

Two Novel *KCNQ2* Mutations in 2 Families With Benign Familial Neonatal Convulsions

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Abstract

Benign familial neonatal convulsion is a rare autosomal dominant inherited epilepsy syndrome characterized by unprovoked seizures in the first few days of life, normal psychomotor development, and a positive intergenerational family history of neonatal seizures. Over 90% of the affected individuals have inherited causal mutations in *KCNQ2*, which encodes for the potassium voltage-gated channel subfamily Q, member 2. Mutations in *KCNQ2* are also associated with a severe neonatal encephalopathy phenotype associated with poor seizure control and neurodevelopmental deficits. The authors report the clinical presentations, response to medication, and intrafamilial phenotypic variability in 2 families with benign familial neonatal convulsions, carrying previously unreported heterozygous missense mutations, c.1066C>G (p.Leu356Val) and c.1721G<A (p.Gly574Asp), in *KCNQ2*. The cases reported herein suggest that inherited missense mutations in *KCNQ2* can be associated with an intermediate phenotype and illustrate the challenges associated with prognosis and counselling for individuals with inherited missense mutations in *KCNQ2*.

Keywords

benign familial neonatal convulsions, BFNC, *KCNQ2*

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Neonatal seizures are defined as those seizures occurring within the first 28 days of life. Identification of the underlying etiology is critical as it has great implications for patient management, family counselling, and prognosis. Benign familial neonatal convulsion (BFNC, online mendelian inheritance in man [OMIM] #121200) is an autosomal dominant disorder with high penetrance that is characterized by unprovoked seizures occurring in the first few days of life, later normal psychomotor development, and a positive family history of neonatal seizures.^{1,2} Mutations within the *KCNQ2* gene (OMIM *602235) are responsible for approximately 90% of cases, with more than 70 mutations reported in this gene to date.³⁻⁵ The BFNC generally carries a favorable prognosis; however, approximately 7% of affected individuals will have mild neurological deficits (mainly mild learning disability and cognitive impairment) and 10% to 15% of affected individuals will have a recurrence of their seizures at a later time.¹ The authors hereby report 2 families with previously unreported missense mutations in *KCNQ2* and expand the phenotype associated with *KCNQ2* mutations.

Case Report

Proband A. The patient is currently a 3-year-old boy born at term by spontaneous vaginal delivery to a 35-year-old primigravida. The pregnancy was uncomplicated, and delivery was vaginal at term with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. On the second day of life, the patient presented with 3 brief episodes of multifocal seizures for which he was loaded with phenobarbital (20 mg/kg) and started on a maintenance dose of 5 mg/kg.

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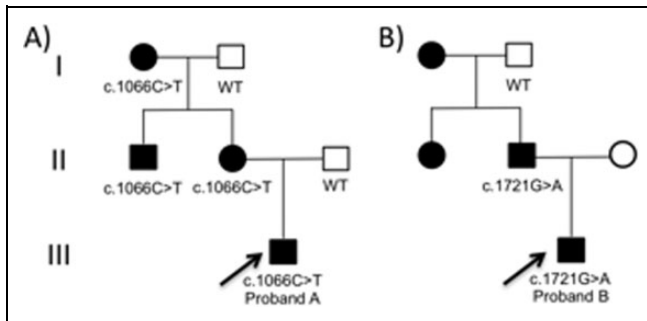


Figure 1. Pedigrees of probands A (A) and B (B) illustrating the affected family members with benign familial neonatal convulsion (BFNC) and segregation of the mutations.

General and neurological examinations were unremarkable apart from mild axial hypotonia. Initial workup, including a complete blood count, electrolytes, glucose, ammonia cerebrospinal fluid analysis, and culture, were unremarkable. Interictal electroencephalogram (EEG) and cerebral magnetic resonance imaging (MRI) were normal. The patient's seizures were poorly controlled, requiring the addition of levetiracetam at escalating doses. The EEG telemetry documented an electrographic seizure originating from the right frontal head region with secondary generalization. Seizures were controlled by day of life 8, and the patient was discharged on levetiracetam only (60 mg/kg).

In the following year, he presented again with breakthrough seizures on 7 occasions. Topiramate (up to 6mg/kg) and clobazam (1 mg/kg) were added for short periods but were ineffective. It was only after phenobarbital was resumed that good seizure control was achieved. Interictal EEGs remained normal, and he is currently seizure free since age 18 months. He has normal gross and fine motor development but moderate to severe expressive and receptive language delay. At the age 3 years, he can make 3-word sentences. He has some mild autistic traits with poor eye contact and unusual fixations and obsessions. His neurological examination is normal. His current medications are levetiracetam (40 mg/kg) and phenobarbital (5 mg/kg).

His family history revealed that individuals from 3 generations were affected with neonatal seizures (Figure 1A). His mother had only neonatal seizures and was not on any antiepileptic medication; his maternal uncle had neonatal seizures that resolved by a few months of life, but he later developed generalized seizures in adolescence for which he was treated with anticonvulsants for 2 years; and his maternal grandmother had neonatal seizures only and didn't require antiepileptic medication. All affected members had normal development and good cognitive outcome.

Sequencing of *KCNQ2* identified a heterozygous missense variant (NM_172107.2, c.1066C>G [p.Leu356Val]). This variant is rare as it is absent in large public databases (Exome Variant Server [EVS] and Broad Institute Exome Aggregation Consortium [ExAC]) and predicted damaging by in silico programs (PolyPhen 2, score = 0.994 and SIFT, score = 0.00). It affects a well-conserved amino acid located in the cytoplasmic carboxyl tail. The cytoplasmic C-tail is important for channel

assembly, gating, regulation, and formation of complexes with various regulating factors.⁷ Segregation studies revealed that this variant was present in the other affected family members (mother, uncle, and maternal grandmother). Thus, this variant is considered pathogenic and responsible for the patient's phenotype.

Proband B. The patient is currently a 3-year-old female infant who was born to a primigravida mother followed for aplastic anemia for 4 years prior to conception; pregnancy was complicated by anemia and thrombocytopenia, requiring blood and platelet transfusions and intravenous immunoglobulin infusion as well. The patient was born at term, by vaginal delivery with Apgar scores of 9 and 10 at 1 and 5 minutes, respectively. She was discharged home on the second day of life, with no complications. On the third day of life, she presented with 7 brief episodes of multifocal seizures. The patient was loaded with phenobarbital (20 mg/kg) and started on a maintenance dose (5 mg/kg). The patient developed mild respiratory depression likely secondary to the phenobarbital and thus was switched to topiramate (4 mg/kg), with no further seizure recurrence. General and neurological examinations were unremarkable. Initial workup, including a complete blood count, electrolytes, glucose, venous blood gas, ammonia, cerebrospinal fluid analysis, and culture, were unremarkable. The EEG was normal and MRI of the brain at 1 week of life showed a small periventricular venous infarct and residual small subdural hematoma, which could have possibly explained the seizures. Topiramate was slowly weaned off at the age 9 months and remained seizure free.

The patient's early gross and fine motor development milestones were normal. However, she had moderate expressive language delay and mild receptive delay for which she received speech therapy. At the age 3 years, she made 2- to 3-word sentences but had difficulty expressing her needs and understanding complex instructions. Occupational therapy assessment at that time concluded that her play was immature for her age and that she had moderate fine motor delays.

Family history revealed individuals spanning 3 generations who had neonatal seizures (Figure 1B). Her father had neonatal seizures treated with anticonvulsants for the first 3 months of life. A paternal aunt had seizures in the neonatal and childhood epochs, which required treatment with anticonvulsants until the age 18 years. Finally, a paternal grandmother had neonatal seizures that were only treated for the first few months of life with no recurrence. All affected family members had a normal psychomotor development. Sequencing of *KCNQ2* identified a novel missense variant (c.1721 G>A [p.Gly574Asp]). This variant is extremely rare as it is absent in EVS and ExAC. In addition, it is predicted damaging by both SIFT (score = 0.00) and PolyPhen-2 (score = 1.00) in silico programs. Father harbored the same *KCNQ2* variant.

Discussion

The authors hereby report on 2 novel missense mutations in *KCNQ2*, c.1066C>G (p.Leu356Val) and c.1721G>A

(p.Gly574Asp) and describe the associated clinical outcome of affected individuals. *KCNQ2* encodes for the voltage-gated potassium channel Kv7.2 and combines with Kv7.3, encoded by *KCNQ3*, to form heteromultimeric channels that mediate the muscarinic-regulated potassium current (M-current).⁵ The M-current is a slowly activating and deactivating K⁺ current that regulates neuronal excitability by affecting the subthreshold range of action potential generation.^{8,9} The BFNC due to mutations in *KCNQ2* is predominantly associated with a favorable outcome, with remission of neonatal seizures and a good response to anticonvulsants when used.⁸ Inherited mutations associated with BFNC are mainly frameshift or nonsense mutations, though missense mutations are also reported.^{6,7} The BFNC-associated *KCNQ2* mutations result in either a loss of function or hypomorphic alleles, resulting in a 20% to 40% reduction in the current across the *KCNQ2/KCNQ3* heteromultimeric channel sufficient to cause electrical hyperexcitability.¹⁰ *KCNQ2* mutations associated with BFNC do not result in a dominant-negative effect. In contrast, all reported patients with the *KCNQ2*-associated severe neonatal epileptic encephalopathy phenotype have de novo missense mutations.^{3,11,12} These missense mutations have been shown to confer a dominant negative effect by stabilizing the activated state of the channel.^{13,14}

Both our patients, who harbored missense mutations in *KCNQ2*, had clinical courses that were less benign than is usually predicted with BFNC: proband A required the use of multiple anticonvulsants for control of his seizures, and both probands had delays in their development. The reported cases herein suggest that missense mutations in *KCNQ2*, although inherited, can carry a more guarded prognosis. Similarly, other reports have also documented a poorer prognosis with inherited missense *KCNQ2* mutations.^{15,16} Soldovieri et al¹⁵ report on 16 *KCNQ2* BFNC families, 8 with heterozygous missense mutations and 8 with heterozygous loss-of-function mutations (such as nonsense and frameshift mutations and large deletions encompassing *KCNQ2*). Interestingly, all 7 families without persistent or drug-resistant seizures had heterozygous loss-of-function mutations. Though precise genotype–phenotype correlations are difficult, in the phenotypic spectrum associated with *KCNQ2* mutations, inherited heterozygous loss-of-function mutations are usually associated with the more benign phenotype of BFNC, and the de novo missense mutations are usually associated with the severe epileptic encephalopathy and developmental delay. The impact of inherited *KCNQ2* missense mutations is more difficult to predict and can be associated with a more variable phenotype, which can include persistent seizures and neurodevelopmental deficits.

Though *KCNQ2* mutations in BFNC are associated with a relatively homogeneous phenotype within an affected family, interfamilial variability has been previously reported.^{15,17,18} The family history of proband A illustrates the possible significant intrafamilial variability in the severity of the clinical phenotype. Indeed, the proband's mother had only a few seizures in the neonatal period without any recurrence, whereas the uncle continued to have yearly seizures even in adulthood. It is interesting to note that the more severely affected individuals were

male. Male gender has not been previously associated with a worse outcome in BFNC. The reasons for the significant intrafamilial variability are unclear, and the effect of other genetic modifiers or environmental factors can possibly play a role.

The observation that the seizures in proband A responded better to phenobarbital than levetiracetam, topiramate, or clobazam is interesting. There are no evidence-based guidelines for the pharmacologic treatment of neonatal seizures. Phenobarbital is the first-line treatment in BFNC and other neonatal seizures based on expert consensus.^{19,20} However, newer anticonvulsants such as levetiracetam and topiramate are increasingly used as the first-line treatment despite lack of efficacy data because these agents do not appear to have phenobarbital's neurotoxic and proapoptotic effects.^{21–23} The case described here raises the question as to whether seizures in BFNC can respond better to phenobarbital. Further studies are required to address optimal BFNC treatment. The efficacy of the *KCNQ2* channel openers, retigabine and ezogabine, has been explored. Animal studies demonstrated that retigabine attenuated seizures in *KCNQ2* knock-in mice more markedly than phenobarbital.²⁴ Ezogabine treatment in 11 infants with *KCNQ2* encephalopathy was well tolerated and showed a potential benefit against refractory epilepsy.²⁵

Mutations in *KCNQ2* are associated with a wide spectrum of phenotypic severity. Some generalities can be made in terms of genotype/phenotype correlation. On one end, inherited truncating mutations usually result in BFNC, and on the opposite end, de novo missense mutations usually result in a severe neonatal encephalopathy. Prognosis and counseling for individuals with inherited missense mutations are more challenging, especially given the significant intrafamilial variability of severity of the phenotype. The cases reported herein suggest that inherited missense mutations in *KCNQ2* can be associated with an intermediate phenotype.

Author Contributions

MSr and GAY contributed to conception and design; acquisition, analysis, and interpretation of data; drafted the manuscript; critically revised manuscript; gave final approval; and agree to be accountable for all aspects of work ensuring integrity and accuracy. MSh contributed to conception; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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