Clinical/Scientific Notes

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PHENYTOIN-RESPONSIVE EPILEPTIC ENCEPHALOPATHY WITH A TANDEM DUPLICATION INVOLVING *FGF12*

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Nonsecreting fibroblast growth factors (FGFs), which are sodium channel–binding proteins, have recently been associated with neurodevelopmental disorders,[1,2] similar to some voltage-gated sodium channel subunits. Recently, a de novo mutation in *FGF12* (p.R52H) was reported in a pair of siblings with epileptic encephalopathies.[3] The affected siblings developed severe seizures within 1 month of age, and their seizures were refractory to multiple antiepileptic drugs. This mutation has a gain-of-function effect on sodium channel gating, which might lead to increased neuronal excitability. Here, we report another case of *FGF12*-related epileptic encephalopathy. In contrast to the previous report, the phenotype in our patient was relatively mild. Of note, his seizures responded to phenytoin, a sodium channel blocker, similar to epileptic encephalopathy associated with *SCN2A*[4] and *SCN8A*,[5] encoding voltage-gated sodium channel subunits.

The boy was born as the first child of a nonconsanguineous Japanese couple at term after an uncomplicated pregnancy with a birth weight of 3,100 g (−0.5 SD), a birth height of 48.2 cm (−1.2 SD), and a birth head circumference of 33.4 cm (−0.4 SD). The patient had been healthy with no seizures before 3 years of age. Fever due to viral infection triggered his first seizure, followed by daily seizures without fever. Three types of seizures, including tonic-clonic seizures, brief tonic seizures with vocalization, and complex partial seizures with repeated blinking, were observed. An EEG showed severe suppression of background activity with paroxysmal bursts (figure e-1B at Neurology.org/ng). His seizures were refractory to multiple antiepileptic drugs, including phenobarbital, clobazam, valproic acid, and potassium bromide, but responded to phenytoin add-on, which was started after 2 years of the onset of seizures. His seizures were completely controlled with phenytoin levels between 20 and 35 µg/mL (figure 1B). Brain MRI showed mild cerebral and cerebellar atrophy at the age of 8 years (figure 1A). Just after the onset of seizures, his development was halted, and he

developed ataxia and aphasia. These clinical symptoms were gradually exacerbated, although his seizures have been suppressed. He can walk, although ataxic, and take a liquid diet himself when his seizures are suppressed; when his seizures are not suppressed, he becomes bedridden with severe feeding difficulties due to dysphagia necessitating tube feeding and presents with sleep disorder and dyspnea. At the last follow-up, he showed profound intellectual disability. He had a long face, bilateral epicanthal folds, long-slitted eyes, a broad nose, straight and broad eyebrows, and ptosis (figure e-1B).

Array comparative genome hybridization revealed a 0.58-Mb gain, arr[hg19] 3q28q29 (191876978_192454675) ×1 (figure 1C). Analysis of the parents confirmed that this 3-copy gain occurred de novo. The copy-number gain was confirmed by fluorescence in situ hybridization and genomic quantitative PCR analysis (figure e-2). Around the edges of the copy-number variation (CNV) gain, there were 2 L1 family long interspersed nuclear elements (LINEs) (L1PA2), suggesting that a tandem duplication occurred via these LINEs. In CNV-specific PCR, a positive PCR was obtained only from the patient's genomic DNA, which therefore confirmed both the existence of the duplication and its tandem arrangement (figure 1D). Sequencing of the PCR product determined the breakpoint regions with a 48-bp overlap (figure 1E). Target sequencing of epileptic encephalopathy–related genes did not detect any candidate mutations (appendix e-1).

Using CNV-specific real-time PCR, aberrant transcripts were detected only in the patient's hair follicle (figure e-3). Sequencing of the PCR products revealed aberrant transcripts with or without an inserted aberrant exon. Complementary DNA analysis showed biallelic expression of *FGF12* with abnormal transcripts (figure e-3). No difference in the expression of *FGF12* between the complementary DNAs of the patient and controls was detected (data not shown).

Because we did not investigate the aberrant protein expression from the duplicated allele, it remains unclear whether the phenotype of our patient occurred because of gain-of-function, haploinsufficiency, or dominant-negative effects of *FGF12*. It is possible that the CNV has gain-of-function effects,

Figure 1   Clinical data and genomic analysis
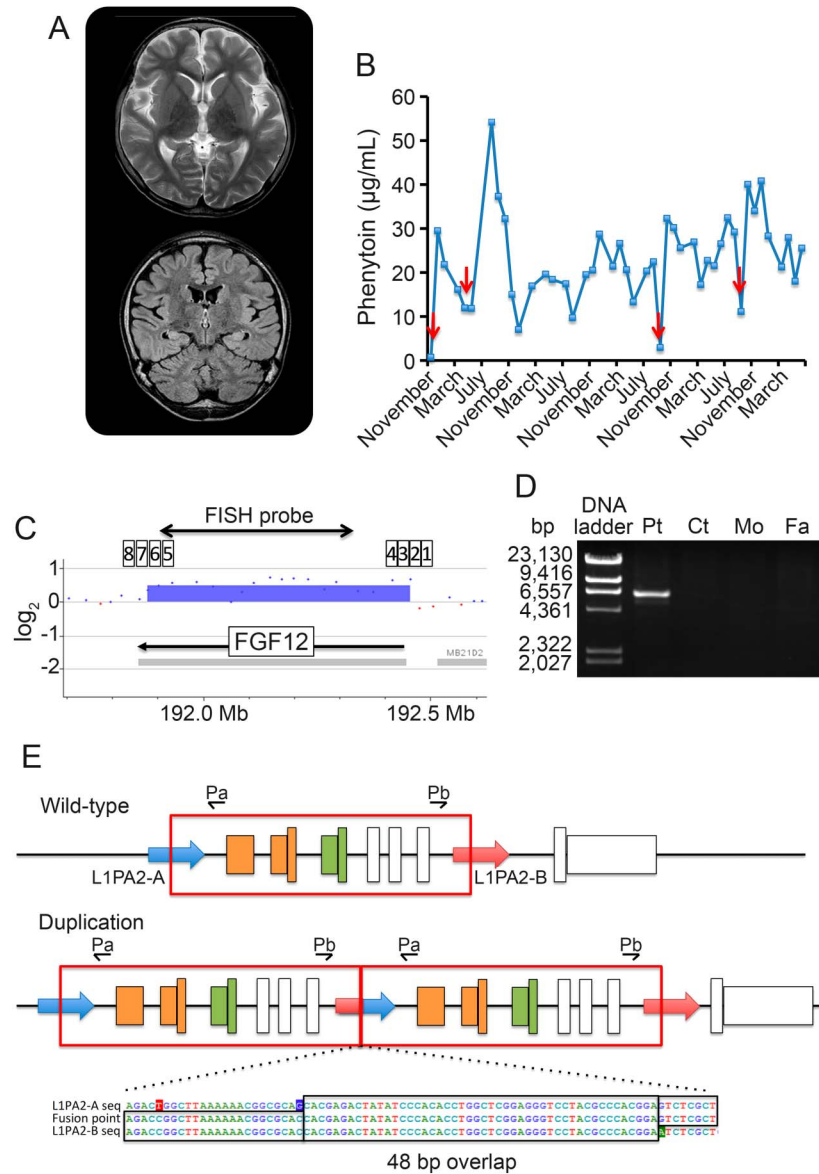
(A) Axial T2-weighted image (top) and fluid-attenuated inversion recovery (bottom) of the brain at age 8 years showed mild cerebral and cerebellar atrophy without signs of associated focal cortical dysplasia or postischemic injury. (B) Phenytoin levels of the patient. Red arrows indicate hospital admissions for increased seizure frequency. (C-E) Genomic analysis revealed a tandem duplication, involving *FGF12*, between 2 long interspersed nuclear elements (LINEs). (C) Array comparative genome hybridization showed a 0.6-Mb gain, which contains nearly the entire *FGF12* gene. A double-headed arrow indicates the position of the fluorescence in situ hybridization (FISH) probe for *FGF12* (see also figure e-2A). The numbers in boxes indicate the primer positions for quantitative PCR analysis (see also figure e-2B). (D) Copy-number variation (CNV)-specific PCR. A ~6-kb junction amplicon was generated from the patient but not from the control and his parents. (E) Schematic map showing the wild-type and duplication alleles. Boxes indicate exons of *FGF12*. Yellow, green, and white boxes indicate exons for *FGF12* isoform 2 (NM_004113.5), *FGF12* isoform 1 (NM_021032.4), and common exons, respectively. Blue and red arrows indicate LINEs. The breakpoints lie within 2 LINE L1PA2s occurring at chr3:192457003-192463031 and chr3:191867105-191873117 with a 48-bp overlap. Pa, Pb = primers for CNV-specific PCR (D). FGF = fibroblast growth factor.

similar to the previous patients. Similar to our patient, phenytoin was used also in most of the previous patients with the R52H mutation in *FGF12*,[6] suggesting that the same mechanism underlies the pathogenicity of the previous cases and our case. The aberrant proteins from the duplicated allele in our patient are predicted to have duplicated sodium channel–binding sites.[7] By contrast, some in silico data indicate that haploinsufficiency of *FGF12* may also be sufficient to invoke a disease phenotype. Loss-of-function mutations are absent except in the last exon or isoform-specific exons in the Exome Aggregation Consortium (ExAC) database (ExAC, Cambridge, MA; URL: exac.broadinstitute.org, last

accessed May 2016). Moreover, the haploinsufficiency score[8] for *FGF12* is extremely low (haploinsufficiency index 3.95%). Our case supports the results of the previous report,[3] highlighting the requirement for fine-tuning of the *FGF12* dosage to maintain normal brain activity. Further functional analysis will enhance the understanding of the genotype-phenotype relationship.

This report provides further evidence for a causative gene for epileptic encephalopathy and expands the phenotypic spectrum of *FGF12*-related epileptic encephalopathy. We also propose the therapeutic use of sodium channel blockers for *FGF12*-related epileptic encephalopathy.

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