Periventricular heterotopia associated with chromosome 5p anomalies

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Abstract—Periventricular heterotopia (PH) is characterized by neuronal nodules along the lateral ventricles. Whereas mutations in X-linked FLNA cause such cortical malformations, the authors report two cases of PH localizing to chromosome 5p. Both subjects have complex partial seizures. MRI demonstrated bilateral nodular PH, with subcortical heterotopia or focal gliosis. FISH identified a duplication of 5p15.1 [46,XX,dup(5)(p15.1p15.1)] and a trisomy of 5p15.33 [46,XY,der(14)t(5;14)(p15.33;p11.2) mat]. These findings suggest a new PH locus along the telomeric end of chromosome 5p.

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Recent studies suggest that periventricular heterotopia (PH) is a clinically and genetically heterogeneous disorder. The best understood form of PH follows an X-linked dominant inheritance pattern and is caused by mutations in *FLNA*.[1] Female patients typically present with seizures and have normal intelligence, whereas a skewed sex ratio for offspring of affected women suggests that this disorder is frequently embryonic lethal for hemizygous males. Mutational analyses in familial PH show that greater than 80% of affected pedigrees have a detectable *FLNA* mutation. Within individual sporadic cases, however, only 20% of the affected patients have *FLNA* mutations.[2,3] More recent findings suggest existence of an autosomal recessive form of PH and a familial PH associated with hydrocephalus (personal observations). Classification of these different syndromes will provide an approach for genetic evaluation.

Here we describe two individuals with PH and seizures, associated with duplications of the distal region of chromosome 5p, suggesting at least a third distinctive PH syndrome.

Methods. *Subjects.* The study includes two affected individuals from unrelated families. The patients were consented according to clinical research programs, approved by the respective institutions' human subjects research review committees. Probands underwent MRI scanning in a 0.5- or 1.5-Tesla magnet. Routine spin-echo sequences (T1- and T2-weighted) and high-resolution T1 volumetric studies were obtained in all planes. EEG studies were obtained according to the International 10–20 system.

FISH analysis. Cytogenetic analysis of peripheral blood lymphocytes from patients and the family of Patient 2 was performed using standard techniques.[4] Labeling of bacterial artificial chromosome (BAC) probes followed standard procedures with deoxyuridine 5-triphosphate containing fluorescent tags (methods outlined in the Vysis nick translation kit, Grove, IL). BAC probes used included D5S23, C84C11T7, D14S308, RP11–20b3, RP11–46O23, RP11–29n3, RP11–88L18, and RP11–253b9. Hybridization was performed by denaturing the slides in 70% formamide/2× saline-sodium citrate buffer, dehydrating the slides with serial ethanol washes, and applying the probe to the slide samples. Posthybridization, the slides were washed, coverslipped, and examined under fluorescence microscopy (Zeiss Axioskop, Thornwood, NY).

Results. *Subjects.* Proband 1 is a 12-year-old girl with developmental delay and complex partial seizures. She had no dysmorphic features and no murmurs were detected on cardiac auscultation. Neurologically, she had an IQ of 40. She had oral-motor apraxia with drooling, a left hemiparesis (secondary to a perioperative brain contusion), and associated hypertonia and spasticity. Her gait was hemiparetic. Peripheral blood counts, electrolytes, liver function tests, and coagulation studies were unremarkable. Interictal EEG demonstrated focal slowing over the right temporal lobe. At seizure onset, rhythmic sharp waves and spikes with arrhythmic slowing localized to the right temporal lobe. MRI of the brain demonstrated bilateral nodular heterotopia, lining both frontal horns of the lateral ventricles (figure 1A). The nodules (<1 cm diameter) appeared continuous and symmetric. Radiographs also showed T2 abnormality in the right hippocampus, consistent with gliosis (see figure 1B, black arrow).

Proband 2 is an 8-year-old boy with partial complex seizures, a repaired atrial septal defect (secundum), mitral and tricuspid valve prolapse and regurgitation, and a history of bilateral equinovarus and inguinal hernia repair. He had minor craniofacial ab-

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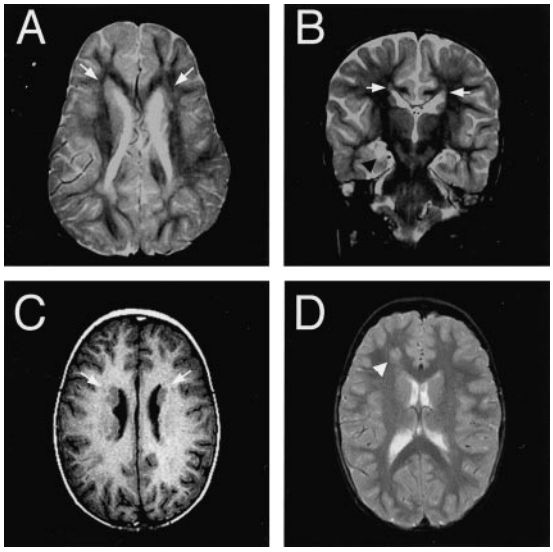

Figure 1. Brain MRI appearance of patients with periventricular heterotopia (PH) associated with trisomy for distal chromosome 5p. (A) Axial T2-weighted image from Proband 1 demonstrates nodular areas of heterotopic gray matter adjacent to the frontal horns of the lateral ventricles bilaterally (white arrows). (B) Coronal T2-weighted image of same patient in (A) shows bilateral periventricular heterotopia (white arrows) and an area of abnormally increased T2 signal in the right hippocampus (black arrowhead) consistent with hippocampal sclerosis. (C) Bilateral noncontinuous heterotopia (white arrows) can similarly be appreciated in Proband 2 on the T1-weighted axial image. (D) Axial T2-weighted image shows the additional finding of a right frontal subcortical heterotopia (white arrowhead).

normalities that included mild plagiocephaly, a low anteriorhairline with fine red hair, and asymmetric downslanting eyes and palpebral fissures. He had mild pectus excavatum, hyperextensibility at multiple joints, soft skin with mild extensibility, and clinodactyly of the fifth toe bilaterally. Neurologically, he had an IQ of 95, verbal IQ of 106, and performance IQ of 84. He had generalized hypotonia. Peripheral blood counts, electrolytes, and liver function tests were unremarkable. Urine organic acids, quantitative amino acids, and homocysteine did not suggest a metabolic disorder. Results of an interictal EEG were also normal. MRI of the brain demonstrated noncontinuous, nodular (<1 cm in diameter), bilateral (R > L) periventricular heterotopic gray matter (see figure 1C). A large heterotopic nodule of subcortical gray matter was also apparent between the frontal horn of the right lateral ventricle and the cortex (see figure 1D).

Fluorescence in situ hybridization (FISH). The duplication in Proband 1 was initially identified by GTG banding. Analysis by FISH demonstrated a duplication of chromosome 5p15.1 on metaphase spread (figure 2). The additional genomic copy of the chromosome 5p15.1 fragment was confirmed on cells arrested in interphase. Further analyses using BAC probes more distal (RP11–29n3) and proximal (RP11–253b9) to the RP11–88L187 marker showed no signal, indicating that the duplication was limited to 5p15.1. In contrast, GTG banding results (>550 bands) were normal for Proband 2. Analysis by FISH in Proband 2 demonstrated a cryptic unbalanced translocation t(5;14) resulting in a trisomy of chromosome 5p15.33 (figure 3). Further analyses using BAC probes (RP11–46O23) proximal to the RP11–20b3 probe indicated that the additional genetic material was limited to a region within chromosome 5p15.33. Finally, FISH studies showed that the proband's mother and brother were carriers of the balanced translocation t(5;14)(p15.33;p11.2) (see figure 3B). No BAC probes could be identified that were trisomic in both Probands 1 and 2.

Discussion. Other than the shared features of PH and seizures, the clinical and radiographic features

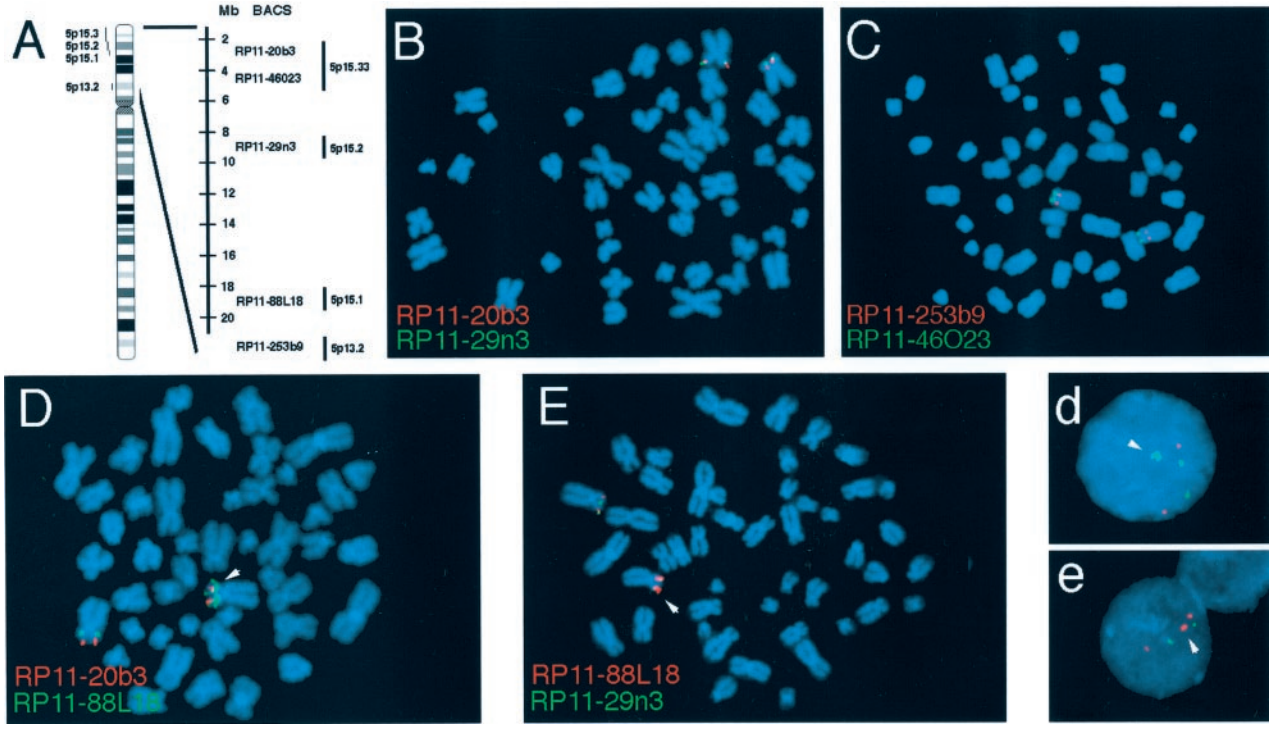

Figure 2. Analysis by fluorescence in situ hybridization in Proband 1 demonstrates duplication of chromosome 5p15.1. (A) Bacterial artificial chromosomes (BAC) used for in situ hybridization to the short arm of chromosome 5. (B, C) Metaphase spread under fluorescence microscopy reveals normal hybridization near the p-terminus (5p15.2 and 5p15.3) and the region proximal to chromosome 5p15.1. (D, E) Duplication of the chromosomal region 5p15.1 is shown under both fluorescein and rhodamine fluorescence without involvement of 5p15.2 or 5p15.3 and confirmed on interphase spread (d, e).

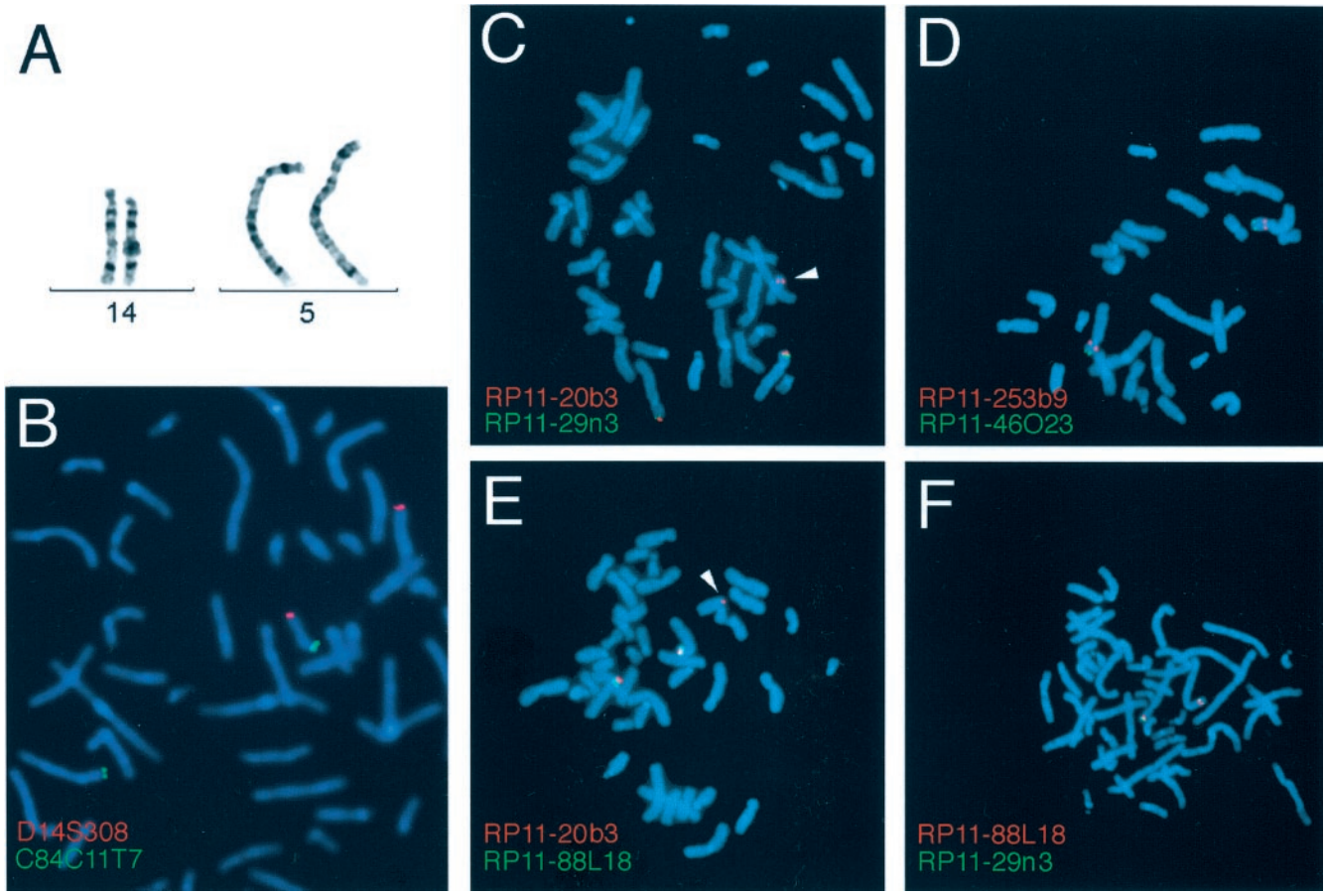

Figure 3. Analysis by high resolution karyotyping and fluorescence in situ hybridization in Proband 2 demonstrates an unbalanced translocation, resulting in trisomy of chromosome 5p15.3. (A) Karyotyping of Proband 2 shows an unbalanced translocation involving the p-terminus of chromosome 14. (B) Metaphase spread of the mother under fluorescence microscopy indicates a balanced translocation between distal 5p and 14p. (C through F) The identical bacterial artificial chromosomes (BAC) used for in situ hybridization on Proband 1 (figure 2A) reveal normal hybridization for the genetic area proximal to and including chromosome 5p15.2. The region of trisomy corresponds to the telomeric end of chromosome 5p15.33 with the additional gene copy detected by the BAC RP11–20b3 and normal hybridization appreciated 1–2 Mb proximally (BAC RP11–46O23).

of the two patients described here are different, compared to one another and to individuals with X-linked PH. Proband 1 has moderate mental retardation, which is not commonly seen in X-linked PH. Proband 2 has normal intelligence but hyperextensibility of the joints and extensibility of the skin; such features are analogous to Ehlers-Danlos syndrome with subependymal PH.[5] Radiographically, the frontal horn predominance of PH in Proband 1 and the noncontinuous PH, as well as the subcortical heterotopia in Proband 2, represent findings not typically appreciated in X-linked PH.[6] Such clinical and radiographic features in these individuals represent characteristics distinct from X-linked PH, whereas the differences between each proband presumably reflect the presence of trisomy for distinct regions of 5p.

Studies have shown that more severe craniofacial malformations, in association with cardiac, renal, intestinal, and limb deformities, arise from duplications of the short arm of chromosome 5 proximal to p14.[7] Less severe deficits are seen in individuals with breaks distal to this band. Both affected individuals in this study have duplications of distal chromosome 5p with primarily neurologic deficits. Proband 1 has no extra-CNS findings; Proband 2 has minor dysmorphic features, cardiac defects, and bilateral clubfeet.

Previously reported chromosomal abnormalities and their corresponding phenotypes suggest that the neuronal migration disorder seen in PH could localize to the distal p-terminus of chromosome 5p. MRI of a child with a de novo complete trisomy 5p detected subependymal heterotopic nodules of gray matter, lining the left lateral ventricle.[8] Heterotopia containing neuroglial tissue in the meninges and pons and heterotopic neurons in the frontal and calcarine cortex were also documented on autopsy in an individual with a more restricted duplication of 5p14 to p15.33.[9] The two probands in this report present with gray matter nodules along the lateral ventricles and in one individual, additional subcortical heterotopia is also appreciated. Their chromosomal dupli-

cations are further restricted to distal 5p. These findings suggest that trisomies of distal chromosome 5p alone may be sufficient for PH formation.

The current mapping studies of the probands clearly suggest that nonoverlapping regions of duplication, involving chromosomes 5p15.1 and 5p15.33, can result in PH. Despite the shared radiographic findings, this disparity could merely suggest further heterogeneity in PH, perhaps involving different genes in the same molecular pathway. Related families of genes, however, can cluster within the same chromosomal region.[10] Thus, the proximity of the two chromosomal regions may reflect inappropriate expression of homologous genes, which can give rise to heterotopia. Alternatively, the close genomic localization in these cases may lead to differential regulation and expression of the same gene. Finally, the resolution limits of the hybridization may not have detected additional rearrangements in these regions or rearranged areas common to both patients.

References

1. Fox JW, Lamperti ED, Eksioglu YZ, et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. Neuron 1998;21:1315–1325.
2. 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.
3. Moro F, Carrozzo R, Veggiotti P, et al. Familial periventricular heterotopia: missense and distal truncating mutations of the FLN1 gene. Neurology 2002;58:916–921.
4. Verma RS, Babu A. Human cytogenetics. Principles and techniques. New York: McGraw-Hill, 1994.
5. Thomas P, Bossan A, Lacour JP, Chanalet S, Ortonne JP, Chatel M. Ehlers-Danlos syndrome with subependymal periventricular heterotopias. Neurology 1996;46:1165–1167.
6. Poussaint TY, Fox JW, Dobyns WB, et al. Periventricular nodular heterotopia in patients with filamin-1 gene mutations: neuroimaging findings. Pediatr Radiol 2000;30:748–755.
7. Riordan D, Vust A, Wickstrom D, et al. Identification of a dup(5)(p15.3) by multicolor banding. Clin Genet 2002;61:277–282.
8. Grosso S, Cioni M, Garibaldi G, et al. De novo complete trisomy 5p: clinical and neuroradiological findings. Am J Med Genet 2002;112: 56–60.
9. Harrison KJ, Teshima IE, Silver MM, et al. Partial tetrasomy with triplication of chromosome (5) (p14-p15.33) in a patient with severe multiple congenital anomalies. Am J Med Genet 1998;79:103–107.
10. Holland PW. Beyond the Hox: how widespread is homeobox gene clustering? J Anat 2001;199(pt 1–2):13–23.

Neurotransmitter specificity of sympathetic denervation in Parkinson's disease

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Abstract—In PD, orthostatic hypotension reflects sympathetic noradrenergic denervation. The authors assessed sympathetic cholinergic innervation by the quantitative sudomotor axon reflex test (QSART) in 12 patients who had sympathetic neurocirculatory failure, markedly decreased cardiac 6-[^{18}F] fluorodopamine-derived radioactivity, and subnormal plasma norepinephrine increments during standing. All 12 had normal QSART results. The sympathetic nervous system lesion in PD involves loss of postganglionic catecholaminergic but not cholinergic nerves.

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Patients with PD often have signs or symptoms of autonomic failure. In particular, loss of sympathetic postganglionic noradrenergic nerves appears to underlie orthostatic hypotension in PD.[1]

At postganglionic sympathetic terminals innervating eccrine glands, acetylcholine, rather than norepinephrine, constitutes the main neurotransmitter for thermoregulatory, gustatory, and emotional sweating.[2] Patients with PD may have reduced eccrine sweat responses, but the literature on this topic has been somewhat inconsistent.[3-7] Whether sympathetic cholinergic denervation occurs in PD with orthostatic hypotension has been unknown. We examined the status of sympathetic postganglionic cholinergic innervation in patients with PD who had evidence for sympathetic noradrenergic denervation.

Methods. *Subjects.* The patient population consisted of 12 patients (7 men, 5 women), mean age 66 ± 9.7 years (range 47 to 82 years). The patients fulfilled standard clinical criteria for PD. All had bradykinesia, cogwheel rigidity, and one or more other parkinsonian features such as pill-roll resting tremor, stooped posture, festinating gate, difficulty initiating movement, masked face, micrographia, or marked locomotor improvement while treated with levodopa. None of the patients had signs of neurodegeneration that would suggest a different diagnosis such as multiple system atrophy. Sex-matched normal subjects underwent the same tests. All subjects were studied at the NIH Clinical Center, after giving written informed consent to participate in one or more

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