Epilepsia, **47**(1):211–214, 2006
Blackwell Publishing, Inc.

Mutation in Filamin A Causes Periventricular Heterotopia, Developmental Regression, and West Syndrome in Males

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Summary: *Purpose:* Familial periventricular heterotopia (PH) represents a disorder of neuronal migration resulting in multiple gray-matter nodules along the lateral ventricular walls. Prior studies have shown that mutations in the filamin A (*FLNA*) gene can cause PH through an X-linked dominant pattern. Heterozygotic female patients usually remain asymptomatic until the second or third decade of life, when they may have predominantly focal seizures, whereas hemizygotic male fetuses typically die in utero. Recent studies have also reported mutations in *FLNA* in male patients with PH who are cognitively normal. We describe PH in three male siblings with PH due to *FLNA*, severe developmental regression, and West syndrome.

Methods: The study includes the three affected brothers and their parents. Video-EEG recordings and magnetic resonance image (MRI) scanning were performed on all individuals. Mutations for *FLNA* were detected by using polymerase chain reaction (PCR) on genomic DNA followed by single-stranded conformational polymorphism (SSCP) analysis or sequencing.

Results: Two of the siblings are monozygotic twins, and all had West syndrome with hypsarrthymia on EEG. MRI of the brain revealed periventricular nodules of cerebral gray-matter intensity, typical for PH. Mutational analyses demonstrated a cytosine-to-thymidine missense mutation (c. C1286T), resulting in a threonine-to-methionine amino acid substitution in exon 9 of the *FLNA* gene.

Conclusions: The association between PH and West syndrome, to our knowledge, has not been previously reported. Males with PH have been known to harbor *FLNA* mutations, although uniformly, they either show early lethality or survive and have a normal intellect. The current studies show that *FLNA* mutations can cause periventricular heterotopia, developmental regression, and West syndrome in male patients, suggesting that this type of *FLNA* mutation may contribute to severe neurologic deficits. **Key Words:** Subependymal heterotopia—Periventricular heterotopia—Familial—West syndrome—Male.

Gray-matter heterotopias are collections of neurons in abnormal locations (in areas other than the cortex) secondary to arrest of radial migration during human cerebral cortical development. Heterotopia can be either isolated or seen in association with other structural anomalies (1).

Gray-matter nodules along the lateral ventricular walls characterize periventricular heterotopia (PH), also known as subependymal heterotopia. The first description is attributed to Barkovich and Kjos in 1992 (2,3), although Martin Araguz et al. (4) related the case of a 27-year-old woman with epilepsy that was caused by PH in 1989. Since then, many other cases have been described with the increasing use of magnetic resonance imaging (MRI) .(5).

Familial PH is usually associated with prenatal lethality in male subjects because of severe early truncation mutations (the first seven coding exons) in the X-linked filamin A (*FLNA*) gene (6,7). More recently, mild missense mutations have been detected in male patients with PH, and they appear have a normal intellect (6,8).

We describe a pedigree of three affected male siblings with PH and West syndrome due to a mutation in the *FLNA* gene. We discuss the genotype–phenotype correlation in these individuals, which suggests that the type of *FLNA* mutation may correspond with the severity of neurologic deficits.

Accepted July 24, 2005.
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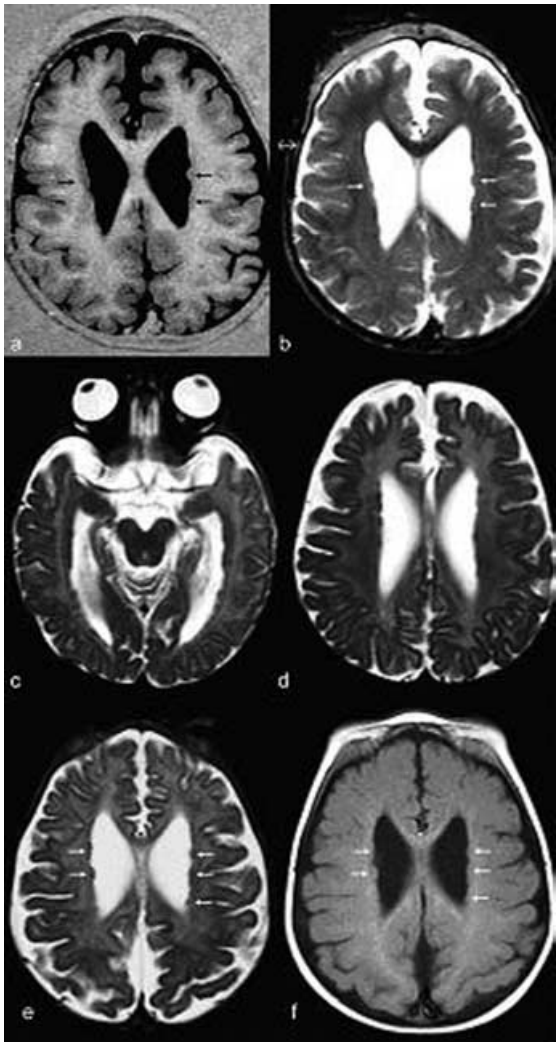

FIG. 1. Brain MRI appearance of affected individuals. First brother: **(A)** Axial T_1-weighted inversion recovery (IR) image reveals multiple nodules of heterotopic gray matter lining the lateral ventricles (*arrows*). **B:** Axial T_2-weighted turbo spin-echo (TSE) image demonstrates that the heterotopia (*arrows*) remain isointense with cortical gray matter. Note enlarged lateral ventricles and prominent sulci indicating atrophic changes. First twin: axial T_2-weighted TSE images at the level of mesencephalon **(C)** and body of lateral ventricles **(D)**. Multiple subependymal nodules of gray matter line the temporal horns and bodies of the lateral ventricles. Second twin: axial T_2-weighted TSE image and T_1-weighted SE image at the level of the body of lateral ventricles in E and F, show subependymal heterotopia lining the lateral ventricles (*arrows*).

METHODS

The study includes the three affected brothers and their parents. Video-EEG recordings (obtained according to the International 10-20 system) and MRI scanning (routine spin-echo sequences and high-resolution T_1 volumetric studies obtained in all planes) were performed on all individuals.

Genomic DNA was extracted from lymphocytes of whole blood by using standard manual technique. Initial linkage studies were performed by using standard ABI microsatellite markers that localized to chromosome Xq28, the *FLNA* locus (DXS1200, DXS1193, DXS1073). Polymerase chain reactions (PCRs) were carried out by using the published primer sequences for the *FLNA* gene (6). Greater than 95% of the *FLNA* gene was analyzed by single-strand conformation polymorphism (SSCP) or sequencing.

RESULTS

The mother's first pregnancy resulted in a miscarriage between the first and second months after conception. The spontaneous abortion was attributed to an accidental maternal abdominal contusion.

The second pregnancy had an uncomplicated prenatal course, resulting in an apparently healthy term boy weighing 3,040 g. The child was discharged from the hospital on the second day of life. At age 4 months, flexor spasms developed and clustered at times when he would awaken. His neurologic development, which had been normal until this date, underwent slow and progressive regression. Over the course of 1 year, the spasms were gradually replaced by tonic–asymmetrical seizures. Currently 4 years old, the child has severe neurologic deficits, requiring a nasogastric tube for feeding, is unable to sit without support, and fails to express verbal language. He has occasional tonic–asymmetrical seizures, usually of short duration, which are controlled with valproic acid (VPA; 50 mg/kg/day) and nitrazepam (NTZ; 1 mg/kg/day). MRI of the brain (Fig. 1A and B) showed characteristic bilateral periventricular gray-matter nodules. Video-electroencephalogram monitoring (video-EEG) demonstrated discrete periods of disorganization in background activity but no paroxysmal epileptiform activity. The diagnosis of West syndrome secondary to PH was established with the onset of similar symptoms in his younger brothers.

The third and most recent pregnancy was again accompanied by an uncomplicated prenatal course. Monozygotic twin boys were born by cesarean delivery, term, weighing 2,980 (first twin) and 2,830 g (second twin). Both children were discharged on the third day of life. At age 4 months, the first twin had the same clinical symptoms as his older brother. Treatment with adrenocorticotropic hormone (ACTH; 25 IU/day, IM) over 7 days yielded no improvement in seizure pattern, although administration of the drug was interrupted because of a febrile state. Complete remission in seizures was obtained with VPA (50 mg/kg/day) and NTZ (1 mg/kg/day). MRI demonstrated bilateral PH (Fig. 1C and D), and video-EEG showed fragmented hypsarrhythmia. After treatment initiation, the EEG normalized (Fig. 2). The second twin had the same clinical picture 1 month later. MRI (Fig. 1E and F), and video-EEG monitoring (Fig. 2) findings were similar to those observed in the twin brother. He also responded to the same drug treatment.

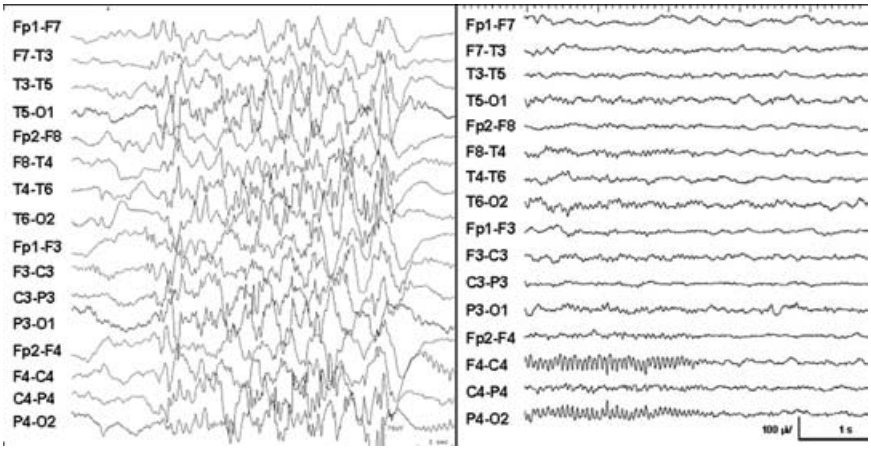

FIG. 2. Video-EEG telemetry in both twins showed the same electrographic pattern. *Left:* Fragmented hypsarrhythmia visualized before treatment. *Right:* After treatment institution, the EEGs returned to normal.

The parents are first-degree cousins. The mother (23 years old) and the father (22 years old) are both healthy and have no personal history of seizures. Their MRI and EEG studies were normal. The family history is negative for seizures, mental retardation, or congenital anomalies.

Initial linkage studies did not exclude the *FLNA* locus. Sequencing of genomic DNA from the affected individuals demonstrated a mutation in exon 9 of the *FLNA* gene (Fig. 3). The mutation corresponded to a cytosine-to-thymidine (C-to-T) substitution at base pair 1286, resulting in a threonine-to-methionine amino acid substitution. The base-pair change was not detected in the NCBI SNP database and was not evident on prior sequencing of >100 control alleles.

DISCUSSION

The severity of mutation seen in the *FLNA* gene appears to correspond to the severity of clinical presentation in male patients with PH. The 48 exons of *FLNA* encode for a 280-kDa cytoplasmic phosphoprotein (2,648 amino acids), consisting of a receptor-binding domain (the amino terminus), multiple IG repeats, and an actin-binding domain (the carboxyl terminus). Presumably, interactions along the amino terminus are modulated and transduced by *FLNA* onto the actin cytoskeleton. Previous studies have demonstrated that severe early truncation mutations, typically within the first seven exons of the *FLNA* gene, lead to early male lethality (6,7). Similarly, severe missense mutations that cause a change in molecular charge and polarity/hydrophobicity lead to loss in male fetal viability (7). Conversely, distal truncation mutations or mild missense mutations that cause no change in charge or polarity/hydrophobicity lead to male patients with PH who are intellectually normal (6,8). In this report, the cytosine-to-thymidine (c.C1286T) missense mutation leads to a threonine (polar and hydrophilic) to methionine (nonpolar and hydrophobic) amino acid substitution. This moderate missense mutation does not lead to a charge change but does alter polarity/hydrophobicity and thus may allow male viability with severe neurologic deficits.

Previous studies have suggested that *FLNA* undergoes homodimerization with itself or heterodimerization with

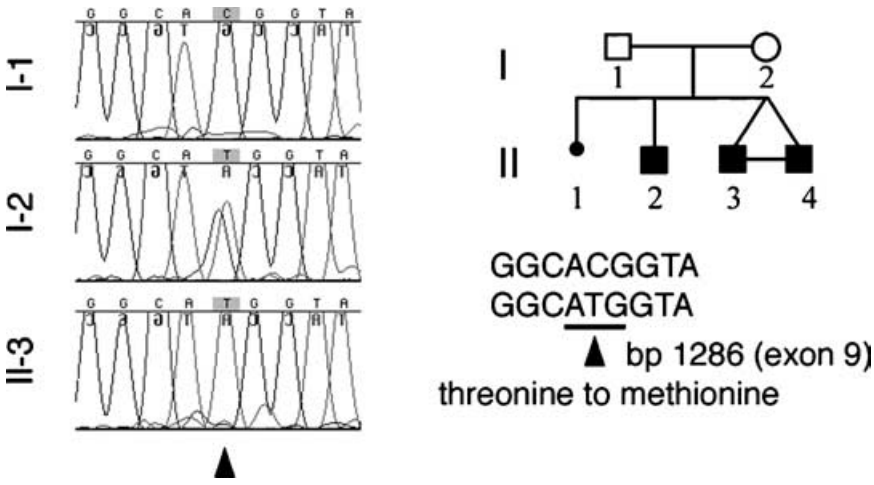

FIG. 3. Sequencing of the *FLNA* exons demonstrates a mutation in exon 9 of the gene. The father (I-1) carries the normal genomic sequence GG**C**ACGG; the mother (I-2) is heterozygous at base pair 1286 and is likely a mosaic carrier, given that she is not affected. All the affected children (II-2-4) harbor the cytosine-to-thymidine mutation, resulting in a threonine-to-methionine amino acid substitution.

a highly homologous FLNB protein (9). Dimerization is thought to occur along the multiple IG repeat domains of the filamin protein. The exon 9 mutation reported here is within one of the IG repeat domains, suggesting that formation of FLNA-FLNA homodimers or potentially FLNA-FLNB heterodimers may be very important for the function of these proteins.

Point mutations in the IG repeat domains of the *FLNA* gene have previously been shown to cause malformations in the otopalatodigital (OPD) spectrum (10). Several recurrent mutations in the rod domain repeats 3,10, and 14/15 result in the craniofacial and skeletal abnormalities characteristic of OPD syndrome and do not result in PH. These defects have been ascribed to gain-of-function mutations. The point mutation in the current study occurs in repeat 2 of the IG repeat domains, but the affected individuals do not have OPD features. These discrepancies in clinical presentation may merely reflect the fact that the cytosine-to-thymidine (c.C1286T) missense substitution in repeat 2 may be a loss-of-function mutation. Alternatively, disruption of the *FLNA* gene may lead to various phenotypes [PH and OPD and Ehlers–Danlos (11)], and degrees of penetrance will determine the phenotypic presentation.

Genes like *FLNA*, which are located on the X chromosome, undergo random X-inactivation. In heterozygous female patients, single cells express either normal or abnormal copies of *FLNA,* depending on which of the two X-alleles is inactivated. This variability may alter the genotype–phenotype correlation, making it difficult to interpret the pathologic mechanisms underlying this gene. Further characterization of the genetic mutations in *FLNA* and the corresponding phenotypes in hemizygous males will therefore allow greater insight into the various functional domains of this gene.

Acknowledgment: This study was sponsored by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). We thank Dr. Iara Leda Brandão Almeida and Dr. Fábio Rossi Torres for helping with the genetic tests. V.L.S. is supported by a grant from the NIMH (1KO8MH/NS63886) and the Milton fund. V.L.S. is a Charles A. Dana fellow. C.A.W. is supported by grants from R37 NS35129 and the March of Dimes (6-FY00-132). C.A.W. is an Investigator of the Howard Hughes Medical Institute.

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