Periventricular Heterotopia May Result From Radial Glial Fiber Disruption

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Abstract. Periventricular heterotopia (PVH) are collections of neurons and glia heterotopically located adjacent to the ventricles. The pathogenesis of periventricular heterotopia is believed to be a failure of cells to migrate from the ventricular zone. Mutations in filamin-1 (FLN1) have recently been identified as a genetic defect that results in an X-linked dominant form of PVH. In addition to this X-linked form, PVH may be found sporadically or occasionally as part of other syndromes. The pathogenesis(es) of PVH has not been entirely elucidated for patients with or without FLN1 mutation. In an attempt to better understand the pathogenesis of PVH, we examined 5 fetuses (gestational ages 21 to 34 wk), 3 females and 2 males, with PVH. Neuropathologic examination of these 5 fetuses revealed several to multiple periventricular nodules. No case showed the extensive periventricular heterotopia most commonly found in females with FLN1 mutations. By immunohistochemistry, neurofilament-positive cells were identified within the PVH in 3 of 5 cases and glial fibrillary acidic protein-positive cells surrounded the nodules in all 5 cases, but positive cells were only found within the nodules of 3 cases. Surprisingly, small collections of CD68-positive macrophages were found at the base of the nodules in 4 of the 5 cases. Moreover, in all cases, the radial glia highlighted with vimentin, showed disorganization specifically around the nodules. These data suggest that at least one pathogenesis for PVH is a disruption of the radial glial organization, resulting in a failure of cells to migrate from the ventricular zone.

Key Words: Epilepsy; Filamin-1; Neuronal migration; Periventricular heterotopia; Radial glia.

INTRODUCTION

One of the hallmarks of central nervous system (CNS) development is that many cells migrate great distances to their final location in the mature brain. Progenitor cells proliferate in a specialized zone adjacent to the central canal of the neural tube known as the ventricular zone (VZ) and later the subventricular zone (SVZ), a second specialized zone of cell proliferation (1, 2). The 2 daughter cells that result from a cell division with these proliferative zones can either both re-enter the cell cycle, both exit the cell cycle, or one may re-enter the cell cycle and one may exit to G0. Those cells exiting the cell cycle migrate away from the VZ by first associating themselves with a specialized glial cell, known as a radial glial cell, and then use this as a guide to migrate upon (3–5).

A failure to migrate from the VZ or a defect in migration along the radial path of migration results in neuronal migration anomalies (1, 6). This class of anomalies includes the lissencephaly/subcortical band heterotopia/pachygyria spectrum, isolated or multifocal pachygyria, nodular heterotopia, and periventricular heterotopia (PVH). Patients with these anomalies frequently show mental retardation and/or epilepsy (7, 8). Recently the molecular basis for several of these migrational abnormalities has been elucidated (6).

The X-linked dominant inheritance pattern for bilateral PVH (9) is one of the syndromes where the underlying molecular defect has been identified (10). Affected females may have normal intelligence or may be mentally retarded. Epilepsy ranges from mild to intractable. In addition, pleomorphic extra-CNS signs may also exist, particularly involving the vascular system (9, 10). The disorder is most often lethal in males.

Mutations in filamin-1 have been identified as the genetic basis for the X-linked PVH (10). Filamin-1 is a cytoplasmic structural protein that is anchored to cell surface receptor and has actin-binding domain that is likely to links the cell surface and the F-actin cytoskeleton (12–15). In addition, it has been shown that filamin-1 is involved in the formation of filopodia through the Cdc42-mediated pathway (16). Together, these data suggest that PVH due to filamin-1 mutation results in the loss of signal transduction to the cytoskeleton and, potentially, a cell migration-specific defect. Through a presumed random X-inactivation 2 populations of neurons exist; those expressing the mutant allele that in all probability fail to migrate and reside in the periventricular region as heterotopia, and those that express the normal allele and migrate to the correct position in the cortical plate.

The pathogenesis of PVH in some cases can not be explained by FLN1 mutation. PVH may be found sporadically in male and female patients (17) exhibiting other CNS anomalies, including hippocampal abnormalities (18), or as part of other syndromes (19, 20). Neither the molecular or cellular pathogenesis of these other forms of PVH has been defined.

In the present study we examine 5 fetuses with bilateral PVH, 3 females and 2 males, with gestational ages from 34 to 21 wk. We found the radial glial scaffold to be
disrupted specifically around the heterotopic nodules. Moreover, we describe the presence of scattered groups of CD68-positive macrophages surrounding the nodules. We suggest the possibility that disruption of the radial glia could represent a novel pathogenetic mechanism of the failed neuronal migration.

MATERIALS AND METHODS

All cases were found in the pathology files from the Brigham and Women's Hospital, Boston, Massachusetts or the Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, based on the presence of PVH in the final diagnosis. The demographics of the cases used are detailed in the Results section and in Table 1. Gestational age was estimated using the crown-heel, crown-rump, foot length, and fetal weight measurements.

Hematoxylin and eosin (H&E)-stained microscopic sections of brain areas showing PVH were reviewed. No pathologic abnormalities were seen in the brains (e.g. periventricular leukomalacia, hemorrhages, or inflammation) other than those described in this report and in Table 1. Additional sections were immunostained with antibodies to neurofilament (NFP; RMDO20 kindly provided by Dr. V. Lee; 1:10 dilution), vimentin (monoclonal antibody, 1:50 dilution; Dako, Carpinteria, CA), glial fibrillary acidic protein (GFAP; monoclonal antibody, 1:800 dilution; Dako) and CD68 (monoclonal antibody, 1:200 dilution; Dako). Immunohistochemistry was performed using standard methods as previously described and di-amino benzidine as a chromogen (21). Sections incubated with antibodies to NFP and GFAP were microwaved for 10 min in a citrate buffer (pH 6). Sections incubated with the antibody to CD68 were pretreated with 2 mg/ml Pepsin for 10 min prior to the immunohistochemistry. Vimentin staining was done without any form of antigen retrieval. All immunolabeled sections were counter stained with hematoxylin. Images were viewed on a Nikon E400 microscope equipped with a Nikon FDX-35 camera.

RESULTS

Clinical Data

Two cases were from the Brigham and Women's Hospital and 3 from the Children's Hospital of Philadelphia. Information regarding family history, gestational age, sex, and associated abnormalities were collected from clinical and pathological records and are summarized in Table 1. The fetuses ranged in age from 21 to 34 wk and there were 2 males and 3 females. Age- and sex-matched cases without brain or systemic malformations were selected as controls. No family history of females with epilepsy or mental retardation was found for the 3 female fetuses; genetics studies looking for mutations in filamin-1 were not performed.

Pathology

The PVH were present on gross examination of the formalin-fixed brains in all but one case (case 4). Case 4 was a partially disrupted fetal brain, possibly contributing the failure to recognize the PVH in this case. The heterotopia were recognized as small protrusions into the ventricle (Fig. 1). The PVH were bilateral in all cases (recognized microscopically in case 4) and ranged from a few to numerous. They ranged from microscopic to approximately 3 mm in greatest dimension. No cases

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**TABLE 1**

<table>
<thead>
<tr>
<th>Case</th>
<th>Gestational Age</th>
<th>Sex</th>
<th>Other Abnormalities</th>
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<tbody>
<tr>
<td>1</td>
<td>34 wks</td>
<td>F</td>
<td>dysmorphic face, renal agenesis, ambiguous genitalia, and hypoplastic lungs</td>
</tr>
<tr>
<td>2</td>
<td>24 wks</td>
<td>M</td>
<td>myelomeningocele, Chiari II malformation</td>
</tr>
<tr>
<td>3</td>
<td>21 wks</td>
<td>F</td>
<td>hydrocephalus, aqueductal stenosis</td>
</tr>
<tr>
<td>4</td>
<td>22 wks</td>
<td>F</td>
<td>hydrocephalus</td>
</tr>
<tr>
<td>5</td>
<td>21 wks</td>
<td>M</td>
<td>Arhinencephaly; sibling with caudal regression syndrome and holoprosencephaly</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Macroscopic appearance of PVH in a 24-wk-old fetus (case 2). Note the small protrusions into the ventricle from the lateral wall (arrow).
Low-power photomicrograph from the parieto-occipital lobe of a 21-wk fetus with PVH (case 5). The PVH (arrows identify examples) are present as well-defined nodules scalloping the normally smooth ventricular lining. (H&E).

showed the near continuous bands seen in most females with filamin-1 mutations.

The PVH were easily recognized as discrete nodules of small cells at all ages examined. The H&E-stained sections from periventricular areas of the brain clearly demonstrated the nodular collection of cells located adjacent to, and deforming the contour of the lateral ventricular wall, at all ages examined (Fig. 2). The cells were homogenously small with small nuclei and scant cytoplasm and only rare cells labeled with the proliferation marker MIB-1. We next asked if the cells within the nodules had differentiated along glial and/or neuronal lines. In order to answer this question, we performed immunochemistry with a panel of primary antibodies (see Materials and Methods section). The NFP labeled cells within the nodules in 3 of the 5 cases (Fig. 3; Table 2). The reactivity was similar to that observed in the overlying cerebral cortex, implying an age appropriate degree of differentiation (data not shown). GFAP immunoreactivity was seen in cells surrounding the nodules in all cases and within the nodules of 2 cases (Fig. 3; Table 2). Only in our oldest case were the PVH cells negative for both markers. The significance of this finding is unclear and will have to be studied in additional cases of this age and older as they become available.

Several cell types were clearly observed surrounding the PVH. In all 5 cases the PVH were surrounded by GFAP-positive astrocytes (Fig. 3; Table 2). In addition to the astrocytes, a population of cells with the cytological features of macrophages was found in several cases by H&E staining. To further characterize this population of cells, immunohistochemistry was performed with an antibody directed against the CD68 antigen. In 4 of the 5 cases, CD68-positive cells were found adjacent to the nodules (Fig. 4). CD68-positive cells were not identified in significant numbers in regions distant from the PVH or in the periventricular region of age matched control embryos (data not shown). The one case that did not show macrophages around the PVH was the oldest fetus examined (34 wk gestation).

The presence of macrophages at the margins of the periventricular heterotopia makes an etiologic relationship plausible. One hypothesis we generated is that a disruption in the local organization, number, or architecture of the radial glia interferes with the ability of cells to migrate away from the VZ. To study the radial glia we used an antibody to vimentin that labels these cells. In all 5 cases and in the control cases the vimentin immunolabeling highlighted a series of parallel tracks of radially oriented glial cells, which stretched from the ventricular zone to the pial surface (Fig. 5A). In contrast, the radial glial system is in complete disorganization in the periventricular adjacent to the periventricular heterotopia. These areas show various patterns from focal disruption (Fig. 5B) to swirling (Fig. 2C) of the radial glial fibers around the nodules. The disruption of the radial glia was found in all 5 cases; although in one case the findings were quite subtle. Radial glia that were not adjacent to the PVH showed a normal morphology and course in all fetuses.

DISCUSSION

We have studied 5 fetuses (gestational ages 21 to 34 wk) with bilateral PVH. The PVH were present by gross examination in 4 of the 5 cases. Microscopically the cells were similar in all cases, showing a small immature morphology that resembled the cells in the overlying cerebral cortex. Further characterization of the cells within the
Fig. 3. Representative examples of PVH immunolabeled with antibodies to neurofilament protein (NFP) and glial fibrillary acidic protein (GFAP). The cells within the PVH are strongly positive for NFP, however, adjacent cells, including those of the germinal matrix are not labeled. In contrast to the NFP, GFAP shows few cells within the PVH, but strong staining surrounding the nodules.

### TABLE 2

<table>
<thead>
<tr>
<th>Case</th>
<th>NFP in PVH nodules</th>
<th>GFAP in PVH around nodules</th>
<th>GFAP in nodules</th>
<th>CD68 around nodules</th>
<th>Vimentin in radial glia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>disrupted</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>disrupted</td>
</tr>
<tr>
<td>3</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>disrupted</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>? disrupted</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>disrupted</td>
</tr>
</tbody>
</table>

PVH indicated that neuronal and glial lineages are represented. In 2 cases, the cells within the heterotopic nodules were negative for NFP, and in one of those cases the cells were negative for both NFP and GFAP. Several explanations exist for these findings. First, the cells in the nodules remained in an undifferentiated state. A second possibility is that the cells in the nodules did not express the class of neurofilament proteins recognized by the RMDO-20 neurofilament antibody. The RMDO-20 antibody that was used specifically recognizes an unphosphorylated form of the medium (160 kD) and high molecular (200 kD) weight neurofilament proteins, but does not recognize the low molecular weight form (22).

While characterizing the cells within the PVH, we identified a population of cells adjacent to the PVH. Evaluation of this population of cells indicated that they are macrophages. Similar collections of macrophages were not seen within other regions or outside the germinal zone. These data suggested that the presence of the macrophages might be pathologically linked. Given that the macrophages are frequently associated with necrosis or degeneration, we hypothesized that microscopic foci of cellular degeneration or necrosis could underlie the pathogenesis of the PVH. One plausible etiologic explanation for this hypothesis is the loss or derangement of radial glia, thus removing the cellular guidance for radial cell migration from the VZ. In order to test this hypothesis, we evaluated the radial glial architecture using an antibody to vimentin that labels the radial glial fibers. Our
data document a qualitative derangement of the radial glial processes. We were not able to determine if a quantitative difference in the number of radial glia existed in the region of the PVH, however, the radial fibers were clearly disrupted and occasionally showed anomalous projection patterns. These data support a disruption of the radial glia as a possible pathogenetic mechanism underlying PVH.

Another possible explanation of this data is that the radial glial cells are not primarily disrupted by some injury, rather, the radial glial are secondary displaced by the heterotopic nodules. Although this cannot be excluded, the one case showing swirling of vimentin-positive fibers around the nodules (Fig. 2C) would seem inconsistent with this hypothesis. Another possibility is a direct radial glia defect resulting from a mutation in FLN1. Given that the mechanism by which mutations in filamin-1 disrupt the ability of cells to migrate out of the ventricular zone awaits further functional characterization of this protein, a direct influence on radial glia cannot be excluded at this time.

We believe that none of our 5 cases is due to filamin-1 mutations. First, filamin-1 mutations appear to be embryonically lethal in males (11), and 2 of our cases are males, ages 21 and 24 wk, respectively. Neither one presented with vascular abnormalities or the fatal bleeding disorder that has been described in a single live-born male with a FLN1 mutation (11). One of our male cases presented with myelomeningocele and Chiari II malformation and the other with unilateral arhinencephaly and with a family history of a sibling with caudal regression and holoprosencephaly. The possibility, in particular for the latter case, being a familial form with abnormalities of chromosome Xq28, cannot be completely excluded. It seems unlikely, however, that these 2 cases represent one of the syndromes described by Dobyns and Fink (23–25). In those cases, males may have bilateral periventricular nodular heterotopia, mental retardation, epilepsy, syndactyly, cerebellar hypoplasia, possible cortical dysplasia, cataracts, hypospadias, and at least in some cases, abnormalities of chromosome Xq28.

The 3 female cases also do not appear to fit the spectrum of females with filamin-1 mutations (10, 11). One female (case 1) is a 34-wk-old female with dysmorphic face, ambiguous genitalia, hypoplastic lungs, but no other CNS abnormalities—features not commonly associated with FLN1 mutations. Cases 3 and 4 have hydrocephalus, a feature also not associated with FLN1 mutations. None of our female cases have cerebellar anomalies, defects of the corpus callosum, vascular system abnormalities, or a family history of epilepsy. Nonetheless, in the absence of genetic studies, we cannot completely exclude the possibility that one or several of our cases represent new mutations in the filamin-1 gene.
Fig. 5. Vimentin immunolabeling highlights the radial glial architecture. A: Radial glia in a 21-wk fetus without pathological anomalies. The radial glial processes form delicate, parallel arrays extending from the VZ (bottom) to the cortical plate (top, not in view). B, C: The radial glial architecture is disrupted in the VZ around the PVH. In (B) the vimentin antibody labels a disorganized set of processes radiating around the heterotopic nodule and no parallel processes moving radially out from the VZ (to the left in the image). Some of the immunolabeled processes may represent astrocytes; however, the normal radial organization is clearly disrupted. A disruption in parallel radial processes is also noted in (C). PVH to the right, radial processes should be extending to the left.

We suggest that one possible mechanism for the disorganization of the radial glial fascicles is a localized injury to the radial glial cells. Our data, showing focal disruption and swirling, mainly around the nodules, with groups of macrophages would support this hypothesis. We do not know the nature of the injury to the radial glia, although a vascular or toxic insult is possible. Support for this hypothesis comes from data in rats where it is possible to induce brain abnormalities, including heterotopia, along the border of the lateral ventricles after prenatal injection of a toxic substance, such as methylazoxymethanol (MAM), (26, 27). On the basis of the immunohistochemical profiles, at least
some cells in the heterotopic nodules of these rats were hypothesized to be destined for the cerebral cortex (27). This observation is in agreement with our data that many cells in the periventricular nodules are NFP-positive, with reactivity similar to that of the overlying cortex. Others have also suggested that periventricular nodules might result from disruption of radial glial scaffolding and consequent formation of rosettes of radial glia (28, 29). However, our data cannot prove incontrovertibly that a local injury disrupts the radial glia, it remains possible that the groups of macrophages are a response and not the cause.

PVH is a relatively uncommon form of cerebral dysgenesis that is frequently associated with seizures (9, 17, 30, 31). It can be familial (9, 11) or sporadic, isolated or associated with other developmental defects of the brain (32), or syndromes including hydrocephalus, agenesis of the corpus callosum, micropolygyria, megalencephaly, cerebellar hypoplasia, various forms of agyria/pachgyria, encephalohole, Aicardi syndrome, Chiari II malformation, aplasia cutis congenital, and 17p13.3 syndrome (20, 26). Even though PVH is present in so many and different conditions, the molecular basis of the pathogenetic mechanism has been identified only for the X-linked form associated to FLN1 mutations (10). Our data raise the possibility that at least one other mechanism for the development of PVH is a disruptive process that occurs focally along the ventricles and directly affects the radial glia.

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Received March 28, 2001
Revision received May 29, 2001
Accepted June 1, 2001