A Nonsense Mutation of the *MASS1* Gene in a Family with Febrile and Afebrile Seizures

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A naturally occurring mutation of the *mass1* (monogenic audiogenic seizure-susceptible) gene recently has been reported in the Frings mouse strain, which is prone to audiogenic seizures. The human orthologous gene, *MASS1*, was mapped to chromosome 5q14, for which we previously have reported significant evidence of linkage to febrile seizures (*FEB4*). We screened for *MASS1* mutations in individuals from 48 families with familial febrile seizures and found 25 DNA alterations. None of nine missense polymorphic alleles was significantly associated with febrile seizures; however, a nonsense mutation (S2652X) causing a deletion of the C-terminal 126 amino acid residues was identified in one family with febrile and afebrile seizures. Our results suggest that a loss-of-function mutation in *MASS1* might be responsible for the seizure phenotypes, though it is not likely that *MASS1* contributed to the cause of febrile seizures in most of our families.

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Febrile seizures (FSs) are relatively common and represent most childhood seizures. Studies in the developed nations indicate that 2 to 5% of all children will experience an FS before 5 years of age.[1] In the Japanese population, the incidence rate is as high as 7%.[2] Extensive genetic studies have shown that at least four loci are responsible for FS: *FEB1* on chromosome 8q13-q21, *FEB2* on 19p, *FEB3* on 2q23-q24, and *FEB4* on 5q14-q15.[3–6] A small proportion of individuals with FS has additional generalized epilepsy or afebrile seizures.[7] Genes for the β-subunit[8] and the α1-subunit[9] of the neuronal voltage-gated Na^+ channel and the $GABA_A$ receptor γ2-subunit gene[10] have been shown to be responsible for generalized epilepsy with FS plus.[11] However, the causative gene has not been found in most patients with FS or generalized epilepsy with FS plus.

A naturally occurring mutation of the *mass1* (monogenic audiogenic seizure-susceptible) gene has been reported in the Frings mouse strain, which is prone to audiogenic seizures.[12,13] The mutation is a deletion of nucleotide 7009G of the cDNA (within exon 27), converting amino acid V2250 to a stop codon. The human orthologous gene, *MASS1*, was mapped to chromosome 5q14, on which we previously have reported *FEB4*.[6] Therefore, *MASS1* is a good candidate gene for *FEB4*. In this study, we screened for mutations in the *MASS1* gene in families with FS.

Subjects and Methods

Subjects

Study participants were FS probands and their family members of 58 familial FS and 100 unrelated healthy controls. These FS families numbered 231 individuals in total including 117 affected children. Among these affected children, 9 had afebrile seizures and 15 had complex FS. All participants were Japanese. We screened mutations in *MASS1* in probands of 48 FS families; 47 of these families were included in another report.[6] Diagnosis of FS was performed by analyzing medical records and collecting detailed information about convulsive disorders in family members who were interviewed by trained pediatricians.[14] We performed the transmission disequilibrium test in all families. A full verbal and written explanation of the study was given to all participants. Informed consent for participants under school age was provided by their parents. The study was approved by the ethics committee of the University of Tsukuba.

Methods

DNA was extracted from peripheral blood leukocytes of the subjects. Human *MASS1* mRNA was cloned. The intron–exon structure of the *MASS1* gene was elucidated by comparisons between sequences of the human *MASS1* mRNA clone and the genomic clone (GenBank accession numbers AC093529). Primers were designed to amplify all 35 coding exons and the flanking intronic splice sites. The primer sequences are available on request. Polymerase chain reaction was performed with AmpliTaq or AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA) according to the manufacturer's recommended protocol. Potential mutations in exons and exon–intron boundaries of the *MASS1* gene were screened in the 48 probands by direct sequencing with BigDye terminator chemistry and an ABI 3100 Genetic Analyzer (Applied Biosystems). The genotype for each DNA alterations was determined by restriction fragment length polymorphism analysis and direct sequencing.

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Statistical Analysis

Transmission disequilibrium testing was applied by means of the ASPEX program (ftp://lahmed.stanford.edu/pub/aspex/index.html). Comparison between FS patients and control subjects were made by Fisher's exact test. A p value of less than 0.05 was considered statistically significant.

Results

Analysis of the *MASS1* gene showed 25 DNA alterations (Fig 1). Of these variants, 14 were in exons, 11 were nonsynonymous, and 3 were synonymous. Eleven of the variants were in introns. We examined the association between the 11 nonsynonymous mutations and FS (Table). Transmission disequilibrium test analysis showed no evidence of nominally significant transmission disequilibrium. Case–control comparisons did not yield significant values. Because the other 3 exonic and 11 intronic polymorphisms identified in this study were in strong linkage disequilibria with at least 1 of the 9 nonsynonymous polymorphisms genotyped ($D' > 0.7$), we did not genotype these synonymous or intronic polymorphisms.

Because the S2584P and S2652X mutations were each found in only one FS family and none of the controls had the mutation, we could not evaluate the effect of these mutations. The S2584P mutation was observed in a family (FS 31) in which the unaffected mother, an affected sister, an unaffected brother, and the proband were heterozygous for the mutation, and the unaffected father and an unaffected brother did not have the mutation. The nonsense mutation (S2652X) causes a deletion of C-terminal 126 amino acid residues. This mutation was observed in a family (FS 17) in which the father, an affected brother, and the proband were heterozygous for the mutation (Fig 2). The proband in this family was a 12-year-old girl who had an FS with generalized tonic-clonic seizures lasting 5 minutes at age 2 years 3 months. At 3 years 2 months, she also had an afebrile seizure with generalized clonic seizures of left side dominance lasting less than 1 minute. Brain magnetic resonance imaging showed no abnormal findings, and electroencephalograph showed sharp waves in the right central area that disappeared by the time the child was 10 years 8 months of age. Her brother, 11 years old at the time of this study, experienced generalized tonic-clonic seizures associated with fever twice, once at age 6 years and once at age 7 years. His electroencephalograph showed single spike and wave discharges in the right hemisphere that disappeared by the time he was 7 years 10 months of age. Both affected children had normal mental and motor development. The proband's paternal aunt also had recurrent episodes of FSs during childhood. Unfortunately, she declined to be examined.

Discussion

In this study, we searched for *MASS1* mutations and identified nine missense polymorphisms and one rare missense mutation. We did not observe significant association between any of the nine missense polymorphisms and FS. Although we did not genotype the other synonymous or intronic polymorphisms, they were in linkage disequilibria with at least 1 of the 9 missense polymorphisms. Therefore, an association between these ungenotyped polymorphisms and FS is unlikely. In addition, the SpliceView program (http://l25.itba.mi.cnr.it/~webgene/wwwspliceview.html) did not predict

Fig 1. Genomic structure and the MASS1 *gene mutations identified in this study.*

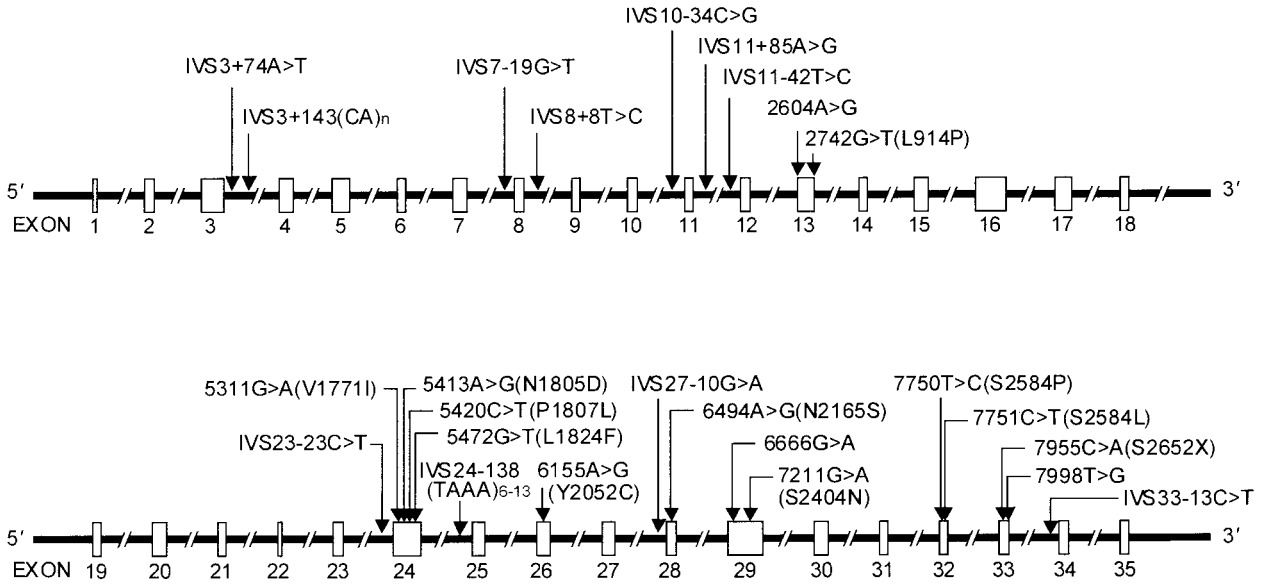

Table. Case–Control Study and Transmission Disequilibrium Test for Febrile Seizures

Mutation	Minor Allele	Allele Frequencies			TDT		
		Control (n = 200)	Patients (n = 116)	*p*	Transmitted	Not Transmitted	*p*
L914P	L	0.133	0.140	0.86	17	25	0.34
V177II	V	0.133	0.140	0.86	17	25	0.34
N1805D	D	0.366	0.336	0.47	53	39	0.20
P1807L	P	0.468	0.474	0.92	51	41	0.44
L1824F	F	0.366	0.336	0.47	53	39	0.20
Y2052C	Y	0.468	0.474	0.92	51	41	0.44
N2165S	N	0.468	0.474	0.92	51	41	0.44
S2404N	N	0.133	0.140	0.86	17	25	0.34
S2584P	P	0.000	0.009	0.37	2	0	0.16
S2584L	L	0.125	0.121	0.91	28	19	0.32
S2652X	X	0.000	0.009	0.37	2	0	0.16

TDT = transmission disequilibrium testing.

splice alternation caused by any intronic or exonic polymorphisms. Thus, the results of this study do not suggest a major role for *MASS1* in the genetic cause of FS in our families. However, we could not rule out the possibility of a small effect of a detected polymorphism or unknown gene variant that is not in linkage disequilibrium with the polymorphisms investigated here. It also remains possible that polymorphism(s) of the larger *MASS1/VLGR1* gene described below could be associated with FS.

The S2584P mutation was found in only one family and not in healthy controls. The S2584 is within one of the *MASS1* repetitive motifs. The S2584L polymorphism causes a substitution of the same serine residue, and no significant deviation in transmission of the L2584 allele to FS patients was observed, indicating that the S2584P polymorphism is not likely to confer liability to FS. However, serine to leucine is a much less drastic change than the serine to proline. Although this mutation was not cosegregating with the seizure phenotype, an association between S2584P and FS remains possible.

In one FS family, we detected a nonsense mutation that was not found in the 200 control chromosomes. This S2652X mutation is expected to produce a truncated MASS1 protein. A small repetitive motif from MASS1 shares homology with numerous sodium–calcium exchangers. This motif occurs 18 times within the sequence. The 18th motif would be missing from the truncated MASS1 protein of the S2652X gene product.

Recently, a new member of the G protein–coupled receptor family, very large G protein–coupled receptor–1 (VLGR1) was identified.[15,16] *MASS1* has several transcripts, the longest of which actually includes exon 5 to 39 of *VLGR1*. VLGR1 has a large ectodomain containing multiple calcium exchanger β repeats that resemble regulatory domains of the sodium–calcium exchanger protein. VLGR1 has three transcripts: VLGR1a, VLGR1b, and VLGR1c. The longest gene product, VLGR1b, comprises 6,307 amino acids containing 35 calcium exchanger β repeats and a pentaxin homology domain. It encompasses more than 600kb of genomic sequence, comprising 90 exons. The function of VLGR1 remains unclear, but the presence of multiple calcium exchanger β repeats in the ectodomain suggests a role in protein–protein interaction that is perhaps calcium mediated. In situ hybridization studies with mouse embryo sections have shown that high-level expression of VLGR1 is restricted to the developing central nervous system and eye. Strong expression in the ventricular zone, home of neural progenitor cells during embryonal neurogenesis, suggests a fundamental role for VLGR1 in the development of the central nervous system.[16] The S2652X mutation of *MASS1* in our patients corresponds to S2832X in exon 37 of *VLGR1*. The mutation is predicted to prevent synthesis of VLGR1b protein, but it does not influence VLGR1a encoded by exons 65 to 90 and VLGR1c encoded by exons 1 to 32. The S2652X mutation may cause dysfunction of MASS1 and VLGR1.

Frings mice are a model of generalized epilepsy and have seizures in response to loud noises. This phenotype is caused by a deletion of nucleotide 7009G of the cDNA resulting in V2250X of *Mass1* in the autosomal recessive mode of inheritance.[13] The S2653X mutation found in this study also may be a *MASS1* loss-of-function mutation. However, FS-affected siblings with S2653X identified in this study were heterozygous for the mutation. Whether haploinsufficiency of *MASS1* is related to temperature-sensitive convulsive phenotype is an open question. Another possibility is that the FS-affected siblings were compound heterozygotes with the S2653X mutation and an unknown mutation not identified in this study.

Unfortunately, the number of members for the family FS 17 that harbors the S2653X mutation was not sufficient to establish unambiguously the cosegregation of

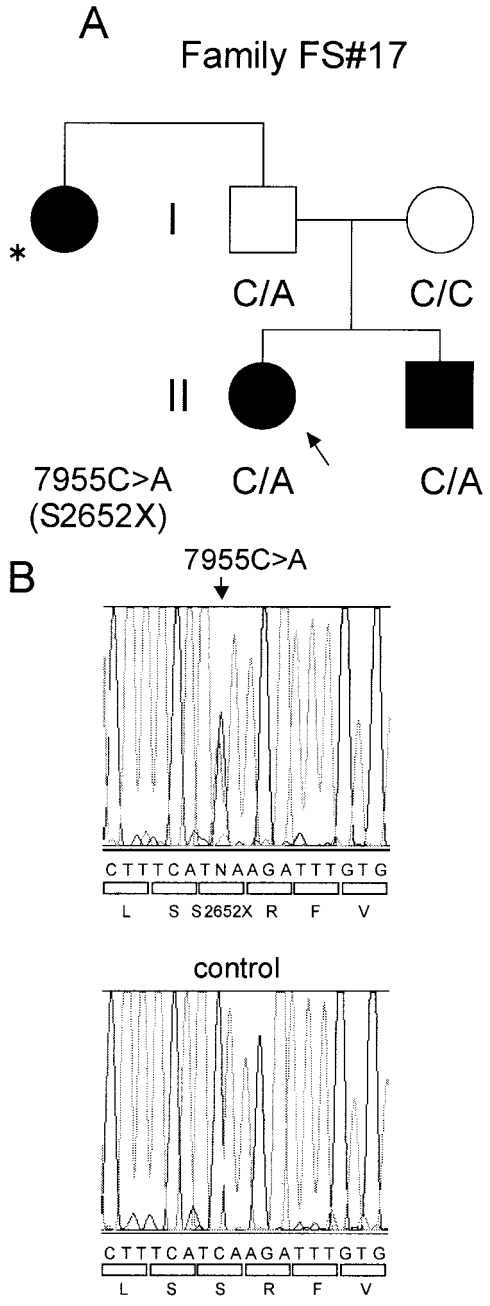

Fig 2. The nonsense mutation identified in a Japanese family with febrile seizures (FSs) associated with afebrile seizures. (A) Pedigree of a Japanese family FS 17 with FS and afebrile seizures (proband), fever-associated seizures (brother), and FS only (paternal aunt). 7955C→A (S2652X) mutations were identified in the proband, her brother, and her father. (asterisk) *Paternal aunt was not examined. The* arrow *indicates the proband. (B) Electropherogram of the mutation in the gene for MASS1 identified in the same family. The nucleotide sequence of the relevant region of exon 33 of the proband is shown.* Arrow *indicates nucleotide 7955, where a heterozygous C-to-A transition resulted in termination codon S2652X.*

the mutation with the seizure phenotype. Further investigation of *MASS1* in association with FS is warranted.

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