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FLNA genomic rearrangements cause periventricular nodular heterotopia

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ABSTRACT

Objective: To identify copy number variant (CNV) causes of periventricular nodular heterotopia (PNH) in patients for whom *FLNA* sequencing is negative.

Methods: Screening of 35 patients from 33 pedigrees on an Affymetrix 6.0 microarray led to the identification of one individual bearing a CNV that disrupted *FLNA*. *FLNA*-disrupting CNVs were also isolated in 2 other individuals by multiplex ligation probe amplification. These 3 cases were further characterized by high-resolution oligo array comparative genomic hybridization (CGH), and the precise junctional breakpoints of the rearrangements were identified by PCR amplification and sequencing.

Results: We report 3 cases of PNH caused by nonrecurrent genomic rearrangements that disrupt one copy of *FLNA*. The first individual carried a 113-kb deletion that removes all but the first exon of *FLNA*. A second patient harbored a complex rearrangement including a deletion of the 3' end of *FLNA* accompanied by a partial duplication event. A third patient bore a 39-kb deletion encompassing all of *FLNA* and the neighboring gene *EMD*. High-resolution oligo array CGH of the *FLNA* locus suggests distinct molecular mechanisms for each of these rearrangements, and implicates nearby low copy repeats in their pathogenesis.

Conclusions: These results demonstrate that *FLNA* is prone to pathogenic rearrangements, and highlight the importance of screening for CNVs in individuals with PNH lacking *FLNA* point mutations. **Neurology**® 2012;78:269-278

GLOSSARY

CGH = comparative genomic hybridization; **CMT1A** = Charcot-Marie-Tooth disease type 1A; **CNV** = copy number variant; **FoSTeS** = Fork Stalling and Template Switching; **LCR** = low copy repeat; **MLPA** = multiple ligation dependent probe amplification; **MMBIR** = microhomology-mediated break-induced replication; **NAHR** = nonallelic homologous recombination; **NHEJ** = nonhomologous end joining; **PMD** = Pelizaeus-Merzbacher disease; **PNH** = periventricular nodular heterotopia; **PTLS** = Potocki-Lupski microduplication syndrome; **qPCR** = quantitative PCR.

Congenital disorders of human brain development represent a diverse group of conditions, clinically and genetically. Loss-of-function mutations in *FLNA*, encoding the actin cross-linking protein Filamin A, cause one of the most prevalent brain malformations encountered clinically: X-linked periventricular nodular heterotopia (PNH).¹⁻³ In PNH, the neurons accumulate as nodules along the surface of the lateral ventricles,¹ resulting in seizures by early adulthood, and sometimes impeding normal psychomotor development.¹ Since loss-of-function mutations in *FLNA* are most often lethal prenatally in males, X-linked PNH is clinically encountered primarily in females.^{1,4}

FLNA point mutations are found in most, but not all, patients with X-linked familial PNH,^{5,6} and in only 26% of sporadic patients with bilateral PNH.⁵ Some of the missing heritability may be

Supplemental data at
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Supplemental Data



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due to genetic heterogeneity.⁷⁻¹⁰ Alternatively, lesions in Filamin A undetectable by traditional sequencing may also cause disease. One category of such lesions is copy number variants (CNVs), which are an increasingly recognized cause of disease^{11,12} and trait variation.¹³⁻¹⁵ *FLNA* resides in a rearrangement-prone region of the X chromosome at Xq28.^{16,17} We hypothesized that CNVs in this region might represent a previously unrecognized cause of PNH.

Here, we describe 3 patients with pathogenic *FLNA* CNVs as a cause of otherwise unexplained PNH. Custom tiling oligo comparative genomic hybridization (CGH) revealed distinct CNV boundaries consistent with rare, nonrecurrent events, likely stimulated by nearby genomic low copy repeats (LCRs). These results demonstrate that genomic rearrangements are an important cause of *FLNA* mutation, and establish the clinical importance of screening for such rearrangements in PNH.

METHODS **Diagnosis.** Clinically suspected PNH in patients was confirmed by brain MRI.

Affymetrix 6.0 microarray analysis. DNA samples were analyzed on an Affymetrix genome-wide SNP Array 6.0. Data were processed using Birdsuite¹⁸ to identify candidate copy number changes. Birdsuite calls were loaded into a MySQL database and filtered by score ($\text{lod} > 6$) to generate a list of candidate CNVs. Separately, SNP calls were analyzed using PennCNV¹⁹ with the software's recommended default settings.

Multiple ligation dependent probe amplification analysis. Multiple ligation dependent probe amplification (MLPA) analysis was performed in individuals from 3 families using the Lissencephaly probe kit (SALSA P061-B2, MRC Holland, Amsterdam, the Netherlands), including 7 paired probes specific for the *FLNA* gene (exons 4, 11, 22, 25, 29, 39, and 46). The resulting products were separated and sized on an ABI 3130XL sequencer (Applied Biosystems). Analysis was performed according to the manufacturer protocol.

Quantitative PCR validation. Taqman copy number assays from Applied Biosystems were used to validate CNVs (table e-1 on the *Neurology*[®] Web site at www.neurology.org) with RNaseP as the internal control. DNA samples from neurologically normal Caucasian controls were used to calibrate the data. The data were analyzed, plotted, and 95% confidence intervals calculated using CopyCaller software from Applied Biosystems.

Oligo microarray CGH. A custom CGH array (Nimblegen) with 137,408 probes was used to detect copy number of genomic DNA in areas of interest. The probes were designed to span the entire *FLNA* genomic locus, including neighboring

genes (chrX:152,943,207–153,559,506, hg18), with an average spacing of 30 bp. The data were plotted using the statistical package *R*. Copy number values were calculated by $\log_2(\text{Cy3}/\text{Cy5})$, where Cy3 is the probe signal of the proband and Cy5 is the probe signal from a reference female control. To reduce noise, values were normalized to CGH results of a second reference female control, previously established by quantitative PCR (qPCR) to lack copy number variation at the locus. Exon numbering is provided relative to UCSC gene ID uc004fkk.2.

Functional breakpoint sequencing. PCR amplification (Promega *GoTaq*) was employed to sequence the breakpoints of the *FLNA* locus rearrangements. Primer sequences for family 1: 5'-CTCTGTGAGCCGCAAAGTGT and 5'-CCCCCA-CAGCTGTTTAGAGA; family 2: 5'-ATGCAGTGCGAGATGTGGAC and 5'-CAGCCTGAAAATCCCTGGTA. Sequencing results were mapped against the human hg18 genomic reference sequence.

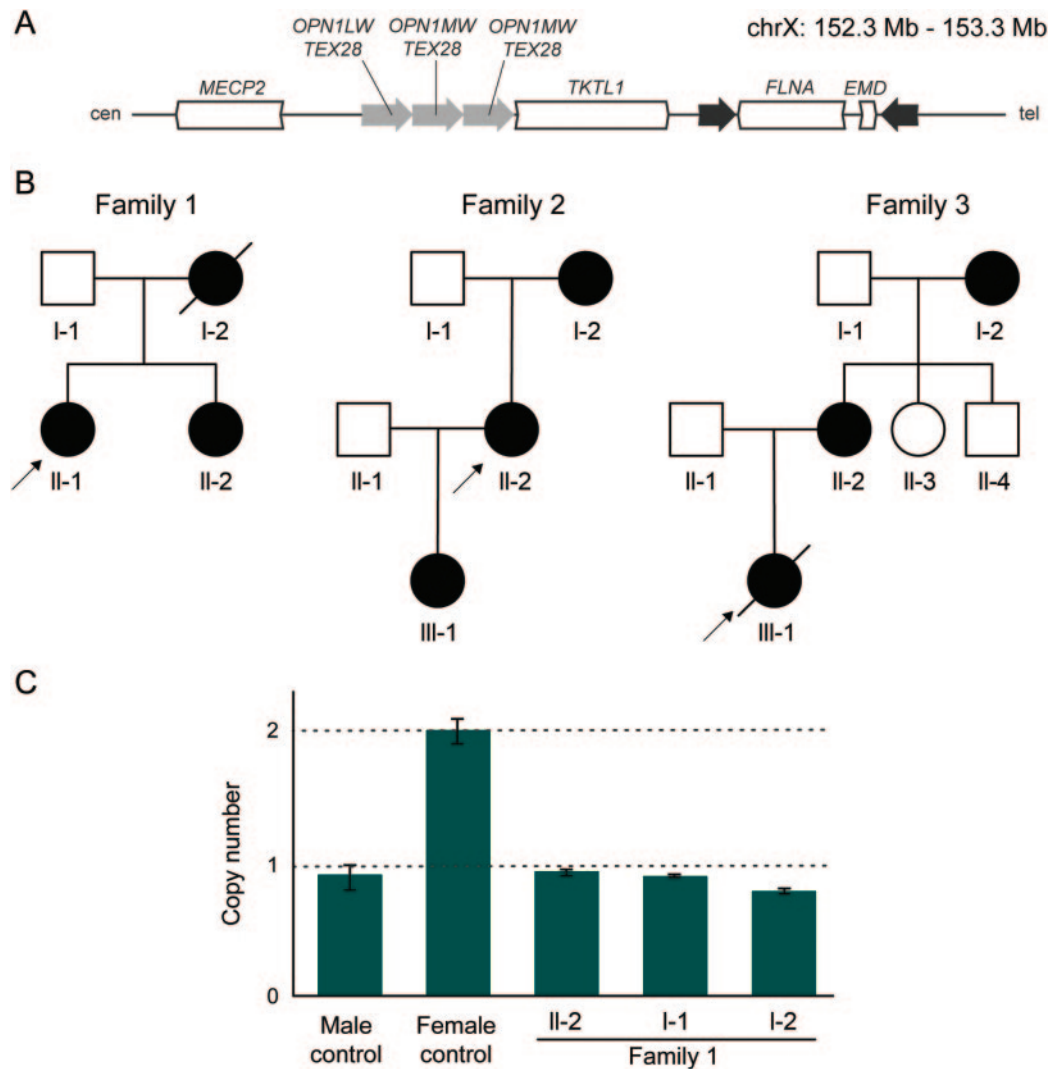
Standard protocol approvals, registrations, and patient consents. Clinical details and biological samples were obtained with written informed consent from the participants or their parents/guardians at Children's Hospital Boston. The study was approved by the Human Research Ethics Committee of all participating institutions and conducted as part of the National Institute of Neurological Disorders and Stroke Trial, Human Epilepsy Genetics–Neuronal Migration Disorders Study #NCT00041600.

RESULTS **Clinical information.** Family 1 is a non-consanguineous family of South Asian ancestry with multiple women affected by PNH (figure 1B, table 1). Screening ultrasound of female II-1 revealed irregular linings of the ventricles bilaterally, and brain MRI established a diagnosis of PNH. Developmental milestones were achieved appropriately with the exception of walking, which was mildly delayed at 18 months. She is currently pursuing a university degree. Her older sister, II-2, had normal early development but developed frequent seizures in childhood requiring antiepileptics. She had slight right-sided weakness. She required special education and is currently in a certificate program. MRI revealed bilateral nodular heterotopia (figure 2). She remains on antiepileptic medications. Their mother, I-2, was diagnosed with PNH by MRI following her daughters' diagnoses. She died at age 49 of undetermined causes.

Family 2 is a multigeneration family affected by PNH (figure 1B, table 1). The proband (II-2) experienced seizures resistant to multiple antiepileptics beginning in childhood. Her cognitive development and capabilities were within normal limits. MRI demonstrated classic, bilateral PNH (figure 2). The proband's mother, I-2, and the proband's daughter, III-1, were both asymptomatic and their diagnoses were ascertained after PNH was diagnosed in the proband, II-2.

Three generations of women in family 3 are affected by bilateral PNH (figure 1B, table 1), with some additional pathogenic features. The proband III-1 was born at 39 weeks to a 40-year-old mother, II-2. The pregnancy was significant for slowed intra-

Figure 1 The *FLNA* locus is prone to rearrangement



(A) The *FLNA* and *EMD* genes are flanked by 11.3-kb inverted repeats at Xq28. *FLNA* encodes for the protein Filamin A, an actin cross-linking protein. *EMD* encodes for Emerin, a protein disrupted in Emery-Dreifuss muscular dystrophy. Recombination at this locus results in benign inversions. *FLNA* also lies telomeric to 3 low copy repeats of the color vision gene *OPN1* and *TEX28*. Nonallelic homologous recombination-mediated deletion of *OPN1-TEX28* repeats is a known cause of human color blindness. (B) Pedigrees of families 1, 2, and 3. Females are represented by circles, affected individuals by dark circles, and deceased individuals by an angled line. Arrows indicate the proband in each family. (C) Quantitative PCR (qPCR) confirmation of *FLNA* copy number loss in II-2 and I-2 of family 1. qPCR results targeting sequence centered on chrX: 153,240,806. When normalized to a female control, it is apparent that the affected mother and daughter have one copy of the allele at that location, whereas the female control has 2 copies. The father, I-1, has one copy, which is expected for an unaffected male. The 95% confidence intervals were calculated using CopyCaller software by Applied Biosystems.

uterine growth from 28 to 29 weeks. At 2 months of age, she became irritable, with decreased feeding, poor sleep, stridor, and weak cry. She was admitted to the hospital where investigations revealed right ventricular hypertrophy, a patent foramen ovale, and pulmonary emphysema affecting multiple lobes. She was seen by the Genetics team because of possible dysmorphic features (blue sclerae, joint hypermobility, and loose skin); routine chromosome analysis and array CGH studies were normal. Her pulmonary function deteriorated progressively, and she died of respiratory insufficiency at 7 months. Autopsy

showed bilateral, symmetric PNH, pulmonary panacinar emphysema, and tricuspid and mitral valve dysplasia. Her mother and grandmother were subsequently also diagnosed with PNH. Her mother also had cardiac valvular abnormalities and apical bullae of the lungs; her grandmother had lobar emphysema. No seizures were evident in any of the 3 affected individuals.

Molecular investigations in family 1. The proband of family 1 was identified in a screen for *FLNA* CNVs in a cohort of 35 PNH patients (from 33 pedigrees) genotyped with Affymetrix 6.0 microarrays. The result-

Table 1 Families with PNH due to *FLNA* deletions resolved by array CGH

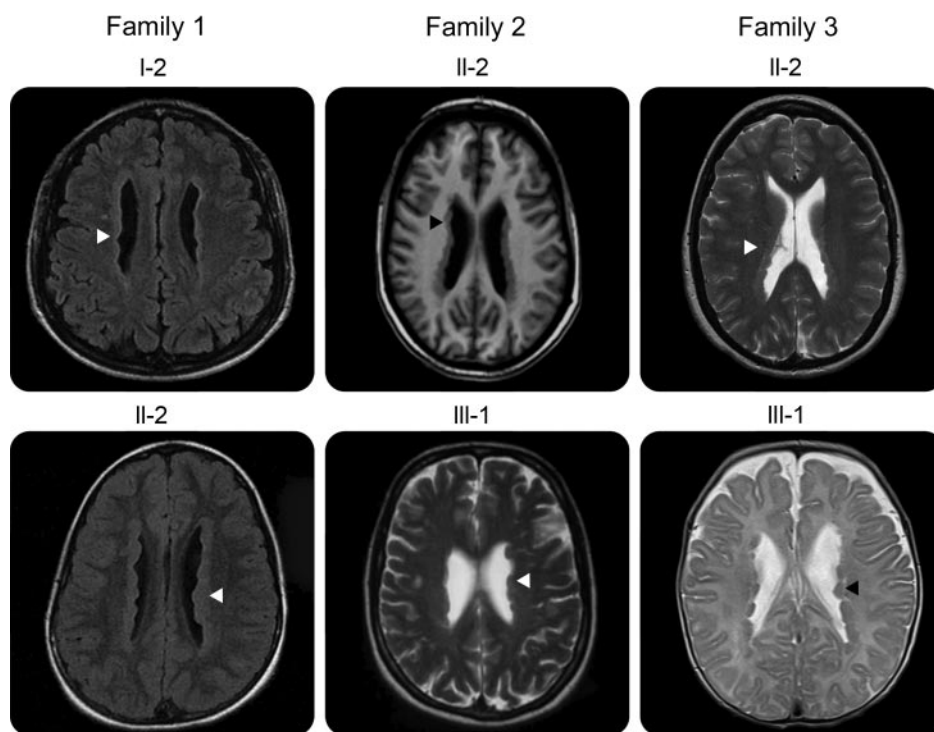
Family	Ethnicity	Phenotype	<i>FLNA</i> mutation
1	South Asian	Sister of proband with seizures, requiring special education	3' <i>FLNA</i> deletion sparing first exon
2	Caucasian (French)	Proband with epilepsy resistant to antiepileptics	3' <i>FLNA</i> deletion and duplication of 5' <i>FLNA</i> and <i>EMD</i>
3	West Indian father, French Canadian mother	Proband with emphysema and respiratory failure at 7 months	<i>FLNA</i> and <i>EMD</i> deletion

Abbreviations: CGH = comparative genomic hybridization; PNH = periventricular nodular heterotopia.

ing data were used to screen for CNVs using 2 algorithms, Birdsuite¹⁸ and PennCNV.¹⁹ Applying Birdsuite, we found 3 patients with evidence suggestive of copy number loss overlapping *FLNA*. Only one of these events was predicted with PennCNV, and qPCR confirmed only the single true positive. In this female individual (figure 1B, family 1, individual II-1), Birdsuite predicted a single copy deletion spanning 19.8 kb (hg18, chrX:153,228,832–153,248,612), disrupting the 3' end of *FLNA*. qPCR in the proband using multiple probes spanning the *FLNA* locus, as well as MLPA, confirmed a copy number of 1 over this interval (data not shown); this copy number change was also found in

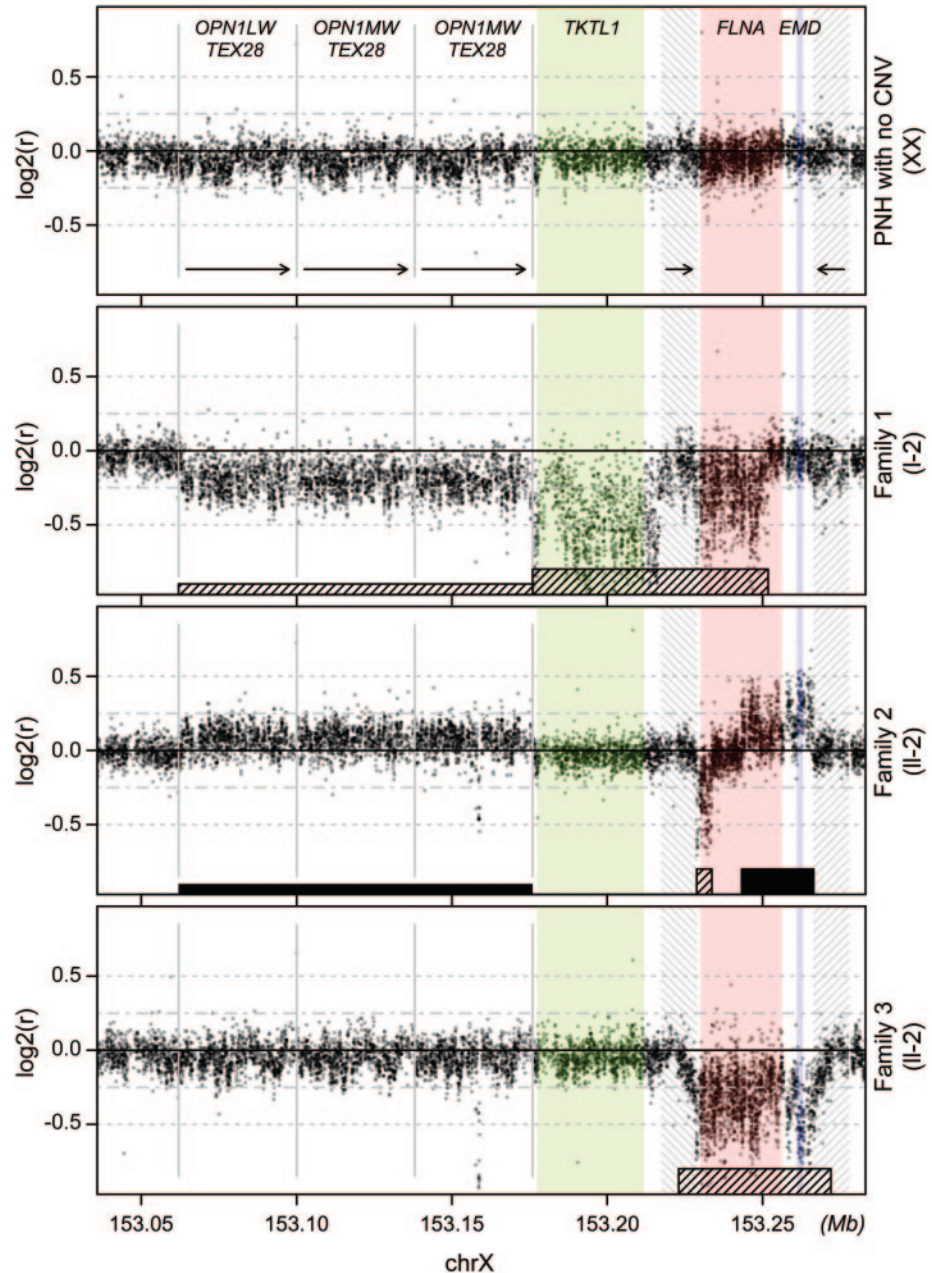
2 other affected family members (figure 1C, family 1, sister II-2, and mother I-2), both of whom had MRI-confirmed PNH (figure 2).

To understand the rearrangement that led to this copy number change, we generated a custom microarray to fine-tile the *FLNA* locus with overlapping 40–60 bp probes at a spacing of 15–30 bp, and performed CGH. CGH of individual I-2 from family 1 confirmed the presence of a *FLNA* 3' deletion, which was significantly larger than originally predicted by the Affymetrix 6.0 data. Decreased signal intensities were observed over the entire *TKTL1* gene and nearly all of *FLNA*, consistent with loss of a single copy (figure 3, second panel). This lesion spares only *FLNA* exon 1 (noncoding) and *FLNA* exon 2 (the first coding exon), and is therefore predicted to abrogate *FLNA* function. The remainder of the locus demonstrated partially depressed signal intensities over the ~120 kb region centromeric to *TKTL1*, as well as over both inverted 11.3 kb LCRs flanking *FLNA* (figure 3, second panel). The former region contains 3 39-kb direct LCRs that include the opsin genes responsible for human color vision.²⁰ Each repeating subunit contains one copy of the opsin gene *OPN1* and one copy of *TEX28* (figure 3, first panel). Since the CGH probes in the *OPN1-TEX28* locus cannot distinguish one direct repeat from another, a

Figure 2 Brain MRI in affected individuals from families 1–3

Representative axial brain MRI images from affected individuals in this study. Bilateral gray matter nodules (arrowheads) line and project into the lateral ventricles, the classic appearance of periventricular nodular heterotopia. Family 1, I-2 and II-2, family 2, II-2: T1-weighted. Family 2, III-1, family 3, II-2 and III-1: T2-weighted.

Figure 3 High-resolution array comparative genomic hybridization (CGH) of the *FLNA* locus delineates distinct genomic rearrangements in each family with periventricular nodular heterotopia (PNH)



Top panel: A female individual with PNH, but without *FLNA* copy number change detected by quantitative PCR (qPCR), demonstrates signal intensities consistent with normal copy number across the locus. Second panel: Affected individual I-2 of family 1 demonstrates single-copy loss of all but the 5' end of *FLNA*, as well as the adjacent *TKTL1* gene. Third panel: Affected individual II-2 of family 2 demonstrates a single copy deletion of the 3' end of *FLNA*, and duplication of the 5' end of *FLNA* and *EMD*. Bottom panel: Affected individual II-2 of family 3 demonstrates deletion of both *FLNA* and *EMD*. The y-axis represents the \log_2 ratio of the signal intensity from the patient compared to a reference female control. Tall striped rectangles = inferred deletions; tall filled rectangle = inferred duplications; short striped and solid rectangles = regions of slightly decreased and increased signal intensity, respectively, across entire *OPN1-TEX28* locus; green, red, blue shading = *TKTL1*, *FLNA*, *EMD* gene loci, respectively; gray hashes and short arrows = boundaries and direction of the inverted repeats flanking *FLNA* and *EMD*; gray vertical lines and long arrows = boundaries and direction of the *OPN1-TEX28* tandem repeats. CNV = copy number variant.

deletion of one of the 3 LCRs on one chromosome would be predicted to cause a one-sixth reduction in observed copy number relative to a diploid control [expected \log_2 ratio of $\log_2(5/6) = -0.26$]. We therefore interpret the slight but uniform decrease in

\log_2 ratio across the *OPN1-TEX28* region to indicate deletion of one of the 3 LCRs on one chromosome.

Molecular investigations in families 2 and 3. Screening additional cohorts of PNH families using MLPA

revealed 2 more pathogenic *FLNA* deletions. Family 2 is a 3-generation family with PNH (figure 1B). DNA was obtained from the proband, II-2 (DNA samples were not available from I-2 and III-1). MLPA revealed the proband to be missing one copy of *FLNA* (data not shown). Fine-tiling array CGH delineated a 4.8-kb deletion removing exons 42–48 of *FLNA* (figure 3, third panel), accompanied by a 23-kb duplication spanning the 5' end of *FLNA* and all of *EMD* (figure 3, third panel; see Discussion). This mutation is likely to be deleterious, since distal truncating mutations as late as exon 47 in *FLNA* cause PNH.²¹ There is also a slight but uniform increase in signal intensity in the *OPN1-TEX28* tandem repeat region, consistent with a duplication of one repeat on one chromosome [expected log₂ratio, log₂(7/6) = 0.22].

Family 3 is another 3-generation family exhibiting X-linked PNH (figures 1B and 2). MLPA screening of individual III-1 and her mother II-2 revealed a *FLNA* copy number loss (data not shown), and fine-tiling array CGH in II-2 demonstrated a 39-kb deletion with breakpoints mapping to the 11.3-kb inverted LCRs (figure 3, fourth panel). The identical deletion was detected by array CGH in individual III-1 (data not shown). This deletion removes one copy of the entire *FLNA* gene, providing an explanation for PNH in this pedigree. The deletion also removes one copy of the neighboring *EMD* gene. Mutations in *EMD* cause X-linked recessive Emery-Dreifuss muscular dystrophy, a degenerative myopathy without involvement of the CNS.²² Female carriers of *EMD* mutations are at risk for cardiac abnormalities, including cardiomyopathy and conduction defects.^{23,24}

Junctional breakpoint analysis. A series of PCR assays were designed to interrogate the rearrangement breakpoint junctions. We successfully identified breakpoint PCR products in 2 of the 3 families. In family 1, PCR was attempted under the assumption of a simple, contiguous 113-kb deletion extending from *TKTL1* to *FLNA* (see Discussion). A junctional amplification product was obtained of the approximate size predicted (data not shown), but sequence analysis revealed evidence of a more complex mechanism. Instead of a contiguous 113-kb deletion, the junctional product contained sequence from *TEX28* intron 1, followed by an apparent 1,700-bp deletion, followed by 10 base pairs of *TEX28* promoter, followed by a 33,403-bp deletion, followed by 43 base pairs of *TEX28* intron 1, followed by a 77,942-bp deletion, followed by sequence from *FLNA* intron 2 (figure 4A). Closer inspection revealed 2 to 3 base pairs of microhomology between adjacent fragments (figure 4A).

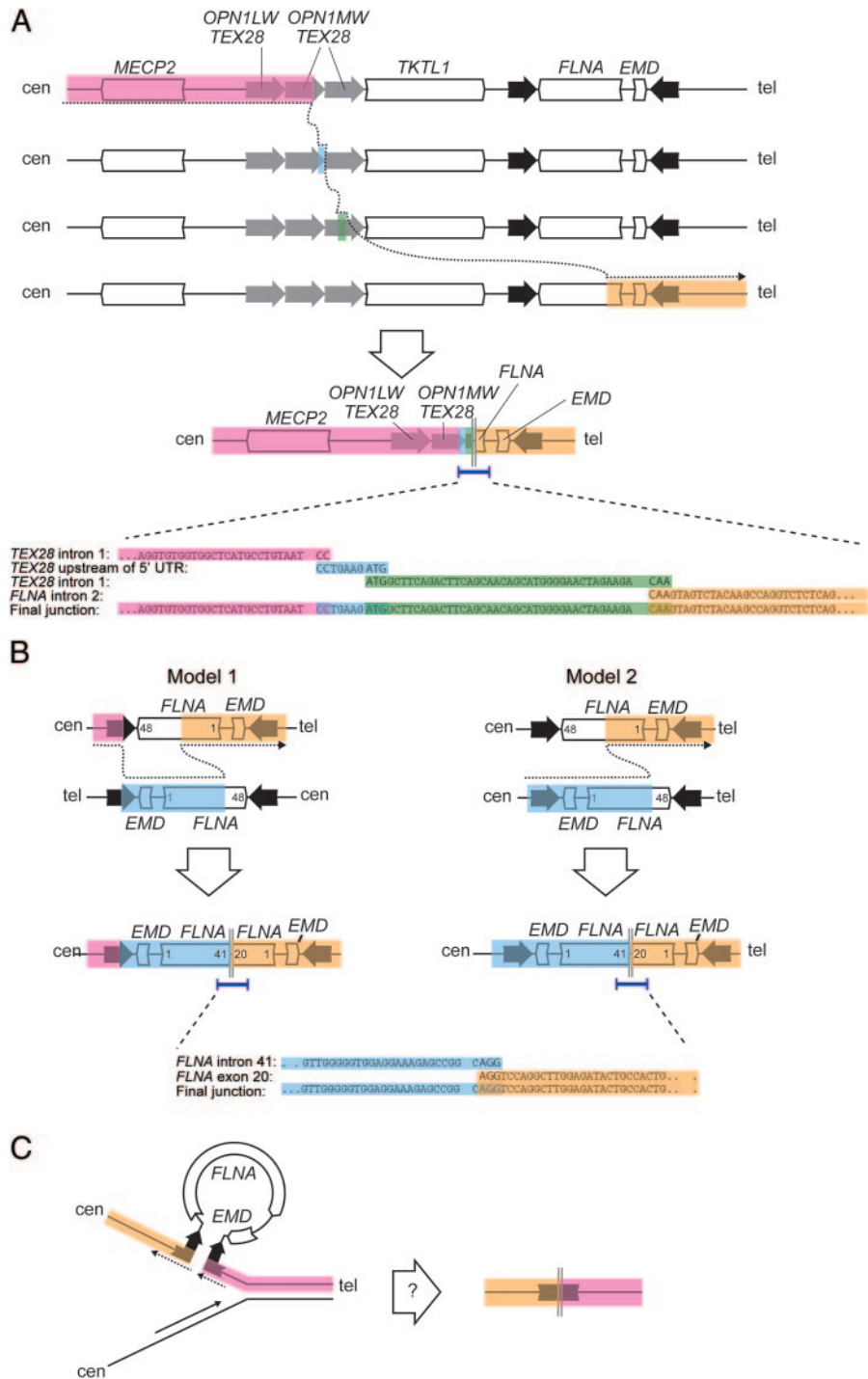
In family 2, array CGH data suggested a head-to-tail partial duplication of *FLNA* (see Discussion). We designed PCR primers extending in the centromeric direction from exon 20 and from exon 41 and successfully obtained an amplification product, analysis of which revealed a junction between *FLNA* intron 41 and exon 20 (figure 4B). Three base pairs of microhomology were shared between the 2 fragments.

DISCUSSION Point mutations in *FLNA* are found in most but not all patients with X-linked familial PNH, and only 26% of sporadic cases,^{5,6} suggesting the existence of other causative genetic lesions. Here, we applied a variety of methods to identify CNVs overlapping *FLNA* in patients with developmental brain disorders. Three families harboring pathogenic *FLNA* deletions are described: family 1, carrying a deletion of the majority of the 3' end of *FLNA*; family 2, with a deletion of the 3' end of *FLNA* and partial duplication of the 5' end and *EMD*; and family 3, with a deletion of the entire *FLNA* gene and *EMD*. Deletions of *EMD* have been previously described,^{16,17} but to our knowledge, this is the first report of deletion involving both *EMD* and *FLNA*.

The *FLNA-EMD* locus has been reported to be prone to genomic rearrangements, likely stimulated by multiple LCRs in the region.¹⁶ Two 11.3-kb inverted repeats flank *FLNA* and *EMD*. Inverted repeats promote genomic instability²⁵ by a variety of mechanisms,^{26,27} one of which is nonallelic homologous recombination (NAHR). NAHR between the inverted repeats results in benign inversion of *FLNA-EMD* in up to 18% of patients of European descent.¹⁶ Mispairing between the highly homologous 11.3-kb inverted repeats, followed by nonhomologous end joining (NHEJ) between an intervening *Alu* element and *FLNA* intron 28, was shown to result in deletion of *EMD* (and partial duplication of *FLNA*) in a patient with Emery-Dreifuss muscular dystrophy.¹⁶ Inspection of the copy number pattern observed in family 2 (figure 3) suggests a similar mechanism of rearrangement (figure 4B): NAHR between 2 misaligned inverted repeats, followed by NHEJ between exon 20 of *FLNA* and intron 41 of *FLNA* on the opposite chromatid, resulting in deletion of the 3' end of *FLNA* and head-to-tail duplication of the 5' end of *FLNA* and *EMD*. Alternatively, a second model is possible in which this rearrangement first occurred in a maternal ancestor heterozygous for one copy of the benign *FLNA-EMD* inversion (figure 4B). NAHR between properly aligned LCRs, followed by NHEJ between *FLNA* exon 20 and intron 41, would result in the same final product.

The boundaries of the *FLNA* deletion observed in family 1 suggest a distinct, more complicated mecha-

Figure 4 Copy number changes at the *FLNA* locus in 3 families with periventricular nodular heterotopia implicate distinct molecular mechanisms



(A) Breakpoint sequencing supports a mechanism for family 1 rearrangement involving replication fork stalling and template switching/microhomology-mediated break-induced replication with template switches occurring between the second *OPN1-TEX28* tandem repeat, the junction between the first and second *OPN1-TEX28* tandem repeats, the third *OPN1-TEX28* repeat, and *FLNA* intron 2. The result is loss of 1 *OPN1-TEX28* tandem repeat and a deletion of all but the 5' end of *FLNA*. (B) Breakpoint sequencing in family 2 supports 2 alternative models. In model 1, nonallelic homologous recombination between mispaired inverted repeats is followed by nonhomologous end joining (NHEJ) between 2 inverted copies of *FLNA* on opposite chromatids, leading to deletion of the 3' end of *FLNA* and duplication of the 5' end of *FLNA* and *EMD*. Model 2 presupposes a heterozygous background for the benign *FLNA-EMD* inversion, in which case a single NHEJ event between *FLNA* intron 41 on one chromatid and *FLNA* exon 20 on the second chromatid is sufficient to generate the observed rearrangement. (C) A speculative, replication-based model for *FLNA* rearrangement in family 3, in which the inverted repeats flanking *FLNA* and *EMD* form a single-strand secondary structure that results in contiguous deletion of both genes and the inner half of both repeats.

nism. While array CGH results (figure 3) initially suggested a single 113-kb deletion, breakpoint sequencing revealed that the expected contiguous deletion was actually interrupted by 2 sequences, 10 and 43 base pairs long, corresponding to sequences upstream of the *TEX28* 5' UTR and sequences from *TEX28* intron 1, respectively, with 2–3 base pairs of microhomology at each sequence junction (figure 4A). This pattern, in which the resulting product appears as if the polymerase “skipped” several times across the template with evidence of microhomology at each junction, suggests that the family 1 rearrangement arose via a replication Fork Stalling and Template Switching (FoSTeS)/microhomology-mediated break-induced replication (MMBIR).²⁸ FoSTeS/MMBIR has been observed to underlie other CNV-associated disorders such as Pelizaeus-Merzbacher disease (PMD),²⁹ Potocki-Lupski microduplication syndrome (PTLS)/Smith-Magenis microdeletion syndrome, and Charcot-Marie-Tooth disease type 1A (CMT1A).³⁰ The resulting product in family 1 is an allele deleting one *OPN1-TEX28* unit, all of *TKTL1* and the centromeric 11.3-kb inverted repeat, and the 3' end of *FLNA* (figure 4A). We believe the PNH phenotype in this family is explained entirely by the *FLNA* truncation; to our knowledge, no clinical diseases have been ascribed to *TKTL1* haploinsufficiency, although there are hypothesized links to susceptibility to Wernicke-Korsakoff syndrome.^{31–34}

In family 3, array CGH predicts a clean excision of *FLNA* and *EMD*, as well as the inner half of each flanking repeat. This is despite the fact that direct homologous recombination between 2 inverted repeats is expected to cause inversion, not deletion. Examination of the Alu-rich inverted repeats for direct repeats that could lead to deletion revealed no such sequence (data not shown). We speculate that a mechanism for the family 3 rearrangement might be one akin to replication slippage,²⁸ in which the inverted DNA repeats—exposed during replication as single-stranded DNA—form a secondary structure that leads to deletion of the intervening DNA (figure 4C). This explanation is not wholly satisfactory, however, as the size of this deletion (39 kb) is considerably larger than those typically observed in replication slippage. Since we were unable to amplify the breakpoint sequence, we cannot rule out the possibility of FoSTeS/MMBIR in this family as well.

The reported spectrum of clinically relevant *FLNA* variations includes not only loss-of-function mutations (resulting in PNH) but also gain-of-function mutations (resulting in otopalatodigital spectrum disorders) and partial loss-of-function mutations (associated with milder PNH and male survival).³⁵ The clinical presentations of the patients in

this study are generally indistinguishable from those of other loss-of-function alleles. This is consistent with these lesions acting as molecular nulls.

Affected individuals in family 3 exhibited additional pulmonary abnormalities, a finding that has been recently reported in 2 cases, in the setting of a missense mutation (p.G74R) and a truncating mutation (p.K331X).^{36,37} Our finding that deletion can phenocopy these changes indicates that the pulmonary phenotype is the result of *FLNA* loss-of-function, rather than a novel gain-of-function effect specific to the 2 other alleles. Although the true incidence of pulmonary disease in individuals with *FLNA* mutation remains to be established, it is unclear why pulmonary abnormalities are more severe in some individuals. In this respect, while affected individuals in family 3 are unique in that they harbor a concomitant *EMD* deletion, this is probably insufficient to account for their pulmonary presentation: *EMD* mutations are not known to affect the lung, and the previously reported pulmonary cases^{36,37} are not known to have additional mutations in *EMD*.

Here we have described 3 cases of *FLNA* genomic rearrangements leading to PNH, each with a unique pattern of copy number loss or gain that implies distinct, nonrecurrent mutational mechanisms. These results add to the rich variety of human variation documented at the Xq28 locus—opsin gene deletion causing color blindness, benign *FLNA-EMD* inversion, *EMD* deletion causing muscular dystrophy, and now *FLNA* disruption causing PNH—and underscore the importance of molecular screening for such rearrangements.

AUTHOR CONTRIBUTIONS

K.R.C. performed Birdsuite copy number analyses, QPCR experiments, and wrote the manuscript. T.W.Y. designed and performed Birdsuite copy number analyses, QPCR and breakpoint localization experiments, and wrote the manuscript. V.S.G. helped perform copy number analyses, helped design and perform breakpoint localization experiments, and helped write the manuscript. B.B. organized clinical information and patient samples. Y.C. performed PennCNV copy number analyses. D.M., B.F., and E.P. identified and clinically characterized family 2, and D.M. and E.P. performed MLPA analysis of family 2. L.D. and M.N. identified and characterized family 1. C.S. and C.K. identified and clinically characterized family 3. R.G. directed the overall research and wrote the manuscript. C.A.W. directed the overall research and wrote the manuscript.

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DISCLOSURE

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REFERENCES

- Ekşioğlu YZ, Scheffer IE, Cardenas P, et al. Periventricular heterotopia: an X-linked dominant epilepsy locus causing aberrant cerebral cortical development. *Neuron* 1996;16:77–87.
- Fox JW, Lamperti ED, Ekşioğlu YZ, et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* 1998;21:1315–1325.
- Zenker M, Rauch A, Winterpacht A, et al. A dual phenotype of periventricular nodular heterotopia and frontotaphyseal dysplasia in one patient caused by a single *FLNA* mutation leading to two functionally different aberrant transcripts. *Am J Hum Genet* 2004;74:731–737.
- Guerrini R, Mei D, Sisodiya S, et al. Germline and mosaic mutations of *FLN1* in men with periventricular heterotopia. *Neurology* 2004;63:51–56.
- Parrini E, Ramazzotti A, Dobyns WB, et al. Periventricular heterotopia: phenotypic heterogeneity and correlation with Filamin A mutations. *Brain* 2006;129:1892–1906.
- Sheen VL, Dixon PH, Fox JW, et al. Mutations in the X-linked filamin 1 gene cause periventricular nodular heterotopia in males as well as in females. *Hum Mol Genet* 2001;10:1775–1783.
- Sheen VL, Topçu M, Berkovic S, et al. Autosomal recessive form of periventricular heterotopia. *Neurology* 2003;60:1108–1112.
- Sheen VL, Wheless JW, Bodell A, et al. Periventricular heterotopia associated with chromosome 5p anomalies. *Neurology* 2003b;60:1033–1036.
- Sheen VL, Basel-Vanagaite L, Goodman JR, et al. Etiological heterogeneity of familial periventricular heterotopia and hydrocephalus. *Brain Dev* 2004;26:326–334.
- Sheen VL, Ganesh VS, Topcu M, Sebire G, et al. Mutations in *ARFGEF2* implicate vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex. *Nat Genet* 2004;36:69–76.
- Zhang F, Gu W, Hurles ME, Lupski JR. Copy number variation in human health, disease, and evolution. *Annu Rev Genomics Hum Genet* 2009;10:451–481.
- McCarroll SA, Altshuler DM. Copy-number variation and association studies of human disease. *Nat Genet* 2007;39(7 suppl):S37–S42.
- Carvalho CMB, Zhang F, Lupski JR. Evolution in health and medicine: Sackler colloquium: genomic disorders: a window into human gene and genome evolution. *Proc Natl Acad Sci USA* 2010;107(suppl 1):1765–1771.
- Lee JA, Lupski JR. Genomic rearrangements and gene copy-number alterations as a cause of nervous system disorders. *Neuron* 2006;52:103–121.
- Walsh CA, Engle EC. Allelic diversity in human developmental neurogenetics: insights into biology and disease. *Neuron* 2010;68:245–253.
- Small K, Iber J, Warren ST. Emerin deletion reveals a common X-chromosome inversion mediated by inverted repeats. *Nat Genet* 1997;16:96–99.
- Small K, Warren ST. Emerin deletions occurring on both Xq28 inversion backgrounds. *Hum Mol Genet* 1998;7:135–139.
- Korn JM, Kuruvilla FG, McCarroll SA, et al. Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nat Genet* 2008;40:1253.
- Wang K, Li M, Hadley D, et al. PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. *Genome Res* 2007;17:1665–1674.
- Nathans J, Thomas D, Hogness DS. Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. *Science* 1986;232:193–202.
- Moro F, Carozzo R, Veggioni P, et al. Familial periventricular heterotopia: missense and distal truncating mutations of the *FLN1* gene. *Neurology* 2002;58:916–921.
- Bione S, Maestrini E, Rivella S, et al. Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. *Nat Genet* 1994;8:323–327.
- Funakoshi M, Tsuchiya Y, Arahata K. Emerin and cardiomyopathy in Emery-Dreifuss muscular dystrophy. *Neuromuscul Disord* 1999;9:108–114.
- Karst ML, Herron KJ, Olson TM. X-linked nonsyndromic sinus node dysfunction and atrial fibrillation caused by emerin mutation. *J Cardiovasc Electrophysiol* 2008;19:510–515.
- Gordenin DA, Lobachev KS, Degtyareva NP, Malkova AL, Perkins E, Resnick MA. Inverted DNA repeats: a source of eukaryotic genomic instability. *Mol Cell Biol* 1993;13:5315–5322.
- Lobachev KS, Shor BM, Trai HT, et al. Factors affecting inverted repeat stimulation of recombination and deletion in *Saccharomyces cerevisiae*. *Genetics* 1998;148:1507–1524.
- Voineagu I, Narayanan V, Lobachev KS, Mirkin SM. Replication stalling at unstable inverted repeats: interplay be-

- tween DNA hairpins and fork stabilizing proteins. *Proc Natl Acad Sci USA* 2008;105:9936–9941.
28. Hastings PJ, Lupski JR, Rosenberg SM, Ira G. Mechanisms of change in gene copy number. *Nature Reviews Genetics* 2009;10:551–564.
 29. Lee JA, Carvalho CMB, Lupski JR. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell* 2007;131:1235–1247.
 30. Zhang F, Khajavi M, Connolly AM, Towne CF, Batish SD, Lupski JR. The DNA replication FoSTeS/MMBIR mechanism can generate genomic, genic and exonic complex rearrangements in humans. *Nat Genet* 2009;41:849–853.
 31. Blass JP, Gibson GE. Abnormality of a thiamine-requiring enzyme in patients with Wernicke-Korsakoff syndrome. *N Engl J Med* 1977;297:1367–1370.
 32. Mukherjee AB, Svoronos S, Ghazanfari A, et al. Transketolase abnormality in cultured fibroblasts from familial chronic alcoholic men and their male offspring. *J Clin Invest* 1987;79:1039–1043.
 33. Leigh D, McBurney A, McIlwain H. Erythrocyte transketolase activity in the Wernicke-Korsakoff syndrome. *Br J Psychiatry* 1981;139:153–156.
 34. Leigh D, McBurney A, McIlwain H. Wernicke-Korsakoff syndrome in monozygotic twins: a biochemical peculiarity. *Br J Psychiatry* 1981;139:156–159.
 35. Feng Y, Walsh CA. The many faces of filamin: a versatile molecular scaffold for cell motility and signalling. *Nat Cell Biol* 2004;6:1034–1038.
 36. Masurel-Paulet A, Haan E, Thompson EM, et al. Lung disease associated with periventricular nodular heterotopia and an FLNA mutation. *Eur J Med Genet* 2010;54:25–28.
 37. de Wit MCY, Tiddens HAWM, de Coo IFM, Mancini GMS. Lung disease in FLNA mutation: confirmatory report. *Eur J Med Genet* 2011;54:299–300.

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