Focal cortical dysplasia (FCD) is a developmental malformation of the human cerebral cortex that is closely associated with epilepsy. Dysplastic, ‘balloon’ and heterotopic neurons that comprise FCD are heterogeneous cell populations that exhibit abnormal morphologies. A pivotal unanswered question is how these cell types are generated during cortical development. As a strategy to define the lineage relationships between cells in FCD, the size of a heterozygous trinucleotide (CAG) repeat sequence within the X-chromosome encoded androgen receptor (XAR) mRNA was determined by RT-PCR and direct sequencing in single, microdissected cells from six female patients with FCD compared with control cortex. As a consequence of X-chromosome inactivation, only one of the two XAR alleles is expressed in all somatic cells, and cell types derived from distinct progenitors will express XAR CAG repeat lengths of differing size. Disparate XAR CAG repeat lengths were detected with equal frequency in single dysplastic, ‘balloon’ and heterotopic neurons in FCD whereas the XAR CAG repeats lengths in control cortex were identical in 70–80% of closely apposed neurons. These results support a random X-inactivation pattern in FCD. We propose that dysplastic, ‘balloon’ and heterotopic neurons in FCD derive from a population of progenitor cells or post-mitotic neurons during cortical development.

Introduction

Focal cortical dysplasia (FCD) (Taylor et al., 1971) is a developmental brain malformation that is closely associated with medically intractable epilepsy (Palmini et al., 1995; Whiting and Duchowny, 1999). FCD is defined histologically by disorganized cortical lamination and the presence of dysplastic neurons (DNs) and ‘balloon’ neurons (BNs) as well as heterotopic neurons (HNs) in the subcortical white matter (Mischel et al., 1995; Andermann, 2000). The abnormal morphological features of DNs, BNs and HNs include cytomegaly, with BNs exhibiting the largest cell soma, extension of aberrant dendritic arbors, and loss of radial orientation within the cortex in relation to the pial surface. The histological features of FCD suggest developmental abnormalities affecting select steps during neural proliferation, differentiation or migration (Crino and Eberwine, 1997). Defining the molecular etiology of FCD has been problematic because FCD appear to be a sporadic disorder and their FCD family pedigrees have not been identified. Furthermore, monozygotic twins are discordant for FCD (Senel et al., 2000; Briellmann et al., 2001) and thus FCD may instead arise through non-inherited and potentially multifactorial mechanisms.

A pivotal question in defining the pathogenesis of FCD is how DNs, BNs and HNs are derived from progenitor cells during cortical development? One hypothesis is that DNs, BNs and HNs are derived from a single abnormal neural progenitor cell that has sustained a somatic gene mutation. Several candidate genes have been identified in animal strains and other human malformations that could cause FCD (Walsh, 1999). In this scenario, FCD would be comprised of a clonally derived yet morphologically heterogeneous population of cells. A second hypothesis is that only single cell types within FCD, i.e. DNs, BNs or HNs, are the progeny of the progenitor cell that has sustained a gene mutation and thus FCD would in effect be a mixed lineage lesion consisting of clonally and non-clonally derived cell types. A third hypothesis is that DNs, BNs and HNs are derived from multiple progenitor cells whose proliferative regulation, migratory pathways or lamination cues have been disrupted. For example, deleterious effects of hypoxia, viral infection, a toxin or traumatic brain injury in utero or the neonatal period (Rakic, 1975; Sarnat, 1992; Lombroso, 2000) could damage multiple neural progenitor cells, multiple post-mitotic neurons en route to the cortical plate, or even radial glial cells that guide migrating neurons and result in disorganized cortical cytoarchitecture. Clearly, defining the lineage of DNs, BNs and HNs as clonally or non-clonally derived cell populations would provide a key insight into the mechanism of FCD formation during brain development.

The X-androgen receptor (XAR) inactivation assay (Mutter and Boynont, 1995; Grant and Chapman, 1998) using genomic DNA extracted from tissue specimens can be used to define cellular clonality in tissue samples. This method takes advantage of the highly heterozygous and polymorphic CAG repeat sequence (ranging from 10 to 30 repeats) present within the X-chromosomes of female patients. As a consequence of random X-chromosome inactivation, one XAR allele is transcriptionally silenced during embryogenesis and all progeny of a single precursor cell will contain the same active and inactive X chromosomes. Thus, only one of two XAR CAG repeat lengths will be expressed in each female somatic cell and any two cells derived from distinct precursor cells will contain XAR CAG repeats of disparate size. Clonal growth patterns have been identified in human tumors by demonstrating skewed, i.e. non-random, inactivation patterns of one XAR allele across multiple cells. Polyclonal tissues such as the brain have both XAR alleles approximately equally, i.e. randomly, represented (Mashal et al., 1993; Zhu et al., 1995). X-chromosome inactivation patterns in human brain have not been directly analyzed. However, it is likely that X-chromosome inactivation in human females is completed at least by 5 weeks post-conception (Migeon and Kennedy, 1975; Kattar et al., 1997; Ray et al., 1997; Taylor et al., 2001) and may be initiated at the 10–20 cell stage of embryonic development (Monteiro et al., 1998; Uehara et al., 2000), well before formation of the telencephalic ventricular zone (Sidman and Rakic, 1973). X-inactivation patterns have been used to define clonality of cortical neurons in the mouse (Tan et al., 1995) and can provide insights into developmental lineage relationships between human cortical neurons.

There are technical limitations to the XAR inactivation assay using genomic DNA from human tissue sections (Grant and Yue Hua and Peter B. Crino

Department of Neurology and PENN Epilepsy Center, University of Pennsylvania Medical Center, Philadelphia, PA 19104, USA

Cerebral Cortex Jun 2003;13:693–699; 1047–3211/03/$4.00 © Oxford University Press 2003. All rights reserved.
Chapman, 1998) and defining the XAR CAG repeat sizes in whole FCD sections does not provide a methodology to definitively determine the lineage relationships between individual cell types. Thus, we defined the CAG repeat length in XAR mRNA amplified from single microdissected DNs, BNs and HNs in human FCD specimens. Amplification of mRNA from single cells in fixed tissue specimens (Crino et al., 1996) provides a method to generate sufficient template concentration for analysis of the XAR CAG repeat length by RT-PCR and to assess lineage relationships between individual cells in FCD.

**Patients and Methods**

**Human Brain Tissue Specimens**

Six FCD specimens (five temporal lobe, one occipital lobe) were resected from female patients (ages 19–56 years) for the treatment of complex partial epilepsy. Two temporal neocortical specimens exhibiting normal cytoarchitecture were obtained post-mortem from patients (one male age 33 years and one female age 41 years) without seizures or FCD. A single brain tumor specimen was obtained from a female (age 37 years) with a pathologically defined glioblastoma. The use of surgically resected or post-mortem tissue was in accordance with protocols approved by the University of Pennsylvania Institutional Review Board and Committee on Human Research.

**Immunohistochemistry**

Fixed, paraffin embedded FCD and control sections (7 μm) were immunolabeled with anti-MAP2B antibody (courtesy J. Trojanowski, University of Pennsylvania, Philadelphia, PA) overnight at 4°C. Immunolabeling was visualized using the avidin–biotin conjugation method (Vectastain ABC Elite, Vector Labs, Burlingame, CA) and 3,5-diaminobenzidine.

**In Situ Transcription**

Following immunolabeling, tissue sections were treated with Proteinase K (50 μg/ml) at 37°C for 30 min. Two distinct 3′-primers and a specific XAR primer: 5′-CTCTACAGTTGCGCTTTGGGAGAAC-3′ each coupled to a T7 RNA polymerase promotor were annealed to cellular poly(A) mRNA overnight. cDNA was generated from tissue poly(A) mRNA via in situ reverse transcription (IST) directly on the tissue section in IST reaction buffer (10 mM HEPES buffer pH 7.4, 120 mM KCl, 1 mM MgCl₂, 250 μM dATP, dCTP, dGTP, dTTP) with avian myeloblastosis reverse transcriptase (AMVRT; 0.5 U/μl). cDNA was extracted from the tissue section with 0.2 N NaOH/0.1% SDS, 5 M KAc, phenol:chloroform (1/1 v/v), and ethanol precipitation (Kacharmina et al., 1999). cDNA served as template for mRNA amplification or for RT-PCR to assess the XAR CAG repeat length (see below).

**Microdissection of Single Immunolabeled Cells**

Single MAP2B immunolabeled DN, BNs and HNs in FCD, or pyramidal neurons in control cortex were characterized morphologically using computer-assisted image acquisition and analysis software (Phase 3 Imaging System/ImageProPlus; Media Cybernetics, Silver Spring, MD). The diameter of the BNs exceeded 90 μm and these cells exhibited a laterally displaced nucleus and minimal polarization into dendritic segments (absence of definitive MAP2 immunoreactive dendritic arbors). DN were smaller (50–80 μm) and exhibited MAP2 immunoreactive dendrites that were characteristic of neurons. HNs exhibited similar features as DN but were defined by their location (at least 300 μm) within the subcortical white matter. Layer III pyramidal neurons were identified by their characteristic morphology in control cortex. Single DN, BNs, HNs or pyramidal neurons were microdissected from FCD or control specimens using a stainless steel microscalpel and joystick micromanipulator. Retrovirial tagging experiments in rodent and primate cortex have demonstrated that neurons separated by greater distances are more likely to derive from distinct populations of progenitor cells (Kornack and Rakic, 1995; Reid et al., 1995). Thus, single cells were dissected from a defined region of FCD or control cortex in attempt to provide the most representative view of the cell lineage mixture.

A representative 0.4 mm² sector of layer III was selected in control cortex, and eight pairs (n = 16 neurons) of directly adjacent pyramidal neurons were microdissected from layer III. In FCD specimens, cells (n = 90 total neurons) were microdissected from a representative 0.4 mm² sector from sections in each case that contained DN, BNs and HNs in close anatomic proximity. Single cells were aspirated into a plastic microelectrode and then were transferred to a microfuge tube where cDNA synthesis was initiated with the oligo-dT-24 primer, a specific XAR primer (5′-CTCTACAGTTGCGCTTTGGGAGAAC-3′) both coupled to a T7 RNA polymerase promotor and AMVRT (0.5 U/μl).

**mRNA Amplification**

Double-stranded template cDNA was generated with T4 DNA polymerase I (Boehringer-Mannheim) from cDNA made in whole sections or single cells (Crino et al., 1996; Kacharmina et al., 1999). mRNA was amplified (aRNA) from the double-stranded cDNA template with T7 RNA polymerase (Epitcience Technologies, Madison, WI). aRNA served as a template for a second round of cDNA synthesis with AMVRT, dNTPs and N(6) random hexamers (Boehringer-Mannheim). cDNA generated from aRNA was made double stranded and served as template for a second aRNA amplification. Radiolabeled aRNA (incorporating 32P[CTP]) was used to probe cDNA arrays.

**cDNA Array Analysis**

Linearized plasmid cDNAs encoding XAR, α-internexin (dINX), glial fibrillary acidic protein (GFAP), and neurofilament (low molecular weight isoform, NFL) were adhered to nylon membranes (cDNA slot arrays) via UV cross-linking. pBlueScript plasmid cDNA was included to define background levels of hybridization. Blots were hybridized as described previously (Crino et al., 1996). Blots were washed in 2× SSC and apposed to phosphorimaging screen cassette for 24–48 h to generate an autoradiograph.

**XAR PCR**

Specific XAR CAG repeat flanking primers (5′-AGGCTGTGAACCTC- TCGAGGC-3′; 5′-GCCTGGAAGTGTGGTTCTC-3′; 5′-CCAGAATCTGT- TCCAGACTGTTAGG-3′; 5′-CTCTACAGTTGCGCTTTGGGAGAAC-3′) –250 bp apart were used. PCR amplification conditions were 93°C for 1 min, 55°C for 30 s, and 72°C for 1 min for 35 cycles. A single extension step at 72°C for 5 min was used. 32P ATP was used to end-radiolabel the primer. PCR products were assessed on agarose or polyacrylamide gels. The XAR CAG repeat size was confirmed by direct sequencing (ABI Prism). Statistical comparisons of XAR CAG lengths between control and FCD cases were determined by Chi-squared analysis (StatView 5.0).

**Results**

The expression of XAR mRNA was equivalent in the FCD and control sections (Fig. 1) as determined by probing the cDNA arrays with 32P-radiolabeled aRNA amplified from whole tissue sections. Hybridization of aRNA from FCD or control sections to α-internexin, NFL and GFAP cDNAs confirmed the fidelity of aRNA amplification from cells in the whole sections.

Two XAR CAG repeat sequence lengths were identified in each whole FCD or control tissue section for each patient by RT-PCR using primer pairs flanking the XAR CAG repeat sequence. These experiments defined the size of the XAR CAG repeats represented in the section that we could expect to identify from the single cells (below). Two XAR CAG repeat lengths of differing size were amplified from each of the six female FCD specimens (Table 1) and the one female control specimen and were confirmed by direct sequencing (Fig. 2). The XAR CAG repeat lengths differed across the six FCD cases ranging from 15 to 24 repeats (45–72 bp), and were within the normal range for the XAR CAG (10–30 repeats). The repeat lengths detected in the normal female control cortex were CAG 17 and CAG 23. The detection of two XAR CAG repeat sizes in control female cortex supported previous work demonstrating...
that the population of mature neurons in any cortical region derives from progenitor cells of mixed, i.e. non-clonal, lineage (Tan et al., 1995; Reid et al., 1995). The identification of two XAR CAG repeat lengths in whole FCD sections supported the hypothesis that the cellular constituents of FCD (BNs, DNs and HNs) are likely derived from multiple progenitor cells.

The RT-PCR assay was verified in two additional experiments. As expected, only a single XAR CAG repeat length was amplified by RT-PCR from the male control cortex section (CAG 18 repeats; Fig. 2). A single XAR CAG repeat length sequence was amplified from a brain tumor specimen (glioma) obtained from a female patient (CAG 15 repeats; not shown) consistent with prior XAR gene inactivation studies demonstrating the clonality of glial tumors (Zhu et al., 1995). These results demonstrated that spurious amplification of non-XAR CAG sequences did not contaminate the results.

Next, the XAR CAG repeat lengths were defined in single microdissected DNs, HNs and control neurons to directly assay the X-inactivation patterns in distinct cell types. Hybridization to α-internexin and NFL but not GFAP cDNAs

<table>
<thead>
<tr>
<th>Age</th>
<th>Location</th>
<th>CAG repeat size</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>temporal</td>
<td>CAG 19</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAG 22</td>
<td>47</td>
</tr>
<tr>
<td>22</td>
<td>temporal</td>
<td>CAG 19</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAG 24</td>
<td>47</td>
</tr>
<tr>
<td>31</td>
<td>occipital</td>
<td>CAG 19</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAG 20</td>
<td>48</td>
</tr>
<tr>
<td>37</td>
<td>temporal</td>
<td>CAG 15</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAG 18</td>
<td>46</td>
</tr>
<tr>
<td>39</td>
<td>temporal</td>
<td>CAG 22</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAG 24</td>
<td>58</td>
</tr>
<tr>
<td>56</td>
<td>temporal</td>
<td>CAG 19</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAG 20</td>
<td>54</td>
</tr>
</tbody>
</table>

Numbers reflect percentage of microdissected cells (DNs, HNs and BNs combined from each case) expressing repeat sizes detected in each case.
confirmed the neuronal phenotype of the microdissected control neurons (see Fig. 3). RT-PCR amplification of XAR CAG repeats from DNs, BNs, HNs or control neurons yielded amplicons that matched one of the two XAR CAG repeat sequences identified in whole sections from that case. Only one XAR CAG repeat length was detected in each dissected cell and the XAR CAG repeat lengths were confirmed by direct sequencing. The percentage of directly adjacent cells that exhibited XAR CAG repeat lengths of identical size was used as an index of the lineage relationship between anatomically proximate cells in control cortex and FCD. The XAR CAG repeat lengths determined in directly adjacent control pyramidal neurons (Fig. 3) were identical and skewed to one repeat size (CAG 23) in seven out of eight neuron pairs (14/16 neurons or 87.5% of cells) whereas the XAR CAG repeat length disparate (CAG 17, CAG 23) in only one cell pair (Fig. 3). In contrast, no definitive XAR CAG repeat length distribution pattern could be discerned for directly adjacent DNs, BNs or HNs in the restricted sector of the FCD (Table 1). Both XAR CAG repeat lengths were equally represented (40–60% of cells expressing either repeat size) among selected DNs, BNs and HNs in each of the six FCD cases. Chi-squared analysis of XAR CAG repeat lengths demonstrated that the differences in XAR CAG repeat sizes between the control cortex, individual FCD specimens, and all FCD cases together were statistically significant (P < 0.025). For example, in one FCD case where the identified repeat sizes in the whole section were CAG 19 and CAG 22, among five microdissected DN, three DN expressed CAG 19 and two DN expressed CAG 22 (Fig. 4). In another FCD case, two XAR CAG repeat sizes (CAG 18 and CAG 20) were represented equally in five adjacent DNs and five HNs, i.e. two DNs and three HNs with CAG 18 and three DNs and two HNs with CAG 20 (Fig. 5A). Among 10 DNs, six expressed CAG 19 and four expressed CAG 20 (Fig. 5B) and among nine DNs, four DNs were CAG 19 and five DNs were CAG 24 (Fig. 5C). Two BNs present in this region each expressed a distinct repeat length. Among six DNs, four HNs, and five BNs in another FCD specimen, there was equal representation of CAG 15 and CAG 18 in each cell group (not shown). In one case the distribution of CAG repeat lengths was particularly intriguing (Fig. 5D); among five BNS, one BN expressed CAG 22 and four BNS expressed CAG 24. Thus, the detection of a disparate CAG repeat length in a single BN among a cluster of cells of similar phenotype supported a distinct lineage for this cell despite the fact that it shared a virtually identical morphology with the adjacent cells. Overall, the detection of different XAR CAG repeat lengths in

![Figure 3. XAR CAG repeat sizes in single microdissected control neurons. Top, Single MAP2 immunolabeled pyramidal neurons in layer III selected for microdissection. Numbers depict 16 representative cells (eight cell pairs) selected for PCR amplification of XAR CAG repeat. Middle, PCR amplicons from each cell pair. Note similar CAG 23 repeat size in seven cell pairs (1–14). Despite close anatomic proximity, XAR CAG repeats of disparate size were identified in neurons 15 and 16 (arrow). Bottom, expression of neural mRNAs αINX and NFL but not GFAP mRNA, in dissected neurons. PBS, pBlueScript control plasmid.](image-url)

![Figure 4. Single cell microdissection and PCR amplification in FCD. Top, Cluster of DNs in FCD (labeled 1–5). Middle, Representative microdissection of DNs 1–3 (box). Bottom, PCR products (incorporating 32PdCTP) in lanes 1–5 (6% polyacrylamide denaturing gel) demonstrate disparate CAG repeat sizes in DNs 1–5 depicted in panel A (lanes/cells 1, 3, 4 express CAG 19, lanes/cells 2 and 5 express CAG 22).](image-url)
each FCD specimen and across all cases combined, suggested a random X-inactivation profile that was not skewed towards one particular cell type or repeat size.

**Discussion**

Insights into how FCDs are formed during brain development may yield new therapies for seizures associated with FCD and may even suggest treatment strategies to prevent the formation of FCD during embryogenesis. The ability to identify different XAR CAG lengths in single human neurons provides a methodology to assess the lineage relationships among phenotypically defined single cells. Our results demonstrate that FCD exhibit a random pattern of X-chromosome inactivation and support the hypothesis that DNs, BNs and HNs are derived from multiple progenitor cells or multiple post-mitotic neurons during brain development.

The clonal lineage and boundary distribution of neurons in mature mouse cerebral cortex has been defined as a function of X-chromosome inactivation (Tan et al., 1995). A lacZ transgene was inserted into the X chromosome and the ensuing random X-inactivation pattern in female mice yielded blue or white columns or ‘stripes’ in cortex that defined clonal and non-clonal cell population boundaries. Approximately 70% of cortical neurons within each cortical stripe exhibited similar lacZ transgene expression demonstrating that the majority of neurons within a restricted cortical region exhibit clonal (skewed or non-random) X-inactivation patterns and were clonal progeny derived from a common progenitor cell. In contrast, 30% of the neurons within each cortical stripe exhibited a disparate color reaction suggesting that these cells were derived from a clonally distinct population. Clonally derived cell clusters consisting of both post-mitotic neurons and radial glial cells remain closely apposed during dynamic phases of brain development (Noctor et al., 2001) and retroviral tagging assays in rodent and primate cortex have demonstrated that clonally derived neurons tend to migrate and ultimately reside in close anatomical proximity within mature cortex (Kornack and Rakic, 1995; Reid et al., 1995).

The detection of two XAR CAG repeat lengths in whole control or FCD sections indicated that the cellular constituents of both tissue types were derived from non-clonal progenitor cell populations during development since both XAR CAG repeat

---

**Figure 5.** Distribution of XAR CAG repeats in four FCD cases. Numbers depict XAR CAG repeat size in single cell. (A) HNs are red, DNs are black. Note equal expression of both XAR CAG repeat sizes among DNs and HNs. (B) Differential expression of XAR CAG repeats in DNs (black) and two cells morphologically defined as BNs (blue). (C) Among nine DNs, equal representation of both XAR CAG repeats identified in this specimen (CAG 19 and CAG 24). (D) Five BNs of similar morphology in which one of five XAR CAG repeat sizes are disparate. Inset, lane 1 shows size of XAR CAG repeats in tissue section. Similar XAR CAG repeat lengths (lanes 2–5) while lane 6 demonstrates disparate XAR CAG repeat size (8% polyacrylamide denaturing gel).
lengths were represented by the cells within these tissue types. Interestingly, closely apposed neurons in layer III of control cortex exhibited XAR CAG repeat lengths of identical size consistent with skewed pattern of X-inactivation and cellular clonality. Only one pair of assayed control neurons exhibited disparate XAR CAG repeat lengths, suggesting a non-clonal lineage for these two cells. In contrast, among six FCD specimens, the distribution of XAR CAG repeat lengths in single DNs, BNs and HNs was essentially random, even in cells that were clustered in direct contact or that exhibited similar morphologic features, suggesting a non-clonal lineage for these cell types. When compared with cell lineage boundaries defined by XAR CAG repeat expression in control cortex, FCD represents a restricted cortical region in which the organized pattern of non-random X-inactivation has been disrupted.

There are several important considerations regarding lineage analysis in human FCD specimens. First, a particular cell type in FCD, rather than all cells present in the FCD, may be selectively derived from a single progenitor cell. For example, a gene mutation occurring in a single progenitor cell could yield cells of a particular phenotype, e.g. BNs, that represent the truly ‘dysplastic’ cells within FCD. The surrounding cell types, e.g. DNs or HNs, may be merely ‘innocent bystanders’ whose ability to migrate or achieve laminar destination has been disrupted. In this case, we would have expected to identify a single XAR CAG repeat length within a subset of cells in each FCD case suggesting a skewed (non-random) pattern of X-inactivation. However, a skewed pattern of XAR CAG repeat expression was not identified in any of the morphological cell types in FCD and in fact, DNs, BNs and HNs categorically exhibited a random pattern of XAR CAG repeat sizes suggestive of a non-clonal lineage. Second, a pivotal issue in experimental analysis of FCD is the accuracy of phenotypic distinctions between DNs, BNs and HNs since the classification scheme for DNs, BNs and HNs in FCD is predicated solely upon morphological features and a definitive phenotypic marker for these cell types has not yet been defined. Thus, a large DN and a more diminutive BN may share certain morphologic features that could result in selection or classification error. Similarly, precise delineation of a large cell located in the subcortical white matter as a BN or HN can be a matter of debate. Thus, identification of a selective cellular marker in the future may aid in lineage analysis. However, differences in mRNA and protein expression have been defined in DNs, BNs and HNs categorized on the basis of morphological features (Garbelli et al., 1999; Crino et al., 2001) and in the present study we implemented rigorous morphologic criteria to distinguish DNs, BNs and HNs. Thus, the possibility that a single cell subtype is clonally derived within FCD cannot be completely discounted. Clearly, however, the population of cell types within FCD is quite heterogeneous and appears to be distinct from most normal cortical neurons. The detection of disparate XAR CAG repeat lengths in DNs, BNs and HNs supports the hypothesis that FCD reflects a mixed cell population derived from multiple progenitor cells.

**How Might FCD Be Formed?**

Why then, do directly adjacent dysplastic, ‘balloon’ and heterotopic neurons exhibit random X-inactivation patterns and how do these results provide insight into the pathogenesis of FCD? While the inciting event responsible for FCD remains to be defined, candidate external agents including hypoxia–ischemia, intrauterine infection, nutritional deficit or environmental toxins such as cocaine or ethanol have been proposed (Baraban et al., 1999; Andermann, 2000) which would impact on the developmental programs of multiple progenitor cells in the brain. In one clinical series, 32% of patients with cortical malformations had identifiable pre- or perinatal risk factors including birth trauma, asphyxia, infection or drug exposure (Raymond et al., 1995) and another study has suggested that early postnatal traumatic brain injury can induce FCD (Lombroso, 2000). A recent study has suggested that polymorphisms in the TSC1 (tuberous sclerosis complex) gene is associated with some FCD cases, perhaps providing a genetic backdrop for environmental events to alter cortical lamination (Becker et al., 2002). Thus, prenatal or in utero effects resulting on a population of progenitor cells may lead to the cytoarchitectural features of FCD. For example, gene mutations or altered gene expression induced by environmental events in multiple progenitor cells could alter the structural integrity of nascent neurons leading to the heterogeneous and aberrant cell morphologies characteristic of DNs, BNs and HNs. As a consequence, these cells may lose the ability to migrate appropriately to their laminar destination. This possibility is supported by the effects of the toxin methylazoxymethanol on developing rodent brain that induces cellular dysmorphology and laminar disorganization likely through direct mutagenic effects on multiple progenitor cells (Colacitti et al., 1999). Alteration of guidance or trophic signals necessary for neural migration affecting a population of migrating neurons could result in a focal region of cortical laminar disorganization consisting of mixed lineage cells as has been demonstrated in the fetal rodent brain following exposure to neurtrophin-4 (Brunstrom et al., 1997). One compelling theoretical consideration is that guidance cues provided by radial glial cells or even the structural integrity of radial glial fibers themselves may be disrupted in FCD so that radially migrating neurons ‘lose their way’ as they move into the cortical plate. Support for this hypothesis has been marshaled in nodular heterotopia (Santi and Golden, 2001). Disruption of radial glial guidance of migrating neurons as a primary pathogenic mechanism in FCD would be compatible with the observations of disparate XAR CAG repeat lengths in DNs, BNs and HNs since it is very likely that altered function of multiple radial glial cells would impact the migrational pathways of multiple neurons with non-clonal lineage relationships.

Studying the lineage relationships between DNs, BNs and HNs provides insights into the developmental pathogenesis of human FCD and raises several important questions regarding the mechanisms responsible for abnormal cortical cytoarchitecture. A rigorous investigation of prenatal or early post-natal events in FCD patients may identify potential risk factors or causative agents for FCD. In addition, a gene mutation or susceptibility loci for FCD may yet be identified. These studies may help initiate the first steps towards designing new therapeutic strategies to prevent the formation of these highly epileptogenic brain lesions in utero and to treat intractable epilepsy associated with FCD.

**Notes**

This work was supported by funding to P.B.C. from MH01658, NS39938, the Esther A. and Joseph Klingenstein Fund, and Parents Against Childhood Epilepsy (PACE). The authors thank J. Eberwine, D. Kolson and S. Scherer.

Address correspondence to Peter B. Crino, PENN Epilepsy Center and Department of Neurology, University of Pennsylvania Medical Center, 3 West Gates Bldg, 3400 Spruce St., Philadelphia, PA 19104, USA. Email: crinop@mail.med.upenn.edu.

**References**
