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Periventricular Heterotopia: An X-Linked Dominant Epilepsy Locus Causing Aberrant Cerebral Cortical Development

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Summary

Periventricular heterotopia (PH) involves dramatic malformations of the human cerebral cortex. Here we show that PH is closely linked to markers in distal Xq28 (maximal two-point lod score = 4.77 for *F8C* at $\theta = 0$; maximal multipoint lod score = 5.37), so that affected females are obligatory mosaics for the mutation; that PH is lethal to at least some affected males; that PH malformations consist of well-differentiated cortical neurons filling the adult subependymal zone; and that individuals with PH are at high risk for epilepsy, though they have no other neurological or external stigmata. The PH gene may represent an important epilepsy susceptibility locus in addition to playing a key role in normal cortical development.

Introduction

Although identification of genes responsible for epilepsy has profound implications for therapy as well as neurophysiology, it has proven a difficult task. Whereas evidence for inherited factors in epilepsy is strong, epilepsy appears to be genetically and phenotypically heterogeneous, and there are nongenetic causes of epilepsy that complicate linkage analysis (Delgado-Escueta et al., 1994). Recently, several epileptic syndromes have been mapped genetically (Greenberg et al., 1988; Ottman et al., 1995; Phillips et al., 1995), although some of these have been controversial (Whitehouse et al., 1993; Ryan, 1995). An alternative approach is to understand the genetic basis of cerebral cortical malformations, since developmental malformations of the cerebral cortex have long been observed in the brains of many humans with epilepsy (Alzheimer, 1907). Postmortem studies have suggested that up to 40% of epileptic brains show subtle or more obvious developmental malformations (Meencke and Janz, 1984), and human brain tissue that has been removed for the clinical treatment of intractable epilepsy shows developmental malformations in 30%–50% of specimens (Hardiman et al., 1988), especially in cases of severe epilepsy in children (Farrell et al., 1992).

Genetic analysis of two inherited epileptic cortical malformations has yielded genes apparently active in signal transduction in both the developing and adult brain. Tuberous sclerosis (TSC) shows two types of cortical malformations (Gomez, 1988). The *TSC2* gene, responsible for many cases of TSC, encodes a protein expressed in both the developing and adult cortex with structural homology to rasGAP (The European Chromosome 16 Tuberous Sclerosis Consortium, 1993). Lissencephaly of the Miller–Dieker type is a profound disorder of neuronal migration into the cortex (Dobyns et al., 1992). The *LIS1* gene has also recently been cloned (Reiner et al., 1993) and encodes a protein involved in signaling by platelet-activating factor, a bioactive lipid implicated as a neurotransmitter in the adult CNS (Hattori et al., 1994). Therefore, cloning genes involved in genetically inherited malformations of the cerebral cortex may provide insight into other signal transduction pathways active both in development and in the normal adult.

Periventricular heterotopia (PH) is a disorder of cortical development that has been recognized for some time, but only recently suggested to represent a genetic trait. PH is a descriptive term applied to a variety of disorders that all result in masses of neural cells along, or protruding into, the lateral ventricles beneath the cerebral cortex. A unique, genetically inherited form of isolated PH was recently reported (DiMario et al., 1993; Kamuro and Tenokuchi, 1993; Oda et al., 1993; Huttenlocher et al., 1991, 1994). Affected individuals in these pedigrees showed heterogeneous types of epilepsy, but had no other cognitive or neurological markers and no peripheral stigmata. Recognition of the inherited nature

of this ''isolated'' PH has emerged only from the increasingly widespread use of magnetic resonance imaging (MRI) in epileptic patients, because PH is easily seen on MRI images.

Since patients with isolated PH usually develop seizures in adolescence (see below), PH may represent a significant genetic cause of uncomplicated epilepsy, and hence identify an important epilepsy susceptibility locus. In contrast to many epilepsy susceptibility genes, PH appears to be highly penetrant (perhaps 100%), when assayed by MRI, and does not have any known nongenetic disorders that resemble it. Therefore, the developmental malformation serves as an objective marker for the epilepsy locus.

This paper defines the genetics and biology of inherited PH and identifies an X-linked locus associated with PH. Our data show that PH is linked to Xq28 and shows expression in females with perinatal or prenatal lethality in males. Thus, affected females are genetic mosaics for the PH mutation. We also describe the microscopic appearance of PH, indicating the key role of the PH gene in human cerebral cortical development.

Results

Pedigree Analysis

Four pedigrees with PH were available for linkage analysis.

Chicago Pedigree

A PH pedigree with 6 affected individuals has been previously reported (Huttenlocher et al., 1991, 1994), but for the purposes of this study, the pedigree was extended further (Figure 1). We analyzed MRI (or computerized tomography [CT]) images from siblings (Figure 1A, I-2 and I-3) of the oldest affected member of the pedigree (I-1) and reviewed medical histories of the offspring of these siblings. MRI images of a female cousin were also reviewed, and medical histories were taken concerning 14 other cousins and their offspring. Death certificates of the mother, grandmother, and grandfather of patient I-1 were reviewed. No evidence was found for the appearance or segregation of epilepsy in these additional family members, suggesting that PH might have arisen as a new mutation in individual I-1 and been transmitted since then. Individual III-4 was a male who died (in 1963) of a severe neonatal hemorrhagic disorder, diagnosed clinically as hemophilia. Factor VIII analysis was not done, however, and examination of the brain was not undertaken. Thus, his disease status is unknown, and he was considered not informative in linkage calculations, although postmortem sections of spleen and liver were analyzed for some DNA markers. Individual III-5 has had seizures but refused evaluation and participation in the study.

Connecticut Pedigree

Two members of a pedigree with PH were previously published (DiMario et al., 1993). For the purposes of genetic analysis, a pedigree was constructed (Figure 1B) and consisted of a nuclear family with an affected mother (II-1) and daughter (III-1). Two clinically unaffected siblings (III-2 and III-3) had normal imaging studies. One sister of the mother (II-4) had a normal CT scan, no seizures, and normal children. The grandmother (I-1) died 15 years ago without imaging studies or autopsy. She suffered from epilepsy, but it is not known whether she showed PH; she is considered as ''status unknown'' in all linkage calculations, though she may have been affected and though postmortem tissue was analyzed for DNA markers. An additional pregnancy of individual I-1 resulted in spontaneous abortion of a male fetus in the third trimester. Two brothers of the affected mother (II-2 and II-3) and siblings of I-1 (I-2, I-3, I-4, and I-5) refused participation in the study but are included in the pedigree to indicate male:female ratios.

Kagoshima Pedigree

A three generation pedigree of PH was published previously (Kamuro and Tenokuchi, 1993), but the pedigree was extended further for linkage analysis (Figure 1C). Individual II-1 had 2 brothers, 1 of whom (II-2) was normal by MRI and had 2 clinically normal children. A second brother (II-3) refused participation in the study but is included in the pedigree to indicate male:female ratios.

Australia Pedigree

A new two generation pedigree of PH has not been reported before (Figure 1D). It consists of an affected mother and 4 daughters, 3 of whom have PH. The mother also had four miscarriages, of which two were sufficiently late in gestation to be identifiable as males. Several siblings of the affected mother have not yet been studied with MRI.

Imaging of PH

PH could be diagnosed clearly and unambiguously by imaging modalities at all ages studied (Figure 2) and as early as 6 months of postnatal age, the earliest age at which CT or MRI has been attempted. The malformations showed remarkably similar characteristics even when studies from different pedigrees were compared (Figures 2b and 2c). The lesions formed continuous bands through the periventricular region, although in some individuals the malformations were arrayed as discontinuous nodules with the appearance of ''beads on a string.'' The nodules showed MRI imaging characteristics similar to cortical gray matter in all sequences, and were very different from white matter. The MRI appearance is consistent with the malformations containing mainly neurons, and this has been confirmed by microscopic analysis (see below). So far, no individuals in any of the pedigrees have developed PH following normal imaging studies, and repeated studies in several individuals have shown no change in the lesions with time.

Inherited PH Is Frequently Associated with Epilepsy

Whereas PH is inherited in all four pedigrees as a dominant trait with apparently full penetrance in females (see Figure 1), epileptic features of individuals affected with PH were less consistent (Table 1). Of 15 individuals with PH by MRI in four pedigrees, 10 suffered from documented seizures; one woman, now in her seventies, was given a diagnosis of ''pseudoseizures,'' since her episodes of altered consciousness were not related to changes in a simultaneously recorded electroencephalograph (EEG). The average age of onset of bona fide seizures was 14.3 years of age (range 4–24), with an age-corrected risk of seizures of 14% at age 5, 21% at 10, 31% at 15, 54% at 20, and 75% at 25 and thereafter.

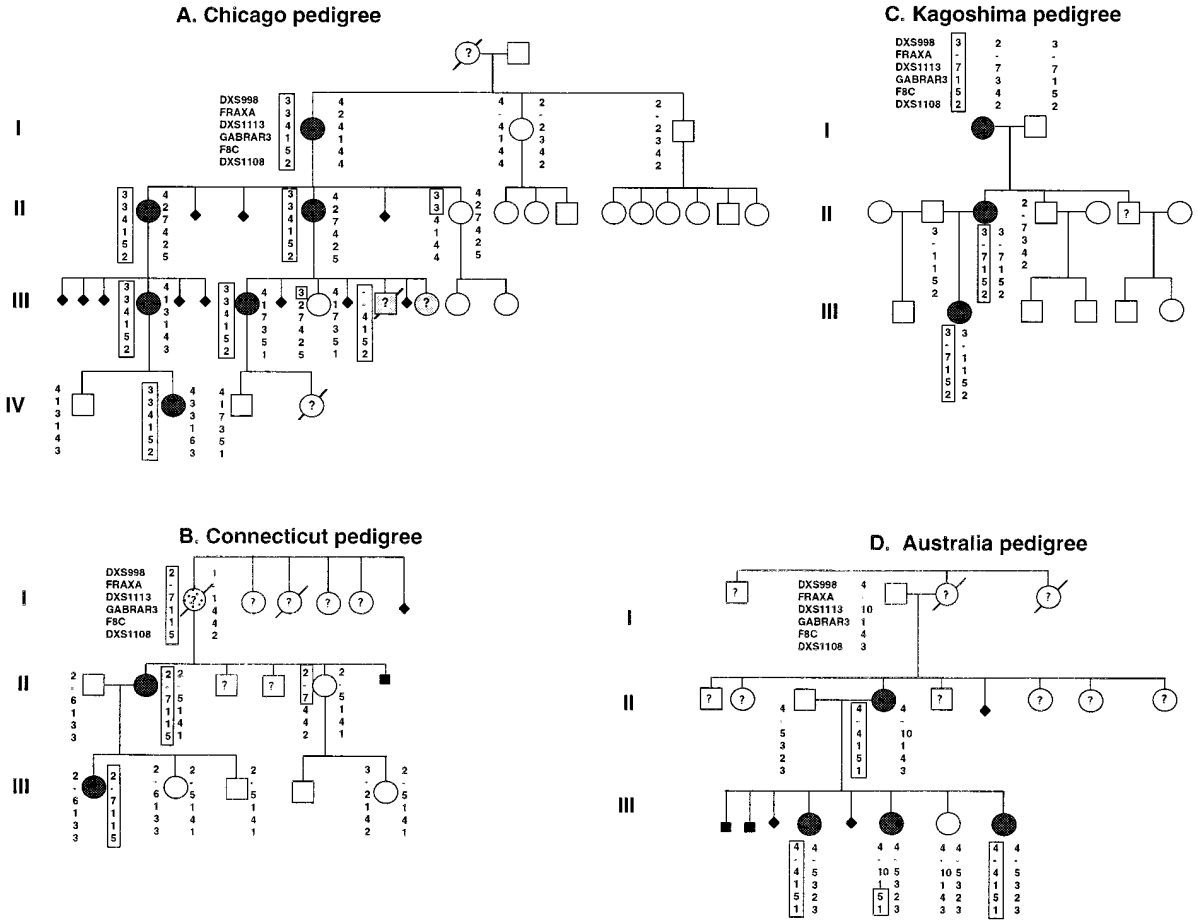

Figure 1. Pedigree Analysis of Periventricular Heterotopia in Four Pedigrees

Three pedigrees were originally published between May, 1993, and January, 1994, and the fourth is unpublished. Shading represents affected individuals, defined by MRI. Alleles of each marker are indicated by numbers and are matched to published allele identities and allele frequencies. The haplotype associated with PH is shown by an outline, and recombinant breakpoints are indicated. Dashes indicate markers not typed. Individuals with clinical features that might suggest PH but for whom definitive MRI information is not available are indicated with light shading, while unshaded symbols with question marks indicate no knowledge at all about phenotype; all of these individuals were considered as unknown phenotype when calculating lod scores. In all pedigrees, males are shown with squares, females with circles, and unknown or uncertain gender with diamonds. Spontaneous abortions or stillbirths are indicated with smaller closed symbols.

Therefore, although the MRI abnormality in PH appears to show very high penetrance, PH also represents an epilepsy susceptibility locus. However, the epilepsy phenotype itself has an age-related penetrance that is significantly less than 100%.

PH was associated with several types of epilepsy, including temporal lobe epilepsy, generalized tonic-clonic epilepsy, and mixed seizure types (Table 1). There is a tendency for seizure types within a family to be similar, which may reflect ascertainment bias, allelic heterogeneity of the disorder in different families, diagnostic differences, or actions of different contributory genes. The severity of seizures ranged from a single seizure in a lifetime to severe, medically refractory seizures. EEGs were similarly nonspecific, ranging from normal to focally or globally abnormal.

Linkage Analysis of PH

The expression of PH exclusively in females in one pedigree led Huttenlocher et al. (1994) to suggest that PH was either an X-linked trait that is dominant in females and lethal to males, or that it was due to an autosomal or mitochondrial gene associated with some other sex-specific effect (e.g., imprinting or hormonal effects) that accounted for the sex-specific phenotype. We distinguished between these hypotheses by analyzing linkage of the MRI abnormality to 54 polymorphic X-chromosomal markers (average interval < 5 cM; largest interval $= 20$ cM) in the four pedigrees. Most markers consisted of simple sequence length polymorphisms, while a few were restriction fragment length polymorphisms that were analyzed using PCR. Markers were analyzed in the Chicago pedigree (see Figure 1) across the entire chromosome. Markers that gave positive lod scores were then tested in the rest of that pedigree and in the other pedigrees as well. Lod scores were calculated using the LINKAGE programs (Lathrop and Lalouel, 1984, 1988), assuming X-linked as well as autosomal inheritance of PH, with a penetrance of 95% in females and 100% in males, a gene frequency of 2:10,000 and

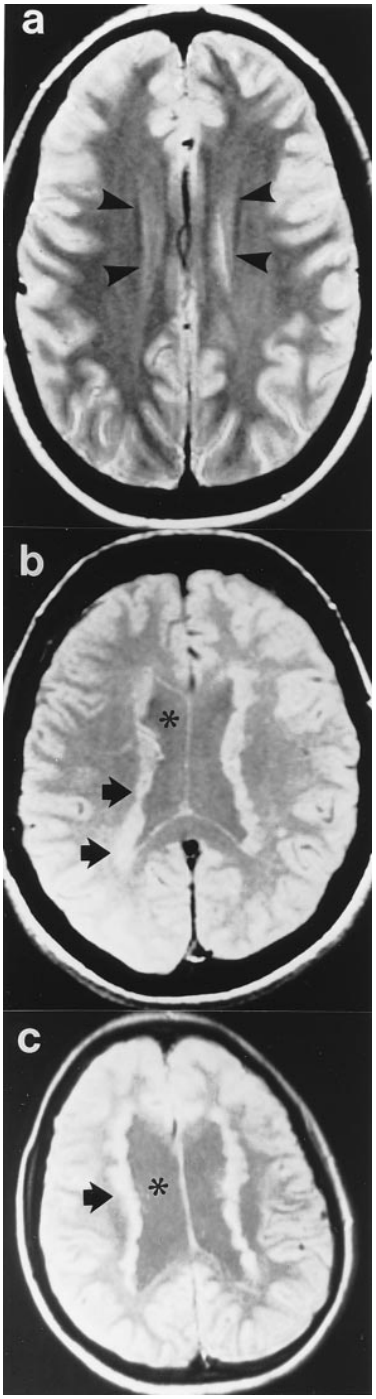

Figure 2. MRI of Periventricular Heterotopia

T1-weighted MRI (TE 22, TR 3526, SE technique) of a normal individual (a) shows good definition of white matter from gray matter and shows that the periventricular zone is normally extremely thin and indistinct. MRIs of individuals with PH from the Chicago pedigree (b) and the Connecticut pedigree (c), performed with a similar technique (TE 16, TR 450, SE technique), are very similar to one another, given differences in reproduction, but are distinctly abnormal. The periventricular zone of both lateral ventricles is distorted and enlarged by increased signal intensity that forms a continuous lining without clear asymmetry (arrows). The heterotopic nodules have MRI signal characteristics identical to gray matter, suggesting that they correspond to neurons. The ventricles (asterisks) are also somewhat enlarged relative to normal.

a mutation rate of 1:10,000. No evidence for linkage was seen to markers anywhere on the p arm or to markers on the proximal portion of the q arm. Cytogenetic studies were also performed at high resolution on 2 affected members of each pedigree (as well as on 5 other sporadic cases) and were normal in every case.

Consistently positive lod scores were obtained between PH and markers in distal Xq, in the Xq28 region. Two-point lod scores (Table 2) ruled out close linkage of PH to markers in Xq27 such as *DXS998*, or to the *FRAXA* gene itself. However, no obligatory recombinant events were observed between PH and several markers in distal Xq28 (*GABRA3*, *F8C*, *F8C-HindIII*, *DXS1108*, and *DXYS154*) in the four pedigrees. The most highly informative single marker in all pedigrees was F8C, which gave a maximal two-point lod score of 4.77 (at $\theta = 0.0$). A nearby marker, *DXS15*, listed at $\sim$0 cM from *F8C* in several recent linkage maps of the X chromosome (Donnelly et al., 1994; Wang et al., 1994; Fain et al., 1995), could be combined with *F8C* in pedigrees in which neither marker alone was fully informative. The combined marker made all meioses informative and provided a lod score of 5.37 (at $\theta = 0.0$). A two-point lod score that exceeded 3.0 was also seen for *DXS1108*.

Multipoint linkage analysis and haplotype analysis (see Figure 1) also suggested a most probable location for the PH gene in distal Xq28, giving a maximal lod score of 5.37 near *F8C*, with slightly lower scores over the telomere of the q arm. At present, lod scores and further gene localization of the PH gene are limited by the small number and size of pedigrees. Recombination events (Figure 3) suggest a location for the PH gene distal to *DXS1113*, a region that comprises $<$7 Mb of DNA (Sedlacek et al., 1993).

Inherited PH Is Associated with Spontaneous Abortion and Is Lethal to Some Males

Male offspring of females with PH are normal and so far have not transmitted PH. However, affected females showed a shortage of male offspring and an excess of spontaneous abortion. Spontaneous abortions generally occurred at 2–4 months of gestation, and the sex of the miscarried fetuses was unknown. However, three pregnancies progressed longer, and in all three cases the aborted fetus was male. The offspring of affected females included 17 females and 6 males, a ratio significantly different from 1:1 ($p < .02$ compared with a binomial distribution). Affected females exceeded normal females (11:6), probably owing to ascertainment bias since pedigrees were selected on the basis of having more than 1 affected female. Furthermore, affected females showed a miscarriage rate (15/37 pregnancies) that was approximately 2-fold the expected rate of 20%–25% of pregnancies. To demonstrate directly that PH is lethal to males, we analyzed DNA markers from a male, born at term to an affected female, that died within 1 week after birth from overwhelming spontaneous bleeding (see Figure 1). The structure of the brain in this case was not studied, and therefore the phenotype of this individual is considered as unknown for the purposes of linkage analysis. However, the baby carried the haplotype linked to PH for markers covering all of

Table 1. Clinical Features of Patients with X-Linked Periventricular Heterotopia

Patient	Age	Gender	Seizure Type	Age at Seizure Onset	EEG Findings
Chicago pedigree					
I-1	72	F	''Pseudoseizures''	–	Normal (including long-term monitoring)
II-1	49	F	Generalized TC	18	ND
II-2	46	F	Generalized TC	4	Bitemporal epileptiform discharges
III-1	27	F	Generalized TC (1)	24	Normal
III-2	22	F	Generalized TC (2)	5	Right fronto-temporal slowing
IV-2	3	F	None	–	ND
Connecticut pedigree					
II-1	36	F	TLE	21	ND
III-1	17	F	TLE; generalized TC	10	R > L bicentral paroxysmal activity
Kagoshima pedigree					
I-1	61	F	None	–	ND
II-3	35	F	TLE; secondarily generalized	15	R anterior-temporal spikes
III-2	14	F	None	–	Normal
Australia pedigree					
II-2	53	F	None	–	Normal
III-1	24	F	Generalized TC (1)	16	Normal
III-2	17	F	Generalized TC (2)	16	Normal
III-3	21	F	None	–	Normal

Data were obtained from previous publications (DiMario et al., 1993; Kamuro and Tenokuchi, 1993; Huttenlocher et al., 1994) and were supplemented as needed by additional interviews with pedigree members. TC, tonic-clonic seizures; TLE, temporal lobe epilepsy; ND, not determined.

Xq28, suggesting that this male carried the affected X chromosome. These data suggest directly that PH is lethal to males carrying the mutation in some pedigrees, although the male lethality could reflect the effect of an adjacent gene rather than the PH gene itself.

The morphological and clinical appearance of PH is quite striking. Analysis of more than 40 sporadic cases (Y. Z. E. and C. W., unpublished data) and review of the literature indicate that the only inherited form of PH that is not associated with other neurological or other deficits has the following characteristics: female gender, bilaterally symmetrical periventricular nodules, MRI appearance consistent with gray matter, and seizures with no other neurological symptoms. Therefore, since all analyzed pedigrees so far show linkage to the same locus, since all other inherited cases in the literature involved females only, and since >90% of sporadic cases of PH occur in females (Raymond et al., 1994; Y. Z. E. and C. W., unpublished data), the present data suggest that PH cases that fit the above criteria relate to the same X-linked gene.

PHs Consist of Well-Differentiated Cerebral Cortical Neurons in Striking Patterns

Histological analysis has been performed on several brains that show periventricular lesions (Barkovich and

Table 2. Two-Point lod Scores for Four Periventricular Heterotopia Pedigrees

	$\theta = 0$	$\theta = 0.05$	$\theta = 0.10$	$\theta = 0.20$	$\theta = 0.30$	Z_{max}	Total Z_{max}
DXS998							
Chicago	−9.99	−1.14	−0.66	−0.27	−0.10	−0.10	
Connecticut	−0.17	−0.14	−0.10	−0.05	−0.02	−0.02	
Kagoshima	0.30	0.26	0.21	0.13	0.06	0.30	
Australia	0.00	0.00	0.00	0.00	0.00	0.00	$\Sigma = 0.18$
DXS1113							
Chicago	1.09	1.06	1.01	0.84	0.61	1.09	
Connecticut	0.42	0.43	0.42	0.35	0.24	0.43	
Kagoshima	0.00	0.00	0.00	0.00	0.00	0.00	
Australia	−2.62	−0.29	−0.06	0.10	0.12	0.12	$\Sigma = 1.64$
F8C							
Chicago	2.49	2.26	2.03	1.55	1.03	2.49	
Connecticut	0.90	0.81	0.71	0.51	0.31	0.90	
Kagoshima	0.30	0.26	0.21	0.13	0.06	0.30	
Australia	1.08	0.99	0.90	0.69	0.46	1.08	$\Sigma = 4.77$
DXS1108							
Chicago	1.90	1.76	1.60	1.26	0.87	1.90	
Connecticut	0.90	0.80	0.71	0.51	0.31	0.90	
Kagoshima	0.00	0.00	0.00	0.00	0.00	0.00	
Australia	1.08	0.99	0.90	0.69	0.46	1.08	$\Sigma = 3.88$

Two-point linkage analysis was performed using the LINKAGE package (Lathrop and Lalouel, 1984, 1988; Terwilliger and Ott, 1994) using previously published allele frequencies, obtained from the Genome Data Base (Fasman et al., 1994).

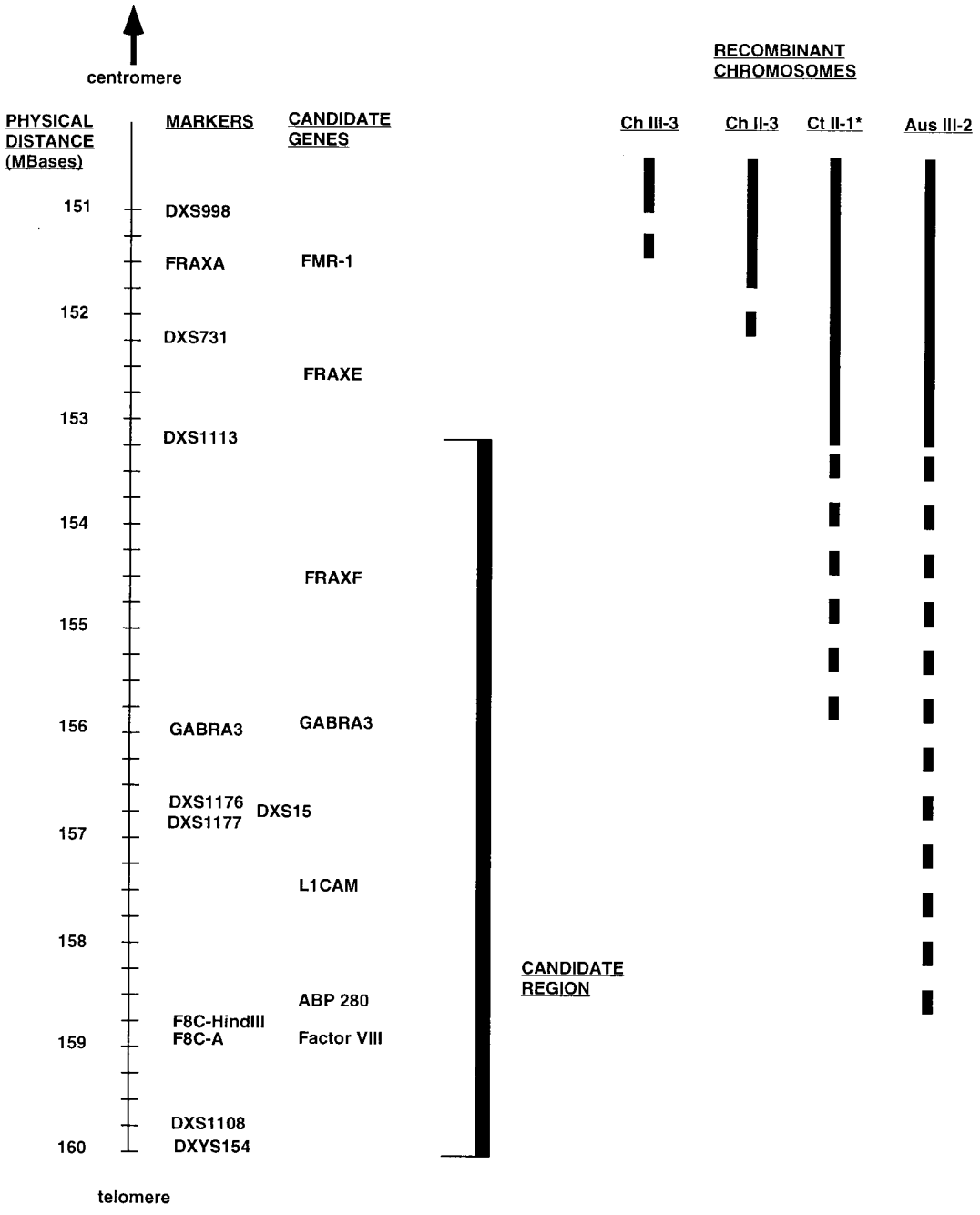

Figure 3. Location of Genetic Markers Tested and Candidate Region for Periventricular Heterotopia

Physical distance along the X chromosome is indicated at the left; the locations of markers and candidate genes are indicated. Regions from which the PH gene is excluded by informative recombination events are shown in heavy black; regions in which markers are not informative are shown in interrupted bars. One individual (Ct II-1) is illustrated, but the precise phenotype of her mother is not known. Therefore, it is uncertain whether the recombination event further refines the gene. Data for constructing the Xq28 map are taken from Willard et al., 1994.

Kjos, 1992; J. Golden, Y. Z. E., J. Joseph, and C. W., unpublished data), but one of these cases shares the distinctive features of the genetically inherited PH described above. The individual was a woman with onset of seizures at age 16 that became intractable by the time of her death at age 27. The periventricular lesions filled the entire subependymal zone symmetrically beneath the cerebral cortex (Figure 4a), matching almost precisely the appearance of PH by MRI (see Figure 2). The lesions consisted of confluent nodules 2–10 mm in diameter (Figures 4b and 4c).

Microscopically, the nodules consisted of highly differentiated neurons that looked remarkably normal, except that they were oriented in multiple directions. Neuron–glia relationships were indistinguishable from normal gray matter (Figures 4d–4f). Multiple neuron

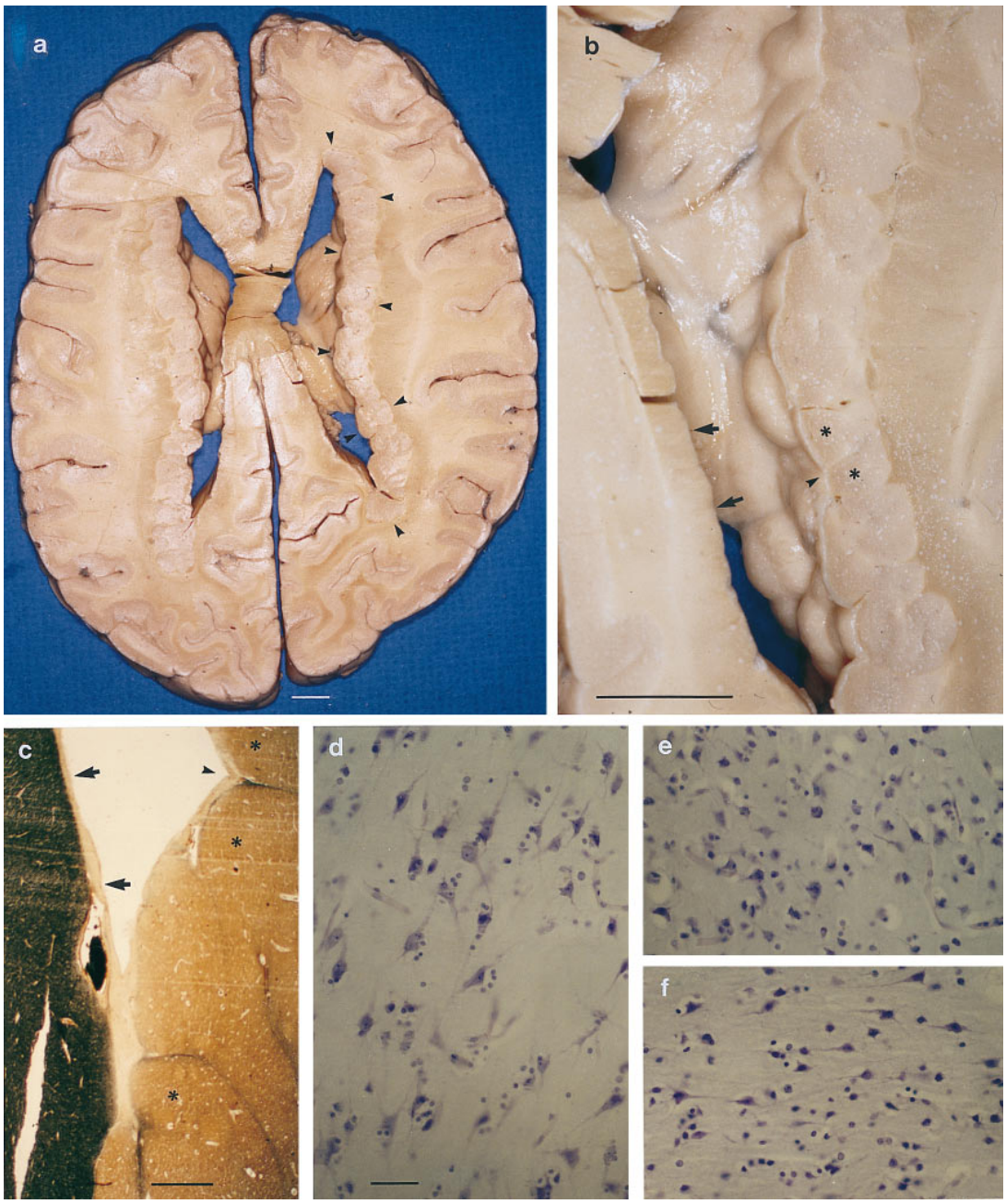

Figure 4. Postmortem Appearance of Periventricular Heterotopia

Photographs are taken from the brain of a 27-year-old female with intractable seizures that began at age 16.
(a) A gross section of the brain, taken in a plane similar to that shown in the MRIs in Figure 2, shows that the periventricular nodules (arrowheads) are indistinguishable in appearance from those seen with MRI.
(b) A higher magnification view illustrates the continuous involvement of the lateral margin of the periventricular zone (arrowhead), while the medial margin of the lateral ventricle (arrows) is uninvolved.
(c) A Luyoz myelin stain shows the lateral ventricle with the nodules (asterisks) along its lateral border, separated by myelinated septae (arrowhead), while the medial border of the lateral ventricle (arrows) is normal.
(d) A high magnification photomicrograph illustrates one nodule that contains predominantly large pyramidal neurons, identifiable by their large apical and basal dendrites, large pale nuclei, and abundant Nissl substance.
(e) Another nodule from the same section shows numerous smaller neurons with small dendrites that are not obviously oriented consistent with nonpyramidal neurons.
(f) Another nodule from the same section shows small pyramidal cells with apical and basal dendrites, but darker staining perikarya and smaller, darker nuclei than in (d). Bars, 1 cm (a and b), 1 mm (c), 100 μm (d–f).

types were evident. Some microscopic fields contained primarily medium to large pyramidal neurons (Figure 4d) with large pale-staining nuclei, prominent nucleoli, and large, oriented apical and basal dendrites. Other regions in the same section contained primarily nonpyramidal neurons with small nuclei and round cell bodies without obviously stained or oriented dendrites (Figure 4e). Still other regions contained primarily small to medium-sized pyramidal cells with prominent, oriented apical and basal dendrites, but smaller cell bodies and smaller, darker nuclei than the large pyramids (Figure 4f).

Fiber staining and immunohistochemical analysis demonstrated that the heterotopic neurons were richly innervated, although the source of this innervation is not yet clear. Myelinated fibers (Figure 5a) and neurofilament-positive fibers (data not shown) course near and through the nodules. Immunohistochemistry for synaptophysin showed dense presynaptic terminals throughout the nodules and surrounding individual heterotopic neurons (Figure 5b). The borders of the nodules were typically sharply defined by the absence of synaptophysin staining from adjacent white matter (Figure 5c). Whether the synaptic input arises exclusively from within the nodules, or shows a source in the normal cortex as well, is not known. Tracing the source of the synaptophysin-positive innervation of the nodules is not possible anatomically with current technology: DiI has been used in human tissue but never for the large distances necessary to trace from heterotopias to cortex. Determining the relationship of the nodules to epilepsy is an interesting project that may require functional MRI imaging.

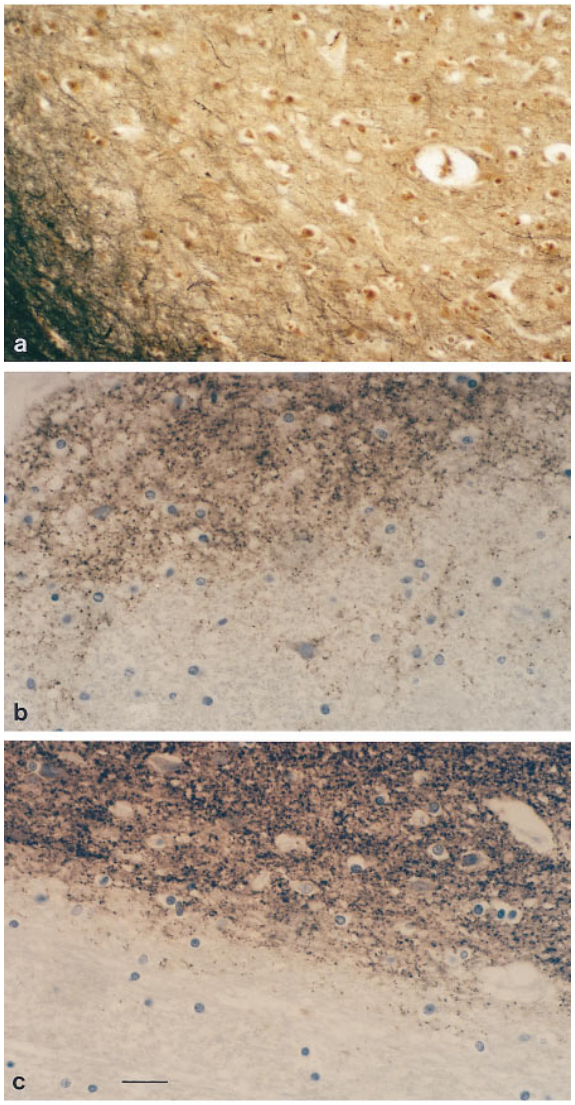

Figure 5. Fiber Staining and Immunohistochemistry of Periventricular Heterotopia

Luyoz myelin stain (a) shows myelinated fibers that course near and through the heterotopic nodules. Immunohistochemistry with an antibody against synaptophysin (b and c) illustrates that the heterotopic neurons are typically outlined by synaptophysin-positive terminals (b). The dense synaptophysin immunoreactivity of the nodules contrasts sharply with the absence of immunoreactivity from the neighboring white matter (c), so that the nodules are sharply outlined. Bar, 100 μm.

Discussion

PH Represents a Novel Epilepsy Susceptibility Locus

PH represents a potentially important epilepsy susceptibility locus, since affected individuals show uncomplicated epilepsy and normal intelligence, and lack other distinctive features that suggest a specific diagnosis. Before the widespread use of MRI analysis, PH pedigrees would be interpreted simply as pedigrees with inherited epilepsy and no other findings. The ease with which PH can be imaged today, however, greatly simplifies its genetic analysis compared with other epilepsy susceptibility genes, because of the simple, objective means for diagnosis of PH. It is unclear whether epilepsy in PH arises from some direct effect of the lesions or is due to abnormalities in signaling systems that are important during both cortical development and adult function. Further insight into the epileptogenesis of the lesions might come from tracing connections between the lesions and normal cortex in appropriately preserved postmortem human material, or by direct analysis of the electrical activity of the nodules using functional MRI.

Consequences of X-Linked Dominant Inheritance of PH

Since PH is a lethal disorder in males, new mutations should be required for PH to be maintained in a population. Distinct haplotypes are linked to PH in the pedigrees illustrated, and there is no evidence that the families are related through previous generations. The Chicago pedigree has been traced by interviews and review of death certificates back to the time of the Revolutionary War in the United States. Epilepsy was not manifested as an observable trait before the generation depicted in Figure 1, suggesting a new mutation that arose relatively recently.

PH joins several known X-linked disorders of human cerebral cortical development. Aicardi syndrome, a developmental disorder of the cerebral cortex that maps to Xp22, is also apparently lethal to males (Aicardi, 1988). Recently, an X-linked form of pachygyria/lissencephaly has been suggested by pedigree studies (Zollino et al.,

1992; Berry-Kravis and Israel, 1994) and by the existence of a lissencephaly patient carrying an X-chromosomal translocation at Xq22 (Dobyns et al., 1992). In X-linked lissencephaly, females generally show mental retardation, while males show very severe mental retardation, seizures, and death usually by 2 years of age. Recent pedigrees have also shown affected males with lissencephaly, while female carriers show a milder migrational disturbance called ''double cortex syndrome'' (Pinard et al., 1994). However, there is little to suggest that PH is allelic to any of these disorders: PH has a distinctive radiographic appearance and shows an extremely mild phenotype in females with a severe, prenatal lethal effect in males. Moreover, linkage of PH to Xq22 can be ruled out.

Incontinentia pigmenti (IP), another X-linked dominant, male-lethal disorder, shows two genetic forms that are clinically indistinguishable, and analysis of large pedigrees has suggested that one form of IP (IP2) is linked to markers in Xq28 (Sefiani et al., 1991). PH has not been clearly described in IP patients, although seizures or mental retardation occur in 30% of IP patients. It is conceivable that IP and PH may be allelic disorders, though careful skin examination showed no skin lesions in affected PH patients.

Xq28 is one of the most gene-rich regions in the human genome, with several human disease genes and cDNAs mapped to this region (see Figure 3). Two forms of fragile X-linked mental retardation that map to Xq28 include *FRAXA* and *FRAXE*. Both *FRAXA* and *FRAXE* appear to map outside of the candidate region for PH (Willard et al., 1994). Another candidate gene of interest within the region for PH is the α3 subunit of the $GABA_A$ receptor (*GABRA3*). GABA receptor mutants may play a role in inherited epilepsy syndromes, and deletion of other GABA receptor subunits is associated with intractable seizures in the neurological mutants of the p mouse (Nakatsu et al., 1993). Another gene that has been implicated in several developmental disorders of the human brain is the L1 cell adhesion molecule (*L1CAM*), and the *L1* gene is within the candidate interval for PH. Mutations in *L1* are associated with X-linked hydrocephalus (Rosenthal et al., 1992), the MASA syndrome (mental retardation, aphasia, shuffling gait, and adducted thumbs; Vits et al., 1994), and X-linked spastic paraparesis (Jouet et al., 1994). Although none of these disorders is known to be associated with PH, *L1* has been implicated in neuronal migration (Lindner et al., 1983), and it will be of great interest to see whether mutations in *L1* underlie PH. The identification of the PH gene should be facilitated by systematic cloning efforts directed at isolating most expressed cDNAs in Xq28 (Bione et al., 1993; Sedlacek et al., 1993).

PH and Cerebral Cortical Development

The strikingly well-developed neuronal nodules in PH brains clearly indicate a major role for the PH gene product in guiding human cerebral cortical development. The PH phenotype could be explained by a failure of migration of cortical neurons, but the hint that different nodules contain different neuronal types indicates some additional mechanism to segregate cortical neuronal types that does not require migration into the laminated cerebral cortex. An alternative model is that the PH gene is actually involved in proliferation of neuronal precursor cells, with loss of PH function resulting in formation of excessive numbers of cortical neurons that are secondarily unable to migrate properly. The apparent segregation of neuronal subtypes within the PH nodules bears intriguing similarity to the clustered cortical clones (Parnavelas et al., 1991; Walsh and Cepko, 1992; Luskin et al., 1993) or subunits of widespread cortical clones (Reid et al., 1995) in rodents, or the horizontal clusters described in primates (Kornack and Rakic, 1995), which often contain multiple cortical neurons of similar type or laminar location. Further understanding of the genesis of the PH malformations will come with identification of the specific PH gene and study of its role in cortical development.

Experimental Procedures

Patients

Families were studied after obtaining informed consent and in accordance with a Human Studies Protocol approved by the Beth Israel Hospital. Skin exam, X-ray studies, renal ultrasound, and cardiac ultrasound were performed in several patients to rule out tuberous sclerosis or other disorders (DiMario et al., 1993; Huttenlocher et al., 1994). Routine MRI or CT scans were used to type unknown members of each pedigree, since both studies visualized PHs well. Peripheral blood samples were taken, and DNA was isolated from leukocytes using Qiagen columns according to the manufacturer's instructions.

Genetic Markers

Most markers consisted of highly polymorphic dinucleotide repeats. The entire X chromosome was screened using markers from Généthon (Weissenbach et al., 1992; Weissenbach, 1993; Donnelly et al., 1994; Gyapay et al., 1994) purchased from Research Genetics. Additional markers were obtained from GDB or from specific publications, and appropriate primers were synthesized. *GABRA3* (Hicks et al., 1991), *DXS1108*, and *DXYS154* (sDF-2 and sDF-1; Freije et al., 1992), p26 (*DXS1177*; Wehnert et al., 1993), and *F8C* (Lalloz et al., 1991) were also analyzed as described. Markers were analyzed after radioactive labeling of one primer with [γ-^{32}P]ATP and T4 polynucleotide kinase (GIBCO–BRL). PCR reactions were run using standard conditions (usually 92°C for 30 s, 55°C for 2 min, 72°C for 1 min for 30 cycles, followed by 72°C for 5 min), or with conditions specific to a few individual primer pairs, on a Hybaid Geneamp thermal cycler. PCR reaction products were separated on urea/formamide polyacrylamide gels (Litt et al., 1993). The gels were then fixed in 20% methanol/10% acetic acid, transferred to filter paper, dried, and autoradiographed in the dark overnight. Allele sizes were determined by running size standards in parallel. Markers for the entire X chromosome were tested initially in the Chicago pedigree. When positive evidence for linkage was obtained to markers in Xq28, additional markers were obtained in this region and tested in all four pedigrees.

Linkage Analysis

Linkage analysis was performed using the LINKAGE package on a VAX computer (Lathrop and Lalouel, 1984, 1988; Terwilliger and Ott, 1994). For initial screening, equal allele frequencies were used. For the calculations illustrated, allele sizes and frequencies were obtained from GDB (Fasman et al., 1994). PH was modeled as a dominant trait with 95% penetrance in females and 100% penetrance in males. Since PH appears to be lethal in males, the mutation frequency was considered to be one-half of the gene frequency, assuming that Hardy–Weinberg equilibrium was achieved. An estimated gene frequency for PH of 2:10,000, and a mutation frequency of 1:10,000 were used.

Immunohistochemistry

Histological analysis of a human postmortem specimen was performed after embedding blocks of tissue in paraffin and sectioning at 10–15 μm. Sections were mounted on glass slides, and the paraffin was removed in xylenes. Sections were then stained with cresyl violet or with the Luyoz myelin stain. Other sections were hydrated and stained in a Ventana 320 automated slide stainer using the following primary antibodies: a rabbit anti-human monoclonal antibody to synaptophysin (DAKO) at 1:75 dilution, a mouse anti-bovine monoclonal antibody to vimentin (Ventana), and a mouse anti-human neurofilament antiserum (Bio Genex Laboratories) at 1:50 dilution. Histochemical detection of primary antibodies was performed using the Ventana Medical Systems DAB detection kit (250-001), which employs a universal biotinylated immunoglobulin (anti-mouse and anti-rabbit antibodies) as a secondary antibody.

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