RESEARCH ARTICLE

**PRIMA1 mutation: a new cause of nocturnal frontal lobe epilepsy**

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**Abstract**

**Objective:** Nocturnal frontal lobe epilepsy (NFLE) can be sporadic or autosomal dominant; some families have nicotinic acetylcholine receptor subunit mutations. We report a novel autosomal recessive phenotype in a single family and identify the causative gene. **Methods:** Whole exome sequencing data was used to map the family, thereby narrowing exome search space, and then to identify the mutation. **Results:** Linkage analysis using exome sequence data from two affected and two unaffected subjects showed homozygous linkage peaks on chromosomes 7, 8, 13, and 14 with maximum LOD scores between 1.5 and 1.93. Exome variant filtering under these peaks revealed that the affected siblings were homozygous for a novel splice site mutation (c.93+2T>C) in the PRIMA1 gene on chromosome 14. No additional PRIMA1 mutations were found in 300 other NFLE cases. The c.93+2T>C mutation was shown to lead to skipping of the first coding exon of the PRIMA1 mRNA using a minigene system. **Interpretation:** PRIMA1 is a transmembrane protein that anchors acetylcholinesterase (AChE), an enzyme hydrolyzing acetycholine, to membrane rafts of neurons. PRIMA knockout mice have reduction of AChE and accumulation of acetylcholine at the synapse; our minigene analysis suggests that the c.93+2T>C mutation leads to knockout of PRIMA1. Mutations with gain of function effects in acetylcholine receptor subunits cause autosomal dominant NFLE. Thus, enhanced cholinergic responses are the likely cause of the severe NFLE and intellectual disability segregating in this family, representing the first recessive case to be reported and the first PRIMA1 mutation implicated in disease.
**Introduction**

Nocturnal frontal lobe epilepsy (NFLE) is characterized by frequent, sometimes violent, and often brief seizures at night, that usually commence during childhood. It can arise sporadically or be inherited in an autosomal dominant fashion (ADNFLE). Clinical features associated with nocturnal seizures include vocalizations, complex and often violent automatisms, and ambulation, making the condition sometimes difficult to distinguish from parasomnias. Electroencephalography (EEG) is often unrevealing, and magnetic resonance imaging (MRI) typically shows no lesions in patients with NFLE. In some cases NFLE is accompanied by intellectual disability and psychiatric disorders, the pathogenic mechanisms of which remain unclear. A minority of ADNFLE cases are due to mutations in three genes (CHRNA2, CHRNA4 and CHRNB2) that encode the α2, α4 and β2 subunits of the neuronal nicotinic acetylcholine receptor. More recently, mutations in the sodium-activated potassium channel KCNT1 were shown to cause a small number of severe familial and sporadic cases. Mutations in the mTOR signaling protein DEPDC5 may also present with NFLE, including in 13% of families in one study. The etiology of other familial NFLEs remain to be elucidated, but additional genes are expected to be involved. Gain of function of mutated nicotinic cholinergic receptors appears to be the common mechanism in in vitro studies of human mutations. Based on this electrophysiological data, and the genetic data above, other proteins of the cholinergic nervous system may be involved in the pathogenesis of NFLE.

Herein we report a small Australian family of Italian origin segregating autosomal recessive NFLE (ARNFLE) and intellectual disability. Using a methodology we recently reported we performed linkage analysis with whole exome sequencing data to map the disorder to multiple genomic loci, and then, using the same sequence data, we identified the causative gene mutation on chromosome 14. The mutated gene, PRIMA1, encodes a proline-rich transmembrane protein that efficiently transforms secreted acetylcholinesterase (AChE) into an enzyme anchored on the outer cell surface.

**Materials and Methods**

**Family and sporadic cases**

A two-generation Australian family of Italian origin with NFLE and intellectual disability was studied (Fig. 1). The Human Research Ethics Committee of Austin Health, Melbourne, Australia, approved this study. Informed consent was obtained from living subjects or their relatives.

**Whole exome sequencing**

Exome sequencing was performed using 3 μg of venous blood-derived genomic DNA from each of four family members (I:1, II-1, II-2 and II-3; Fig. 1). Genomic DNA was sonicated to approximately 200 base pair (bp) fragments and adaptor-ligated to make a library for paired-end sequencing. Following amplification and barcoding, the libraries were hybridized to biotinylated complementary RNA oligonucleotide baits from the SureSelect Human All Exon 50 Mb Kit (Agilent Technologies, Santa Clara, CA) and purified using streptavidin-bound
magnetic beads as described previously. Amplification was conducted prior to sequencing on the Illumina HiSeq 2000 system (San Diego, CA). The exome design covers 50 megabases of human genome that includes all exons annotated in the GENCODE project (reference annotation for the ENCODE project; Wellcome Trust Sanger Institute) and consensus CDS database (CCDS – March 2009) as well as 10 bp of flanking sequence for each targeted region and small noncoding RNAs from miRBase (http://www.mirbase.org/) (v.13) and Rfam (http://rfam.xfam.org/).

Whole exome analysis and linkage mapping
Exome sequencing reads were aligned with Novoalign version 3.02.00 (http://www.novocraft.com/) to the human genome assembly with ambiguous SNPs (hg19 dbsNP132-masked, UCSC Genome Browser). PCR duplicates were removed using MarkDuplicates from Picard (http://picard.sourceforge.net).

For linkage analysis, a previously described method was used. Briefly, genotypes of SNPs at HapMap Phase II loci were inferred using GATK UnifiedGenotypes with a required minimum depth of 10 reads per sample, to produce variant call format (VCF) files. LINKDATAGEN produced files for linkage, including removing Mendelian errors. To satisfy linkage equilibrium assumptions markers were chosen so that they were approximately 0.3 cM apart when available. Inbreeding coefficients (F) were estimated using the FEstim algorithm. The estimated inbreeding coefficients were used to generate an appropriate pedigree with an inbreeding loop (Fig. 1) for homozygosity mapping. Multipoint parametric linkage analysis using exome genotypes was performed using MERLIN with a fully penetrant recessive disease model with disease allele frequency 0.00001, with the pedigree described in Figure 1 for homozygosity mapping, and the nuclear family for recessive disease model mapping.

Variant detection was performed with GATK HaplotypeCaller and variant annotation using ANNOVAR. Exome variants were filtered and selected according to the following criteria for the homozygous and compound heterozygous recessive models: location within a linkage region, genotypes fitting the disease model, a minor allele frequency ≤0.01 (0.05) in the exome variant server dataset (http://evs.gs.washington.edu/EVS/) and 1000 Genomes, a minor allele frequency of ≤0.05 in the Exome Aggregation Consortium database, appearance in <10 alleles of our in-house exome dataset, and mutation type (missense, nonsense, coding indel or splice site variant). Compound heterozygous variants were filtered at the gene level, where candidate genes had to have at least two remaining variants after filtering. Variants in the same gene were assessed pairwise requiring that exactly one variant was heterozygous in the father (I:1), no more than one variant was observed in the unaffected sibling (II:1), and not present in cis-phase as assessed in IGV when possible.

PCR and sanger sequencing
The PRIMA1 gene and exome variants were amplified using gene-specific primers (Table 1) designed to the reference human gene transcripts (NCBI Gene; http://www.ncbi.nlm.nih.gov/). Amplification reactions were cycled using a standard protocol on a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA) at 60°C annealing temperature for 1 min. Bidirectional sequencing of all exons and flanking regions was completed with a BigDye v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer’s instructions. Sequencing products were resolved using an 3730× L DNA Analyzer (Applied Biosystems). All sequencing chromatograms were compared to published cDNA sequence; nucleotide changes were detected, using Codon Code Aligner (CodonCode Corporation, Dedham, MA).

Generation and analysis of lymphoblastoid cells
Immortalized lymphoblastoid cell lines were established from venous blood of family members II:1 and II:3 using Epstein–Barr virus and maintained in RPMI-1640 medium supplemented with 20% fetal calf serum. RNA was extracted from the cells using an RNeasy Minikit (Qiagen) and cDNA generated using a SuperScript III First-Strand Synthesis System (LifeTechnologies, Grand Island, NY) according to the manufacturer’s instructions. PCR was performed as described above using oligonucleotides designed to PRIMA1 mRNA and products were resolved on 2% agarose gels.

Minigene assay
Minigenes were generated using Exontrap vector (MoBiTec Molecular Biotechnology, Goettingen, Germany), which includes a 5′ and a 3′ exon separated by a 600 bp current issue of  Annals of Clinical and Translational Neurology. Published on behalf of the American Neurological Association. 

Table 1. Oligonucleotides used in this study.

<table>
<thead>
<tr>
<th>Target</th>
<th>Oligonucleotide (5′-3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMA1 exon 1</td>
<td>CTGACCCCTAGCCTTGCTCTC (forward) AGGAGGGAGGGACAGCT (reverse)</td>
</tr>
<tr>
<td>PRIMA1 exon 2</td>
<td>CAGCTCTAGTGGCTTATGGTC (forward) CTCACTGGATCTGGCGGG (reverse)</td>
</tr>
<tr>
<td>PRIMA1 exon 3</td>
<td>CATGACCACCTCATCCAGG (forward) ATGTGGTAGGACCCCACA (reverse)</td>
</tr>
<tr>
<td>PRIMA1 exon 4</td>
<td>ACTAATGGTGTCCTTGCTCTC (forward) ACCTGCTTTCCATGTCCAC (reverse)</td>
</tr>
</tbody>
</table>
intron containing a poly linker for cloning of DNA of interest. Wild-type and mutant (c.93+2T>C) PRIMA1 fragments were amplified from genomic DNA of family members II:1 and II:3, respectively, using PCR with the primers 5'–AGGCTTGGTTTACTAGGGTG (forward) and 5'–GAGAAACACTAGTGCGTTGGCTGCAAGA (reverse, introduced SpeI site is highlighted in bold). The PCR products were digested with Xmal (restriction site is located in intron 1–2) and SpeI, and cloned into Xmal/ SpeI-digested poly linker of Exon trap vector. The resulting minigenes contained 307 bp fragment of PRIMA1: exon 2 (124 bp) with flanking intronic sequences (83 bp of intron 1–2 and 100 bp of intron 2–3). The minigene sequences were confirmed by Sanger sequencing.

HEK293T cells (a human embryonic kidney cell line stably transfected with the SV40 large T antigen) were maintained in Dulbecco’s Modified Eagle’s Medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine. The cells were transfected with wild-type or mutant PRIMA1 minigenes, or empty Exon trap vector, using Lipofectamine-2000™ (Invitrogen). Twenty-four or 48 h post-transfection the cells were harvested and total RNA was extracted using an RNeasy Minikit (Qiagen). cDNA was generated from 1 μg of total RNA using SuperScript III reverse transcriptase and random hexamer primers (Invitrogen). PCR was performed on the cDNA using Platinum High Fidelity Taq (Invitrogen) with the primers binding to the 5' and 3' exons of Exon trap vector. The resulting PCR products were analyzed by electrophoresis on a 2% agarose gel.

Results

Clinical details

Two of three siblings with unaffected parents, who were born in the same small Italian village, were diagnosed with NFLE and intellectual disability (Fig. 1). Both required long-term institutional care. The 53 year-old female proband (II:3) had a history of motor and speech delays, ataxic gait, congenital nystagmus, and a squint. Chromosome analysis, metabolic screening and computed tomography (CT) were normal in childhood. During early childhood she developed seizures that persisted throughout adult life. The predominant attacks were nocturnal hyperkinetic seizures with duration ~1–2 min and a frequency of 1–2 per week characterized by loud vocalizations, bilateral asymmetrical limb movements, often with incontinence and postictal confusion. Occasional generalized convulsions also occurred. Examination revealed nystagmus, moderate intellectual disability, and a broad-based gait without other specific cerebellar signs. Video electroencephalogram (EEG) confirmed frontal lobe nocturnal hyperkinetic attacks, and diurnal staring attacks thought to be behavioral. CT scans and MRI brain showed no specific lesions; there was a suggestion of mild cerebellar atrophy. Her clinical course was relatively stable over the last two decades.

Her 61-year-old affected brother (II:2) shares many clinical features with his sister. He had congenital nystagmus and developed epilepsy in early childhood characterized by monthly ~1–2 min nocturnal seizures with vocalization, asymmetrical limb movements and postictal drowsiness as well as brief generalized tonic-clonic seizures in the early morning or late evening. Clinical evaluation revealed moderate intellectual disability, nystagmus, and a mild-ataxic gait. EEG showed slow, poorly sustained posterior dominant rhythm, delta slowing, with bifrontal epileptiform discharges. Brain CT revealed mild cerebral and cerebellar atrophy. He required a number of hospitalizations for poor seizure control.

Candidate gene screening

The coding regions and splice sites of three known ADNFLE genes – CHRNA2, CHRNA4, and CHRN2 – were Sanger sequenced without identification of a causal mutation. The KCNT1 and DEPDC5 genes were discovered to be causal NFLE genes only after this study commenced and no mutation of these genes was found in the subsequent exome sequencing analysis.

Linkage mapping using exome data detects multiple genomic loci

The inheritance pattern in the family was unclear although a recessive mode appeared most likely. We performed linkage analysis with a fully penetrant recessive model using 7102 SNP markers generated from the exomes of four family members (I:1, II:1, II:2 and II:3; Fig. 1). We tested the hypothesis that the family was consanguineous and FEstim analysis indicated that the parents were related (F estimates 0.034, 0.021, 0.036 (SE 0.010, 0.009, 0.014) for II:1, II:2, and II:3, respectively), with an inferred relationship of second-cousins. The father (I:1) also showed evidence of consanguinity (F = 0.022 [SE 0.009]), but his inbreeding loop is not relevant to the detection of autozygosity for this phenotype. Multipoint parametric linkage analysis using a pedigree with an inferred inbreeding loop revealed multiple linkage peaks including four with a parametric LOD score >1.5 (Fig. 2A; Table 2), and using the nuclear pedigree produced 36 regions with LOD score >0.5 (Fig. 2B; Table 3), allowing us to exclude 98 and 74% of the autosome, respectively.
Exome variant analysis reveals a mutation in the PRIMA1 gene

We generated high coverage exomes for all four family members (Table 4). Exome variants under the linkage peaks were filtered as described in the Methods section where one homozygous recessive and twelve compound heterozygous variants in one and six genes, respectively, were identified that passed filtering (Tables 5, 6). The homozygous variant on chromosome 14 in the PRIMA1 gene is a novel splice site mutation (c.93+2T>C) that substitutes the invariant ‘T’ allele of the 5’ splice site of the intron following exon 2 (the first coding exon) for a ‘C’ (Fig. 2C). This mutation is predicted to cause skipping of exon 2 during PRIMA1 pre-mRNA splicing as supported by splice prediction software (Analzyer Splice Site Tool, Tel Aviv University, Israel) indicating the splicing machinery will fail to recognize the mutant splice site. Since individual I:2 was not exome sequenced, her c.93+2 genotype was determined by Sanger sequencing of genomic DNA derived from her breast tissue (Fig. 1). Although PRIMA1 lymphocyte expression was not reported in publicly available expression databases, we obtained a fresh blood sample from affected (II:3) and unaffected (II:1) sibs and generated lymphoblastoid cell lines (LCLs) to check for mRNA expression. However, PRIMA1 expression was not detected in cell lines from either family member (data not shown).

Table 2. Chromosomal regions with LOD > 1.5 identified by homozygosity mapping.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Flanking genetic markers</th>
<th>Begin of region</th>
<th>End of region</th>
<th>Peak LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chr Marker</td>
<td>Base pairs</td>
<td>cM</td>
<td>Chr Marker</td>
</tr>
<tr>
<td>7</td>
<td>rs17241389</td>
<td>83,764,309</td>
<td>101.32</td>
<td>rs2106432</td>
</tr>
<tr>
<td>8</td>
<td>rs3935174</td>
<td>131,165,086</td>
<td>144.37</td>
<td>rs2292781</td>
</tr>
<tr>
<td>13</td>
<td>rs7997193</td>
<td>56,574,363</td>
<td>55.7</td>
<td>rs9541675</td>
</tr>
<tr>
<td>14</td>
<td>rs3818263</td>
<td>92,588,002</td>
<td>90.12</td>
<td>rs1047351</td>
</tr>
</tbody>
</table>

chr, chromosome; cM, centimorgan.
None of the six genes containing compound heterozygous variants (Table 6) are convincing candidates. For **LOC120824**, nothing is known while little functional information is available for **DNHD1**, other than it encodes the dynein heavy chain domain 1 that is predicted to be a chaperone or mitotic protein,28 and **CLEC16A**, other than it is a susceptibility gene for diabetes, multiple sclerosis, and immunoglobulin A deficiency.29 **ABCA8** is a xenobiotic transporter gene of the ABC subfamily that transport foreign chemicals, such as antibiotics, across cellular membranes by ATP hydrolysis.30 **PPL** encodes periplakin that is expressed in brain but its known function is to associate with desmosomal plaques and keratin filaments in the epidermis, and mice null for the gene are phenotypically normal.31 A tumor suppressor gene, **LLGL1** regulates basal protein targeting in both embryonic and larval Drosophila neuroblasts,32 and newborn homozyous knockout Lgl1 pups develop severe hydrocephalus and die neonatally.33

**Confirmation of PRIMA1 exon skipping in vitro**

Since **PRIMA1** was not expressed in lymphocytes and we did not have access to other tissues from family members, we examined the effect of the mutation using a minigene
PRIMA1 Mutation Causes Epilepsy

Table 4. Exome coverage statistics.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total reads</th>
<th>Mean read depth</th>
<th>% bases covered by 2 reads</th>
<th>% bases covered by 5 reads</th>
<th>% bases covered by 10 reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>I:1</td>
<td>2278520917</td>
<td>44</td>
<td>94</td>
<td>91</td>
<td>85</td>
</tr>
<tr>
<td>II:1</td>
<td>3801912357</td>
<td>73</td>
<td>96</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>II:2</td>
<td>1802808122</td>
<td>35</td>
<td>94</td>
<td>89</td>
<td>81</td>
</tr>
<tr>
<td>II:3</td>
<td>5292190911</td>
<td>102</td>
<td>96</td>
<td>94</td>
<td>92</td>
</tr>
</tbody>
</table>

Table 5. Filtered homozygous recessive exome variants under linkage peaks.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Genomic site</th>
<th>Variant</th>
<th>Gene</th>
<th>Amino acid</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>94,253,970</td>
<td>T&gt;C</td>
<td>PRIMA1</td>
<td>–</td>
<td>Splice site</td>
</tr>
</tbody>
</table>

Table 6. Filtered compound heterozygous exome variants under linkage peaks.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Genomic site</th>
<th>Variant</th>
<th>Gene</th>
<th>Amino acid</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>6,519,561</td>
<td>A&gt;G</td>
<td>DNHD1</td>
<td>Q&gt;R</td>
<td>Nonsynonymous SNV</td>
</tr>
<tr>
<td>11</td>
<td>6,568,866</td>
<td>C&gt;T</td>
<td>DNHD1</td>
<td>R&gt;C</td>
<td>Nonsynonymous SNV</td>
</tr>
<tr>
<td>11</td>
<td>48,997,336</td>
<td>C&gt;T</td>
<td>LOC120824</td>
<td>C&gt;Y</td>
<td>Nonsynonymous SNV</td>
</tr>
<tr>
<td>11</td>
<td>49,003,195</td>
<td>C&gt;A</td>
<td>LOC120824</td>
<td>C&gt;F</td>
<td>Nonsynonymous SNV</td>
</tr>
<tr>
<td>16</td>
<td>4,934,157</td>
<td>- &gt; CCACCTTTCCTTGACCT</td>
<td>PPL</td>
<td>V&gt; EVKEKVV</td>
<td>Nonframeshift insertion</td>
</tr>
<tr>
<td>16</td>
<td>4,945,400</td>
<td>CTC&gt;-</td>
<td>PPL</td>
<td>E&gt;-</td>
<td>Nonframeshift deletion</td>
</tr>
<tr>
<td>16</td>
<td>11,097,073</td>
<td>G&gt;C</td>
<td>CLEC16A</td>
<td>G&gt;A</td>
<td>Nonsynonymous SNV</td>
</tr>
<tr>
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<td>11,272,419</td>
<td>C&gt;A</td>
<td>CLEC16A</td>
<td>P&gt;T</td>
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<td>18,129,027</td>
<td>G&gt;A</td>
<td>LLGL1</td>
<td>A&gt;T</td>
<td>Nonsynonymous SNV</td>
</tr>
<tr>
<td>17</td>
<td>18,133,277</td>
<td>A&gt;C</td>
<td>LLGL1</td>
<td>N&gt;T</td>
<td>Nonsynonymous SNV</td>
</tr>
<tr>
<td>17</td>
<td>66,879,927</td>
<td>C&gt;T</td>
<td>ABCA8</td>
<td>–</td>
<td>Splice site</td>
</tr>
<tr>
<td>17</td>
<td>66,914,289</td>
<td>G&gt;C</td>
<td>ABCA8</td>
<td>F&gt;R</td>
<td>Nonsynonymous SNV</td>
</tr>
</tbody>
</table>

assay. PRIMA1 DNA fragments containing exon 2 (124 bp) with flanking intronic sequences (wild-type and c.93+2T>C) were amplified from the genomic DNA of unaffected and affected family members, respectively, and cloned into a splicing-competent minigene vector. HEK293T cells were transfected with the minigenes and the vector, and incubated for 24 or 48 h. Cellular RNA was then extracted and the splicing of minigene-derived RNA was analyzed by RT-PCR using PCR primers binding to the 5’ and 3’ exons of the vector. The results revealed incorporation of exon 2 in RNA produced by the wild-type minigene and skipping of exon 2 in RNA produced by the mutant minigene (Fig. 3).

Re-sequencing of PRIMA1 in additional cases

We designed oligonucleotides (Table 1) to screen the entire coding region and splice sites of the PRIMA1 gene. Three hundred unrelated patients diagnosed with NFLE, including 212 with Italian ancestry, were screened using these oligonucleotides by Sanger sequencing or as part of the exome sequencing studies. No potential recessive or compound heterozygous mutations were detected.

Discussion

Nocturnal frontal lobe epilepsy is an important epilepsy syndrome known to be inherited in an autosomal dominant manner or to occur sporadically. Recessive inheritance has not been previously recognized. Using a whole exome and linkage analysis strategy, we discovered the cause for ARNFLE and intellectual disability in this family, being a mutation in the PRIMA1 gene.

Acetylcholinesterase (AChE) plays a pivotal role as a hydrolase in the central and peripheral nervous systems catalyzing the hydrolysis of acetylcholine (ACh) to maintain neurotransmitter homeostasis. Inhibition of AChE leads to enhanced cholinergic responses due to excess ACh over-stimulating nicotinic and muscarinic receptors.34 This can alter both central and peripheral processes, including control of respiration and seizure activity, autonomic, and somatic motor functions. In brain, AChE is found in its functional form as a tetramer associated with PRIMA1 at neuronal synapses. This association is facilitated by interaction between the tryptophan amphiphilic tetramerization domains on the catalytic subunits of AChE and the proline-rich attachment domain (PRAD) on the extracellular domain of PRIMA1.35

Perturbation of the cholinergic system has been linked to the pathogenesis of ADNFLE, specifically the increased sensitivity of nicotinic acetylcholine receptor (nAChR) subunits to ACh. Dominant mutations in three receptor subunits have been found in families segregating NFLE.5–7 This hypersensitivity was demonstrated for the α2 subunit in vitro by whole-cell recordings of HEK293 cells transfected with wild-type or mutant receptor.5 Loss
of PRIMA1 represents a new mechanism for over-stimulation of nAChRs via reduction in AChE activity leading to accumulation of ACh. A severe reduction in AChE has already been demonstrated in PRiMA knockout mice that lack the critical PRAD domain.34 In the striatum, chosen because it contains the highest level of AChE in the brain, PRiMA knockout mice only exhibit 2–3% of wild-type AChE activity despite having normal AChE mRNA levels. The residual AChE is localized to the endoplasmic reticulum. These data indicate that PRiMA is critical to intracellular processing of AChE, and targeting it to and stabilizing it at the axon. It is noteworthy that PRiMA is only required to anchor AChE at synaptic junctions in the brain, and not in the muscle, consistent with the phenotype observed in the family studied here.

The splice site mutation we found in PRIMA1 results in skipping of the first coding exon that contains the first 93 base pairs of coding sequence. While in-frame, this would result in loss of the first 31 amino acids of the PRIMA1 protein, including the methionine start site and most of the signal peptide. An alternative methionine start site is not present until position 137, close to the C-terminus of the 153 amino acid PRIMA1 protein. Since the affected siblings are homozygous for the mutation, the effect is predicted to be a complete knockout of PRIMA1 in their cells.

We describe ARNFLE, and document a more severe phenotype than usually observed in dominant cases that includes intellectual disability. Our genetic analysis reveals the first cause for this disease being mutation of PRIMA1. Whilst it would have been ideal to identify further independent subjects with mutated PRIMA1, analysis of 300 other unrelated cases was negative. However, the likely loss of function of PRIMA1 caused by this mutation and its biological plausibility because of its effect on the cholinergic system strongly suggest it is a novel, albeit rare, cause of NFLE. This further highlights the role of the cholinergic system in this characteristic nocturnal epilepsy syndrome.

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**Author Contributions**


**Conflict of Interest**

Authors report grant funds that contributed to this project as outlined in the Acknowledgements section. I. E. S. discloses payments from UCB Pharma, Athena Diagnostics and Transgenomics for lectures and educational presentations. S. F. B. discloses payments from UCB Pharma, Novartis Pharmaceuticals, Sanofi-Aventis, and Jansen Cilag for lectures and educational presentations, and a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies.

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