De novo mutation in 2 patients with neonatal-onset epilepsy

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Abstract: OBJECTIVE We describe 2 additional patients with early-onset epilepsy with a de novo mutation. METHODS Whole-exome sequencing was performed in 2 unrelated patients with early-onset epilepsy and their unaffected parents. Genetic variants were assessed by comparative trio analysis. Clinical evolution, EEG, and neuroimaging are described. The phenotype and response to treatment was reviewed and compared to affected siblings in the original report. RESULTS We identified the same de novo mutation reported previously (c.G155A, p.R52H) in 2 additional patients with early-onset epilepsy. Similar to the original brothers described, both presented with tonic seizures in the first month of life. In the first patient, seizures responded to sodium channel blockers and her development was normal at 11 months. Patient 2 is a 15-year-old girl with treatment-resistant focal epilepsy, moderate intellectual disability, and autism. Carbamazepine (sodium channel blocker) was tried later in her course but not continued due to an allergic reaction. CONCLUSIONS The identification of a recurrent de novo mutation in 2 additional unrelated probands with early-onset epilepsy supports the role of p.R52H in disease pathogenesis. Affected carriers presented with similar early clinical phenotypes; however, this report expands the phenotype associated with this mutation which contrasts with the progressive course and early mortality of the siblings in the original report.

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De novo *FGF12* mutation in 2 patients with neonatal-onset epilepsy

**ABSTRACT**

**Objective:** We describe 2 additional patients with early-onset epilepsy with a de novo *FGF12* mutation.

**Methods:** Whole-exome sequencing was performed in 2 unrelated patients with early-onset epilepsy and their unaffected parents. Genetic variants were assessed by comparative trio analysis. Clinical evolution, EEG, and neuroimaging are described. The phenotype and response to treatment was reviewed and compared to affected siblings in the original report.

**Results:** We identified the same *FGF12* de novo mutation reported previously (c.G155A, p.R52H) in 2 additional patients with early-onset epilepsy. Similar to the original brothers described, both presented with tonic seizures in the first month of life. In the first patient, seizures responded to sodium channel blockers and her development was normal at 11 months. Patient 2 is a 15-year-old girl with treatment-resistant focal epilepsy, moderate intellectual disability, and autism. Carbamazepine (sodium channel blocker) was tried later in her course but not continued due to an allergic reaction.

**Conclusions:** The identification of a recurrent de novo mutation in 2 additional unrelated probands with early-onset epilepsy supports the role of *FGF12* p.R52H in disease pathogenesis. Affected carriers presented with similar early clinical phenotypes; however, this report expands the phenotype associated with this mutation which contrasts with the progressive course and early mortality of the siblings in the original report.

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**GLOSSARY**

**EOEE** = early-onset epileptic encephalopathy; **WES** = whole-exome sequencing.

High-throughput sequencing technology, including whole-exome sequencing (WES), has revolutionized gene discovery in epilepsy. It has also demonstrated the significant genetic heterogeneity of epilepsy and the importance of de novo mutations in the epileptic encephalopathies, which are the most severe forms of epilepsy. Recently, WES in a family quintet with 2 affected siblings with early-onset epileptic encephalopathy (EOEE) and cerebellar atrophy identified fibroblast growth factor 12 (*FGF12*, also known as *FHF1*) as a putative gene responsible. A de novo gain-of-function variant (p.R52H) was found in 2 affected siblings with lethal disease. *FGF12* encodes a cytosolic protein that interacts with neuronal sodium channels and increases their voltage-dependent fast inactivation. Here, we performed WES-trio analysis and identified the same de novo missense mutation in 2 patients with severe neonatal-onset epilepsy and variable clinical outcomes.

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Family trio analysis identified the same missense mutation in both patients, identical to the variant reported previously. The mutation is a de novo heterozygous G>A transition at position 155 of the complementary DNA (NM_004113) which results in a p.R52H missense substitution. Sanger sequencing was performed in both trios and confirmed that the mutation was only present in the affected probands. No other pathogenic mutations in known epilepsy genes were present.

Clinical phenotypes. Patient 1 is a developmentally normal 11-month-old baby, born at term after an uncomplicated pregnancy. She presented on day 3 of life with a 2-minute seizure involving extension of all limbs, head, and eye deviation to the right side and apnea with desaturation. On day 3, the interictal EEG showed multifocal spikes. During continuous video-EEG monitoring on day 8, multiple independent left and right frontal seizures were recorded. Seizures occurred from sleep, and 1 typical seizure with corresponding EEG is shown in the video at Neurology.org/ng and in the figure. Seizures continued despite treatment with phenobarbital, levetiracetam, topiramate, and a midazolam infusion. She required intubation and ventilation for 3 days. Her seizures stopped on day 20 of age after the addition of phenytoin. She had 1–3 seizures per month on phenytoin and topiramate. Carbamazepine was added to topiramate at 6 weeks of age, and she has been seizure free since 5 months of age on carbamazepine (30 mg/kg/d). Her initial neurologic examination showed mild hypotonia which later resolved. Her development remains normal. Her head circumference remains at the 85th percentile, and her EEG performed at 10 months of age was normal. Extensive neurometabolic investigations and a head MRI performed at day 4 of life were unremarkable.

Patient 2 is a 15-year-old girl, born at term following an uncomplicated pregnancy and delivery. She presented on day 2 of life with tonic seizures characterized by generalized stiffening which evolved to intermittent stiffening and twitching of the limbs. On day 11, her EEG demonstrated suppression of the background and multifocal spikes. Seizures did not respond to phenobarbital, clonazepam, topiramate, and lamotrigine. At 4 years of age, she developed focal seizures with impaired awareness which could evolve to bilateral tonic-clonic seizures. The interictal EEG showed mild background slowing and left frontotemporal spikes. Carbamazepine was tried at 4 years of age; however, it was stopped immediately following an allergic reaction. Rufina-mide was added to lamotrigine at the age of 8 years and at most recent follow-up, the patient has been seizure free for 1 year. Developmental delay was noted by 1 year of age, and her development regressed at times of increased seizure frequency. She currently has moderate intellectual disability and autism. MRI of the brain at 2 weeks and 2 years of age was normal except for an incidental Chiari 1 malformation.

RESULTS Family trio analysis identified the same FGF12 missense mutation in both patients, identical to the variant reported previously. The mutation is a de novo heterozygous G>A transition at position 155 of the complementary DNA (NM_004113) which results in a p.R52H missense substitution. Sanger sequencing was performed in both trios and confirmed that the mutation was only present in the affected probands. No other pathogenic mutations in known epilepsy genes were present.

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DISCUSSION In this report, we describe 2 additional patients with a de novo p.R52H mutation in FGF12 but with milder phenotypes. The same variant observed in our patients was recently described in 2 siblings with a lethal disorder characterized by EOEE and cerebellar atrophy. All 4 affected individuals presented with early-onset tonic seizures and most had seizures from sleep (table). Patient 1 does not have an epileptic encephalopathy given the cessation of seizures, normal development, and normal EEG. However, patient 2 and the original reported clinical features of the siblings are consistent with an epileptic encephalopathy. In all cases, seizures were refractory to multiple antiseizure medications. However, patient 1 had a good response to sodium channel blockers and has been seizure free since 5 months of age. In patient 2, carbamazepine resulted in an allergic reaction and phenytoin was not tried. Patient 2 is currently seizure free on a combination of lamotrigine and rufinamide, which is possibly due to the effects of these drugs on the sodium channel. The current neurologic examination and development are normal for patient 1. This contrasts with the report of the siblings, which indicates a neurodegenerative course associated with early death. The clinical course of patient 2
Seizure began with arousal from sleep and extension of all 4 limbs followed by clonic jerking of the right arm and bradycardia (see video). Ictal EEG demonstrated (A) generalized attenuation of EEG activity at seizure onset, followed by rhythmic spikes in left central area; (B) spread to right central area; (C) spread to bilateral central frontal parietal regions; and (D) postictal suppression.
also is not as severe as that of the siblings; however, her seizures were refractory to treatment except for the past year, and she did have developmental regression associated with increased seizure activity. She also has intellectual disability and autism. In the original report, progressive cerebellar atrophy was also a prominent feature of the disease phenotype which differs from the normal MRI seen in patient 1 and the incidental Chiari I malformation identified on MRI in patient 2. It is difficult to accurately compare MRI findings given the young age of patient 1; however, MRI of patient 2 showed no sign of cerebellar atrophy at age 2. The milder phenotype with normal clinical outcome seen in patient 1 may be influenced by the early seizure control. However, other genetic and/or environmental factors may also be playing a role. Although the young age of patient 1 limits a clear impression of the natural history of this disorder, there is a striking clinical difference between patient 1 normal clinical course to date and moderate to severe course of patient 2 and the lethal outcome of the previously described siblings. Early seizure control in patient 1 suggests

### Table: Clinical features of patients with de novo p.R52H FGF12 mutation

| Individuals | Patient 1 (this study) | Patient 2 (this study) | Patient 1 | Patient 2 |
|-------------|------------------------|------------------------|-----------|-----------|
| Sex         | Female                 | Female                 | Female    | Male      |
| Seizure onset | 3 d                   | 2 d                    | 14 d      | 28 d      |
| Age or age at deatha | 11 mo                 | 15 y                   | 7 y; status epilepticus | 3.5 y; unknown cause |
| Gestational age | Term                 | Term                   | Term (twin fetus died in utero) | NA |
| Diagnosis   | Early-onset tonic and focal seizures | EOEE | EOEE with cerebellar atrophy | EOEE with cerebellar atrophy |
| Development prior to seizure onset | Normal | Normal | Normal | Normal |
| Seizure type at onset | Tonic | Tonic | Tonic | Tonic |
| Course of seizures | Focal motor seizures; last seizure at 5 mo | Focal with impaired awareness, evolution to generalized tonic-clonic; seizure free for 1 y | Tonic, generalized, and focal | Tonic, generalized, and focal |
| Ictal EEG    | Generalized suppression, then rhythmic spikes in the left, then right central region | Generalized spike wave followed by diffuse depression or electrodecremental events | Low voltage fast activity, followed by long suppression of background | Low voltage fast activity, followed by long suppression of background |
| Interictal  | Multifocal spikes then normal | Suppression of background and multifocal spikes, mild background slowing, and left frontotemporal spikes | Background slowing, multifocal epileptic abnormalities, and later hypsarrhythmia | Background slowing, multifocal epileptic abnormalities, and later hypsarrhythmia |
| Timing of seizure occurrence | Sleep | Waking and sleep | Usually during sleep | Usually during sleep |
| Head circumference | Normal | Normal | Acquired microcephaly | Acquired microcephaly |
| Neurologic findings | Hypotonia which resolved | Normal | Axial hypotonia, ataxia (limbs), severe feeding difficulties | Hypotonia, ataxia, severe feeding difficulties |
| Response to treatment | Responded to phenytoin and carbamazepine. Failed phenobarbital, levetiracetam, topiramate, and midazolam infusion | Seizure reduction on rufinamide and lamotrigine. Failed phenobarbital, clonazepam, and topiramate; rash on carbamazepine | Treatment resistant | Treatment resistant |
| ID          | Normal                 | Moderate               | Profound  | Profound  |
| Development | Normal                 | Speech delay (20 words at 5 y), ambulatory, sensory issues, autism | Sit without support (24 mo), no walking or standing, no speech | NA |
| Visual abnormalities | None                   | Nystagmus, intermittent esotropia, moderately farsighted and wears corrective lenses | Cortical visual impairment | Poor visual contact |
| Funduscopy  | Normal                 | Normal                 | Normal initially, pale optic discs and narrow vessels at 5 y | NA |
| MRI (age)   | Normal (4 d)           | Normal (2 wk), Chiari I malformation (2 y) | Normal (5 mo), cerebellar atrophy (6 y) | Normal (2 mo), cerebellar atrophy (3 y) |

Abbreviations: EOEE = early-onset epileptic encephalopathy; ID = intellectual disability; NA = not available.
possible important treatment implications for this genetic disorder which could affect outcome.

During the preparation of this manuscript, we became aware of 3 additional patients with an epileptic encephalopathy carrying the same FGF12 de novo mutation. The identification of a recurrent de novo mutation in 6 unrelated patients all presenting with early-onset epilepsy strongly supports the involvement of FGF12 in disease pathogenesis. Moreover, the identification of additional patients carrying the same FGF12 variant broadens the phenotypic spectrum associated with this gene.

AUTHOR CONTRIBUTIONS
Dr. Ilaria Guella: conception and design of the study; analysis and interpretation of data; and drafting the manuscript. Dr. Linda Huo: clinical assessment and data acquisition for patient 1. Ms. Marna B. McKenzie: execution of the study; acquisition and analysis of genetic data for patient 1; and drafting the manuscript. Mr. Eric B. Toyota: data acquisition. Dr. E. Martina Bebin: recruitment and clinical characterization of patient 2 and collection of biological samples. Dr. Michelle L. Thompson: acquisition and analysis of genetic data for patient 2. Dr. Gregory M. Cooper: design of the study for patient 2 and guided implementation across all components. Mr. Daniel M. Evans: bioinformatics and data analysis. Dr. Sarah Buerki: data acquisition and revising manuscript. Ms. Shelin Adam: review of literature. Dr. Margot V. Van Allen and Dr. Tanya N. Nelson: review of literature and reviewing manuscript. Dr. Mary B. Connolly: review of patient 1 clinically; revising manuscript; and obtaining funding. Dr. Matthew J. Farrer: conception and design of the study; obtaining funding; and revising manuscript. Dr. Michelle Demos: conception and design of the study; review of patient 1 clinically; obtaining funding; and revising manuscript.

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DISCLOSURE
Dr. Ilaria Guella reports no disclosures. Dr. Linda Huo has served on the scientific advisory boards of the British Columbia Epilepsy Society and Matthew’s Friends (nonprofit). Ms. Marna B. McKenzie and Mr. Eric B. Toyota report no disclosures. Dr. E. Martina Bebin has served on scientific advisory boards for Novartis, GW Pharmaceuticals, FDA Orphan Drug Program, and NIH; has received gifts from Novartis and GW Pharmaceuticals; has received travel funding/speaker honoraria from GW Pharmaceuticals; has served on the editorial board of Pediatric Neurology; has been a consultant for and served on the speakers’ bureau of GW Pharmaceuticals; and has received research support from NIH. Dr. Michelle L. Thompson reports no disclosures. Dr. Gregory M. Cooper has served on the editorial board of PLoS Genetics Genome Research and has received research support from NIH. Mr. Daniel M. Evans, Dr. Sarah E. Buerki, Ms. Shelin Adam, and Dr. Margot V. Van Allen report no disclosures. Dr. Tanya N. Nelson has received research support from the BCCH Foundation and Genome BC. Dr. Mary B. Connolly has served on the editorial board of the Canadian Journal of Neurological Sciences and has served on speakers’ bureaus for Eisai and Bionex. Dr. Matthew J. Farrer has served on the scientific advisory boards of the Michael J. Fox Foundation, Parkinson’s Society Canada, EURAC, and Parkinson’s UK; has served on the editorial boards of Neurobiology of Disease and Parkinsonism & Related Disorders; holds the following patents: (1) International Publication Number WO 2006/045392 A2; (2) International Publication Number WO 2006/068492 A1; (3) US Patent Number 7,544,786; and (4) Norwegian patent 323175— provisionally filed in 2004–2005; has received research support from the Canadian Federal Government (CECRC, CFI, and CIHR) and the Cunhill Foundation; and has received royalty payments from Lundbeck Inc. and Merck for Lrk2 mouse models (2016), and from IJir Pharma. for SNCA mouse model (2015). Dr. Michelle Demos has received research support from the Rare Disease Foundation and the Alva Foundation. Go to Neurology.org/ng for full disclosure forms.

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REFERENCES
1. Epi4K Consortium, Epilepsy Phenome/Genome Project, Allen AS, et al. De novo mutations in epileptic encephalopathies. Nature 2013;501:217–221.
2. Siekierska A, Isrie M, Liu Y, et al. Gain-of-function FHF1 mutation causes early-onset epileptic encephalopathy with cerebellar atrophy. Neurology 2016;86: 2162–2170.
3. Goldfarb M, Schoorlemmer J, Williams A, et al. Fibroblast growth factor homologous factors control neuronal excitability through modulation of voltage-gated sodium channels. Neuron 2007;55:449–463.
4. Steele JC, Guella I, Snu-Tu C, et al. Defining neuredegeneration on Guam by targeted genomic sequencing. Ann Neurol 2015;77:458–468.
5. Sobreira N, Schiettatec F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat 2015;36:928–930.
6. Al-Mehmadi S, Split M, Venkataparan R, et al. The FHF1 (FGF12) epileptic encephalopathy. Neural Genet 2016. In press.
7. MacArthur DG, Manoliko TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. Nature 2014;508:469–476.
8. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–424.
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