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Somatic Variants and Surgical Epilepsies: Search and You Will Find

Detection of Brain Somatic Variation in Epilepsy-Associated Developmental Lesions

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Objective: Epilepsy-associated developmental lesions, including malformations of cortical development and low-grade developmental tumors, represent a major cause of drug-resistant seizures requiring surgical intervention in children. Brain-restricted somatic mosaicism has been implicated in the genetic etiology of these lesions; however, many contributory genes remain unidentified. Methods: We enrolled 50 children who were undergoing epilepsy surgery into a translational research study. Resected tissue was divided for clinical neuropathologic evaluation and genomic analysis. We performed exome and RNA sequencing to identify somatic variation and we confirmed our findings using high-depth targeted DNA sequencing. Results: We uncovered candidate disease-causing somatic variation affecting 28 patients (56%), as well as candidate germline variants affecting 4 patients (8%). In agreement with previous studies, we identified somatic variation affecting solute carrier family 35 member A2 (SLC35A2) and mechanistic target of rapamycin kinase (MTOR) pathway genes in patients with focal cortical dysplasia. Somatic gains of chromosome 1q were detected in 30% (3 of 10) of patients with Type I focal cortical dysplasia (FCD)s. Somatic variation in mitogen-activated protein kinase (MAPK) pathway genes (i.e., fibroblast growth factor receptor 1 [FGFR1], FGFR2, B-raf proto-oncogene, serine/threonine kinase [BRAF], and KRAS proto-oncogene, GTPase [KRAS]) was associated with low-grade epilepsy-associated developmental tumors. RNA sequencing enabled the detection of somatic structural variation that would have otherwise been missed, and which accounted for more than one-half of epilepsy-associated tumor diagnoses. Sampling across multiple anatomic regions revealed that somatic variant allele fractions vary widely within epileptogenic tissue. Finally, we identified putative disease-causing variants in genes not yet associated with focal cortical dysplasia. Significance: These results further elucidate the genetic basis of structural brain abnormalities leading to focal epilepsy in children and point to new candidate disease genes.

Commentary

In cases of epilepsy of unknown cause (formerly known as "idiopathic" epilepsy), the genetic etiology of epilepsy had long been suspected, given the familial clusters and especially twin occurrences. Positional cloning allowed the identification of the first gene associated with one of these epilepsy syndromes in 1995.[1] However, this painstaking methodology required identifying families with several affected individuals. Despite all hurdles, positional cloning helped elucidate Mendelian autosomal dominant (CHRNA4), autosomal recessive (Lafora disease), and X-linked (band heterotopia) patterns of inherited epilepsy. However, all genes found through positional cloning explained only a very small number of epilepsy cases.

A little more than a decade ago, a new methodology, next-generation sequencing (NGS), revolutionized our understanding about the genetic etiology of epilepsies. Through NGS, sequencing exomes or even whole genomes became affordable. Trios (affected individual, plus mother and father) and single individual sequencing became routine. In a short few years, we learned about hundreds of genes causing monogenic epilepsies, especially those associated with developmental and epileptic encephalopathies (DEEs).

The identification of monogenic epilepsies is important as it paves the way to precision therapies. Once the genetic mechanism is uncovered, a focused, possibly disease-modifying treatment can be explored. However, despite the excitement with the new discoveries, most patients with more common forms of epilepsy do not have identifiable monogenic epilepsies. After DEEs, focal cortical dysplasias (FCD) and long-term epilepsy associated tumors ([LEATs], which include ganglioglioma,

dysembryoplastic neuroepithelial tumor [DNET], low-grade glioneuronal tumors, pleomorphic xanthoastrocytoma, and polymorphous low-grade neuroepithelial tumor of the young) cause some of the most drug-resistant epilepsies. Therefore, it would be important to understand the genetic mechanisms underlying FCDs and LEATs in order to design better treatment to these patients. But just how easy is it to identify the genetic causes of FCDs and LEATs?

It all depends on where and how deep you look. The first wave of epilepsy genes discovered through NGS was found in patients with germline mutations, that is, every cell of the body carries the same variant. As such, these abnormal genes could be detected in blood samples. However, the majority of patients with FCDs or LEATs do not have any detectable germline abnormalities. If not in germline, could the abnormal gene be restricted to the cells causing epilepsy? The first endeavors into somatic brain mutations were done in patients with obvious structural abnormalities such as hemimegalencephalies.[2,3] After exciting findings in those brains, researchers started to look for somatic mutations in other, less obvious lesions, such as LEATS, FCDs, and even in nonlesional focal epilepsies.

By examining brain tissue obtained through epilepsy surgery, and comparing that to blood from the same patients some somatic abnormalities became clear. Somatic pathogenic variants has been found in the nucleotide-sugar transporter *SLC35A2* in up to one-third of patients with Type I FCD; *PI3K-AKT-mTOR* somatic abnormalities in the mTOR pathway in up to 50% of patients with Type II FCD. Chromosome 1q gains were also seen in focal epilepsies. RAS-RAF-MAPK and PI3K-AKT-MTOR pathways somatic abnormalities were found in LEATs. *BRAF* somatic mutations have been found in more than half of gangliogliomas, and *FGFR1* in over half of DNET cases.[4,5]

In the study of Bedrosian et al, the authors evaluated somatic and germline variants in 50 patients who had epilepsy surgery for the treatment of drug-resistant epilepsy due to FCD or LEATs. The resected brain tissue was divided for neuropathology and genomic evaluation. This study identified somatic mutations in 40% of FCD and in 100% of LEATs cases.[6]

Patients with FCD were found to carry somatic mutations in *SLC35A2* (n = 2), *RHEB* (n = 1), *PTEN* (n = 1), *MTOR* (n = 1), and chromosome 1q gain (n = 3). Four patients with FCD had germline mutations: *PACS2* (n = 1), *NPRL3* (n = 1), *DEPDC5* (n = 1), and *COL4A1* (n = 1). Interestingly, the patient with germline *DEPDC5* germline mutation did not have a *DEPDC5* somatic variant, but had a somatic variant in *ENPP1*. Autosomal recessive mutations in *ENPP1* gene are associated with infantile arterial calcification, and have not been described in association with epilepsy. Further research will be needed to confirm the role of *ENPP1* in FCDs.

In addition to somatic mutations in genes previously implicated in FCD, this study found novel candidate genes associated with FCDs:

- *PTPN11:* Two patients with Type III FCD had pathogenic/likely pathogenic variants in this gene. The first patient had an extensive area of cortical dyslamination encompassing the frontal, temporal, and parietal regions, associated with hippocampal sclerosis. The second patient also had an extensive cortical dyslamination and a history of intraventricular hemorrhage. Germline (autosomal dominant) mutations in *PTPN11* have been previously observed in patients with Noonan syndrome. This syndrome is characterized by dysmorphism, congenital heart disease, variable degrees of developmental delays, and other systemic findings. Rare patients with Noonan syndrome can have low-grade glioneuronal tumors including DNETs. However, FCD had not been previously observed in patients with Noonan syndrome.
- *EEF2:* One patient with Type I FCD had a likely pathogenic variant in this gene. *EEF2* plays a role in GABAergic signaling and seizure susceptibility. As such, it is a strong candidate gene.
- *NAV2:* One patient with Type II FCD had a likely pathogenic variant in this gene, which plays a role in neuronal migration and cerebellar development. Again, a strong candidate gene, but further research is needed to confirm the role of these genes in FCDs.

A notable finding in this study is that somatic variants were found in 100% of patients with LEATs: BRAF (n = 6), FGFR1 (n = 6), FGFR2 (n = 3), KRAS (n = 1). This success rate was only possible because the authors chose to use both exome sequencing and RNA sequencing. Complex genomic rearrangements may be missed by simple exome sequencing. However, through different algorithms used for RNA sequence interpretation, it was possible to identify fusions and internal tandem duplications in FGFR1 and FGFR2.

Despite the above findings, 60% of FCD cases were not resolved. One possible limitation inherent to this type of work is the uneven distribution of cells carrying the mutant gene. Since somatic variants occur postzygotically, the burden of somatic variants is heterogeneous, that is, some areas of the FCD have more cells with the abnormal gene while other areas have less. The somatic variant allele fractions (VAF) represents the proportion of an abnormal allele compared to the normal one. A high VAF suggests that a high number of cells carry the mutant gene while a low VAF suggests very few cells do. If the VAF is too low, the depth of sequencing used may not be enough to amplify and detect the variant. Variant allele fractions vary widely depending on the anatomic region being sequenced. This poses a sampling bias as the region with higher VAFs may be the one given to the pathologist and not the one sequenced. Interestingly, VAF gradients have been correlated with both image and electrophysiology, that is, the areas with higher VAFs are more epileptogenic and more easily visualized.[7,8]

In conclusion, the study lead by Bedrosian showed that by combining different methodologies one can significantly increase the yield of somatic variants. New candidate genes were also identified. It is very likely that genetic mechanisms

are behind most nonacquired epileptogenic lesions and we just need to keep improving our way of looking for them.

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Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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