics *70*, 171–180.

Letts, V.A., Felix, R., Biddlecome, G.H., Arikkath, J., Mahaffey, C.L., Valenzuela, A., Bartlett, F.S., 2nd, Mori, Y., Campbell, K.P., and Frankel, W.N. (1998). The mouse stargazer gene encodes a neuronal Ca2+-channel gamma subunit. Nat. Genet. *19*, 340–347.

Matsuoka, S., Nicoll, D.A., Hryshko, L.V., Levitsky, D.O., Weiss, J.N., and Philipson, K.D. (1995). Regulation of the cardiac Na(+)-Ca2+ exchanger by Ca2+. Mutational analysis of the Ca(2+)-binding domain. J. Gen. Physiol. *105*, 403–420.

Musumeci, S.A., Bosco, P., Calabrese, G., Bakker, C., De Sarro, G.B., Elia, M., Ferri, R., and Oostra, B.A. (2000). Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. Epilepsia *41*, 19–23.

Nakayama, J., Hamano, K., Iwasaki, N., Nakahara, S., Horigome, Y., Saitoh, H., Aoki, T., Maki, T., Kikuchi, M., Migita, T., et al. (2000). Significant evidence for linkage of febrile seizures to chromosome 5q14-q15. Hum. Mol. Genet. *9*, 87–91.

Neumann, P.E., and Collins, R.L. (1991). Genetic dissection of susceptibility to audiogenic seizures in inbred mice. Proc. Natl. Acad. Sci. USA *88*, 5408–5412.

Nicoll, D.A., Hryshko, L.V., Matsuoka, S., Frank, J.S., and Philipson, K.D. (1996). Mutagenesis studies of the cardiac Na(+)-Ca2+ exchanger. Ann. NY Acad. Sci. *779*, 86–92.

Nikkila, H., McMillan, D.R., Nunez, B.S., Pascoe, L., Curnow, K.M., and White, P.C. (2000). Sequence similarities between a novel putative G protein-coupled receptor and Na+/Ca2+ exchangers define a cation binding domain. Mol. Endocrinol. *14*, 1351–1364.

Noebels, J.L. (2000). The inherited epilepsies. Metabolic and Molecular Bases of Inherited Disease. C.R. Schriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds. (New York: McGraw-Hill), pp. 5807–5832.

Plaster, N.M., Uyama, E., Uchino, M., Ikeda, T., Flanigan, K.M., Kondo, I., and Ptáček, L.J. (1999). Genetic localization of the familial adult myoclonic epilepsy (FAME) gene to chromosome 8q24. Neurology *53*, 1180–1183.

Ptáček, L.J. (1998). Channelopathies: Ion Channels and Paroxysmal Disorders of the Nervous System. Genetics of Focal Epilepsies, P. Genton, ed. (London: John Libbey), pp 203–213.

Ptáček, L.J. (1999). Ion channel diseases: episodic disorders of the nervous system. Semin. Neurol. *19*, 363–369.

Ptáček, L.J., George, A.L., Jr., Griggs, R.C., Tawil, R., Kallen, R.G., Barchi, R.L., Robertson, M., and Leppert, M.F. (1991). Identification of a mutation in the gene causing hyperkalemic periodic paralysis. Cell *67*, 1021–1027.

Ribak, C.E., Roberts, R.C., Byun, M.Y., and Kim, H.L. (1988). Anatomical and behavioral analyses of the inheritance of audiogenic seizures in the progeny of genetically epilepsy-prone and Sprague-Dawley rats. Epilepsy Res *2*, 345–355.

Schreiber, R.A., Lehmann, A., Ginsburg, B.E., and Fuller, J.L. (1980). Development of susceptibility to audiogenic seizures in DBA/2J and Rb mice: toward a systematic nomenclature of audiogenic seizure levels. Behav. Genet. *10*, 537–543.

Seyfried, T.N., and Glaser, G.H. (1981). Genetic linkage between the AH locus and a major gene that inhibits susceptibility to audiogenic seiaures in mice. Genetics *99*, 117–126.

Signorini, S., Liao, Y.J., Duncan, S.A., Jan, L.Y., and Stoffel, M. (1997). Normal cerebellar development but susceptibility to seizures in mice lacking G protein-coupled, inwardly rectifying K+ channel GIRK2. Proc. Natl. Acad. Sci. USA *94*, 923–927.

Singh, N.A., Charlier, C., Stauffer, D., DuPont, B.R., Leach, R.J., Melis, R., Ronen, G.M., Bjerre, I., Quattlebaum, T., Murphy, J.V., et al. (1998). A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat. Genet. *18*, 25–29.

Skradski, S.L., White, H.S., and Ptáček, L.J. (1998). Genetic mapping of a locus (mass1) causing audiogenic seizures in mice. Genomics *49*, 188–192.

Steinlein, O.K., Mulley, J.C., Propping, P., Wallace, R.H., Phillips, H.A., Sutherland, G.R., Scheffer, I.E., and Berkovic, S.F. (1995). A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat. Genet. *11*, 201–203.

Swoboda, K.J., Soong, B., McKenna, C., Brunt, E.R., Litt, M., Bale, J.F., Jr., Ashizawa, T., Bennett, L.B., Bowcock, A.M., Roach, E.S., et al. (2000). Paroxysmal kinesigenic dyskinesia and infantile convulsions: clinical and linkage studies. Neurology *55*, 224–230.

Tecott, L.H., Sun, L.M., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F., and Julius, D. (1995). Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature *374*, 542–546.

Vulpe, C.D., Kuo, Y.M., Murphy, T.L., Cowley, L., Askwith, C., Libina, N., Gitschier, J., and Anderson, G.J. (1999). Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat. Genet. *21*, 195–199.

Wallace, R.H., Wang, D.W., Singh, R., Scheffer, I.E., George, A.L., Jr., Phillips, H.A., Saar, K., Reis, A., Johnson, E.W., Sutherland, G.R., et al. (1998). Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. Nat. Genet. *19*, 366–370.

Zaitsev, V.N., Zaitseva, I., Papiz, M., and Lindley, P.F. (1999). An X-ray crystallographic study of the binding sites of the azide inhibitor and organic substrates to ceruloplasmin, a multi-copper oxidase in the plasma. J. Biol. Inorg. Chem. *4*, 579–587.

Zheng, Q.Y., Johnson, K.R., and Erway, L.C. (1999). Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear Res. *130*, 94–107.

Accession Numbers

The GenBank accession numbers for the *mass1* cDNA sequences reported in this paper are AF405692, AF405693, and AF405694.