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# The Space-Time Continuum of Cortical Dysplasia

**Somatic Mutations Activating the mTOR Pathway in Dorsal Telencephalic Progenitors Cause a Continuum of Cortical Dysplasias**

D'Gama AM, Woodworth MB, Hossain AA, Bizzotto S, Hatem NE, LaCoursiere CM, Najm I, Ying Z, Yang E, Barkovich AJ, Kwiatkowski DJ, Vinters HV, Madsen JR, Mathern GW, Blümcke I, Poduri A, Walsh CA. *Cell Rep*. 2017;21:3754-3766. doi:10.1016/j.celrep.2017.11.106

Focal cortical dysplasia (FCD) and hemimegalencephaly (HME) are epileptogenic neurodevelopmental malformations caused by mutations in mTOR pathway genes. Deep sequencing of these genes in FCD/HME brain tissue identified an etiology in 27 (41%) of 66 cases. Radiographically indistinguishable lesions are caused by somatic activating mutations in *AKT3*, *MTOR*, and *PIK3CA* and germline loss-of-function mutations in *DEPDC5*, *NPRL2*, and *TSC1*/2, including *TSC2* mutations in isolated HME demonstrating a "two-hit" model. Mutations in the same gene cause a disease continuum from FCD to HME to bilateral brain overgrowth, reflecting the progenitor cell and developmental time when the mutation occurred. Single-cell sequencing demonstrated mTOR activation in neurons in all lesions. Conditional *Pik3ca* activation in the mouse cortex showed that mTOR activation in excitatory neurons and glia, but not interneurons, is sufficient for abnormal cortical overgrowth. These data suggest that mTOR activation in dorsal telencephalic progenitors, in some cases specifically the excitatory neuron lineage, causes cortical dysplasia.

## Commentary

Malformations of cortical development (MCDs) are an important cause of epilepsy in childhood. A prominent MCD subgroup is cortical dysplasia, in which disruption of cortical lamination is seen along with abnormal neuronal and glial cellular morphology.[1] In an international survey of children undergoing surgery for intractable epilepsy, 42% were found to have pathologic diagnoses of cortical dysplasia, which includes both focal cortical dysplasia (FCD) and hemimegalencephaly (HME).[2]The etiology of FCD and HME has long been enigmatic. Histologic similarities between type II FCD and the tubers of tuberous sclerosis complex (TSC) were noted from the earliest reports,[3] but studies using standard DNA sequencing techniques were unable to definitively identify pathogenic mutations in the *TSC1* or *TSC2* genes in FCD specimens.[4] However, consistent immunohistochemical findings indicating upregulation of mTOR pathway activity in type II FCD and HME lesions continued to support hypotheses that mutations of *TSC1/TSC2*, or other genes regulating mTOR function, were likely involved in their pathogenesis.[5] Recently, advances in DNA sequencing and analysis technology have begun to unravel the etiological mystery of FCD and HME, identifying low-level mutations in regulators of the mTOR pathway in brain specimens, below the resolution of standard sequencing techniques.[6-11]

In their current article, D'Gama et al expand their previous work investigating mTOR pathway mutations in FCD and HME.[6,12] The goals of the study were to (1) detect pathogenic mutations in a large group of FCD and HME specimens, (2) perform single-cell sequencing to identify the cell types expressing these mutations, and (3) to generate mouse models of mTOR pathway activation limited to dorsal telencephalic progenitors (which generate excitatory neurons and glia) or to cortical interneurons, to see if either of these recapitulated the overgrowth and cortical lamination defects of human cortical dysplasia. DNA extracted from specimens from 90 patients with FCD or HME were subjected to targeted ultra-deep sequencing at a read depth of $>5000\times$. By obtaining this many sequencing reads of each nucleotide in a gene of interest, variants present in a small subset of cells can be identified with high confidence. Brain specimens resected during epilepsy surgery were available from 57 of these patients, while for 33 patients only blood or buccal samples were available. Twelve genes responsible for positive or negative regulation of the mTOR pathway were included in the panel: *AKT1, AKT3, CCND2, DEPDC5, MTOR, PIK3CA, PIK3R2, PTEN, TSC1, TSC2, NPRL2,* and *NPRL3*. Strict criteria were used for the classification of identified variants as pathogenic. In this cohort, mTOR pathway mutations were identified in 8/37 FCD

brain specimens and 8/20 HME brain specimens. When the current results were combined with their previous cohorts,[6,13] an overall yield of 41% (27 of 66) for identification of pathogenic mTOR pathway mutations in FCD/HME brain tissue was established (26% for FCD, 61% for HME).

The identified mutations were either somatic (identified in brain only, affecting less than 50% of alleles, arising after conception in dividing cells) or germline (identified in brain and blood, affecting about 50% of alleles, present in the egg or sperm prior to conception). None of the somatic mutations were detectable in blood. Somatic mutations causing a presumed gain-of-function phenotype in a positive regulator of the mTOR pathway were the most commonly identified mutation type in FCD/HME (*AKT1/3*, *PIK3CA*, *MTOR*), with mutations in the *MTOR* gene itself the most prevalent, similar to previous reports.[11] Somatic loss-of-function mutations were identified in negative regulators of the mTOR pathway, specifically in the *TSC1* and *TSC2* genes, while germline mutations were identified in *DEPDC5* and *NPRL2*, again confirming previous reports.[9,10,14]

The percentage of alleles affected by the identified somatic mutations varied, ranging from as low as 1% of sequenced brain alleles (ie, 2% of cells assuming a diploid genome) to as high as 20% (40% of cells). The authors used these data in combination with data collected from other publications to demonstrate that mutations in the same gene can cause either FCD or HME, and further that localized FCD lesions contain a smaller percentage of affected cells than do HME lesions. In 7 FCD/HME brain specimens, they used fluorescence-activated nuclear sorting to separate neuronal (NeuN+) from non-neuronal (NeuN-) cells, followed by single-cell sequencing to identify the proportion of each cell type containing the pathogenic mutation. They found that in each case the mutation was identified in the neuronal population, with varying involvement of the non-neuronal population. Importantly, in the smallest FCD lesions, the neuronal mutation rate far exceeded the non-neuronal mutation rate, while for larger FCD and HME lesions a greater proportion of non-neuronal cells were affected (although usually to a lesser extent than the neuronal cells). Based on these data, in combination with results from their group investigating the distribution of non-pathogenic somatic mutations in normal brain,[14] the authors proposed that the larger lesions of HME are caused by somatic mutational events in a neural progenitor happening earlier in development, while mutational events in the same cell type happening later in development result in the smaller lesions of FCD.

Additional conceptually important data were obtained from the analysis of FCD/HME lesions containing loss-of-function mutations in genes that inhibit the mTOR pathway, such as *TSC1*, *TSC2*, *DEPDC5*, and *NPRL2*. Germline mutations in any of these genes are known to result in a predisposition to epilepsy. What has been unclear is whether a "second-hit" mutation resulting in loss of function of the normal allele is required for the formation of areas of dysplasia. This is as opposed to mutations causing gain of function in a positive regulator of the mTOR pathway, where mutation of a single copy of the gene is all that is required. In their cohort, two patients with HME were identified with somatic mutation in *TSC2* in resected brain tissue in addition to a germline *TSC2* mutation, consistent with the hypothesis that a second hit is required. Interestingly, neither of these patients had other clinical signs of TSC. In contrast, in 4 other HME/FCD specimens, either a somatic mutation or a germline mutation in *TSC1*, *TSC2*, *DEPDC5*, or *NPRL2* were identified, but not both. Possible explanations for these results include (1) a second-hit mutation is not always necessary for the formation of dysplasia, (2) a second-hit mutation may be present at a frequency below the level of detection, (3) the second hit may be due to a copy number variant undetectable by targeted sequencing, (4) the second hit may be present in an intron, which was not sequenced, (5) expression of the normal allele may be absent due to loss of heterozygosity, or (6) the second hit may be in a different gene also affecting the mTOR pathway.

Finally, the authors further studied the cell-type requirement for formation of dysplastic brain lesions by introducing the constitutively active *Pik3ca* p.H1047R allele into mouse brain using either a construct selective for the dorsal telencephalic lineage (*Emx1-Cre*, active in precursors to cortical excitatory neurons and glia) or the interneuron lineage (*Nkx2.1-Cre*, active in precursors to medial ganglionic eminence-derived interneurons and glia). Similar to previously reported findings using constitutively active forms of *Pik3ca* under control of *Nestin-Cre* or *hGFAP-Cre*,[15] *Emx1-Cre;Pik3ca*$^{H1047R/wt}$ mice demonstrated megalencephaly with abnormal gyrification and cortical laminar disruption, reminiscent of human FCD/HME. In contrast, *Nkx2.1-Cre; Pik3ca*$^{H1047R/wt}$ mice did not have brain overgrowth or apparent dysplasia, although the total interneuron number was reduced. One limitation to this study is that the possible occurrence of seizures in these mice was not investigated, leaving open the possibility that mutation in interneurons could be epileptogenic in the absence of dysplasia.

In summary, this article by D'Gama et al provides valuable information about the high prevalence of mTOR pathway mutations in FCD and HME, and indicates that mutation affecting cortical excitatory neurons is necessary for the formation of these lesions. Larger lesions are more likely to contain a higher percentage of affected cells than smaller lesions, implying a mutational event happening earlier in development. For germline mutations causing loss of function of an mTOR pathway inhibitory protein, a second-hit somatic mutation may be required for the formation of dysplasia. Additional questions still to be answered include the etiology of FCD/HME lesions without identified mutations (60% of their cohort), the contribution of mutated non-neuronal cells to the epileptogenic potential of these lesions, and whether identification of a specific mTOR pathway mutation in an individual patient may allow for personalized therapeutics. Nonetheless, these studies provide solid support for the concept that FCD and HME exist as a continuum of pathology whose manifestation is dictated by space (the cell location and type affected by a somatic mutation) and time (the developmental stage at which the mutation occurs).

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