Distinct Allelic Variants of \textit{TSC1} and \textit{TSC2} in Epilepsy-Associated Cortical Malformations Without Balloon Cells

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Abstract

Epilepsy-associated malformations of cortical development (MCDs) comprise a variety of dysplastic and neoplastic lesions of yet undetermined molecular pathology. Histopathologic similarities between MCDs and dysplastic brain lesions in the autosomal inherited neocutaneous phacomatosis tuberous sclerosis (TSC), which affects the \textit{TSC1} and/or \textit{TSC2} genes, suggest common pathogenetic mechanisms. Previous studies revealed different alterations of \textit{TSC1} and \textit{TSC2} in epilepsy-associated malformations and glio-neuronal tumors despite histopathologic similarities. In order to examine current clinico-pathologic classification systems of cortical malformations on the molecular level, we carried out a mutational analysis of \textit{TSC1} and \textit{TSC2} in a series of surgical specimens obtained from patients with FCD without Taylor type balloon cells (FCDIIa; n = 20), architectural dysplasias (FCDI; n = 15), nodular cortical heterotopias (NCH; n = 4), and heterotopic white matter neurons (WMNH; n = 19). In FCDIIa, abundant genomic polymorphisms were detected in \textit{TSC2} (intron 4) but no allelic variants observed in exon 17 of \textit{TSC1}. This allelic distribution pattern is in contrast to findings in FCDI and WMNH but also to those previously reported in FCDIIb (Taylor's balloon cell type). The latter revealed increased frequencies of specific alleles only in \textit{TSC1}. The determination of characteristic molecular genetic alterations in specific epilepsy-associated malformations will support a comprehensive clinico-pathologic classification system and help to identify molecular pathways with potential pathogenetic relevance.

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Key Words: Epilepsy, Focal cortical dysplasia, Glio-neuronal lesion, Neoplastic transformation, Tuberous sclerosis.

INTRODUCTION

Circumscribed malformative lesions of the central nervous system are frequently associated with drug-refractory epilepsies and comprise a wide spectrum of neuroradiologic and histomorphologic alterations (1–6). Epilepsy surgery has become an important treatment option in many of these patients and access to surgical brain specimens allows a systematic characterization of the histomorphologic lesion. Glio-neuronal lesions, including malformations of cortical development (MCD) and neoplasms such as gangliogliomas, are frequent histopathologic findings (7). These entities share a highly differentiated glio-neuronal architecture and benign biologic behavior (2, 8, 9). The group of MCDs is clinically as well as histologically diverse, ranging from subtle architectural aberrations to obvious cytologic and structural dysplasias. Current classifications on MCDs are based on histologic characteristics including loss of cortical lamination, glio-neuronal and/or neuronal heterotopias, the presence of dysplastic or cytomegalic neurons and Taylor-type balloon cells (2, 4, 10). Although MCDs are related to compromised neuronal migration and differentiation of neuronal precursors during cortical development (11–13), their pathogenetic relationship and molecular genetic basis remain to be determined (14).

Recent clinico-pathologic studies point to a role of genes and pathways in these sporadic lesions (15–17), otherwise associated with rare familial disorders such as tuberous sclerosis (TSC) (18, 19). TSC is caused by germline mutations of the \textit{TSC1} (hamartin) and \textit{TSC2} (tuberin) genes on chromosomes 9q and 16p, respectively (20–22). However, epilepsy patients with gangliogliomas or focal cortical dysplasias (FCDs) do not usually present with additional TSC-associated stigmata such as facial angiofibromata or angiomylipoma of the kidney (23). Hamartin and tuberin form a tumor suppressor complex and play a central role in the phosphatidylinositol 3-kinase (PI3K)/mTOR pathway, affecting morphogenesis, cell adhesion/migration, and cell fate determination (15, 24, 25). Alterations within this signal transduction cascade would thus be compatible with dysplastic lesions of the neocortex and...
presence of balloon cells, i.e. FCD IIb(15). Interestingly, about 66% of FCD IIb lesions carry altered allelic variants of TSC1 or loss of heterozygosity (LOH) of the TSC1 locus (17, 26). The TSC genes also play a role in benign neoplasms, such as subependymal giant cell astrocytomas. In addition, distinct allelic distribution patterns within the TSC2 gene can be identified in the majority of gangliogliomas (26, 27), which share striking histopathologic similarities with cortical tubers or FCD. However, neoplastic transformation appears rather to affect the glial than the neuronal cell component in this peculiar tumor entity (28, 29).

The spectrum of dysplastic and architectural alterations in FCD challenges any clinico-pathologic classification system. In contrast to FCD IIb, FCD IIa lack balloon cells but presents with a misshapen, dysplastic neuronal component characterized by aberrant orientation, enlarged cell size, and abnormal process arborization (Fig. 1). Whereas FCD IIb are frequently localized outside the temporal lobe, FCD IIa and gangliogliomas share a predilection for temporal localization (2). Milder forms of MCDs may be as well related to aberrant neuronal migration and differentiation. This holds true for white matter neuronal heterotopias (WMNH) and nodular cortical

FIGURE 1. Representative malformations of cortical development associated with focal epilepsy. Epilepsy-associated lesions comprise a broad spectrum of histopathologic alterations within cortex and white matter. (A) Patient with histopathologically approved heterotopic white matter neurons. In most patients, coronal T2-weight and FLAIR MR images demonstrate a variable gray/white matter blurring in the anterior temporal lobe (black arrow; accompanying hippocampal Ammon’s horn sclerosis is indicated by white arrow). Histopathologically, numerous heterotopic neurons are interspersed and partially aggregated in the white matter as shown by hematoxylin and eosi staining ([a]; 100×) and synaptophysin immunohistochemistry ([b]; arrowheads, 100×). In nodular cortical heterotopia, circumscribed cortical islands are present in white matter ([c]: H&E-LFB; 40×) and lack regular cortical lamination ([d]: Niss-LFB; 100×). Isolated architectural abnormalities with dyslamination, irregular neuronal arrangement ([e]; arrowheads) and lack of dysmorphic neuronal components are characteristic findings in FCDI ([f]: H&E; 200×; [g]: NeuN-immunohistochemistry, 100×), separating this class of malformations from FCDIIa. (B) In FCDIIa, a widespread spectrum of neuroradiologic alterations can be observed. Using a planar brain surface reformation, irregular gray matter appearance of the insular cortex can be demonstrated (black arrows) (45, 46). Histopathologically, loss of cortical lamination was found. Neuropathologic hallmarks include dysplastic neurons ([h]; arrowheads) with irregular axonal arrangement and lack of Taylor type balloon cells ([i]: H&E, 200×; [j]: NeuN-immunohistochemistry, 100×).
heterotopias (NCH) (Fig. 1). However, ectopic neurons in the white matter represent a physiological hallmark of the temporal lobe (30). In WMNH associated with pharmaco-resistant epilepsy, the abundance of heterotopic neurons in white matter is substantially increased (30, 31) and small clusters of white matter neurons are observed. In contrast, NCH contain coherent cortical islands in the white matter. In these cortical areas, a regular cortical laminar arrangement is missing. FCD2 is characterized by a laminated disarray (Fig. 1), abundant microcolumnar architecture, and solitary giant or immature but not dysplastic neurons (2, 32). Nevertheless, ectopic neurons in white matter can also be observed in these specimens. It remains to be shown, therefore, whether distinct pathogenetic mechanisms operate in individual subgroups of FCDs. Previous findings indicated a high incidence of TSC1 polymorphisms in FCDIIb (17), which prompted us to extend this molecular genetic approach to distinct malformative entities of the neocortex in epilepsy patients by carrying out systematic mutational analyses of the TSC1 and TSC2 genes.

MATERIALS AND METHODS

Surgical Specimens

Biopsy samples were obtained from patients with chronic pharmaco-resistant epilepsy who were surgically treated in the Epilepsy Surgery Programs at the University of Bonn Medical Center (n = 31), Erlangen/Vogtareuth (n = 17), Germany; the Niguarda Hospital, Milano, Italy (n = 8); and the Gilead Hospital, Bielefeld-Bethel, Germany (n = 2). In each patient, surgical removal of the epileptogenic focus was mandatory to achieve seizure control according to established guidelines described in detail before (33, 34). All participants gave informed and written consent and all procedures were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committees of the individual institutions. Surgical specimens were fixed in formaldehyde overnight and embedded into paraffin. Specimens were subsequently subjected to a detailed macroscopic and histopathologic examination by experienced neuropathologists involving the Neuropathologic Reference Center for Epilepsy Surgery at the University of Erlangen, Germany (www.epilepsie-register.de).

Specimens were categorized according to an established classification system (2). Only those specimens in which neuropathologic examination allowed a clear classification as NCH, WMNH, FCD2, or FCDIa groups were included in this study. None of the entities were classified as dual pathology. Particular care was taken to avoid confusion between FCDIa and FCDIb, cohorts by histopathologic examination of multiple areas of the surgical specimen. FCDI cases were obtained from a unique series of young children with severe epilepsies, mental/psychomotor retardation, and multilobar ichogenesis (32). Abnormal cortical architecture with abundant microcolumnar arrangements separated this group from WMNH, which were obtained from a series of adolescent patients suffering from temporal lobe epilepsies (Fig. 1). The number of solitary neurons within temporal lobe white matter sites was microscopically assessed in these specimens and exceeded 15 neurons per mm², a value considered as uppermost margin of the physiological range (32).

DNA Isolation

Starting from paraffin embedded tissue sections, areas comprising the malformative lesions were microdissected by avoiding adjacent normal CNS tissue (10 sections, 10 μm each) and subsequently subjected to DNA isolation. Regional accuracy was obtained by microscopic inspection of an adjacent section stained for hematoxylin and eosin (H&E). DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Leukocyte DNA obtained from 100 unaffected Caucasians with no history of epilepsy or other known CNS diseases served as control and was isolated using a standard protocol as described elsewhere (35).

PCR, Single Strand Conformation Polymorphism, and Sequencing Analyses

PCRs for TSC1 and TSC2 were carried out in an automated thermocycler (Uno Block, Biometra, Goettingen, Germany) as previously described in detail (17, 26). Briefly, in a total reaction volume of 10 μl, amplification was performed using 0.5 μM of each oligonucleotide primer, 200 μM of each dNTP, 50 mM KCl, 10 mM TRIS-HCl pH 8.5, 0.025 U Taq DNA polymerase strand (Invitrogen, Karlsruhe, Germany), and 10 ng DNA template. PCR was carried out as follows: initial denaturation at 94°C for 5 minutes, 32 amplification cycles at 94°C for 30 seconds, a primer-specific annealing temperature for 40 seconds, an extension step at 72°C for 50 seconds, followed by a terminal elongation step at 72°C for 10 minutes. PCR products were electrophoresed on 2 to 3% agarose gels, and visualized by ethidium bromide staining. Primer combinations were used as reported previously (17, 26). The PCR amplification products were subjected to single strand conformation polymorphism (SSCP) analysis. We examined up to 6 different electrophoresis conditions varying the polyacrylamide gel (PAG) concentrations and the acrylamide: bisacrylamide ratios as follows: 14% PAG with a 99:1 ratio, 10% PAG with a 49:1 ratio, and 10% PAG with a 29:1 ratio. In each condition glycerol (5%) was added optionally. All PAGs were run at room temperature or 4°C using a voltage of 60 V (36). SSCP variants as well as corresponding wild type samples were selected for direct sequencing of the PCR products (Fig. 2). Sequencing was performed using an ABI PRISM 373A DNA sequencer (Applied Biosystems, Perkin-Elmer, Weiterstadt, Germany) according to standard protocols described previously (26).

Laser Capture Microdissection

For isolation of heterotopic, malformative and/or dysplastic neuronal elements, as well as adjacent normal CNS tissue/vasculature components as controls, UV-Laser microbeam technology based on a nitrogen laser (PALM, Bernried Germany) was used in order to microdissect and subsequently harvest individual cellular elements (37). Pathological elements could be clearly differentiated according to their heterotopic localization, nuclear and cellular dysplastic morphologies after hematoxylin and eosin staining, and/or Nissl-staining of 10 μm
paraffin sections (Fig. 2). Representative cells were harvested and sampled for each PCR reaction for subsequent use in polymorphism/LOH analyses. Cells from normal cortex as well as vessel components of the same patient outside the malformed lesion served as controls. Harvested samples were further processed as described before (17, 26). The DNA isolation was carried out using the QIAamp DNA Mini Kit according to the manufacturer’s guidelines (Qiagen).

Loss of Heterozygosity Analysis

Examination of LOH status at the TSC1 locus on 9q was realized as described before (17). A total reaction volume of 10 μl containing 10 to 50 ng of DNA, 200 μM of each dNTP, 0.025 U Taq polymerase, 50 mM KCl, 10 mM TRIS-HCl pH 8.5, 1.5 mM MgCl2, 0.01% gelatin, and 0.5 μM of each primer was used. Primer combinations were used for microsatellite markers D9S302 (9q), D9S303 (9q), D9S319 (9cen), and
D9S741 (9p) as reported previously (17). Amplification was performed in an automated thermocycler (Uno Block, Biometra) for 32 cycles at 94°C for 30 seconds, annealing for 40 seconds at 55°C, and 72°C for 50 seconds. Reactions were carried out at least twice. PCR products were separated on denaturing gels (12% polyacrylamide, 5% glycerol, 1× TBE buffer) by electrophoresis (20 W, 15°C) and visualized by a silver staining procedure (36).

Statistical Analysis

Differences of allele frequencies between pathologic specimens and controls were tested by Pearson’s 2-sided χ² test. Cutoff was set 2-sided p ≤ 0.05 for p values to be significant.

RESULTS

Malformations of cortical development were obtained from 58 patients with drug-refractory epilepsy and screened for sequence alterations of the TSC1 and TSC2 genes by SSCP analysis. The lesions included in the present study comprised mild malformations, that is, neuronal WMNH (n = 19) and NCH (n = 4), as well as focal cortical dysplasias subclassified as FCDI and FCDIIa (Fig. 1; Table 1A). Somatic mutations were not identified in this series of cortical malformations. All sequence alterations encountered have been previously described as polymorphisms and were detected either in gangliogliomas and/or FCDIIb (17, 26, 27). In contrast to FCD IIa specimens, we found the polymorphism LA in intron 13/exon 14 to be significantly increased in WMNH (n = 2) (GenBank L48540: 257 GA; GenBank L48521: 3933 GA, silent) (allele frequencies: 5.0% in FCDIIa vs. 5.5% in controls) and exon 40 (n = 5) (GenBank L48521: 5220 TC, silent) previously described in gangliogliomas (26).

In our series of 20 FCDIIa cases, 2 previously described polymorphisms in the TSC1 gene were observed (38, 39) (Table 1B; Fig. 3). One polymorphism was found in 3 FCDIIa patients within the PCR fragment spanning intron 13/exon 14. It represented a combination of a silent base exchange (AG) at position 1556 (Glu, codon 445) and a noncoding base exchange (CG) at position 1555-55 in intron 13 (GenBank AF013168). The allele frequency of 7.5% was not significantly increased in FCDIIa compared to controls (also 7.5%).

Another previously described nucleotide polymorphism was found in exon 22 (17, 38), that is, a silent CT base transition at nucleotide 3050 (Ala, codon 943). The allele frequency of this alteration was 12.5% both in FCDIIa and in controls.

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In contrast to FCDIIa specimens, we found the polymorphism in intron 13/exon 14 to be significantly increased in WMNH and FCDI. The coding polymorphism affecting the interaction domain between hamartin and tuberin (exon 17 of TSC1), which is significantly increased in FCDIIb specimens (17), was not recognized in the present series of cortical malformations.

Rare TSC2 alleles were recognized in FCDIIb, which appear also to accumulate in gangliogliomas. This holds true for a polymorphism in intron 4 (26, 27) (Table 1B), which encodes a CT exchange at nucleotide 174 (GenBank L48521) (26, 27). In 2 FCDIIb patients, a complex cluster of noncoding base pair changes was observed in intron 30, previously described in gangliogliomas as well as FCDIIb (17, 26).

Additional polymorphisms were found in intron 31/exon 32 (n = 2) (GenBank L48540: 257 GA; GenBank L48521: 3935 GA, silent) (allele frequencies: 5.0% in FCDIIa vs. 5.5% in controls) and exon 40 (n = 5) (GenBank L48521: 5220 TC, silent) previously described in gangliogliomas (26).

In the WMNH, NCH and FCDI groups we observed polymorphisms in exons 10 and 41 of the TSC2 gene (Table 1A). The polymorphism in exon 10 is characterized by a base exchange from G to A (GenBank L48521: 1117, codon change 367 R > Q) in combination with an A to G transition (GenBank L48521: 1128, silent) (26, 38, 40, 41). Another silent exonic polymorphism was found in exon 41 (n = 6) (GenBank L48521: 5346 GC). However, none of these additional polymorphisms was significantly increased compared to controls.

Further analysis addressed LOH and microsatellite instability (MSI) at the genomic TSC1 locus, previously described in FCDIIb patients (17, 42). DNA of 18 cortical malformations proved suitable for LOH analysis (Fig. 4). According to established definitions of LOH/microsatellite instability (43), combinations of LOH and MSI were found only in individual NCH as well as FCDIIa cases (Fig. 4). In another FCDIIa, we have observed combined MSI in D9S303 and D9S319. However, in none of these cases, accompanying genomic sequence alterations of TSC1 were present. Vice versa, neither FCDI nor WMNH presented with significant LOH or MSI.

DISCUSSION

Molecular genetic analysis of TSC1 and TSC2 genes indicates a large allelic variability within cohorts of epilepsy

| TABLE 1A. Presence and Frequency of TSC1 and TSC2 Genomic Sequence Alterations in Different Variants of Cortical Malformations |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | TSC1            | TSC2            |                 |                 |                 |                 |                 |
|                 | Number | 5 | 14 | 17 | 22 | 4 | 10 | 30 | 32 | 40 | 41 |
| Heterotopic white matter neurons | 19 | – | 7* | – | – | 2 | – | 2 | – | 4 | 2 |
| Nodular cortical heterotopia | 4 | – | – | – | 1 | 1 | 1 | – | 1 | – | – |
| Focal cortical dysplasia | 15 | 6* | 3 | 3 | 1 | 3 | 5 | 4 | 3 | 5 | 4 |
| FCDI            | 20 | – | 3 | 5 | 5* | – | 2 | 2 | 5 | – | – |
| Total           | 58 | 16 | 9 | 11 | 2 | 4 | 5 | 15 | 6 | – | – |

* The focal cortical dysplasias were classified based on an established classification scheme (2).
patients suffering from MCDs, although no mutations are found in the coding areas of both genes. There is a significant increase of a polymorphism in intron 4 of \( TSC2 \) in FCD\( \text{IIa} \) (Fig. 3), whereas intron 13/exon 14 of \( TSC1 \) contains a complex polymorphism particularly abundant in WMNH and FCD\( \text{I} \). Similar allelic distribution patterns between entities may argue for common pathogenetic mechanisms and appear helpful to corroborate recently established clinico-pathologic classification systems (2–4).

With respect to previous studies in gangliogliomas and FCD\( \text{IIa} \), distinct patterns of polymorphisms and LOH/MSI at \( TSC1 \) and \( TSC2 \) loci can be observed in the present series of cortical malformations. Significantly increased genomic polymorphisms have been reported for \( TSC1 \) with a substantial number of LOH and/or MSI events at the \( TSC1 \) locus on 9q. Furthermore, FCD\( \text{I} \) and WMNH did not exhibit significant genomic sequence alterations of \( TSC2 \). Since the NCH samples under study did not reveal significant sequence alterations in \( TSC1 \) or \( TSC2 \), our data delineate different allelic variants in more subtle cortical malformations. The \( TSC1 \) polymorphism in intron 13/exon 14 is neither coding nor coupled with LOH of the second allele in FCD\( \text{I} \) or WMNH. The increased frequency of this polymorphism might be regarded, therefore, as molecular characteristic but does not point to a pathophysiological role of \( TSC1 \) in these histopathologically mild forms of MCDs. In contrast to our findings in FCD\( \text{IIa} \), the above-mentioned coding polymorphism in exon 17 of \( TSC1 \) is neither present in the WMNH, NCH groups, nor in FCD\( \text{IIb} \). Considering the large spectrum of clinico-pathologic entities to be described, molecular analysis of \( TSC1 \) and \( TSC2 \) alleles support the distinction between FCD type I, IIa, and IIb, as well as WMNH and NCH.

Gangliogliomas reveal a variety of genomic alterations affecting the \( TSC2 \) gene, including abundant complex and silent polymorphisms in exons 4, 40, and 41, respectively (26, 27). The polymorphism in intron 4 of \( TSC2 \) as well as a cluster of polymorphisms in exons 30 and intron 31/exon 32 are also observed in our present series of FCD\( \text{IIa} \) samples (Fig. 3). The mutation detected in intron 32 of laser microdissected glial tumor cells of a ganglioglioma (26) has not been found in the present series of MCDs. Although the patterns described here may be suggestive for a role of FCD\( \text{IIa} \) as potential dysplastic precursor lesion for gangliogliomas (28), coexistence or transition of FCD\( \text{IIa} \) to gangliogliomas have been rarely described in biopsy specimens of pharmacoresistant patients with focal epilepsies (unpublished observation in 418 gangliogliomas, in which only 3 cases revealed distinct signs of cortical dysplasia; www.epilepsie-register.de; see also [7]).

### Table 1B. Type and Frequency of \( TSC1 \) and \( TSC2 \) Sequence Alterations in the Present Collective of Different MCDs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Type of Alteration</th>
<th>Nucleotide Exchange</th>
<th>Amino Acid Change</th>
<th>Effect</th>
<th>Control</th>
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<tr>
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<td>5/20</td>
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<tr>
<td></td>
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<td>14/100</td>
<td>–</td>
<td>6/58</td>
</tr>
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GenBank annotation is as follows: * L48521; † L48540; ‡ AF013168.

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LOH and MSI at the TSC1 locus are frequent findings in FCDIIb specimens and may play a pathophysiologic role for the development of these lesions (42). Notwithstanding, the combination of TSC1 sequence alterations with LOH at the TSC1 locus was confirmed in the laser-microdissected balloon cell component of FCDIIb specimens only (17), which is not present in WMNH, NCH FCD I, or FCDIIa. In our series, we have analyzed chromosome 9q abnormalities in laser-microdissected regions with distinct pathologies obtained either from WMNH, NCH, FCDI, or FCDIIa samples. Neither of these experiments revealed a similar pattern with that observed in FCD IIb specimens. The admixture of dysplastic neurons and balloon cells is, therefore, histopathologically distinctive from FCDIIb and other MCDs, but also with respect to molecular alterations, that is, at the TSC1 locus. It may be reasonable to further conclude that balloon cells represent a specific cell population and are not precursor cells, which sporadically differentiate into glia or dysplastic neurons.

The TSC1/TSC2 complex plays an important role during cortical development and growth control (25). Tuberin and hamartin form a functional complex that is located downstream of Akt and inhibits mTOR signaling by acting as a GTPase-activating protein for small molecular weight-GTPase Rap1 (Rap1Gap) (44). At distinct stages of CNS development, a precise interaction of tuberin and hamartin appears critical. Whether this pathway plays a pathophysiologic role in WMNH and NCH remains to be further determined. We have observed, however, a genomic coding polymorphism in exon 10 in TSC2 intron 4 previously found to be accumulated in gangliogliomas is also significantly increased in FCDIIa. The mutation previously reported in a ganglioglioma was not found in the series of FCDIIa.

FIGURE 3. Sequence alterations of the TSC1 and TSC2 genes in patients with FCDIIa. Sequence alterations of TSC1 in intron 13/exon14 as well as exons 17 and 22, which have been found as significantly increased in FCDIIb, were not accumulated in FCDIIa (gray inserts contain sequence alterations previously found for FCDIIb in TSC1 as well as gangliogliomas in TSC2; χ²; *, p < 0.05). This finding suggests different molecular alteration patterns with respect to TSC1 and TSC2 in FCDIIa versus FCDIIb. A nucleotide exchange in TSC2 intron 4 previously found to be accumulated in gangliogliomas is also significantly increased in FCDIIa. The mutation previously reported in a ganglioglioma was not found in the series of FCDIIa.

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frequencies only for an intron 13/exon 14 polymorphism in \textit{TSC1}. In contrast to FCDIIb, no accumulation of LOH or MSI events was present at this locus. FCD IIa, harboring dysplastic neuronal components, showed increased genomic alterations in \textit{TSC2}, a pattern substantially different from FCDIIb. The correlation between clinical, imaging, neuropathologic, and molecular analyses may help to further develop comprehensive classification systems and treatment strategies in patients suffering from MCDs.

**ACKNOWLEDGMENTS**

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**REFERENCES**


**FIGURE 4.** Cell type specific LOH analysis. LOH status obtained from 20 patients with MCDs using microsatellite markers D9S302, D9S303, D9S319, and D9S741. (O, no amplification; $\ominus$, noninformative; $\Theta$, maintenance of heterozygosity; $\ominus$, loss of heterozygosity; $\gamma$, microsatellite repeat variability). Polymorphisms identified by single strand conformation polymorphism (SSCP) analysis are also listed.

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24. Miller JR, Hocking AM, Brown JD, Moon RT. Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca2+ pathways. Oncogene 1999;18:7860–72