Original article

Etiological heterogeneity of familial periventricular heterotopia and hydrocephalus

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Abstract

Periventricular heterotopia (PH) represents a neuronal migration disorder that results in gray matter nodules along the lateral ventricles beneath an otherwise normal appearing cortex. While prior reports have shown that mutations in the filamin A (*FLNA*) gene can cause X-linked dominant PH, an increasing number of studies suggest the existence of additional PH syndromes. Further classification of these cortical malformation syndromes associated with PH allows for determination of the causal genes.

Here we report three familial cases of PH with hydrocephalus. One pedigree has a known *FLNA* mutation with hydrocephalus occurring in the setting of valproic acid exposure. Another pedigree demonstrated possible linkage to the Xq28 locus including *FLNA*, although uncharacteristically a male was affected and sequencing of the *FLNA* gene in this individual revealed no mutation. However, in the third family with an autosomal mode of inheritance, microsatellite analysis ruled out linkage with the *FLNA* gene. Routine karyotyping and fluorescent in situ hybridization using BAC probes localized to *FLNA* also showed no evidence of genomic rearrangement. Western blot analysis of one of the affected individuals demonstrated normal expression of the FLNA protein. Lastly, sequencing of greater than 95% of the *FLNA* gene in an affected member failed to demonstrate a mutation.

In conclusion, these findings demonstrate the etiological heterogeneity of PH with hydrocephalus. Furthermore, there likely exists an autosomal PH gene, distinct from the previously described X-linked and autosomal recessive forms. Affected individuals have severe developmental delay and may have radiographic findings of hydrocephalus.
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1. Introduction

Periventricular heterotopia represents a heterogeneous group of migrational disorders, characterized by nodules that are composed of neurons and positioned ectopically along the lateral ventricular walls. Prior studies have demonstrated an X-linked form of periventricular heterotopia (PH) caused by mutations in the filamin A gene (*FLNA*) [1–3]. Numerous other PH syndromes have associated findings of a cleft palate, frontal nasal dysplasia, sensorineural hearing loss, and celiac disease [4–6]. Given that mutations in *FLNA* can also lead to the otopalatodigital (OPD) spectrum of disorders with abnormalities in the brain, skeleton, viscera, urogenital tract, and craniofacial structures [7], some of these cases may represent an

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overlapping OPD-PH syndrome, although this remains uncertain. More recently, an autosomal recessive pattern of inheritance has also been seen in some families with PH and microcephaly, while other spontaneous cases show abnormalities of chromosome 5p [8,9]. Further delineation of these various syndromes within the PH spectrum will provide greater refinement towards determining the genetic basis of these neurologic disorders.

PH associated with hydrocephalus has primarily been reported in sporadic cases. Perinatal trauma, probable prenatal onset seizure disorder (episodic rhythmic movements in utero) with additional findings of megalencephaly and lissencephaly [10], or bipolar disorder [11] have been seen with unilateral PH and hydrocephalus. A case of confluent bilateral heterotopia, hydrocephalus, and polymicrogyria resulted in early postnatal lethality [10]. A single individual from a pedigree with sensorineural hearing loss and hydrocephalus (Chudley-McCullough syndrome) also had associated findings of bilateral frontal nodular heterotopia [12]. While these various presentations suggest both heterogeneous and extrinsic causes leading to radiographic features of PHH, the current report of a familial disorder with similar findings argues for the existence of genetic causes of PH with hydrocephalus (PHH) within this spectrum of migrational disorders.

Here we describe three kindreds with PH alone or PH and hydrocephalus. One previously described family has a known *FLNA* mutation with hydrocephalus occurring in the setting of valproic acid exposure. This most likely represented valproate embryopathy. The second family showed suggestive linkage to the Xq28 region including the *FLNA* locus, although the inheritance pattern was not typical of this disorder and no mutation was detected on sequencing. Both genetic and molecular studies on the third pedigree clearly exclude *FLNA* as a causal gene, suggesting that PHH is an etiologically heterogeneous condition that can be caused by at least one as yet unidentified autosomal gene.

2. Case materials and methods

With the exception of the newborn child with PH and hydrocephalus (III-1), clinical descriptions of members from Family 1 have previously been published [1]. In Family 2, individuals across three generations were evaluated. They include the propositus (II-6), her affected offspring (III-1), her unaffected sister (II-8), and the sister's affected son (III-2). The unaffected parents of the propositus (I-1, I-2) were also included in the analyses. Individuals studied in Family 3 include two affected individuals and their parents (Fig. 1).

2.1. Linkage analysis

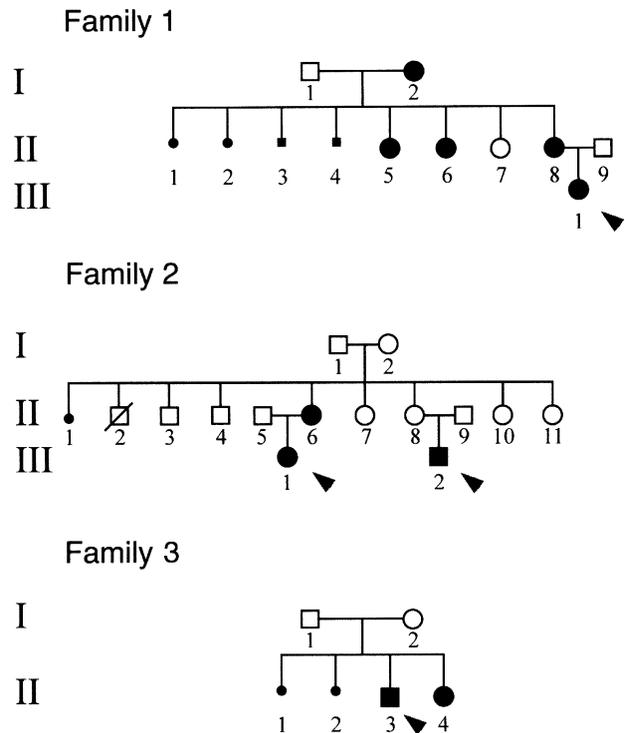Linkage analysis to the *FLNA* gene was performed on two pedigrees. DNA was isolated from peripheral whole

Fig. 1. Pedigrees of the two families. Black symbols = affected individuals with PH; diagonal lines = deceased individuals; black dots = miscarriages, sex unknown; black squares = male miscarriages. Arrowheads = hydrocephalus.

blood using previously described protocols (Qiagen). Human MapPairs with fluorescent labels were obtained within genomic regions flanking the *FLNA* and *FLNB* gene (ResGen, Invitrogen Corp., Applied Biosystems). Polymerase chain reaction (PCR) was performed on the previously isolated DNA using the selected markers (for the X-chromosome: DXS8043, DXS8106, DXS1200, DXS8045, DXS998, DXS1193, DXS8069, DXS8061, DXS8087, DXS1073; for chromosome 3: D3S2402, D3S2452, D3S1300, D3S1600, D3S1287, D3S1285). The samples were run on an ABI Prism 3100 genetic analyzer and alleles were determined using standard software package (Genotyper Analysis). Multi-point and two-point LOD scores were calculated with the GeneHunter statistical program [13].

For analysis of linkage to *FLNA*, the disorder was assumed to be X-linked with a penetrance of 90% in males and 81% in females. For analysis of linkage to *FLNB*, the disorder was considered to be autosomal dominant with a penetrance of 90% for heterozygous individuals, 100% for homozygous individuals. In all families, we assumed a disease allele frequency of 1 in 10,000 and eight alleles per marker at an equal allele frequency (12.5%). A similar analysis for FLNB was performed assuming an autosomal recessive mode of inheritance with a penetrance of 99% in homozygous individuals, 1% for heterozygous individuals, and a disease allele frequency of 1 in 10,000.

2.2. Fluorescent in situ hybridization (FISH) analysis

Cytogenetic analysis of peripheral blood lymphocytes from affected patients were performed using standard techniques [14]. Labeling of the BAC probes followed standard procedures with dUTP containing rhodamine and fluoroscein tags (methods outlined in the Vysis nick translation kit, Grove, IL). BAC probes used included RP11-112H23, CTD-2377O7, CTD-2235M22, RP11-130N6, AND CTD-2511C7 [15]. Hybridization was performed by denaturing the slides in 70% Formamide/$2 \times$ SSC, dehydrating the slides with serial ethanol washes, and applying the probe to the slide samples. Post-hybridization, the slides were washed, coverslipped and examined under fluorescence microscopy (Zeiss Axioskop).

2.3. Western blot analysis

Protein was extracted from peripheral whole blood of an affected male (Family 3, II-3) by previously described methods [16]. Briefly, the sample was solubilized in lysis buffer, separated on a 7.5% SDS-PAGE gel, and transferred onto PVDF membrane. Membranes were probed with anti-filamin A antibody (Novacastra) and binding was detected by enhanced chemiluminescence.

2.4. Sequencing

PCR were performed on genomic DNA from affected individuals (Family 3, II-3 and Family 2, III-2) using previously published primers [2]. Greater than 95% of the FLNA exons were sequenced in each individual. The amplication products were purified (PSIΨClone PCR 96, Adelphia, NJ) and sequenced by the Dana-Farber/Harvard Cancer Center High-Throughput DNA Sequencing Facility using standard techniques. The sequenced exon and intron/exon codes were compared against consensus sequences obtained from the National Center for Biotechnology Information (NCBI, reference #NT 025965) site using standard software for DNA sequencing (Sequencher, version 3.1.1). Additional sequencing of exons was performed for both L1CAM and ARFGEF2, candidate genes for ventriculomegaly and PH, respectively. Primers for these genes were designed using standard software (Primer3).

3. Results

3.1. PH pedigrees

3.1.1. Family 1 (Fig. 1)

The non-consanguineous Caucasian family is of Australian origin. The pedigree, except for the newborn child (III-1), has previously been described [1]. Affected individuals have characteristic bilateral PH (Fig. 2C) and a documented FLNA mutation (A−G substitution at exon $7 - 2$).

Since publication of the original report, the affected individual (Family 1, II-8) gave birth to a female (III-1). Her mother (II-8) was treated with valproic acid (1500 mg/day) and folate during the duration of the pregnancy. The 8 days old neonate underwent repair of a sacral meningomyelocele at 24 h of age and placement of a ventriculo-peritoneal shunt for hydrocephalus. Her early post-natal course was complicated by recurrent seizures, treated with carbamazepine. She also has pulmonary artery stenosis, ventricular septal defect, atrial septal defect, and patent ductus arteriosus. Magnetic resonance imaging (MRI) of the brain demonstrated bilateral nodular heterotopia along the lateral ventricles with thinning of the corpus callosum (Figs. 2A,B). The lateral ventricles were enlarged secondary to hydrocephalus. The cerebellar vermis was inferiorly displaced, consistent with a mild Chiari II malformation.

3.1.2. Family 2 (Fig. 1)

This non-consanguineous Caucasian family is of American origin. The girl (III-1) was examined at 7 years of age. She has a history of obstructive hydrocephalus and bilateral periventricular heterotopia, detected by ultrasound in utero during the second trimester. The mother (II-6) was on carbamazepine (1400 mg/day) for the duration of the pregnancy. Head occipital frontal circumference (OFC) following caesarian delivery was 38.3 cm (90th percentile) and a ventriculoperitoneal shunt was placed. Her development had also been complicated by recurrent infections, which often triggered seizures. The seizure episodes initially began in one arm or leg with mild twitching and subsequent generalization. More recently, she had a brief staring spell, fell to the side and then proceeded to lie on the ground. She stiffened and had eye flutters, lasting only seconds. She is currently on valproic acid and phenobarbitol, with midazolam for breakthrough episodes. She is severely developmentally delayed. At 12 months of age, she had no clear visual fixation, and had a head lag on pull to sit. At 3 years of age, the child was able to sit up with some assistance, able to roll, but unable to walk. She spoke one word responses, and required diapers. On examination, she had a head circumference of 48 cm (20th percentile). No cardiac murmurs were appreciated on auscultation. Bilateral pes planovalgus, bilateral knee recurvatum, and bilateral hip dysplasia were present. Neurologically, she was alert and smiling. She displayed no clear visual fixation, spoke in single words, and was able to point to body parts on request. Her random ocular movements were full, there was a suspicion of optic atrophy, and her facies was symmetric. She had profound hypotonia and weakness on motor examination. Her reflexes were diminished but present. She could not ambulate without assistance. EEG was markedly abnormal with polymorphic high amplitude activity in the right hemisphere, and mixed frequencies and high amplitude activity over the left occipital region. MRI of the brain revealed bilateral heterotopia, contiguous and nodular (< 1.5 cm), that lined the lateral ventricles with
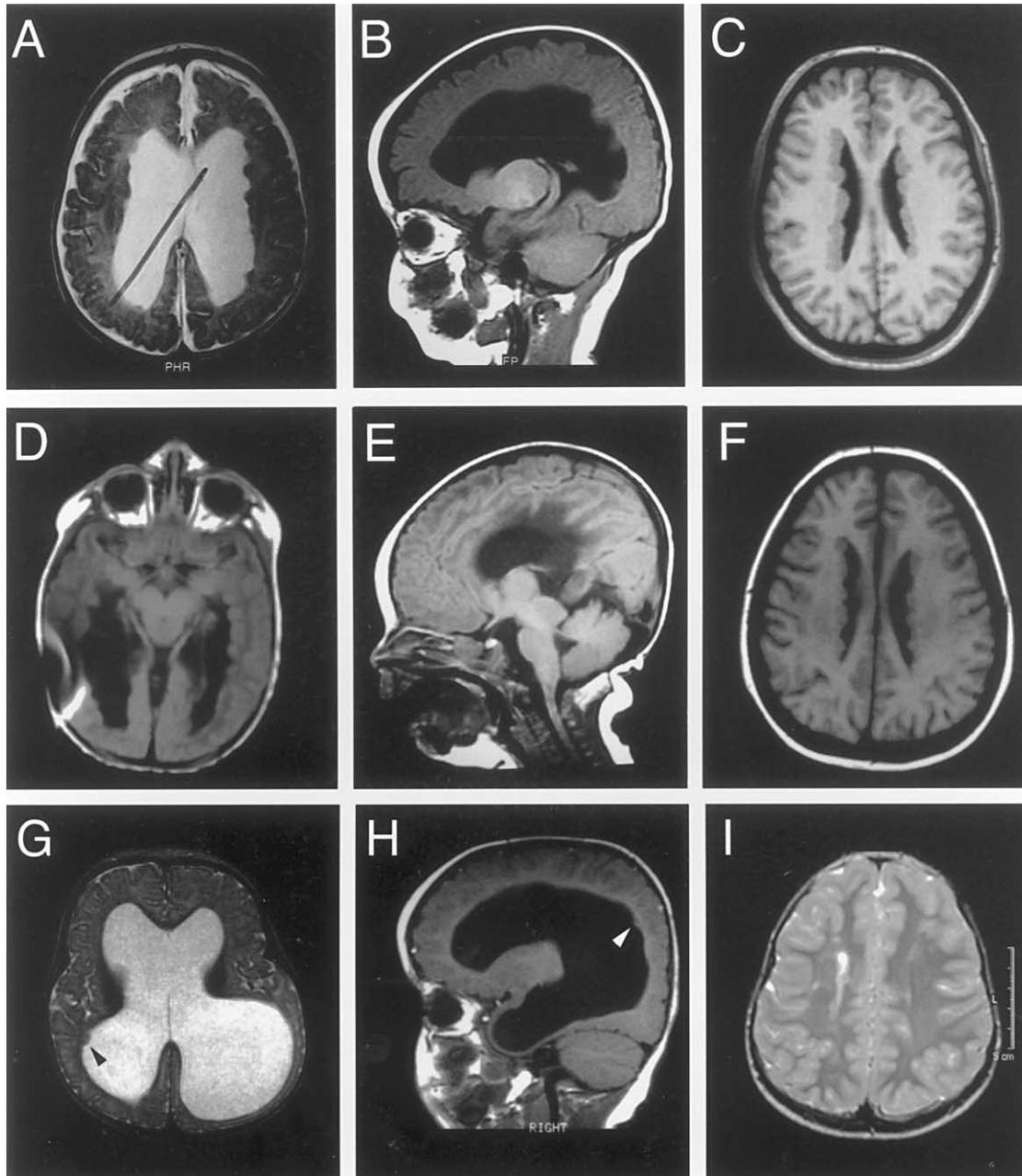
Fig. 2. Brain MRI appearance of affected individuals with PH and hydrocephalus, or PH alone. (A) Axial T2-weighted image of the affected female (Family 1, III-1) demonstrates marked hydrocephalus with PH despite introduction of a shunt. (B) Sagittal T1-weighted image of the same patient in (A) shows a similar widespread distribution of heterotopia. (C) Axial T1-weighted image of the aunt with a known *FLNA* mutation (Family 1, II-5) demonstrates the characteristic bilateral PH seen in this X-linked dominant disorder. (D) Axial T1-weighted image of the patient (Family 2, III-1) demonstrates bilateral contiguous periventricular heterotopia with hydrocephalus. (E) Sagittal T1-weighted image of the same patient in (D) shows that the hydrocephalus is not secondary to a Chiari I or II malformation or aqueductal stenosis. (F) Axial T1-weighted image of the mother (Family 2, II-6) demonstrates bilateral contiguous periventricular heterotopia. (G) Axial T2-weighted image of the male patient (Family 3, II-3) demonstrates hydrocephalus with a few heterotopia (arrowhead) along the margin of the right lateral ventricle. (H) Sagittal T1-weighted image of same patient in (G) shows marked hydrocephalus and a single neuronal heterotopia (arrowhead) lining the ventricle posteriorly. (I) Axial T-2 weighted image of the affected female sibling (Family 3, II-4) displays the characteristic radiographic findings of PH alone.

a question of tectal dysplasia (Figs. 2D,E). The child had hydrocephalus, slightly worse on the right as compared to the left hemisphere with no anterior/posterior gradient of severity, and was not associated with a Chiari I or II malformation or aqueductal stenosis. Subcortical white

matter signal changes were suggestive of delayed myelination and chronic injury due to her hydrocephalus.

The girl's mother (II-6) is currently 29 years of age. She had a long-standing history of intractable complex partial seizures with secondary generalization, beginning at age 12.

Her typical episode has been described as her sitting on a stool at the counter and talking. She would stop talking, begin swaying back and forth and then look side to side. She has had multiple such events, lasting approximately 1 min, over the ensuing 5–10 min. She becomes extremely fatigued afterward. Despite current treatment on valproic acid, she averages one episode every other day with occasional progression to generalized tonic-clonic seizures. She has been diagnosed with major depression. She also has had learning problems throughout her life, requiring special assistance with reading. She completed high school and is currently working in housekeeping. She is married. On examination, she had a Grade I/VI systolic ejection murmur. Neurological exam was unremarkable. Basic laboratory screens were notable for a mild thrombocytopenia (142K plts/mm^3) and normal liver function tests. Echocardiogram revealed a congenitally malformed aortic valve with mild aortic insufficiency. EEG showed very fast low amplitude beta activity, lateralized diffusely over the right greater than left central parietal regions. This pattern was followed by fast repetitive spike activity, lasting some 20 s and evolving into a rhythmic sharply contoured theta/delta higher amplitude discharge. The electrographic seizure episodes lasted 30–90 s in total. MRI of the brain demonstrated bilateral nodular (<1 cm), near-contiguous periventricular heterotopia along the lateral ventricles, including the right temporal horn (Fig. 2F). No hydrocephalus, aqueductal stenosis or Chiari I or II malformation was appreciated on the scan.

The boy (III-2) is currently 7 years old and the cousin of the affected girl (III-1). He was diagnosed with hydrocephalus in utero (OFC 46.5 cm) and a ventriculoperitoneal shunt was placed. His course was complicated by recurrent urinary tract infections and seizures. These seizures began at 8 months of age with twitching of his right foot, arm and then his face. He had rightward turning of his head and developed a glassy eyed appearance. He has had smaller attacks characterized by a quick jerk of one extremity or perhaps both legs together. These events clustered around sleep and occurred up to 30 times per day before initiation of a ketogenic diet and diazepam for breakthrough events. He has had mild developmental delay. At 6 months of age, he had a cognitive score of 86 and motor score of 76. At 3 years of age, he only recognized the color blue, recalled the alphabet up to the letter E, and could count to ten. He was able to walk 20 feet, but had a tendency to walk up on his toes and fall forward. On examination, the child appeared irritable. No appreciable murmurs were detected on auscultation. Examination of his extremities revealed severe contractures of both ankles, which have required botox injections in the past. Neurologically, he was alert, and speaking in short sentences. Facies was symmetric with full extraocular motility and no nystagmus. No weakness was appreciated, but there was increased tone in both lower extremities. The child had no tremor or dysmetria on reaching for objects. Gait was wide based, but steady

without ataxia. He was able to run, jump, and ride a tricycle. Fragile X testing was normal. EEG demonstrated irregular generalized spike and wave activity, initially isolated to the left occipital focus, and more recently, noted to have spread to the right occipital and the right frontal regions. The spike and wave activity was brought on with hyperventilation and superimposed on a slow 2 Hz background. MRI of the brain revealed heterotopia, most apparent along the left lateral ventricle bordering the occipital horn. The scans demonstrated ventriculomegaly of the lateral and third ventricles. Extension of the cerebellar tonsils below the level of the foramen magnum, was consistent with a Chiari I malformation and aqueductal stenosis. The images also suggested functional atrophy or agenesis of the dorsal portion of the corpus callosum.

The boy's mother (II-8) was unaffected and had a normal MRI of the brain.

3.1.3. Family 3 (Fig. 1)

This Jewish family is of Ethiopian origin. The parents deny consanguinity. The younger boy (II-3) is currently 3 years of age. He has a history of hydrocephalus and severe psychomotor retardation. Head OFC at birth was 37 cm (90th percentile). On recent physical examination, OFC was 55.5 cm (98th percentile). He had a short nose, anteverted nares, low set ears and bilateral simian lines, and undescended testes. He was awake but not interactive due to his ability to see or hear. He could not fixate or track. Fundoscopic exam showed evidence of a hypoplastic disc bilaterally. The face was symmetric with no evidence of palsy. He was hypotonic with normal patellar and Achilles reflexes and appropriate Babinski response. Strength and sensation were also normal. He was bedridden and unable to sit or walk. Routine karyotyping and Fragile X testing were normal. Brainstem auditory evoked responses and visual evoked responses in each eye showed complete absence of cortical response, consistent with hearing loss and cortical blindness, respectively. MRI of the brain revealed two to three isolated neuronal heterotopia, less than 1 cm in diameter, along the margin of the right lateral ventricle (Figs. 2G,H). The scans demonstrated marked hydrocephalus, worse on the left as compared to the right hemisphere and affecting the posterior more so than the anterior regions of the brain. The pons was small and the tectum and thalami appeared partially fused.

The girl (II-4) is currently 6 years of age. At 4 months of age, she was hospitalized due to severe enterobacter sepsis that caused renal failure and resulted in her undergoing amputation of fingers and toes. At 6 months of age, she developed seizures. She more recently was toilet trained but dependent in other activities of daily living. On physical examination, OFC was 48 cm (less than the 2nd percentile). She had an atrial septal defect, patent ductus arteriosus (closed surgically), and bronchomalacia of the left main bronchus. There was no dysmorphism. She was able to follow, fixate and track. She was able to speak several

meaningful words and could obey simple commands. Strength and tone, as well as sensation, were unremarkable. Deep tendon reflexes were barely elicited a bilateral dorsiflexion response on Babinski. She was able to walk independently. Karyotyping was normal. MRI of the brain showed multiple small bilateral periventricular nodular (<1 cm), non-contiguous heterotopia (Fig. 2I). A few foci of increased T2 signal were appreciated within the white matter. The anterior right frontal horn was slightly dysmorphic, raising the suspicion of a transmantle dysplasia.

The parents have no healthy children. The deceased siblings (II-1, II-2) represent spontaneous abortions during the 3rd and 4th months of gestation.

3.2. Analysis of Filamin A and Filamin B expression

Only two filamin proteins, *FLNA* and *FLNB*, are known to be expressed in the central nervous system. The X-linked *FLNA* gene can cause periventricular heterotopia. We therefore sought to evaluate both actin-binding proteins as potential causes of PHH by linkage analysis, genomic sequencing, FISH, and protein expression.

Direct evaluation of the *FLNA* locus on the X chromosome was performed using microsatellite markers, which flank the gene (Fig. 3A). Two-point LOD score analyses could not rule out linkage in Family 2 with a maximal LOD of 0.43 (θ recombination frequency of 0), but the size of the family is inadequate to demonstrate positive linkage.

On the other hand, in Family 3, multiple markers flanking *FLNA* shared LOD scores of <-2.0, ruling out linkage to *FLNA* in this family (Fig. 3B). A similar evaluation was performed on the *FLNB* gene in the two pedigrees (Fig. 3C). Multi-point analyses on chromosome 3 with microsatellite markers close to the *FLNB* locus revealed a LOD score of -2.67 and -0.0010 (maximum LOD at $\theta = 0$) for Family 2 and Family 3, respectively (Fig. 3D). In Family 2, the LOD score of <-2.0 ruled out linkage to *FLNB* and the negative LOD score seen in Family 3 does not suggest linkage to this region on chromosome 3. No significant difference in LOD scores was observed with analyses performed for either an autosomal dominant or recessive mode of inheritance.

Since duplications of Xq28 have been associated with some cases of PH [4], we analyzed BAC probes localized to Xq27–Xq28 by FISH, but failed to identify any chromosomal abnormalities in the affected male child (II-3) from Family 3. No duplications, rearrangements, or deletions were appreciated. Furthermore, the BAC probe CTD-2511C7 specifically includes the FLNA genomic region and showed no abnormal hybridization (Fig. 4A). An appropriately sized FLNA protein could also be detected from the peripheral whole blood, further arguing against the actin-binding protein as a causal gene (Fig. 4B). Lastly, genomic sequencing of greater than 95% of the 48 FLNA exons demonstrated a previously detected FLNA mutation (Exon 7-2, AG- > GG) in the child from Family 1 (III-1), but failed to identify any mutations within two affected
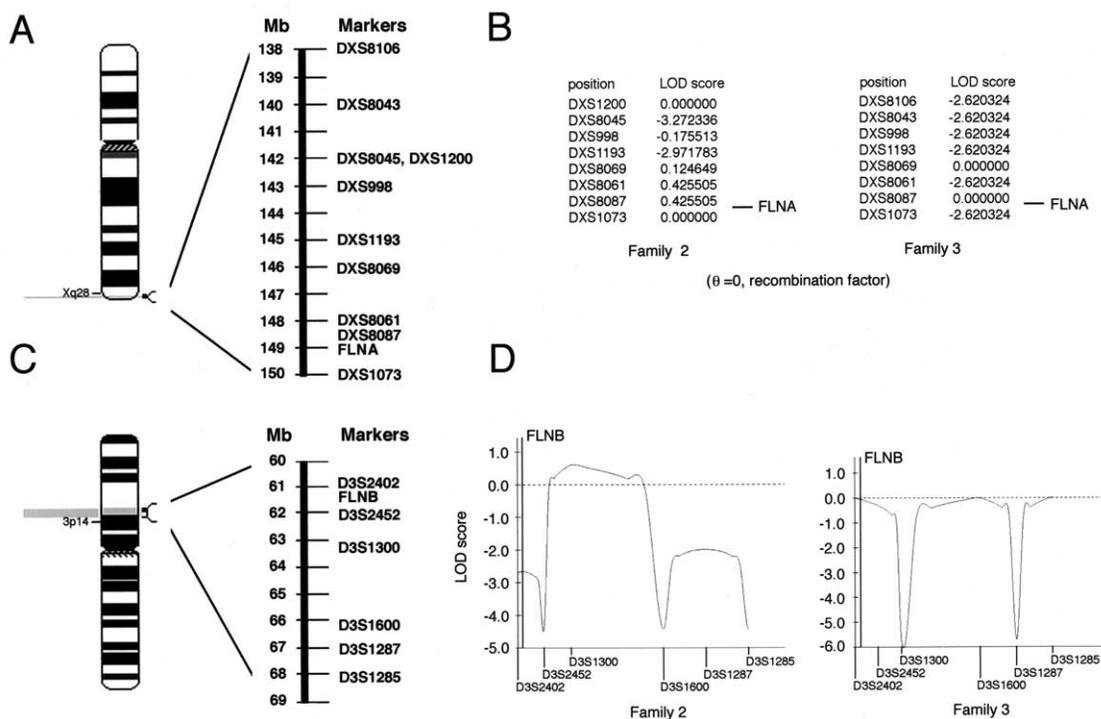

Fig. 3. Linkage analysis of the pedigrees with PHH. (A, D) Microsatellite markers used in evaluation of homozygosity and linkage for *FLNA* and *FLNB*. (B, C) Two-point analyses performed on *FLNA* ($\theta = 0$ recombination fraction) and multi-point analyses performed on *FLNB* suggested no linkage of either gene with Pedigree 1. The LOD score of 0.425 in Pedigree 2 suggests possible linkage with *FLNA*.
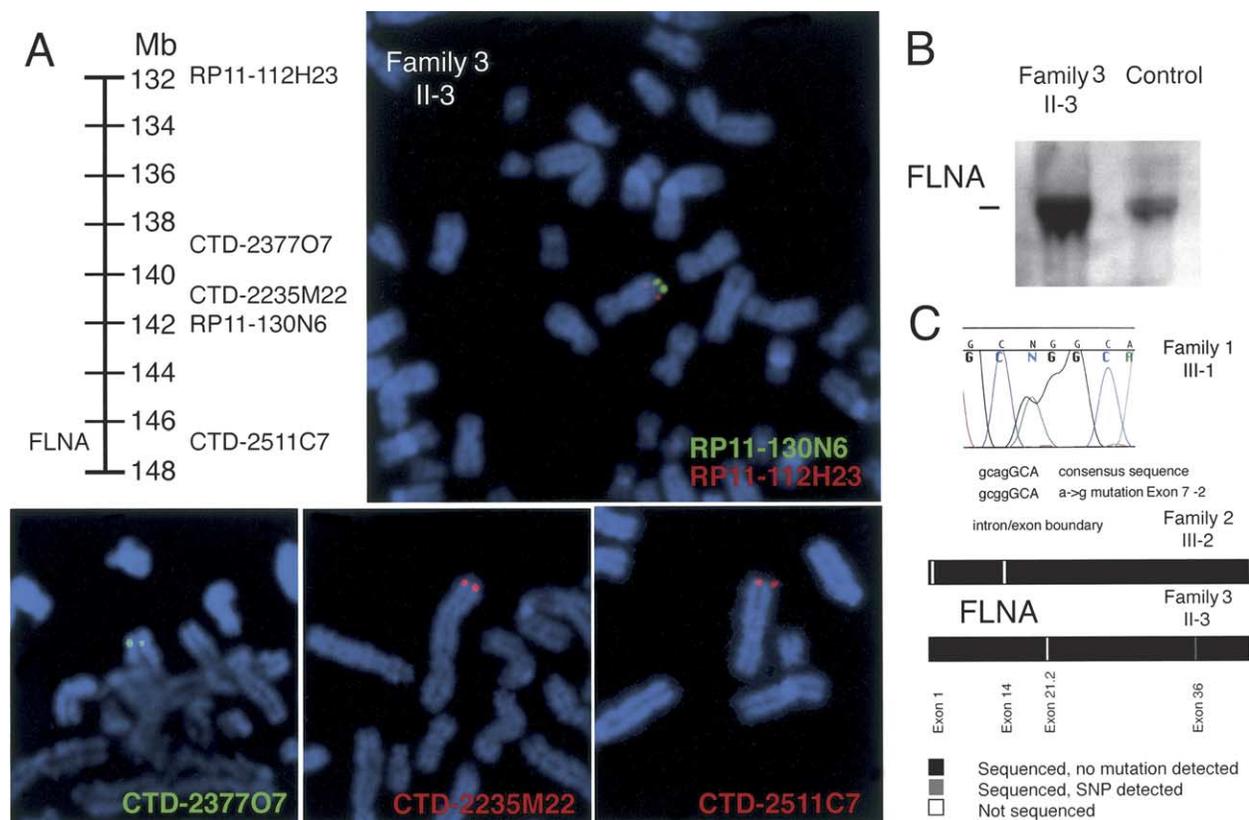
Fig. 4. The PHH syndrome is not associated with *FLNA* in Pedigree 3. (A) Fluorescent in situ hybridization (FISH) analyses using BAC probes localized to Xq27–28 do not detect any chromosomal abnormalities, suggesting that duplications or deletions of FLNA were not likely causes of the PHH phenotype in the affected male (Family 3, II-3). (B) Peripheral blood sample of the same PHH individual confirms expression of an appropriately sized 280 kD actin-binding protein (FLNA) by Western blot analysis. (C) Genomic sequencing of greater than 95% of the *FLNA* gene demonstrates a known mutation (Exon 7-2, AG- > GG) in the child with hydrocephalus (Family1, III-1), but fails to demonstrate any mutation in an affected individual from each of the other two families (Family 2, III-2; Family 3, II-3).

individuals from the other pedigrees (Family 3, II-3 and Family 2, III-2; Fig. 4C). A single nucleotide polymorphism (SNP) was detected in one of the individuals (Family 3, II-3) with a thymidine to cytosine substitution (GC**T** to GC**A**) producing no change in the alanine at AA# 1950. This SNP is a previously reported and non-disease related polymorphism (NCBI Gene Model Site).

Additional candidate genes were evaluated for Families 2 and 3. Given the known causal relationship of *L1CAM* with hydrocephalus, genomic sequencing (>95%) of Family 2 revealed no identifiable mutation. In Family 3, which likely had an autosomal inheritance pattern, a candidate gene for the autosomal recessive form of PH with microcephaly (unpublished observations, Sheen and Walsh), *ARFGEF2*, was sequenced (>85%) but no mutation was identified.

4. Discussion

The current report describes three kindreds with periventricular heterotopia and variably associated hydrocephalus (PHH) that appear to represent a mixture of different causes both genetic and potentially non-genetic.

In Pedigree 1, hydrocephalus occurs for the first time in an infant [III-1] from a pedigree with PH and a known *FLNA* mutation. The affected infant's mother [II-8] was treated with valproate through the pregnancy, such that the new born's hydrocephalus and meningomyelocele may reflect valproate embryopathy. Valproic acid exposure during early pregnancy can result in a 1–2% incidence of spina bifida aperta, a closure defect of the posterior neural tube, as seen in this infant [17]. At least 80% of people with spina bifida also have a Chiari II malformation and associated hydrocephalus [18]. The mechanism of valproic acid teratogenicity is unclear, although its interactions with folate metabolism and fundamental effects on transcriptional regulation through inhibition of histone deacetylase have been proposed [19,20]. While less likely, the presence of PH and a *FLNA* mutation might suggest that a mutation in this gene could cause hydrocephalus in some cases. Furthermore, whether a mutation in *FLNA* increases the likelihood of hydrocephalus and neural tube defects with medication usage remains to be determined. Most neural tube defects, however, are thought to arise from multifactorial causes, involving interactions between genetic predisposition and environmental factors [21].

In Pedigree 2, the slightly positive linkage to Xq28 makes it impossible to definitely rule in or rule out linkage to the *FLNA* locus in Xq28. The fact that two young affected individuals (III-1 and III-2) both have hydrocephalus and PH also argues more strongly for a genetic component, but the mode of inheritance is confusing since the parents of one affected child are normal, while the parent of the other affected child shows PH in a pattern that strongly resembles that seen with *FLNA* mutations. Possible connections to *FLNA* could also not be confirmed by *FLNA* mutational analysis. Although no *FLNA* mutation was found, the DNA sequence analysis probably does not detect all possible mutations. Some kind of genetic link to *FLNA* is also strongly suggested by the presence of typical PH in the mother of one affected child. While this mother was treated with carbamazepine during the pregnancy, carbamazepine embryopathy alone cannot explain the hydrocephalus in this family, since the mother of the second affected child did not receive anti-epileptic medications, making a genetic etiology more likely. With the possibility of an undetected genetic abnormality in Xq28 region, the worsening severity in phenotype over successive generations leads to the possibility of anticipation, as has previously been seen in Fragile X, located in this region [22]. Alternatively, the unaffected parental generations may possess balanced translocations with offspring inheriting either a duplication or deletion. This asymmetric inheritance of alleles could in principle explain the differing radiographic phenotypes. Finally, previous reports have shown that mutations of L1CAM on Xq28 lead to hydrocephalus and can be associated with Hirschsprung disease and pachygyria [23,24]. While no mutation for L1CAM was found on sequencing an affected individual in this family (data not shown), a similar association with gut dysmotility has been seen in individuals with *FLNA* mutations [1], suggesting some genetically linked relationship. Likewise, arrested migration can lead to pachygyria, while mutations in *FLNA* presumably results in impaired neuronal migration. Thus, in this pedigree, hydrocephalus with PH could be associated with some undetected genetic anomaly in the Xq28 region.

Despite the potential association of *FLNA* mutations and PHH in some cases, Pedigree 3 clearly constitutes a new syndrome, different from the autosomal recessive form of PH or the X-linked dominant PH caused by *FLNA* mutations. Genetic and molecular analyses, as well as the clinical phenotype, exclude *FLNA* as the causal gene in pedigree 3. Similarly, the consistent feature of microcephaly in individuals with autosomal recessive PH as well as a failure to detect a mutation in the candidate gene for this disorder, suggest that that Pedigree 3 represents a separate autosomal PH disorder. Clinically, affected individuals have severe developmental delay regardless of hydrocephalus. Radiographically, these affected offspring have bilateral, non-contiguous heterotopia distinct from typical X-linked and autosomal recessive PH [4,8,9,25]. The additional feature of hydrocephalus can be a defining characteristic of an autosomal PHH but as the current studies suggest, may also appear in other PH syndromes.

The current report describes the etiological heterogeneity of familial PH with hydrocephalus. It may comprise, in part, an inherited form of PH exacerbated by anti-epileptic medication. Some genetic abnormality within the Xq28 region may also give rise to PH and hydrocephalus. Finally, PHH can clearly represent a novel autosomal syndrome.

Acknowledgements

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