Bilateral Periventricular Heterotopias in an X-Linked Dominant Transmission in a Family With Two Affected Males

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We report on the case of dizygotic twin boys, born prematurely to an asymptomatic mother. Bilateral periventricular heterotopias with enlarged ventricles were discovered at birth in both twins. One of the twins died prematurely of bronchopulmonary complications, and was shown to have several neuropathological anomalies (microgyria, thin corpus callosum, and reduced white matter). The surviving twin had mental retardation, without epilepsy. MRI of the mother showed asymptomatic periventricular heterotopias without ventricular enlargement. She had two affected daughters also with asymptomatic periventricular heterotopias. A point mutation in the last coding exon 48 of the Filamin A (FLNA) gene (7922c>t) was discovered on sequencing and segregated with the affected individuals. This family has a classical X-linked dominant BPNH pathology, with greater severity in males than females. The location of the FLNA mutation is discussed in light of the neuropathological anomalies and mental retardation in male patients. © 2006 Wiley-Liss, Inc.

Key words: periventricular heterotopias; male; neuronal migration disorder

INTRODUCTION

Bilateral periventricular nodular heterotopias (BPNH) is a recently described neuronal migration disorder in which nodular masses of gray matter line the lateral ventricles and protrude into its lumen [Kamuro and Tenokuchi, 1993]. Genetic studies indicated that the most classical BPNH form (OMIM 300049) is X-linked, and due to mutations in the Filamin A gene (FLNA) [Huttenlocher et al., 1994; Eksioglu et al., 1996; Fox et al., 1998]. FLNA is located within Xq28, and encodes an actin-binding protein, which is expressed in the developing cortex [Fox et al., 1998].

Other rarer cases of bilateral periventricular heterotopia have been described, including an autosomal recessive form of BPNH due to mutations in the ARFGEF2 gene (OMIM 608097), BPNH associated with chromosome 5p duplications (OMIM 608098), and a possible autosomal dominant locus for periventricular heterotopia (PH) [Sheen et al., 2004]. While the FLNA gene is responsible for classical BPNH, mutations in this gene have also been reported to cause oto-palato-digital syndrome (OPD) types 1 and 2, frontometafysial dysplasia and Melnick-Needles syndrome [Robertson et al., 2003]. They are considered mutually exclusive.
because of the different presumed effects of the respective FLNA gene mutations, leading to loss of function in BPNH and gain of function in OPD. In one case, the association of BPNH and frontometaphyseal dysplasia has been described, with a FLNA mutation, giving rise to two functionally different transcripts [Zenker et al., 2004]. Zenker et al. [2004] proposed that the dual phenotype was caused by two functionally different, aberrant FLNA proteins and therefore represented an exceptional case of allelic gain-of-function and loss-of-function phenotypes due to a single mutation event.

The great majority of individuals with classical BPNH related to FLNA mutations are female, who present with partial epilepsy and no mental retardation [Kamuro and Tenokuchi, 1993; Dobyns et al., 1996, Poussaint et al., 2000]. The phenotype of male patients with FLNA mutations is variable and depends upon the severity of the FLNA mutation. The condition is predominantly lethal in males, as suggested by the common occurrence of miscarriages, skewed sex-ratio in the families, and premature male deaths in classical BPNH pedigrees [Kamuro and Tenokuchi, 1993; Huttenlocher et al., 1994; Jardine et al., 1996; Moro et al., 2002]. Some recent publications point to male patients with classical BPNH or BPNH with severe phenotypes [Sheen et al., 2001, 2004; Moro et al., 2002; Guerrini et al., 2004]. Herein, we report on the transmission of BPNH from a clinically asymptomatic mother to two affected dizygotic twin sons and two asymptomatic daughters, in a fully penetrant classical X-linked dominant mode of transmission.

**CLINICAL REPORT**

Twins boys were born to a 22-year-old mother and a 41-year-old father. The mother had previously given birth to a healthy daughter with a different father. She also previously had a miscarriage. The dichorionic diamniotic twin pregnancy reported herein was marked by a mild brain ventriculomegaly (10 mm) found by ultrasound (US) in both fetuses at 23 weeks of gestation (WG). The boys were born at 26 WG after premature amnion rupture and chorioamniotic infection. At birth, the twin boys were eutrophic; T1: BW 870 g (50th centile), BL 35 cm (50th centile), and OFC 24 cm (50th centile). T2: BW 1,025 g (75th centile), BL 36.5 cm (75th centile), and OFC 26.5 cm (90th centile). They had enlarged anterior fontanel without hypertelorism and no other morphological abnormalities. Both twins had patent ductus arteriosus (PDA), medically closed. Brain US, performed at Day 1 in both twins, revealed irregular and nodular ventricular walls. Magnetic resonance imaging (MRI) at 3 months of age in both twins showed bilateral symmetric periventricular nodular heterotopia, and enlarged ventricles (Fig. 1, twin 1 MRI). EEGs, metabolic screening, karyotypic analyses were normal. Severe bronchodysplasia occurred in both preterm twins, resulting in the death of the T2 infant at 8 months of age due to respiratory complications. Due to his extreme prematurity and respiratory distress since birth, his weight and height stagnated at −4 DS (8 months, 3,740 g, 55 cm) 15 days prior his ultimate respiratory compromise. Head circumference was at 38.5 cm (−3 DS in corrected age). Neuropathological examination revealed a microgyric microcephalic brain weighing 484 g at 8 months of age. 5 months of corrected age (normal values at 5 months postnatal = 644 g ± 50 g, normal values at 4 months 540 g ± 50 g) (Fig. 2). On coronal sections, the corpus callosum was thin and the periventricular white matter reduced, filled with heterotopic nodules bulging into the enlarged and irregular ventricular lumen. Histological examination of the periventricular nodules showed irregular pyramidal neurons, intermingled with glial elements (Fig. 3). The cortical plate displayed a normal six layered organization, despite the presence of some inverted large

![Fig. 1. First twin’s MRI. Periventricular linear nodular heterotopias (arrows) and enlarged ventricles.](image1)

![Fig. 2. Second twin’s pathology. Polymicrogyria (at the external view), periventricular heterotopias (circles).](image2)
pyramidal neurons. No malformations of the fourth ventricle, cerebellum, or brainstem were found. The other twin (T1) shows good social interactions although he has mental retardation with delayed milestones (Brunet-Lezine test at 14 months for 24 months corrected for his prematurity). At 21 months, he had a head circumference at 46.5 cm for (−2 DS in corrected age). He had a partial seizure at 26 months, lasting 45 min during a fever for intercurrent respiratory infection. He currently is 6 years of age and has a global IQ level estimated at 50, appropriate for a 2–3-year-old. He has not had any subsequent seizures.

A new pregnancy occurred 2 years later, resulting in the birth of a very premature girl, weighting 680 g at 24 WG +3 days, from a different father, height 32 cm, head circumference 21.7 cm (normal for term). A persistent ductus arteriosus was medically closed. She developed bronchodyplasia. At 6 months, she weighted 4.3 kg, height 56 cm, head circumference at 38 cm (+1 SD for her 3 months of corrected age). Although asymptomatic, she exhibited BNPH, without enlarged ventricles on MRI. Epileptic crisis was suspected twice, but never confirmed, with normal EEGs. Development was considered to be normal at 2½ years of age (in Africa).

The sixth pregnancy, with a fourth partner, gave birth to a girl, born at 35 WG, weighting 2,520 g, height 47 cm, and head circumference at 32 cm (50’P for 35 weeks) also with BNPH, and normal ventricles (Fig. 4). At 2 months, she weighted 5,270 g (M), height 57 cm (−0.8 DS), PC 39.5 cm (+1.8 SD). Her neurological exam was normal. She is being followed for sickle cell disease, both parents being heterozygous AS.

MRI of the asymptomatic mother was done after the birth of the twin boys. Imaging showed less contiguous nodular PH compared to the BPNH seen in the twins, and normal ventricles (Fig. 5). On clinical examination, the mother had normal intelligence and no epilepsy. There also was no family history of epilepsy with nine healthy siblings (four sisters and five brothers) on the maternal side. However, there does appear to be an increased frequency of miscarriages with the grandmother having had two miscarriages out of her 12 pregnancies (Fig. 6). One of the mothers' sisters also had three pregnancies lost at term (two males and one female).
She also lost two daughters, one at 13 years due to malaria, and one at 3 months due to severe hydrocephaly. No investigations could be performed. The mother of these children has normal intelligence, no epilepsy, and there was no consanguinity.

RESULTS

Molecular studies using microsatellite markers on each chromosome confirmed the dizygosity of the twin boys. A homologous recombination was detected just proximal to the Xq28 region, such that both affected twins inherited the same Xq28 region. From centromeric to telomeric, the markers DXS8045, DXS 998, DXS 8091 were different in both twins, and markers DXS 8069 and DXS 1073 were identical. The recombination occurred between DXS 8091 and DXS 8069, indicating that both twins inherited the same FLNA allele.

We performed mutation analysis in individuals II6, III6, III7, and III8. No DNA was available for the newborn affected female. The 48 exons covering the coding region of FLNA and their respective intron-exon boundaries were amplified by PCR. A point mutation in the last exon, exon 48 (7922c > t) of the FLNA gene, was discovered in the mother, both dizygotic twins, and oldest affected daughter. No unaffected individual were available in this family. This pro2641leu substitution is not in the SNP database of either NIH or UC Santa Cruz, and was not found in greater than 120 DNA samples of mixed ethnic origin tested in our lab. In addition, this mutation is located in a highly conserved region of the FLNA protein and involves an invariant residue in several species including a lower vertebrate such as Danio rerio, and non-vertebrates such as Drosophila and Caenorhabditis elegans (Fig. 7a); moreover, the same proline residue is conserved among the alpha, beta, and gamma filamins (Fig. 7b). Altogether, these data strongly suggest that this substitution is not a single nucleotide polymorphism.

DISCUSSION

The BPNH in the presented kindred follows an X-linked dominant mode of inheritance. The mutation found in the FLNA gene segregated with the affected individuals, including the mother, both dizygotic twins, and one of the affected daughters. The cytosine to thymidine substitution results in a proline to leucine substitution. The change in amino acids induces significantly different conformational states, suggesting that this is a pathological mutation. Moreover, the proline in position 2641 is highly conserved among the evolution. The mutation is localized to
the receptor binding region of the FLNA protein, at the very end of the carboxy terminus. Interestingly, the missense mutation would be consistent with a less severe or partial loss of function, and act as a hypomorphic allele, as compared to early truncation mutations in FLNA, which uniformly result in hemizygous male lethality. Correspondingly, the phenotype appears milder in the mother and two girls who are largely asymptomatic and only show evidence of small heterotopia without ventricular enlargement on MRI. These females appear to have normal intelligence and have not developed epilepsy. The dizygotic twin boys shared the same Xq28 region through a recombination event, and are more extensively afflicted than the female siblings. The MRI images show more confluent nodular heterotopia, and ventricular enlargement. Clinically, the one surviving twin has developmental delay and mental retardation.

BPNH—with epilepsy as the sole clinical finding—has been described classically in females [Kamuro and Tenokuchi, 1993; Huttenlocher et al., 1994; Jardine et al., 1996], with very few documented cases of male viability. For example, a pedigree harboring a FLNA missense mutation Glu82Val in exon 2 gives rise to females with BPNH but otherwise very mild consequences. Five males, born to the affected mothers have died unexpectedly early in life [Moro et al., 2002]. Another case of BPNH has been reported in a male newborn who died at 7 days of age because of disseminated coagulopathy; he also displayed a complex cardiovascular malformation (atrial septal defect, ventricular septal defect, persistent superior vena cava draining to coronary sinus, and widely persistent ductus arteriosus), with additional malformations, short and malrotated small intestine, anal stenosis, hypospadias, cryptorchidism, and low-set ears [Guerini et al., 2004]. His mother had BPNH on MRI, and isolated epilepsy. She was shown to carry an 8-bp deletion in the FLNA gene in intron 25 donor splice site. That said, two FLNA missense mutations in male patients were reported in the same article [Guerini et al., 2004]. In the first family, both the mother and her son carried a Met102Val mutation in exon 2, the mother being affected of BPNH with isolated epilepsy, and her son having BPNH and mental retardation. In the other family, the father and daughter carried a Ser149Phe mutation in exon 3, inherited from an asymptomatic grand-mother. The father was mildly affected, with asymptomatic unilateral heterotopias, while his daughter was surprisingly more severely afflicted, with epilepsy and denser unilateral heterotopias [Guerini et al., 2004]. There are reports of mosaicism with FLNA in males with a classical BPNH phenotype [Guerini et al., 2004; Parrini et al., 2004]. Additionally, mutations in FLNA consistent with a partial loss of function have been reported to cause isolated periventricular nodular heterotopias in males, with a less severe outcome [Sheen et al., 2001]. The current report further argues that partial loss of function in the FLNA gene can lead to male viability.

The present case provides significant insight into the function of FLNA. The Filamin protein typically exists as a homodimer with dimerization occurring in the C-terminus of the protein. The C-terminus of each of the dimerized Filamin proteins is open, allowing multiple neighboring proteins to interact with this receptor-binding region. We could suggest that the amino-acid exchange might disturb or destabilize dimerization. The dizygotic twins present with nearly all of the clinical features associated with X-linked dominant PH—namely the neurological features of BPNH thinned corpus callosum, ventriculomegaly, and polymicrogyria; the cardiac findings of PDA; and pulmonary features of bronchodylsplasia, even if extreme prematurity may have added confusing features. The location of the mutation at the very end...
of the carboxyl terminus argues that BPNH and most of its associated features can arise solely from disruption of the very end terminus of the Filamin 1 gene and presumably those proteins which interact with Filamin in this small region (i.e., tissue factor).

Several observations can be made with respect to the neuropathology in the deceased twin with documented brain anomalies. In addition to the gray matter PHs, the cerebral hemispheres displayed a microcephalic, microgyrictic pattern with reduced white matter but a normal six-layered cortex. The microcephaly could be aggravated by his extreme prematurity (26 WG), but reduced white matter and polymicrogyria are not classically premature sequelons. Polymicrogyria has been already described in some brain regions in another BPNH male [Guerrini et al., 2004], in our case, the polymicrogyria was diffuse. The neuropathology from that case also showed a normal cortex but unlike the current study, the cerebellum was noted to be small at 36 weeks by ultrasonography [Guerrini et al., 2004]. Widespread glomeruloid microvascular anomaly and dysplastic cytoarchitecture in the cerebral cortex was reported, with ultrastructural analysis and immunolabelled antibodies [Kakita et al., 2002]. These findings could explain the psychomotor delay present in the surviving twin. Moreover, the findings of microcephaly and reduced myelination would argue that Filamin may also play a role in neuronal differentiation or proliferation- and not just migration.

The current report describes a pedigree with BPNH and male viability due to the classical X-linked dominant FLNA mutation. The neuropathological anomalies and clinical mental retardation seen in the affected males suggest that virtually all of the features associated with the BPNH disorder can result from a disruption of the last exon 45 C-terminal region of the FLNA gene.

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REFERENCES


