BRIEF REPORT

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PRRT2 Mutations Are the Major Cause of Benign Familial Infantile Seizures

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ABSTRACT: Mutations in *PRRT2* have been described in paroxysmal kinesigenic dyskinesia (PKD) and infantile convulsions with choreoathetosis (PKD with infantile seizures), and recently also in some families with benign familial infantile seizures (BFIS) alone. We analyzed *PRRT2* in 49 families and three sporadic cases with BFIS only of Italian, German, Turkish, and Japanese origin and identified the previously described mutation c.649dupC in an unstable series of nine cytosines to occur in 39 of our families and one sporadic case (77% of index cases). Furthermore, three novel mutations were found in three other families, whereas 17% of our index cases did not show *PRRT2* mutations, including a large family with late-onset BFIS and febrile seizures. Our study further establishes *PRRT2* as the major gene for BFIS alone.
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KEY WORDS: synaptic vesicle; epilepsy; choreoathetosis; *PRRT2*

Epileptic seizures and paroxysmal dyskinesias have overlapping clinical features and share a similar pathophysiological background. Idiopathic generalized epilepsy with paroxysmal nonkinesigenic dyskinesia (MIM# 118800) can be caused by mutations in the myofibrillogenesis regulator 1 gene (MIM# 600764) [Lee et al., 2004; Rainier et al., 2004] or in a Ca^{2+}-activated K^+ channel gene (*KCNMA1*; MIM# 600150) [Du et al., 2005]. Mutations in *SLC2A1* (MIM# 138140) encoding the glucose transporter type 1 may cause various forms of epilepsies and movement disorders including absence seizures and paroxysmal exercise-induced dyskinesia (MIM# 612126) [Striano et al., 2012; Suls et al., 2008, 2009; Weber et al., 2008b]. Similarly, benign familial infantile seizures (BFIS) and paroxysmal kinesigenic dyskinesia (PKD; MIM# 128200) can occur either alone or together (then called ICCA for infantile convulsions with choreoathetosis; MIM# 602066) in large pedigrees [Szepetowski et al, 1997]. Recently, mutations in the *PRRT2* gene

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on chromosome 16p11.2, encoding a membrane protein which has been shown to interact with the presynaptic protein SNAP-25, have been described by different independent groups in a broad spectrum of Asian, African, and Caucasian families with PKD alone or with ICCA [Chen et al., 2011; Lee et al., 2012; Li et al., 2012; Wang et al., 2011]. BFIS has been described as an isolated syndrome in many families [Callenbach et al., 2005; Caraballo et al., 2001; Fukuyama, 1963; Striano et al., 2006; Vigevano et al., 1992; Watanabe et al., 1987; Weber et al., 2004], and linkage has been found mainly to the same 16p12-q12 locus [Callenbach et al., 2005; Caraballo et al., 2001; Striano et al., 2006; Weber et al., 2004], or to chromosome 19q12-13.11 [Guipponi et al., 1997].

BFIS (MIM#s 601764, 605751) is characterized by complex partial and generalized tonic–clonic seizures occurring often in clusters between 3 and 12 months of age [Fukuyama, 1963; Vigevano et al., 1992; Watanabe et al., 1987], typically resolving after weeks to months. Two other syndromes share the clinical features of BFIS but have an earlier onset: benign familial neonatal and neonatal/infantile seizures (BFNS, MIM# 121200; BFNIS, MIM# 607745). All three syndromes usually have a good outcome with normal development and rare seizures in adulthood. BFNS and BFNIS are caused by mutations in the potassium channel genes *KCNQ2* (MIM# 602235) and *KCNQ3* (MIM# 602232) and the sodium channel gene *SCN2A* (MIM# 182390), respectively [for review, see Weber et al., 2008a]. Recently, 14 BFIS families of Australian and Asian origin were also described to carry a *PRRT2* mutation [Heron et al., 2012] (MIM# 614386). Here, we analyzed 49 BFIS families and three sporadic cases of a broad ancestry spectrum with a major European background mainly from Italy and Germany, confirming *PRRT2* as the major gene in BFIS.

The analyzed family members or their parents gave written informed consent. All procedures were in accordance with the declaration of Helsinki and approved by the local Ethical Committees. Clinical data were obtained from direct observation or medical charts. A cluster of seizures was defined as three or more seizures in 24 h.

Sequencing of the entire linked chromosome 16 region (22.5–62.5 Mbp) was performed using a custom on-array enrichment design (Roche NimbleGen, Mannheim, Germany) and next-generation sequencing on an IlluminaGAIIx sequencer (see Supp. Methods). All exons of the *PRRT2* (NM_145239.2) gene including the exon–intron boundaries were directly sequenced; polymerase chain reaction conditions and primers are provided in the Supp. Methods. Nucleotide numbering starts with A of the translation initiation codon ATG in the reference sequence (www.hgvs.org/mutnomen).

Forty-nine families of German, Italian, Japanese, and Turkish origin, in which 2–14 members were affected by BFIS alone (without any evidence for an accompanying paroxysmal movement disorder in the whole family; Supp. Fig. S1), and three sporadic cases were studied genetically. The clinical details have been partially published before [Striano et al., 2012; Weber et al., 2004, 2008a] and are summarized in Table 1 and Supp. Table S1. The patients show a very homogeneous clinical phenotype with an onset of seizures at 3–12 months of age in most cases, seizures in clusters in more than 40% of all cases, and a benign outcome in almost all cases without long-term anticonvulsive treatment.

For families BFIS1, BFIS9-11, BFIS13-14, BFIS19, BFIS29, and most Italian families, linkage to the chromosome 16 locus has been reported before [Striano et al., 2006; Weber et al., 2004, 2008a] and for BFIS35 linkage was shown later (unpublished). The other families were either too small for an informative analysis or linkage had not been performed so far. BFIS5 was the only family for which linkage to 16p12-q12 seemed to be excluded [Weber et al., 2004]. *SCN2A* was excluded in all families except BFIS39-43 [Striano et al., 2006 and unpublished] by direct sequencing.

To identify the underlying gene, we first designed a custom array to enrich genomic DNA of the whole linked region on chromosome 16p12-q12, excluding the centromeric gaps. DNA was enriched from two distant affected members each of the two largest linked families (BFIS10/BFIS35) and sequenced using a custom on-array enrichment design and Illumina paired-end sequencing. The mutation c.649dupC was not detected in our patients. Despite a robust coverage in this region (58x), several G/C homopolymers resulted in higher sequencing error rates and thus a relatively low base quality. Manual inspection allowed us to identify several reads with the C-insertion (7/40; 5/30; and 0/33, respectively), which have not been detected by standard parameters for samtools due to low base and mapping qualities. Moreover, we did not find other sequence variations in our analysis of exonic and intronic regions suspected to be disease relevant. After the gene had been published [Chen et al., 2011; Lee et al., 2012; Li et al., 2012; Wang et al., 2011], we directly sequenced *PRRT2* in all 49 BFIS families and the three sporadic cases. Mutations in *PRRT2* were detected in 42 families and one sporadic case, 40 of which showed the c.649dupC mutation which has been identified previously in PKD and ICCA [Chen et al., 2011; Lee et al., 2012; Li et al., 2012; Wang et al., 2011] and predicts a truncated protein (p.R217Pfs*8). Three novel mutations were identified in family BFIS19 (c.629delC/p.P210Qfs*19), BFIS30 (c.968G>A/p.G323E), and BFIS44 (c.291delC/p.N98Tfs*17) (Fig. 1). For p.G323E, which was not detected in the GenBank and the 1000 genome databases, a PolyPhen-2 analysis predicted a probable damaging effect of the mutation. All variants were submitted to the locus-specific database (http://www.lovd.nl/PRRT2).

Cosegregation was studied using previously determined haplotypes and direct sequencing (Supp. Fig. S1). All clearly affected individuals carried one of the mutations confirming their pathogenicity. Thirty asymptomatic healthy individuals also carried one of the *PRRT2* mutations revealing a reduced penetrance of 82%. In sporadic case BFIS17 and the family BFIS50, the c.649dupC mutation was not identified in the parental DNA, further strengthening the hypothesis that this mutation represents a mutational hotspot. The parental status in families BFIS17 and BFIS50 was proven using AmpFISTR Profiler plus kit (Applied Biosystems, Foster City, CA) in BFIS50 and in BFIS17 by STR markers (Promega, Madison, WI, PowerPlex 16). We did not have the possibility to test the parents for mosaicism.

We here describe *PRRT2* as the major disease gene for BFIS in a large cohort of mainly European descent. BFIS is a rare autosomal dominant epilepsy syndrome of childhood, which has been first mentioned in the literature almost 50 years ago [Fukuyama et al., 1963; Vigevano et al., 1992; Watanabe et al., 1987]. *PRRT2* has been recently discovered as the major gene for PKD and ICCA [Chen et al., 2011; Lee et al., 2012; Li et al., 2012; Wang et al., 2011], which share infantile seizures/convulsions as a clinical feature and are linked to the same region as BFIS. Very recently, mutations in 14 pure BFIS families were also described in Australian and Asian families [Heron et al., 2012]. Therefore, BFIS, PKD, and ICCA are allelic diseases and should be regarded as clinical variations of the same syndrome. We here examined 49 families of German, Italian, Turkish, and Japanese origin with BFIS and three sporadic cases without evidence for co-occurring PKD, many of them with positive linkage to the critical chromosome 16p12-q12 region [Striano et al., 2006; Weber et al., 2006, 2008a]. We have a long-term follow up of these families and most of the affected individuals have reached the age in which PKD usually develops, which distinguishes our families

Table 1. Overview of the Clinical Details of the Analyzed Families

Family code (origin)	Number of individuals	Age range (years)	Sex	Onset of seizures (m)	Type of seizures	Individuals with seizure clusters	Individuals with febrile seizures	Additional symptoms	*PRRT2* mutation
BFIS1 (G)	7	12–68	5F, 2M	6	GTCS	6	None	None	c.649dupC
BFIS5 (J)	4	5–46	4M	4–6	CPS, GTCS	3	None	None	c.649dupC
BFIS9 (J)	3	6m–26	3F	3–5	CPS, GTCS	3	None	None	c.649dupC
BFIS10 (G)	7	3–70	5F, 2M	4–6	GTCS	1	None	None	c.649dupC
BFIS11 (T)	6	3–30	3F, 3M	3–6	CPS, GTCS	4	1	None	c.649dupC
BFIS12 (G)	6	8–60	4F, 2M	3–16	CPS, GTCS	3	1	None	c.649dupC
BFIS13 (G)	4	17–79	3F, 1M	8	CPS	0	None	None	c.649dupC
BFIS14 (G)	6	4–65	3F, 3M	5–6	CPS, GTCS	3	None	None	c.649dupC
BFIS15 (G)	3	3–65	1F, 2M	4–6	CPS, GTCS	0	None	None	c.649dupC
BFIS17 (G)	1	8m	1M	8	CPS	0	None	None	c.649dupC
BFIS18 (G)	7	9–73	2F, 5M	3–7	AS, CPS, GTCS	0	1	M	c.649dupC
BFIS19 (G)	6	2–42	3F, 3M	4–6	CPS, GTCS	1	None	None	c.629delC
BFIS20 (G)	2	3m–NA	2F	7	CPS, GTCS	1	None	None	c.649dupC
BFIS21 (G)	1 (+1)	9	1F	4	CPS	1	None	None	c.649dupC
BFIS22 (G)	3	7–38	1F, 2M	6–8	CPS	1	None	None	c.649dupC
BFIS24 (G)	3	5m–30	3F	NA	NA	NA	NA	NA	c.649dupC
BFIS25 (G)	2	7m–Na	2M	7	CPS, GTCS	2	None	None	c.649dupC
BFIS26 (G)	1 (+1)	7	1F	5	CPS, GTCS	1	None	None	c.649dupC
BFIS27 (G)	1	3m	1M	3	NA	NA	NA	NA	None
BFIS28 (G)	8	2–56	5F, 3M	2–7y	CPS, GTCS	4	None	None	c.649dupC
BFIS29 (G)	8	3–45	7F, 1M	10–16	AS, CPS, GTCS	3	None	None	None
BFIS30 (G)	1 (+3)	1.5	1F	7	GTCS	1	None	None	c.968G>A
BFIS31 (G)	1	NA	NA	NA	NA	NA	NA	NA	None
BFIS32 (G)	1 (+1)	32	1F	12	NA	0	None	None	None
BFIS34 (G)	2	22–NA	2F	3	NA	NA	NA	NA	c.649dupC
BFIS35 (G)	13	6–75	9F, 4M	3–3y	CPS, GTCS	5	None	M	c.649dupC
BFIS36 (G)	2	11–32	2F	6–11	GTCS	2	None	M	c.649dupC
BFIS37 (G)	1 (+2)	7	1F	NA	GTCS	NA	None	None	c.649dupC
BFIS38 (G)	4 (+1)	10m–41	3F, 1M	3–9	GTCS	1	None	M	c.649dupC
BFIS39 (T)	14	NA	11F, 3M	7	CPS, GTCS	0	None	None	c.649dupC
BFIS41 (T)	2 (+4)	1–26	1F, 1M	11	CPS, GTCS	0	None	None	None
BFIS42 (G)	2	12–NA	2F	6	CPS, GTCS	1	None	None	c.649dupC
BFIS43 (G)	4	2–82	4F	5–9	CPS, GTCS	2	None	None	c.649dupC
BFIS44 (I)	3	6	1F, 2M	6	GTCS	0	None	None	c.291delC
BFIS45 (I)	4	3–71	2F, 2M	5–6	CPS, GTCS	3	None	None	c.649dupC
BFIS46 (I)	3	10–78	2F, 1M	4–5	CPS	2	None	None	c.649dupC
BFIS47 (I)	6	6–38	4F,2M	5–6	CPS, GTCS	3	None	None	c.649dupC
BFIS48 (I)	2	7	2M	4–5	CPS	2	None	None	c.649dupC
BFIS49 (I)	3	11–15	3M	6–8	CPS	1	None	None	c.649dupC
BFIS50 (I)	2	5–6	1F, 1M	5	CPS	1	None	None	c.649dupC
BFIS51 (I)	4	7–46	2F, 2M	4–6	CPS, GTCS	2	None	None	c.649dupC
BFIS52 (I)	2	9–37	2F	6–7	CPS	2	None	None	None
BFIS53 (I)	3	8–69	2F, 1M	7–NA	CPS	1	None	None	c.649dupC
BFIS54 (I)	2	4–35	1F, 1M	6–7	CPS, GTCS	1	None	None	c.649dupC
BFIS55 (I)	8	8–60	3F, 5M	5–6	CPS, GTCS	4	2	1 congenital hemiparesis	c.649dupC
BFIS56 (I)	3	5–35	1F, 2M	3–4	GTCS	0	None	None	None
BFIS57 (I)	5	13–91	5F	6–8	CPS, GTCS	1	None	None	c.649dupC
BFIS58 (I)	3	6–NA	2NA, 1M	7–NA	GTCS	0	None	None	None
BFIS59 (I)	6	8–92	5F, 1M	5–8	CPS, GTCS	1	None	None	c.649dupC
BFIS60 (I)	4	6–59	4M	4–6	CPS	2	1	None	None
BFIS61 (I)	2	7–12	2F	7–8	CPS	2	None	None	c.649dupC
BFIS62 (I)	4	3–32	2F, 2M	NA	NA	NA	None	None	c.649dupC

Individuals are denoted according to the pedigrees in the Supplementary material.
y, years; m, months; F, female; M, male; NA, not applicable; M, migraine; (G), German origin; (J), Japanese origin; (T), Turkish origin; (I), Italian origin; GTCS, generalized tonic clonic seizure; CPS, complex partial seizure; FS, febrile seizure; AS, atonic seizure.
GenBank RefSeq for *PRRT2*: NM_145239.2. The variants are described using the journal-approved nomenclature. Nucleotide numbering starts with A of the translation initiation codon ATG in the reference sequence.

as by far the largest cohort of "BFIS alone" from those that have been described previously.

PRRT2 mutations were found in 83% of the investigated cases. As in the other reports, the majority (up to 80% of all families) carried an insertion of an additional cytosine in a stretch of nine cytosines, without evidence for a founder effect. The c.649dupC mutation has thus to be arisen independently in different families of German, Italian, Japanese, and Turkish origin, also within the different countries. This suggests that the nine cytosines represent a highly unstable DNA sequence behaving as a mutational hotspot, frequently leading to an insertion of an additional cytosine. Moreover, the particular genomic structure there spoiled our next-generation sequencing approach probably because the relatively high Guanosine Cytosine content and a short palindromic motif greatly impaired efficient capturing of the mutant allele. To our knowledge, similar mutations have not been described up to now in autosomal dominant diseases but a highly variant poly-C stretch of mitochondrial DNA seems to be associated with different cancer forms [Hung et al., 2008]. Since

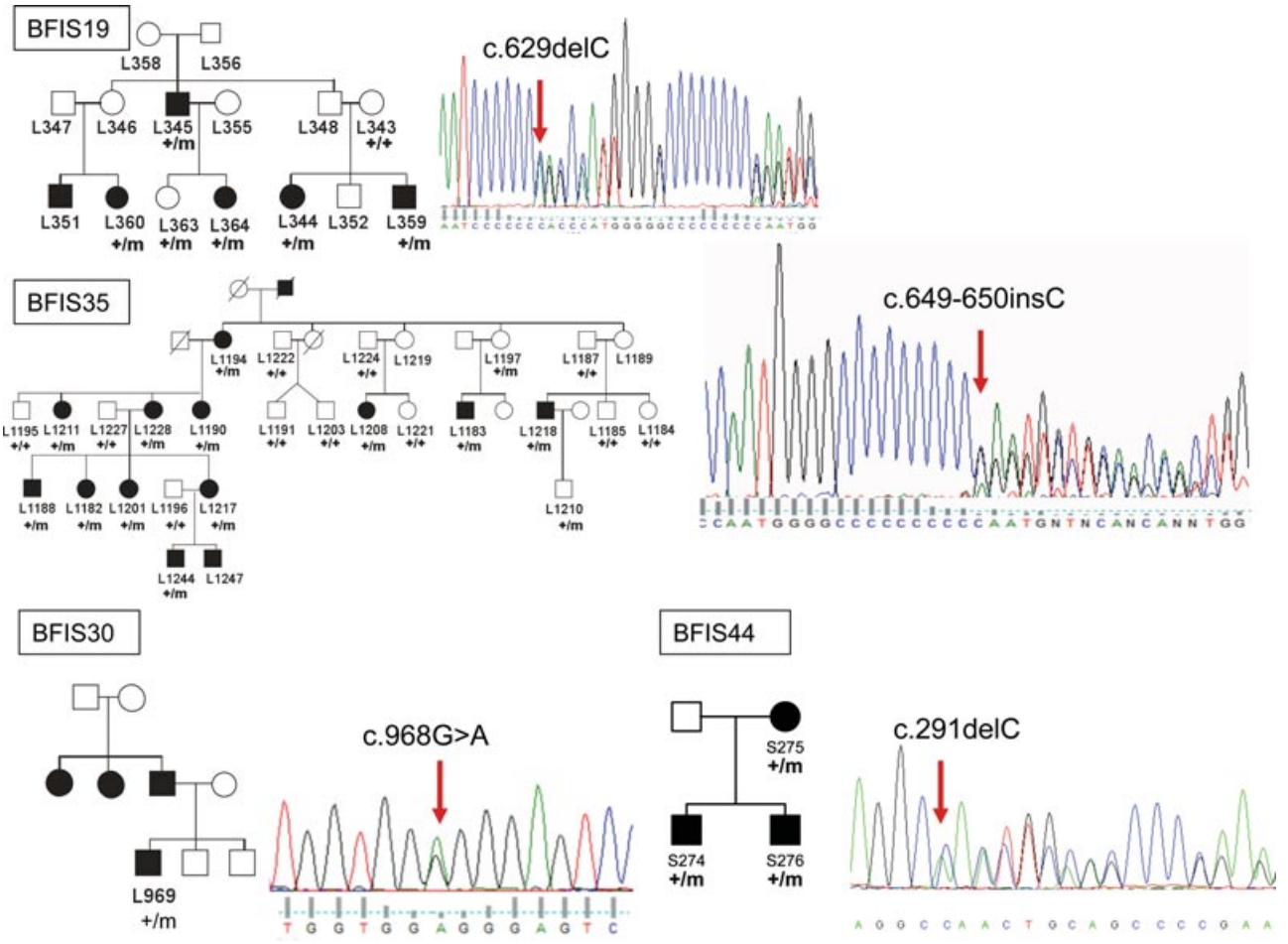

Figure 1. Cosegregation in selected families with *PRRT2* mutations and original sequences showing the detected mutations. The novel mutation c.629delC detected in BFIS19 was also found in the other affected family members and predicts a frame shift and stop codon (p.P210fsX18). The mutation c.649dupC indicating prolongation by one additional cytosine of an unstable series of nine cytosines, which predicts the premature truncation p.R217fsX8 and occurred in 40/52 index cases as described here in BFIS35. The novel point mutation found in BFIS30 predicting the amino acid exchange p.G323E. PolyPhen-2 analysis predicts a possible damaging effect in the resulting protein. The novel mutation c.291delC found in BFIS44 cosegregates with the phenotype in the family and predicts a frame shift and stop codon (p.N98TfsX17). Filled symbols denote affected and open symbols nonaffected family members; squares, males; circles, females; the numbers below the symbols indicate the sample numbers which are identical to those in Table 1 and Supp. Table S1; m, mutated allele; +, wild-type allele.

77% of our large sample of BFIS cases carries this mutation, direct sequencing of the cytosine stretch will be an easy, readily available, and cheap diagnostic genetic test, which can be performed in clinical practice with a high chance to detect the disease-causing mutation. Therefore, BFIS patients should be screened for the c.649dupC mutation first and, if negative, in the complete *PRRT2* gene. On the contrary, unclear epilepsy history demands the genetic analysis of a broader gene spectrum such as *SCN2A, KCNQ2*, and *KCNQ3*. As in other idiopathic epilepsies, an initial diagnostic using a gene panel can avoid multiple and cost intensive sequencing [Lemke et al., 2012].

In addition to this major mutation, we described three new mutations, which also predict deleterious effects on the PRRT2 protein. Allowing incomplete penetrance in several mutation carriers, all mutations cosegregated completely with the phenotype, leaving no doubt from a genetic point of view that these mutations are the monogenetic cause of the familial epilepsy syndrome in our pedigrees.

Nevertheless, BFIS seems to be heterogeneous, since 9/52 families and sporadic cases did not reveal *PRRT2* mutations. As BFNIS can be indistinguishable from BFIS in individual families [for review, see Weber and Lerche, 2008], *SCN2A* mutations could be one reason for the heterogeneity, which were however excluded in all but one family by direct sequencing. In one case (BFIS5), linkage seemed to be excluded to 16p12-q12 [Weber et al., 2004] but a *PRRT2* mutation was found now, probably since the closest microsatellite marker (D16S753) was uninformative and a double recombination occurred in this small family. Fifty-one of our 52 families and sporadic cases presented with typical BFIS, but one family had a different phenotype with late onset between 14 and 20 months of life and frequent febrile seizures. We previously found suggestive linkage to the same 16p12-q12 locus [Weber et al., 2008a], but *PRRT2* sequencing now proved to be negative, revealing that this slightly different syndrome does not belong to the same entity. On the contrary, two *PRRT2* positive patients showed a late onset of seizures: III.1 (BFIS28) and V.2 (BFIS35), both without clusters.

PRRT2 is predicted to be a membrane protein with two transmembrane domains. Interaction has been shown with the presynaptic protein SNAP25, which is known to play an important role in synaptic vesicle handling and neuronal exocytosis [Lee et al,

2012]. Previous reports have shown that truncated PRRT2 proteins do not reach the membrane in heterologous and neuronal expression systems maybe based on rapid degradation [Chen et al., 2011; Lee et al., 2012]. A disturbance of synaptic vesicle function could lead to changes in transmitter release and well explain the occurrence of epileptic seizures, but the exact mechanism still has to be determined.

It has to be assessed how deleterious mutations within the same gene, even the same mutation, cause PKD, ICCA, or BFIS in different families. Possible reasons include differences in the genetic background or methylation procedures during aging, as PKD develops much later in life than infantile convulsions. The age-dependent occurrence of seizures with remission after weeks to months also remains elusive for now. Age-dependent expression of the PRRT2 protein in neuronal compartments, as has been observed in BFNIS for the mutated Na^+ channel $Na_V1.2$. [Liao et al., 2010], could be an explanation.

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