Periventricular heterotopia, mental retardation, and epilepsy associated with 5q14.3-q15 deletion

ABSTRACT

Background: Periventricular heterotopia (PH) is an etiologically heterogeneous disorder characterized by nodules of neurons ectopically placed along the lateral ventricles. Most affected patients have seizures and their cognitive level varies from normal to severely impaired. At present, two genes have been identified to cause PH when mutated. Mutations in FLNA (Xq28) and ARFGEF2 (20q13) are responsible for X-linked bilateral PH and a rare autosomal recessive form of PH with microcephaly. Chromosomal rearrangements involving the 1p36, 5p15, and 7q11 regions have also been reported in association with PH but the genes implicated remain unknown. Fourteen additional distinct anatomoclinical PH syndromes have been described, but no genetic insights into their causes have been gleaned.

Methods: We report the clinical and imaging features of three unrelated patients with epilepsy, mental retardation, and bilateral PH in the walls of the temporal horns of the lateral ventricles, associated with a de novo deletion of the 5q14.3-15 region. We used microarray-based comparative genomic hybridization to define the boundaries of the deletions.

Results: The three patients shared a common deleted region spanning 5.8 Mb and containing 14 candidate genes.

Conclusion: We identified a new syndrome featuring bilateral periventricular heterotopia (PH), mental retardation, and epilepsy, mapping to chromosome 5q14.3-15. This observation reinforces the extreme clinical and genetic heterogeneity of PH. Array comparative genomic hybridization is a powerful diagnostic tool for characterizing causative chromosomal rearrangements of limited size, identifying potential candidate genes for, and improving genetic counseling in, malformations of cortical development.

GLOSSARY

CGH = comparative genomic hybridization; CNV = copy number variation; FISH = fluorescent in situ hybridization; PH = periventricular heterotopia; SNP = single nucleotide polymorphism.

Periventricular heterotopia (PH) is a brain malformation caused by defective neuronal migration that leads to abnormal positioning of post mitotic neurons. Consequently, nodules of heterotopic gray matter accumulate along the walls of the lateral ventricles and can usually be detected using MRI. There is a wide spectrum of anatomic and clinical presentations of PH, ranging from asymptomatic small unilateral or bilateral nodules to extensive agglomerates of heterotopia lining the lateral ventricles in patients with intractable epilepsy and intellectual disabilities. There is also a...
range of associated cerebral and systemic malformations. Two genes, FLNA and ARFGGEF2, have been found to cause PH when mutated. FLNA maps to Xq28 and encodes for FILAMIN A, a very large (280 kD) cytoplasmic protein which binds to actin and a wide range of cytoplasmic signaling proteins involved in cell adhesion and migration. Almost 100% of families with X-linked bilateral PH and about 26% of sporadic patients harbor FLNA mutations. Heterozygous women have normal to nearly normal intelligence and epilepsy. Although a few living men with bilateral PH due to FLNA mutations have been reported, most male fetuses are not viable. Congenital heart block and cardiovascular abnormalities have been observed in some patients.

Mutations in ARFGGEF2, in 20q13.1, are responsible for a very rare autosomal recessive form of PH associated with microcephaly and abnormal cortical folding. ARFGGEF2 encodes a protein called BIG2 (or brefeldin-A inhibited GEF2 protein) involved in vesicular trafficking. PH has also been associated with deletions of the 1p36 or 7q11 regions and with duplications involving 5p15. To date, 14 additional distinct PH syndromes have been described but no genes identified.

Here we report three unrelated children, two boys and one girl, with severe mental retardation, epilepsy, and bilateral PH limited to the subependymal region of the temporal horns, associated with a de novo deletion of the q14.3-15 region on chromosome 5. The deletions range from 6.3 to 17 Mb and share a common deleted region spanning 5.8 Mb. Computational analysis of the critical region identified 14 candidate genes. This phenotype-genotype association recognizes a new, genetically identifiable, syndrome.

METHODS Patients. The study includes three patients from unrelated families from Italy, France, and Australia. These patients were identified in three centers where array comparative genomic hybridization (CGH) is being used to identify, or accurately characterize, chromosomal rearrangements in individuals with developmental brain abnormalities, mental retardation, and epilepsy. Clinical information, brain MRI, and blood or DNA samples were obtained after informed consent.

Fluorescent in situ hybridization (FISH) analysis. In patients 1 and 3, an initial molecular cytogenetic study was executed using FISH with BAC clones carried out on interphase or metaphase chromosome preparations by standard methods. For patient 1, whose unbalanced chromosomal translocation also included a breakpoint at 1q31 (GenBank accession numbers for genes and BAC clones will be provided on request), we used different prediction softwares in order to identify potential coding sequences in this genomic region (see appendix e-1 on the Neurology® Web site at www.neurology.org).

Single nucleotide polymorphism (SNP) array CGH. Patient 3 had initially been studied using a Mapping 100K Set, which comprises two arrays each with >50,000 SNPs, as previously described.

Oligonucleotide array CGH. Array CGH was performed in all three patients using a microarray kit (Human Genome CGH Microarray Kit 244A; Agilent Technologies, Santa Clara, CA), according to the manufacturer’s instructions. This kit uses an oligonucleotide-based platform that allows genomewide survey and molecular profiling of genomic aberrations with an average spacing of 6.5 kb. Labeling and hybridization were performed following the manufacturer’s protocols. Briefly, 500 ng of purified DNA from a patient and a control (Promega Corporation, Madison, WI) were double-digested with Rsal and Alul for 2 hours at 7°C. After 20 minutes at 65°C, DNA of each digested sample was labeled by random priming (Invitrogen, Carlsbad, CA) for 2 hours using Cy5-dUTP for the patient DNA and Cy3-dUTP for the control DNA. Labeled products were column-purified and prepared according to the manufacturer’s protocol. After probe denaturation and pre-annealing with 50 mg of Cot-1 DNA, hybridization was performed at 65°C with rotation for 40 hours. After two washing steps, the arrays were analyzed with the Agilent scanner and Feature Extraction software (V.9.1.3.1). A graphical overview was obtained using CGH analytics software (V.3.4.27).

FLNA mutation analysis. The 47 exons covering the coding regions of FLNA (Entrez Gene, GeneID: 2316) and their respective intron-exon boundaries were amplified by PCR. Primer sequences and PCR conditions are available on request. PCR amplicons were purified using PCR clean-up kits (Sigma Aldrich, St. Louis, MO, and Agencourt, Beverly, MA) and cycle sequenced on both strands using the BigDye Terminator v.1.1 chemistry (Applied Biosystems, Foster City, CA). The products were analyzed on 3100 ABI Prism DNA sequencer (Applied Biosystems).

RT-PCR experiments. Human tissue RNA samples were purchased from BD Biosciences Clontech (Palo Alto, CA). Human fetal brain RNA was isolated from normal fetal brain pooled from 21 spontaneously aborted male/female Caucasian fetuses ages 26–40 weeks. Human adult brain RNA was obtained from individuals who had died suddenly and unexpectedly with no evidence of disease. Whole brain RNA of two Caucasian men aged 47 and 55 years was obtained; cerebral cortex RNA was obtained from a pool of cerebral cortex tissues from 10 Caucasian men/women aged 20–68 years. Human lung, heart, skeletal muscle, and lymphocyte RNA was obtained from healthy tissues of a pool of 20 individuals who had died between ages 15–68 years. Total lymphocyte RNA was extracted from human control lymphoblastoid cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized from human RNA samples using Superscript II (Invitrogen) according to the manufacturer protocol. PCR conditions were 50 µL reaction containing 10X PCR buffer, 2 mM MgCl2, 0.2 mM of each dNTP, 0.1 µM of each primer, and 1 U of Taq polymerase (Invitrogen) polymerase (Applied Biosystems). The reactions were denatured at 96°C for 4 minutes, followed by 25–30–35 cycles of 94°C for 35 seconds, 56–62°C for 35 seconds, and 72°C for 55 seconds. Primer sequences are available on request.
RESULTS Subjects. Clinical and imaging findings are summarized in table e-1.

Patient 1. This 7-year-old boy was born to unrelated healthy parents. At birth, a coloboma of the left iris was noted. Generalized hypotonia and developmental delay were reported in the first 2 years of life. At 3 years, clinical examination revealed minor cranial and facial dysmorphic features including a high forehead, hypertelorism, high arched eyebrows, mild downward slanting of the palpebral fissures, depressed nasal bridge, thick columella, and a flat long philtrum (figure e-1A). Left eye exotropia was also present. The boy walked independently at 5 years but remained markedly hypotonic and developed no language skills. At age 1 year, febrile seizures occurred. At 6 years, generalized tonic-clonic seizures appeared but were soon controlled with antiepileptic drugs. EEG showed normal background activity with no epileptiform discharges. Brain MRI revealed bilateral PH, involving the temporal and occipital horns (figure 1A). Mild dilatation of the frontal horns was also present (figure 1A).

Conventional chromosome analysis of peripheral blood lymphocytes showed a 46, XY, der(5) del(5) (q14;q21) t(1,5) (q31;q14) karyotype (figure 2, A-I) which had arisen de novo. FISH and array CGH were used to further characterize the rearrangement. First, we mapped the translocation breakpoint on chromosome 1 using BAC clones as FISH probes. BAC clones were chosen, on the Ensembl and the UCSC Genome databases, to cross the relevant region q31 on chromosome 1. FISH signals for BAC RP11-191J5 were observed on the derivative 1 and 5 chromosomes as well as the wild-type chromosome 1 (figure 2, A-I), localizing the 1q31 breakpoint to a position within this clone. Sequence analysis of BAC RP11-191J5 using gene prediction softwares failed to identify any coding sequences in this genomic clone. In addition, there are no annotated genes near the breakpoint region as the most telomeric gene (RGS18) is located 597 kb from the breakpoint and the most centromeric gene (FAM5C) is located 910 kb from the breakpoint. Taken together, these data indicate that the 1q31 breakpoint does not interrupt
or alter the function of any gene. Array CGH revealed a 5q interstitial deletion and localized the breakpoint to the 5q14.3-q15 region, spanning 17 Mb with 88,945,075-134 bp being the first oligomer deleted, and 105,929,496-555 bp the first oligomer present (genome assembly May 2004, hg17) (figure 2, A-III, and figure 3B). Mutation analysis of the FLNA gene gave negative results.

**Patient 2** This 5-year-old girl was born to unrelated healthy parents. At birth, bilateral pes talus was observed; developmental delay was apparent in early infancy. At 9 months, infantile spasms appeared and proved resistant to antiepileptic medication. EEG showed poorly organized background activity and multifocal epileptiform discharges. At 3 years, epileptic spasms were still present. Clinical examination revealed severe developmental delay, absence of speech, and minor dysmorphic features including a high forehead, frontonasal hypotelorism, anteverted nostrils, high arched eyebrows, depressed nasal bridge, thick columella, long philtrum, thin lips, and micrognathia (figure e-1B). At age 5, cognitive development was severely impaired and no language skills had developed. Brain MRI revealed bilateral PH, involving the temporal and occipital horns (figure 1, B-I, B-II). Standard chromosome analysis of periph-

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**Figure 2** Molecular karyotyping and array comparative genomic hybridization (CGH) in patients 1, 2, and 3 showing a 5q14.3-q15 deletion

(A-I) High resolution karyotype of patient 1 shows an unbalanced translocation between 1q31 and 5q14.3 (arrowhead), resulting in a deletion of the 5q14.3-q21.3 region. (A-II) FISH analysis on metaphase chromosomes using bacterial artificial chromosomes (BAC) RP11-191J5 (in red) reveals hybridization on the derivative 1 and 5 chromosomes, as well as the wild-type chromosome 1, localizing the 1q31 breakpoint within this BAC. (A-III) Oligonucleotide array CGH profile of patient 1 showing 5q14.3-q15 deletion. (B, C) Oligonucleotide array CGH profiles of patients 2 and 3 showing 5q14.3-q15 deletions.
eral blood lymphocytes showed a normal 46,XX karyotype. Array CGH revealed a 5q interstitial deletion and localized the breakpoint to the 5q14.3-q15 region, spanning 8.4 Mb with 87,086,298-357 bp being the first oligomer deleted, and 95,538,640-699 bp the first oligomer present (genome assembly May 2004, hg17) (figures 2B and 3B). The deletion occurred de novo. Mutation analysis of the FLNA gene gave negative results.

**Patient 3.** This 5-year-old boy was born to non-consanguineous healthy parents. At birth, right post-axial polydactyly of his toes was noted. Early clinical evaluation revealed a triangular shaped head and poor truncal tone with variable tone of the limbs. From 8 months, episodes of unresponsiveness lasting 10–20 seconds occurred many times a day. At 18 months, these episodes ceased but isolated myoclonic jerks appeared. Valproate treatment reduced the myoclonic seizures, which completely ceased by 3 years. Several EEG recordings showed bursts of multifocal and bilaterally synchronous epileptiform activity. The patient walked at 3 years; at 5 years, he was severely delayed and had no speech. Clinical examination revealed macrocephaly (>98th centile).

**Brain MRI revealed bilateral PH,** involving the temporal and occipital horns (figure 1, C-I, C-II). In addition, there were under rotated hippocampi, more severely on the right (figure 1, C-II) and irregular thickening and folding of the cortex in the posterior perisylvian regions, consistent with polymicrogyria (figure 1, C-III).

Molecular karyotyping for this patient was previously reported by Slater and collaborators.13 By conventional chromosome analysis of peripheral blood lymphocytes an abnormal 46,XY, del(5)(q14.2q15) karyotype was identified, which had occurred de novo. A high-density synthetic oligonucleotide array confirmed a 5q14.3-q15 interstitial deletion, span-
Cognitive delay was severe in all three patients as distant cortical areas, which can in turn be dysplastic to involve the heterotopic neuronal aggregates as well. The complexity of the epileptogenic networks which tend to involve the heterotopic neuronal aggregates as well. This variability is expected on the sole basis of the detectable heterotopia and likely indicates widespread cortical impairment. Polymicrogyria was clearly visible in patient 3 but no cortical abnormality was detected on MRI in the remaining two.

Experimental evidence in rats, and neuropathologic studies in humans, suggest that exogenous factors acting during pregnancy can cause PH. However, no definite anatomoclinical patterns of PH have been definitely linked to exogenous factors. Conversely, considerable evidence has been gathered about the genetic causes and genetic heterogeneity of PH. Mutations of the FLNA gene are the main cause of classic bilateral X-linked PH, which is the most commonly recognized phenotype. Rare FLNA mutations have been identified in patients with minor variants of PH such as unilateral PH, bilateral PH associated with Ehlers-Danlos syndrome, and with severe constipation. In the three patients described here, clinical and brain MRI features differ from those due to FLNA mutations. In FLNA mutations, mental retardation is never severe and PH lines the walls of the lateral ventricles, especially the frontal horns and ventricular bodies, with limited or no extension to the temporal-occipital horns, and mega cisterna magna is often observed. Autosomal recessive PH due to ARFGEF2 mutations also exhibits different characteristics in that it is associated with profound neurologic impairment, early onset seizures, microcephaly, and a simplified gyral pattern.

PH and mental retardation have also been described in association with known genetic syndromes or rare chromosomal rearrangements, mainly in isolated cases (table e-2). Several PH syndromes have also been reported for which the genetic bases are still unclear (table e-2). Conversely, there is in vitro and in vivo functional evidence that loss of function of Mekk4 (a mitogen-activated protein kinase) causes PH in mice, but no mutations of the Mekk4 gene have been identified in humans.

Hemizygous deletions of a specific chromosome are often associated with a contiguous gene deletion syndrome, in which the inactivation of several genes is involved in producing a complex phenotype. Molecular karyotyping in our patients established the size of interstitial deletions involving the 5q14.3-q15 region; the clinical and imaging features appear to be sufficiently homogeneous to define a syndrome that can be assigned to the deleted region.

About 40 cases of constitutional interstitial deletion 5q have been reported, mostly before array CGH became available, and only a few were studied with molecular markers, FISH, or both. Overall, 20 patients have been reported whose deletions partially overlap the 5q14.3-q15 region. Their phenotypic features include developmental delay and nonspecific dysmorphic facial features. No brain abnormalities were reported, although high resolution neuroimaging was not performed.

Unless the critical region is very small, it is usually difficult to identify whether a major gene exists for...
Table: Expression analysis of the genes localized in the periventricular heterotopia critical region by semiquantitative RT-PCR

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<th>CETN3</th>
<th>POLR3G</th>
<th>LYSMD3</th>
<th>GPR98 (MASS1)</th>
<th>ARRD3</th>
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<th>CSORF21 (FLJ12078)</th>
<th>POU5F2* (FLJ25680)</th>
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<th>MCTP1</th>
<th>FAM81B (FLJ25333)</th>
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**++** indicates that a signal is detectable after 25 PCR cycles; **+** indicates that a PCR fragment was detectable after 30 cycles of PCR; **+/-** indicates that a PCR fragment was detectable after 35 cycles of PCR; **−** indicates that we did not detect any expression after 35 cycles of PCR. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene was used as a positive control.

*POU5F2 expression is restricted to testis tissues.
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