Am. J. Hum. Genet. 61:379–387, 1997

Identification of a Duplication of Xq28 Associated with Bilateral Periventricular Nodular Heterotopia

James M. Fink,[1] William B. Dobyns,[2] Renzo Guerrini,[3] and Betsy A. Hirsch[1]

Departments of [1]Laboratory Medicine and Pathology and [2]Neurology and Pediatrics, University of Minnesota, Minneapolis; and [3]Divisione Clinica di Neuropsichiatria Infantile, Fondazione Stella Maris, Universita Degli Studi di Pisa, Pisa

Summary

Bilateral periventricular nodular heterotopia (BPNH) is a malformation of neuronal migration and is characterized by nodules of heterotopic gray matter lining the lateral ventricles of the brain. The majority of BPNH patients are female and have epilepsy as a sole clinical manifestation of their disease. Familial BPNH has been mapped to Xq28 by linkage analysis. A multiple congenital anomaly–mental retardation syndrome (BPNH/MR) was recently delineated in three unrelated boys with BPNH, cerebellar hypoplasia, severe mental retardation, epilepsy, and syndactyly. High-resolution chromosome analysis revealed a subtle abnormality of Xq28 in one of the boys with BPNH/MR syndrome. FISH with cosmids and YACs from Xq28 further characterized this abnormality as a 2.25–3.25-Mb inverted duplication. No abnormality of Xq28 was detected by G-banding or FISH in the other two boys. These data support the linkage assignment of BPNH to band Xq28 and narrow the critical region to the distal 2.25–3.25 Mb of Xq28.

Introduction

Development of the brain is a complex process that starts at the beginning of the 3d wk of gestation and continues into postnatal life. Formation of the cerebral cortex begins soon after closure of the rostral end of the neural tube. It may be divided into three overlapping stages, including (1) proliferation of neuroepithelial precursor cells in the ventricular zone—and their differentiation into immature neurons and glia, (2) migration of postmitotic immature neurons from the ventricular zone to the emerging cerebral cortex, and (3) development of a mature cortex by formation of cortical lamination, synaptogenesis, and apoptosis (Norman et al. 1995, pp. 223–277; Barkovich et al. 1996).

In humans, cortical neuronal migration occurs in two major waves during the 6th–16th wk of gestation (Sidman and Rakic 1982; Norman et al. 1995, pp. 223–277). Most neurons are guided to the cortex by climbing along radial glia fibers, although recent studies have shown both radial and tangential migration (O'Rourke et al. 1995), which indicates that several mechanisms for control of migration must exist. Failure of the young neurons to migrate normally results in malformations of neuronal migration, such as lissencephaly and subcortical-band heterotopia (agyria-pachygyria–band spectrum), polymicrogyria, and nodular heterotopia (Barkovich and Kjos 1992; Dobyns and Truwit 1995).

Bilateral periventricular nodular heterotopia (BPNH) is a recently described malformation of neuronal migration (Barkovich and Kjos 1992) and consists of nodular masses of gray matter that line the lateral ventricles and protrude into the lumen (Barkovich and Kjos 1992; Raymond et al. 1994; Dubeau et al. 1995; Dobyns et al. 1996). It can be detected by neuroimaging of the brain. BPNH is sometimes associated with mild hypoplasia of the corpus callosum and/or cerebellum. The majority of individuals with classical BPNH are female and are developmentally and intellectually normal. The most frequent phenotype associated with classical BPNH is epilepsy with multiple seizure types that may prove difficult to control. Several asymptomatic individuals have been found during family evaluations. Family studies indicate that classical BPNH is X linked and that there is significant prenatal lethality in males (Kamuro and Tenokuchi 1993; Huttenlocher et al. 1994). Linkage analysis has localized the BPNH gene to the distal long arm of the X chromosome, in band q28, with a maximal multipoint LOD score of 5.37 near F8C (factor VIII) and with a suggested critical region of <7 Mb (Eksioglu et al. 1996).

Males with BPNH are rare and may present with mental retardation and congenital anomalies in addition to epilepsy. We recently evaluated three boys with BPNH, cerebellar hypoplasia, severe mental retardation, epi-

Received December 10, 1996; accepted for publication June 3, 1997.

Address for correspondence and reprints: Dr. Betsy A. Hirsch, Department of Laboratory Medicine and Pathology, University of Minnesota, Box 609 UMHC, 420 Delaware Street S.E., Minneapolis, MN 55455.

0002-9297/97/6102-0017$02.00

lepsy, and syndactyly; we have designated this the "BPNH/MR syndrome" (Dobyns et al., in press).

In the present study, we utilize high-resolution G-banded chromosome analysis and FISH to demonstrate that one of the boys with the BPNH/MR syndrome has a 2.25–3.25-Mb inverted duplication of distal Xq28. This represents the first description of an association between BPNH and a structural chromosome rearrangement and helps to refine the critical region for the BPNH gene.

Subjects and Methods

Subjects

The three boys included in this study—BPNH-02, BPNH-03, and BPNH-12—were evaluated by W.B.D. or R.G. (Dobyns et al., in press) and had the BPNH/MR syndrome. The mother of BPNH-02 was also studied. All protocols were approved by a Human Subjects Committee Institutional Review Board at the University of Minnesota.

High-Resolution Chromosome Analysis

Peripheral blood lymphocytes were cultured and harvested by use of standard high-resolution cytogenetic techniques (Yunis 1976). Metaphase cells were spread on glass slides, were aged by being heated at 90°C for 2 h, and were G-banded by use of pancreatin and Wright's stain (Gustashaw 1991; Lawce and Brown 1991). For each subject, ⩾20 G-banded metaphase cells were analyzed at the 550–850-band-level resolution.

Probe Preparation

YACs were kindly provided by Drs. Anand Srivastava and David Schlessinger (Greenwood Genetic Center, Greenwood, SC, and Washington University, St. Louis, respectively) and previously had been mapped to Xq28 (Palmieri et al. 1994). YAC strains were cultured overnight and were embedded in low-melting-temperature agarose microbeads (Koob and Szybalski 1992). Intact yeast chromosomes were obtained by lysis of the embedded YAC strains within the agarose microbeads. Pulsed-field gel electrophoresis was used to verify that the strains contained the appropriate-size YACs. PCR template was prepared from the microbead-embedded yeast chromosomes and was subjected to inter-Alu PCR using primers CL1 and CL2 (Lengauer et al. 1992), with an annealing temperature of 37°C. PCR products were passed through a G50 spin column, were ethanol precipitated, and were resuspended in deionized water. Cosmids from Xq28 were kindly provided by Drs. David Nelson and Julia Parrish (Baylor College of Medicine, Houston), and are from the Lawrence Livermore Library, LLOXNCO1. A plasmid clone containing a 4.4-kb L1CAM cDNA was obtained from the American Type Culture Collection (number 65996). After overnight culture in Luria-Bertani medium with either kanamycin (for cosmids) or ampicillin (for L1CAM plasmid), cosmid or plasmid DNA was purified by use of a PERFECT prep plasmid DNA purification kit (5 Prime→3 Prime). The YAC PCR products, cosmids, and plasmid were then labeled by nick-translation using biotin- or digoxigenin-labeled nucleotides and were purified by passage through a G50 spin column and ethanol precipitation. Probe size was verified by gel electrophoresis, as 200–300 bp.

Sequential G-banding→FISH

G-banding was performed as described above, with the exception that slides either were aged for <1 h at 90°C or were allowed to age at room temperature for 5–7 d. G-banded metaphase cells were located and photoimaged by use of GeneVision software (Applied Imaging) linked to a Zeiss Axioscop outfitted with a high-performance charge-coupled-device camera. In preparation for FISH, the slides were then destained in methanol; dehydrated in 70%, 80%, and 95% ethanol; fixed in 3:1 methanol:acetic acid for 10 min and in 3.7% formaldehyde in PBS for 10 min; washed twice in PBS; and dehydrated in 70%, 80%, and 95% ethanol.

FISH

FISH was performed according to routine in situ suppression techniques (Ward et al. 1995). Overnight hybridization was followed by a 5-min wash in 2 × SSC at 72°C. Hybridized probes were detected by use of fluorescein-labeled avidin and/or rhodamine-labeled anti-digoxigenin antibodies (Oncor), according to the manufacturer's instructions; no amplification was performed. Slides were counterstained with propidium iodide in antifade (Oncor); were visualized by use of a Zeiss Axioskop outfitted for fluorescence with FITC, rhodamine, and triple-pass (DAPI/FITC/Texas Red) Chroma filters; and either were photographed with a Zeiss MC-80 camera or were photoimaged with a high-performance CCD and ProbeVision software (Applied Imaging). All cosmids and YACs were confirmed to map to Xq28 by FISH on chromosomes from unaffected male controls.

Results

G-Banded Chromosome Analysis: Indication of a Subtle Abnormality of Xq28

BPNH-03 and BPNH-12 had normal 46,XY male karyotypes at 850-band-level resolution. In contrast, BPNH-02 was found to have a very subtle chromosomal abnormality consisting of a slightly darker-than-average G-band at distal Xq28, with a frequently "pinched"

appearance resembling a fragile site (fig. 1). The same abnormality was detected in one of the X chromosomes of his mother. Cytogenetic and molecular genetic analyses for FRAXA and FRAXE were negative (molecular genetic analyses were performed by D. Nelson, personal communication).

Metaphase FISH: Identification of a Duplication of Xq28

To further characterize the chromosomal abnormality seen in BPNH-02 and his mother, FISH utilizing YACs mapping to Xq28 (fig. 2) was performed on metaphase chromosomes. YACs yWXD766 and yWXD824 each revealed both a single hybridization signal (defined as one fluorescent spot per chromatid) on distal Xq in BPNH-02 and a single hybridization signal on each of the two X chromosomes at band q28 in the boy's mother, indicating that the sequences corresponding to these two YACs were present only once on each X chromosome. When yWXD250 was used to probe BPNH-02, a larger-than-normal fluorescent signal could be seen at distal Xq28 in most of the examined metaphases. In a few early prometaphase cells (>850-band level) from BPNH-02, two fluorescent signals per chromatid were detected, suggesting a small duplication of Xq28. Sequential G-banding to FISH was then performed on metaphase spreads from BPNH-02's mother. In >95% of metaphase cells analyzed, the signal on the abnormal X chromosome was larger than the signal on the normal X chromosome (fig. 3). Again, in some of the early prometaphase cells, the abnormal X chromosome was seen to have a duplicated signal, confirming the presence of a small duplication. The fact that the duplication in both BPNH-02 and his mother could be resolved only on prometaphase chromosomes implied that the size of the duplication was small (<3 Mb). In order to more clearly resolve the duplicated signals, all subsequent analyses were performed on interphase cells. Interphase FISH has previously been shown to be useful for resolving signals that are 50 kb–3 Mb apart (Trask et al. 1991).

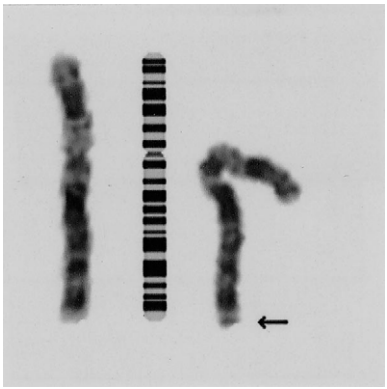

Figure 1 High-resolution G-banded X chromosomes demonstrating abnormality of Xq28 in BPNH-02. Shown are a normal 850-band X chromosome (*left*), an 850-band X-chromosome ideogram (*middle*), and an 850-band abnormal X chromosome from BPNH-02 (*right*). The arrow points to an abnormal dark band at distal Xq28.

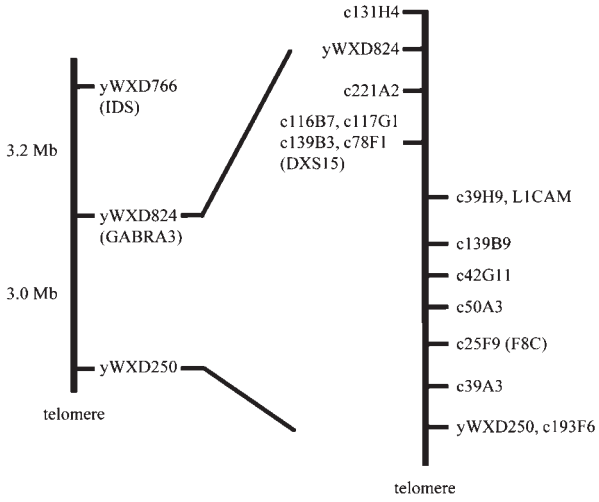

Figure 2 Physical map of distal Xq28. Physical distances are approximate and are based on the work of Palmieri et al. (1994). The region between YAC yWXD824 and yWXD250 is expanded, on the right. The cosmids (denoted by a "c" no.) and YAC that are on the left of the expanded physical map are not duplicated in BPNH-02, whereas the cosmids and YACs that are on the right of the expanded physical map are duplicated in BPNH-02. The distance between the telomere and L1CAM is ~2.5 Mb.

Interphase FISH Characterizing the Extent of Duplication in Xq28

The results of the interphase FISH analyses using cosmid probes mapping between yWXD824 and the telomere of the long arm of the X chromosome are shown in table 1 and figure 4 and are summarized in figure 2. Each of these cosmids gave similar results when applied to a normal male control. A single hybridization signal, corresponding to either a G1- or an early S-phase cell containing one normal unreplicated X chromosome, was seen in the majority (mean 61%) of the scored interphase nuclei from the normal male control. Two hybridization signals, corresponding to either a late S- or a G2-interphase cell containing a normal replicated X chromosome, were seen in 28%–39% of the scored interphase nuclei from the male control. Three or more signals, corresponding to nonspecific hybridization, were seen in a minority (mean 6%) of the interphase nuclei from the male control. With each of the cosmid probes mapping to Xq28, both BPNH-03 and BPNH-12 showed distributions of hybridization signals indistinguishable from those in the male control. Thus, none

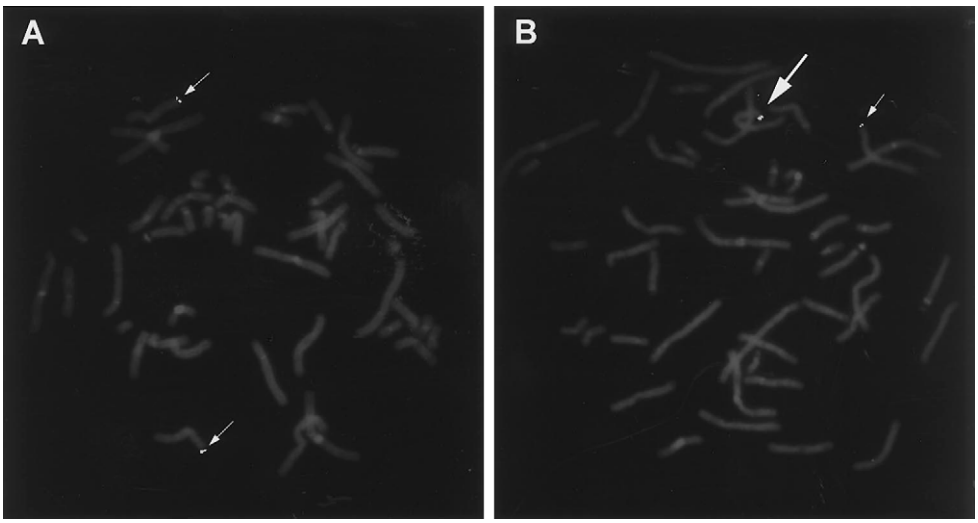

Figure 3 Metaphase FISH with biotin-labeled yWXD250, which hybridizes to Xq28. *A*, Control female metaphase demonstrating equivalent-size signals on the two X chromosomes (*small arrows*). *B*, Metaphase from BPNH-02's mother, demonstrating a signal of normal intensity on one X chromosome (*smaller arrow*) and a signal of double intensity on the other X chromosome (*larger arrow*).

of the sequences contained within the cosmids tested were duplicated or deleted within these boys.

For cosmid probes proximal to and including DXS15, BPNH-02 showed results similar to these other males (fig. 2). However, cosmid probes distal to DXS15 showed a different profile. A single hybridization signal was detected in 2%–10% (mean 5%) of the interphase nuclei; two hybridization signals were detected in 30%–54% (mean 43%) of the interphase nuclei; three hybridization signals were detected in 13%–32% (mean 21%) of the interphase nuclei; four hybridization signals were detected in 13%–34% (mean 27%); and five or more hybridization signals were detected in 0%–16% (mean 5%) of the interphase nuclei. Here, the appearance of two hybridization signals

Table 1

Interphase FISH with Cosmid Probes from Distal Xq28

Subject(s) and Hybridization Signals	Metaphase Cells Scored[a] (%)	
	Cosmids 131H4→78F1	Cosmids 39H9→193F6
Male controls (n = 15):		
1	62	60
2	32	35
3	5	3
4	1	2
>4	0	0
BPNH-02:		
1	60	5
2	34	43
3	4	21
4	2	27
>4	0	5

[a] Average of percentages for all cosmids within the group. Data supporting duplication are underlined.

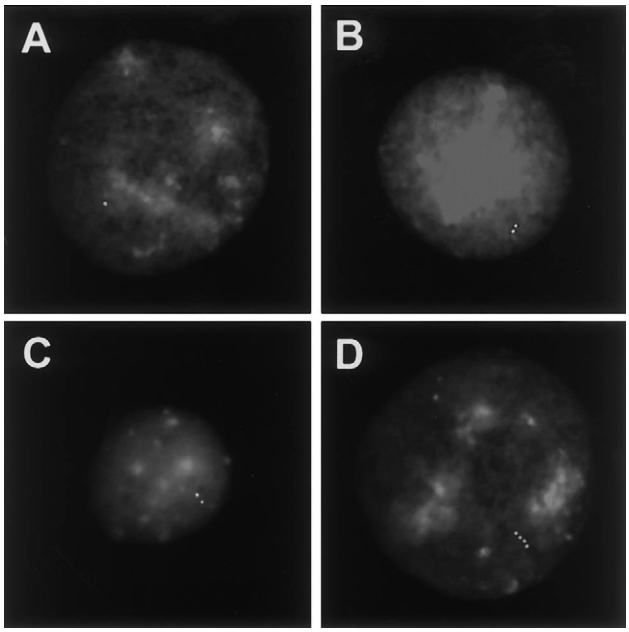

Figure 4 Interphase FISH using biotin-labeled cosmid 39A3, demonstrating the presence of a duplication in BPNH-02. *A* and *C*, G1-interphase nuclei from a normal male and from BPNH-02, respectively. *B* and *D*, G2-interphase nuclei from a normal male and from BPNH-02, respectively.

is interpreted as representing a G1-phase (unreplicated) X chromosome with a duplication of the sequences included in the cosmid, whereas the appearance of four hybridization signals is interpreted as representing a late S- or G2-phase (replicated) X chromosome with a duplication. Interphase cells containing one signal probably represent G1-phase X chromosomes containing the duplication in which the two signals overlap each other and are thus not resolved. Interphase cells with three signals represent either (1) G2-phase X chromosomes containing the duplication in which two of the signals overlap each other and are thus not resolved, (2) cells containing the duplicated X chromosome in S phase, in which only one copy of the duplicated region has been replicated, or (3) G1-phase cells with background nonspecific hybridization. Nuclei with five or more signals probably reflect background nonspecific hybridization.

The minimum size of the duplication is the distance between L1CAM and yWXD250 (fig. 2), or ~2.25 Mb, based on the most recent consensus physical map of the X chromosome (Palmieri et al. 1994; Nelson et al. 1995). The maximum size of the duplication is the distance from DXS15 to the telomere of the long arm, or ~3.25 Mb. These mapping data corroborate the metaphase FISH data, which suggested that the duplication was <3 Mb in size.

Interphase FISH: Demonstration of an Inverted Duplication of Xq28

In order to determine the orientation of the duplication in BPNH-02, interphase FISH utilizing both yWXD250, labeled with digoxigenin and detected with rhodamine (red), and cosmid 25F9, labeled with biotin and detected with FITC (green), was performed. In the majority of interphase nuclei examined, the order of the fluorescent signals was red-green-green-red (fig. 5), indicating that the duplication was inverted and had the following order of markers: tel-yWXD250-cos25F9-cos25F9-yWXD250-//-cen (fig. 5).

Interphase FISH with a Candidate Gene, L1CAM

Interphase FISH utilizing the L1CAM cDNA probe on normal male controls revealed a single hybridization signal in the majority of nuclei analyzed. With the L1CAM probe, results indistinguishable from those in the male control were obtained for BPNH-03 and BPNH-12. Thus L1CAM was neither duplicated nor deleted in these two boys. However, the hybridization pattern obtained with L1CAM on interphase nuclei from BPNH-02 was similar to the pattern obtained with the duplicated cosmids (fig. 2).

Discussion

Mapping of Both Classical BPNH and BPNH/MR to Xq28

Classical BPNH occurs predominantly in females and is generally associated with epilepsy as the sole clinical finding. It is X linked and is lethal in most males (Kamuro and Tenokuchi 1993; Huttenlocher et al. 1994). The gene responsible for classical BPNH recently has been mapped to Xq28 by linkage analysis in four multiplex families, with a maximal multipoint LOD score of 5.37 near F8C (Eksioglu et al. 1996). Unfortunately, as suggested by Eksioglu et al. (1996), further gene localization by linkage analysis will be difficult because of the paucity of families with BPNH. We recently described the BPNH/MR syndrome, which presents with BPNH and severe mental retardation among other abnormalities (Dobyns et al., in press).

We studied three boys with BPNH/MR, using high-resolution G-banding and FISH. One of these boys had an Xq28 abnormality that was detected by G-banding and that was further defined, by FISH, to be a 2.25–3.25-Mb inverted duplication of the most telomeric portion of band Xq28. Neither FISH with a series of 17 probes from within and around the duplicated region nor G-banding identified any abnormalities in the other two boys. The duplication of Xq28 in BPNH-02 represents the first description of a chromosomal abnormality associated with BPNH and corroborates the linkage-analysis data mapping a BPNH gene to Xq28. Furthermore, this helps to narrow the critical region of the gene to the distal-most 2.25–3.25 Mb of the long arm of X.

Possible Mechanisms for the BPNH/MR Syndrome

The 2.25–3.25-Mb inverted duplication in BPNH-02 could cause the BPNH/MR phenotype by either an increased-gene-dosage mechanism or a structural alteration of the BPNH gene at the duplication breakpoint—similar to the model system for Charcot-Marie-Tooth type 1A, which can be caused by increased dosage or mutation of PMP22 (Lupski et al. 1992; Patel et al. 1992; Timmerman et al. 1992; Valentijn et al. 1992*a,* 1992*b;* Roa et al. 1993). A gene-dosage model for BPNH/MR is supported by observations in boys with the XY_{Xq} syndrome (Lahn et al. 1994). The XY_{Xq} syndrome results from aberrant meiotic exchange between Xq and Yq in the fathers, which produces translocation of a portion of distal Xq28 to the Y chromosome inherited by each boy. Three of eight boys with the XY_{Xq} syndrome had a large duplication of the distal 4 Mb of Xq28, including all of the loci duplicated in BPNH-02. The XY_{Xq} syndrome shares many clinical manifestations with the BPNH/MR syndrome, including severe mental retardation, microcephaly, aphasia, seizures, hypotonia, and short stature, which suggests that some of these clinical findings may result from increased gene dosage.

However, not all of the manifestations of BPNH/MR can be accounted for by this duplication mechanism. Importantly, there is no mention of syndactyly or BPNH in the boys with the XY_{Xq} syndrome (Lahn et al. 1994).

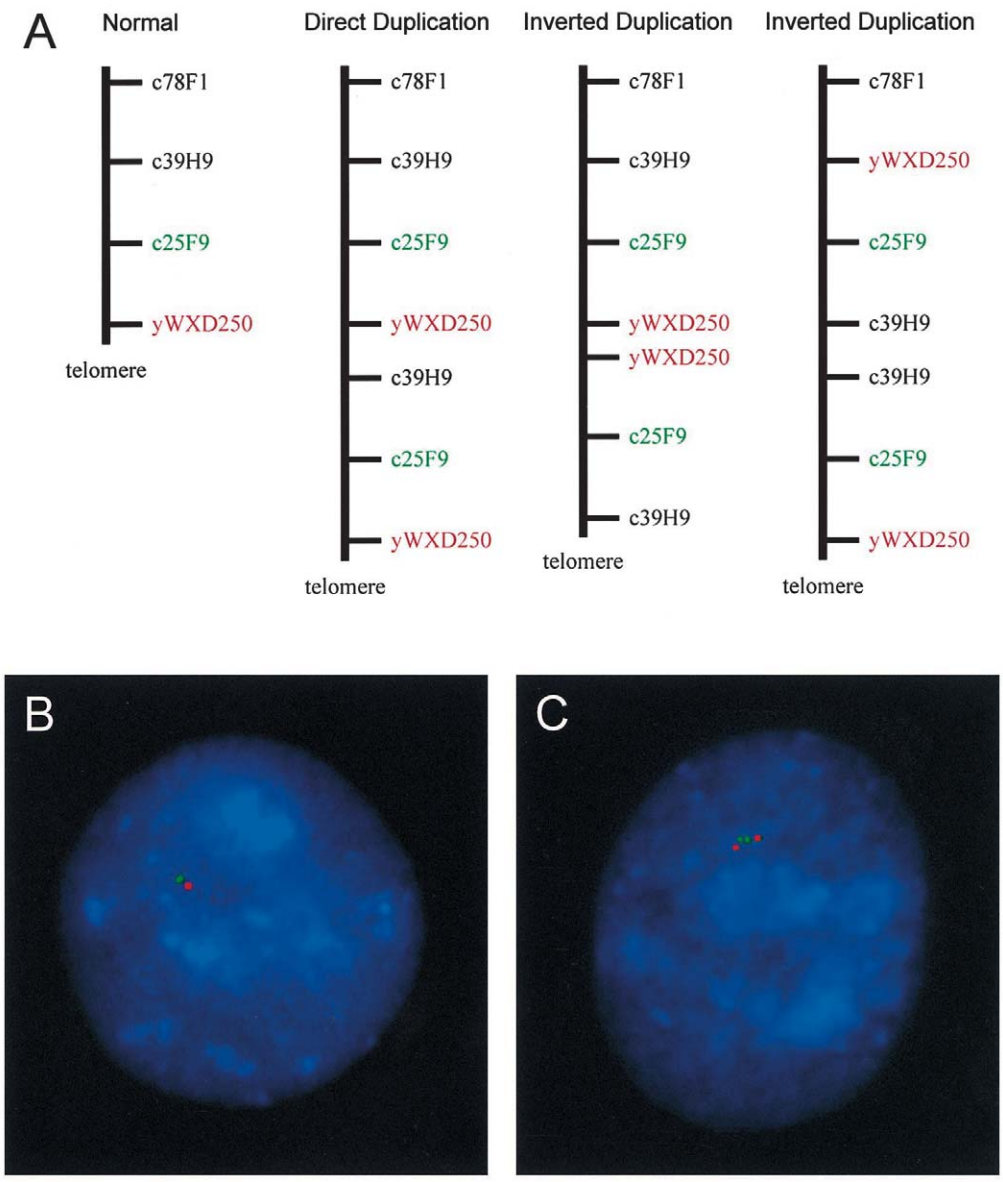

Figure 5 Demonstration that BPNH-02 contains an inverted duplication. *A,* Physical maps of distal Xq28, demonstrating the linear pattern of hybridization signals that would be expected from a normal X chromosome (*left*), from an X chromosome with a direct duplication (2d), or from two alternate types of inverted duplication (3rd and 4th), when cosmid 25F9 (*green*) and YAC 250 (*red*) are used as probes. *B,* FISH on a control male, showing one red hybridization signal and one green hybridization signal. *C,* FISH on BPNH-02, demonstrating an inverted duplication, with the orientation depicted in the physical map (*A*), at the far right.

Cranial magnetic resonance imaging (MRI) studies were not reported by Lahn et al. (1994), so BPNH may have been undetected. However, cranial MRI of the brain of one of these boys and cranial computed tomography in another were subsequently reported to be normal (R. F. Stratton and M. Engel, personal communications). As an alternative to a gene-dosage model, BPNH/MR may be caused by different mutations of a single BPNH gene; for example, different mutations in the fibroblast growth factor–receptor gene 2, FGFR2, can lead to the different craniosynostosis syndromes, including Crouzon, Jackson-Weiss, Pfeiffer, and Apert syndromes (Muenke and Schell 1995). Although craniosynostosis and midface hypoplasia are common to all four of these syndromes, each syndrome differs in the type of foot and hand abnormalities that are present. In this scenario, patient BPNH-02, with BPNH/MR syndrome and the inverted duplication, would have his disease as a result of a BPNH-gene alteration at one of the duplication breakpoints. The clinical similarities between this pa-

tient and those with the XY_{Xq} syndrome would be incidental (most of the shared phenotype consists of nonspecific abnormalities such as mental retardation, microcephaly, hypotonia, and short stature). This could account for our inability to detect a duplication in the other two BPNH/MR patients in our study, who could then be postulated to have a different type of mutation of the BPNH gene. Support for this hypothesis is derived by analysis of a previously published BPNH family (Huttenlocher et al. 1994); this family was subsequently used for the linkage-analysis studies that mapped BPNH to Xq28 (Eksioglu et al. 1996). This family consisted of six females affected with BPNH and no affected males. There were 11 miscarriages among the affected women; among the live-born offspring there were nine daughters and three sons. Two of the boys were normal. The third died at age 7 d, because of multiple systemic hemorrhages that, we speculate, may have been due to an F8C-gene mutation causing hemophilia (Huttenlocher et al. 1994; Eksioglu et al. 1996). Since the F8C gene is located in distal Xq28, it does not seem unreasonable that this family may be carrying a deletion that encompasses both the putative BPNH gene and the F8C gene.

Since classical BPNH and BPNH/MR syndrome both map to Xq28, it seems likely that one gene is responsible for the BPNH component of both diseases. Yet, surprisingly, classical BPNH, which causes only seizures in females, is usually lethal in males, whereas BPNH/MR syndrome has been described only in males and is associated with other phenotypic abnormalites in addition to seizures. These findings can be explained if expression of the BPNH gene is essential for survival. In this scenario, mild mutations such as duplication or some missense mutations would produce excess normal or slightly altered gene product, which would disrupt normal development in males with only one copy of the gene and would cause BPNH/MR syndrome. Females would survive and be normal, because of (1) preferential inactivation of the abnormal X chromosome, (2) random X inactivation with intercellular complementation or selection, or (3) intracellular complementation if the X-linked gene escapes inactivation. This is seemingly the case for the mother of BPNH-02, who is clinically unaffected and has a duplication of Xq28 of one of her X chromosomes. Males with more severe mutations, such as nonsense mutations or deletions, would make either a nonfunctional BPNH gene product or, perhaps, no product at all, leading to death in utero. Females with severe mutations would be expected to have either very mild disease (classical BPNH with normal intelligence) or, perhaps, no disease at all, because of the possible variations in X-inactivation patterns, as described above.

BPNH Candidate Genes in Distal Xq28

Xq28 is one of the most well-characterized regions of the human genome (Nelson et al. 1995). A YAC and partial cosmid contig have been described, as have compositional, transcription, sequence-tagged site, and expressed-sequence-tags maps; a large portion of Xq28 has been sequenced (reviewed in Nelson et al. 1995). The area is extremely gene rich, with ~40 genes identified (see the Genome Database).

The proximal breakpoint of the duplication in BPNH-02 maps between L1CAM and DXS15, and the distal breakpoint is very close to the telomere of the long arm. The duplicated region contains several candidate genes for BPNH. L1CAM is one of these; it is a member of the immunoglobulin superfamily of genes and codes for a neural cell–adhesion molecule critical for neuronal migration (Hlavin and Lemmon 1991). Mutations in L1CAM have been described in syndromic patients with severe X-linked mental retardation and hydrocephalus with stenosis of the aqueduct of Sylvius, aphasia with shuffling gait and adducted thumbs (MASA), complicated spastic paraparesis, or agenesis of the corpus callosum (Fransen et al. 1995). These syndromes are characterized by variable phenotypic findings, but common cardinal features include hypoplasia of the corpus callosum, severe mental retardation, adducted thumbs, spastic paraplegia, and hydrocephalus (Schrander-Stumpel et al. 1995).

The BPNH/MR syndrome phenotype overlaps with several of the cardinal manifestations in these other syndromes, including severe mental retardation, dysplasia of the corpus callosum, and ventriculomegaly. In addition, syndactyly, which is an invariant finding in the BPNH/MR syndrome, is also seen in some cases of MASA syndrome. Our FISH analyses with the L1CAM cDNA probe indicated that L1CAM was duplicated in BPNH-02 but was not either duplicated or deleted in BPNH-03 or BPNH-12.

A second candidate gene is the X-linked chronic idiopathic intestinal pseudoobstruction (CIIPX) gene. Mutations in CIIPX cause a failure in the normal development of enteric argyrophilic neurons in the myenteric plexus, resulting in a syndrome of short small bowel, malrotation, and pyloric hypertrophy, leading to chronic idiopathic intestinal pseudoobstruction. This gene recently has been mapped to Xq28, between DXS15 and DXS1108, by linkage analysis of 26 members of a single extended family (Auricchio et al. 1996). Eight males of this family "died in the first months after birth with gastroenterological symptoms of intestinal pseudoobstruction" (Auricchio et al. 1996, p. 744). No further clinical information was provided on these infants. It is intriguing that there are a large number of prenatal or perinatal deaths in female carriers of either CIIPX or classical BPNH, since both disorders map to the same region of Xq28 and both affect normal nerve-cell development. Interestingly, in three boys a syndrome was

described that included short small bowel, pyloric hypertrophy, malrotation, and periventricular heterotopia (Nezelof et al. 1976), suggesting either that both syndromes are caused by a mutation in a single gene or that the genes map close together. In order to further investigate the genetic mechanism giving rise to BPNH/MR, our future investigations will focus on studying the aforementioned candidate genes and identifying the breakpoints of the inverted duplication in BPNH-02.

Acknowledgments

We gratefully acknowledge Dr. Mary Ella Pierpont for referring patient BPNH-02 for analysis. We also acknowledge Drs. Julia Parrish and David Nelson for providing us with cosmid clones in Xq28, from the LLOXNCO1 library, and we acknowledge Drs. Anand Srivastava and David Schlessinger for providing us with YAC clones in Xq28. The chromosome-specific library LLOXNCO1 used in this work was constructed at the Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA, under the auspices of the National Laboratory Gene Library Project sponsored by the U.S. Department of Energy. We also acknowledge Dr. David Nelson for molecular analyses of FRAXA and FRAXE and acknowledge Dr. Mike Koob for technical assistance with the agarose microbead technique. This work was supported by Minnesota Medical Foundation grants FPK-13-96 and LNF-9-96.

References

Auricchio A, Brancolini V, Casari G, Milla PJ, Smith VV, Devoto M, Ballabio A (1996) The locus for a novel syndromic form of neuronal intestinal pseudoobstruction maps to Xq28. Am J Hum Genet 58:743–748

Barkovich AJ, Kjos BO (1992) Gray matter heterotopias: MR characteristics and correlation with developmental and neurologic manifestations. Radiology 182:493–499

Barkovich AJ, Kuzniecky RI, Dobyns WB, Jackson GD, Becker LE, Evrard P (1996) A classification scheme for malformations of cortical development. Neuropediatrics 27:59–63

Dobyns WB, Andermann E, Andermann F, Czapansky-Beilman D, Dubeau F, Dulac O, Guerrini R, et al (1996) X-linked malformations of neuronal migration. Neurology 47: 331–339

Dobyns WB, Guerrini R, Czapansky-Beilman DK, Pierpont MEM, Breningstall G, Tock DH, Bonanni P, et al. Bilateral periventricular nodular heterotopia (BPNH) with mental retardation and syndactyly in boys: a new X-linked mental retardation syndrome. Neurology (in press)

Dobyns WB, Truwit CL (1995) Lissencephaly and other malformations of cortical development: 1995 update. Neuropediatrics 26:132–148

Dubeau F, Tampieri D, Lee N (1995) Periventricular and subcortical nodular heterotopia: a study of 33 patients. Brain 118:1273–1287

Eksioglu YZ, Scheffer IE, Cardenas P, Knoll J, DiMario F, Ramsby G, Berg M, et al (1996) Periventricular heterotopia: an X-linked dominant epilepsy locus causing aberrant cerebral cortical development. Neuron 16:77–87

Fransen E, Lemmon V, Van Camp G, Vits L, Coucke P, Willems PJ (1995) CRASH syndrome: clinical spectrum of corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraparesis and hydrocephalus due to mutations in one single gene, L1. Eur J Hum Genet 3:273–284

Gustashaw KM (1991) Chromosome stains. In: Barch MJ (ed) The ACT cytogenetics laboratory manual, 2nd ed. Raven Press, New York, pp 205–296

Hlavin ML, Lemmon V (1991) Molecular structure and functional testing of human L1CAM: an interspecies comparison. Genomics 11:416–423

Huttenlocher PR, Taravath S, Mojtahedi S (1994) Periventricular heterotopia and epilepsy. Neurology 44:51–55

Kamuro K, Tenokuchi Y (1993) Familial periventricular nodular heterotopia. Brain Dev 15:237–241

Koob M, Szybalski W (1992) Preparing and using agarose microbeads. Methods Enzymol 216:13–20

Lahn BT, Ma N, Breg WR, Stratton R, Surti U, Page DC (1994) Xq-Yq interchange resulting in supernormal X-linked gene expression in severely retarded males with 46,XYq− karyotype. Nat Genet 8:243–250

Lawce HJ, Brown MG (1991) Harvesting, slide-making, and chromosome elongation techniques. In: Barch MJ (ed) The ACT cytogenetics laboratory manual, 2d ed. Raven Press, New York, pp 31–66

Lengauer C, Green ED, Cremer T (1992) Fluorescence in situ hybridization of YAC clones after Alu-PCR amplification. Genomics 13:826–828

Lupski JR, Wise CA, Kuwano A, Pentao L, Parke JT, Glaze DG, Ledbetter DH, et al (1992) Gene dosage is a mechanism for Charcot-Marie-Tooth disease type 1A. Nat Genet 1: 29–33

Muenke M, Schell U (1995) Fibroblast-growth-factor receptor mutations in human skeletal disorders. Trends Genet 11:308–313

Nelson DL, Ballabio A, Cremers F, Monaco AP, Schlessinger D (1995) Report of the Sixth International Workshop on X Chromosome Mapping 1995. Cytogenet Cell Genet 71:308–336

Nezelof C, Jaubert F, Lyon G (1976) Syndrome familial associant grele court, malrotation intestinale, hypertrophie du pylore et malformation cerebrale. Ann Anat Pathol 21:401–412

Norman MG, Mcgillivray BC, Kalousek DK, Hill A, Poskitt KJ (1995) Congenital malformations of the brain: pathological, embryological, clinical, radiological and genetic aspects. Oxford University Press, New York

O'Rourke NA, Sullivan DP, Kaznowski CE, Jacobs AA, McConnell SK (1995) Tangential migration of neurons in the developing cerebral cortex. Development 121:2165–2176

Palmieri G, Romano G, Ciccodicola A, Casamassimi A, Campanile C, Esposita T, Cappa V, et al (1994) YAC contig organization and CpG island analysis in Xq28. Genomics 24:149–158

Patel PI, Roa BB, Welcher AA, Schoener-Scott R, Trask BJ, Pentao L, Snipes GJ, et al (1992) The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type IA. Nat Genet 1:159–165

Raymond AA, Fish DR, Stevens JM, Sisodiya SM, Alsanjari N, Shorvon SD (1994) Subependymal heterotopia: a distinct

neuronal migration disorder associated with epilepsy. J Neurol Neurosurg Psychiatry 57:1195–1202

Roa BB, Garcia CA, Suter U, Kulpa DA, Wise CA, Mueller J, Welcher AA, et al (1993) Charcot-Marie-Tooth disease type 1A: association with a spontaneous point mutation in the PMP22 gene. N Engl J Med 329:96–101

Schrander-Stumpel C, Howeler C, Jones M, Sommer A, Stevens C, Tinschert S, Israel J, et al (1995) Spectrum of X-linked hydrocephalus (HSAS), MASA syndrome, and complicated spastic paraplegia (SPG1): clinical review with six additional families. Am J Med Genet 57:107–116

Sidman RL, Rakic P (1982) Development of the human central nervous system. In: Haymaker W, Adams RD (eds) Histology and histopathology of the nervous system. Charles C Thomas, Springfield, IL, pp 3–45

Timmerman V, Nelis E, Hul WV, Nieuwenhuijsen BW, Chen KL, Wang S, Othman KB, et al (1992) The peripheral myelin protein gene PMP-22 is contained within the Charcot-Marie-Tooth disease type IA duplication. Nat Genet 1:171–175

Trask BJ, Massa H, Kenwrick S, Gitschier J (1991) Mapping of human chromosome Xq28 by two-color fluorescence in situ hybridization of DNA sequences to interphase cell nuclei. Am J Hum Genet 48:1–15

Valentijn LJ, Baas F, Wolterman RA, Hoogendijk JE, van den Bosch NHA, Zorn I, Gabreels-Festen AAWM, et al (1992*a*) Identical point mutations of PMP-22 in Trembler-J mouse and Charcot-Marie-Tooth disease type 1A. Nat Genet 2: 288–291

Valentijn LJ, Bolhuis PA, Zorn I, Hoogendijk JE, van den Bosch N, Hensels GW, Stanton VP, et al (1992*b*) The peripheral myelin gene PMP-22/GAS-3 is duplicated in Charcot-Marie-Tooth disease type IA. Nat Genet 1:166–170

Ward DC, Boyle A, Haaf T (1995) Fluorescence in situ hybridization techniques. In: Verma RS, Babu A (eds) Human chromosomes: principles and techniques, 2d ed. McGraw-Hill, New York, pp 184–187

Yunis JJ (1976) High resolution of human chromosomes. Science 191:1268–1270