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Open Sesame: Door to Enriched Somatic Variants Underlying Sporadic mTLE

Contribution of Somatic Ras/Raf/Mitogen-Activated Protein Kinase Variants in the Hippocampus in Drug-Resistant Mesial Temporal Lobe Epilepsy

Khoshkhoo S, Wang Y, Chahine Y, Erson-Omay EZ, Robert SM, Kiziltug E, Damisah EC, Nelson-Williams C, Zhu G, Kong W, Huang AY, Stronge E, Phillips HW, Chhouk BH, Bizzotto S, Chen MH, Adikari TN, Ye Z, Witkowski T, Lai D, Lee N, Lokan J, Scheffer IE, Berkovic SF, Haider S, Hildebrand MS, Yang E, Gunel M, Lifton RP, Richardson RM, Blümcke I, Alexandrescu S, Huttner A, Heinzen EL, Zhu J, Poduri A, DeLanerolle N, Spencer DD, Lee EA, Walsh CA, Kahle KT. *JAMA Neurol*. 2023;80(6):578-587. doi:10.1001/jamaneurol.2023.0473

Importance: Mesial temporal lobe epilepsy (MTLE) is the most common focal epilepsy subtype and is often refractory to antiseizure medications. While most patients with MTLE do not have pathogenic germline genetic variants, the contribution of postzygotic (i.e., somatic) variants in the brain is unknown. Objective: To test the association between pathogenic somatic variants in the hippocampus and MTLE. Design, Setting, and Participants: This case-control genetic association study analyzed the DNA derived from hippocampal tissue of neurosurgically treated patients with MTLE and age-matched and sex-matched neurotypical controls. Participants treated at level 4 epilepsy centers were enrolled from 1988 through 2019, and clinical data were collected retrospectively. Whole-exome and gene-panel sequencing (each genomic region sequenced more than 500 times on average) were used to identify candidate pathogenic somatic variants. A subset of novel variants was functionally evaluated using cellular and molecular assays. Patients with nonlesional and lesional (mesial temporal sclerosis, focal cortical dysplasia, and low-grade epilepsy–associated tumors) drug-resistant MTLE who underwent anterior medial temporal lobectomy were eligible. All patients with available frozen tissue and appropriate consent were included. Control brain tissue was obtained from neurotypical donors at brain banks. Data were analyzed from June 2020 to August 2022. Exposures: Drug-resistant MTLE. Main Outcomes and Measures: Presence and abundance of pathogenic somatic variants in the hippocampus vs the unaffected temporal neocortex. Results: Of 105 included patients with MTLE, 53 (50.5%) were female, and the median (IQR) age was 32 (26-44) years; of 30 neurotypical controls, 11 (36.7%) were female, and the median (IQR) age was 37 (18-53) years. Eleven pathogenic somatic variants enriched in the hippocampus relative to the unaffected temporal neocortex (median [IQR] variant allele frequency, 1.92 [1.5-2.7] vs 0.3 [0-0.9]; $P = .01$) were detected in patients with MTLE but not in controls. Ten of these variants were in PTPN11, SOS1, KRAS, BRAF, and NF1, all predicted to constitutively activate Ras/Raf/mitogen-activated protein kinase (MAPK) signaling. Immunohistochemical studies of variant-positive hippocampal tissue demonstrated increased Erk1/2 phosphorylation, indicative of Ras/Raf/MAPK activation, predominantly in glial cells. Molecular assays showed abnormal liquid-liquid phase separation for the PTPN11 variants as a possible dominant gain-of-function mechanism. Conclusions and Relevance: Hippocampal somatic variants, particularly those activating Ras/Raf/MAPK signaling, may contribute to the pathogenesis of sporadic, drug-resistant MTLE. These findings may provide a novel genetic mechanism and highlight new therapeutic targets for this common indication for epilepsy surgery.

Commentary

Focal onset seizures are the most common type experienced by people with epilepsy, making up for more than half of all seizures. Focal seizures can be focal aware or focal unaware previously called partial complex seizures, representing over one-third of all seizures.[1] One such common focal epilepsy subtype occurs in mesial temporal lobe epilepsy (mTLE), which is often resistant to anti-seizure medications and frequently requires neurosurgical resection of the seizure foci to achieve remission of epilepsy. The association of mTLE with somatic genetic variants has been hypothesized since most patients with mTLE do not have a history of an excitotoxic or precipitating insult that could explain focal mesial temporal sclerosis (MTS) reported as the classic imaging and

histopathological feature of mTLE.[2] Genetic mosaicism is defined by the presence of at least 2 genetically distinct cell populations in the same organism and results from postzygotic mutations.[3] A substantial increase in genetic studies of brain malformations in recent years, bolstered by the availability of improved technologies to study surgical tissue, has helped address the long-simmering hypothesis that focal lesions arise from focal, post-zygotic genetic disruptions. These studies have demonstrated that somatic variants that arise post-zygotically underlie a much larger proportion of brain malformations than estimated before. Additionally, studies of nondiseased individuals have revealed that somatic variation occurs routinely during cell division, including during early brain development when the rapid proliferation of neuronal precursor cells provides an environment for somatic mutation to occur and for these variants to accumulate. These pathogenic groups of variants are now being revealed through advanced and high-throughput genetic techniques that contribute to the "open sesame genetics" of clinically well-classified neurological diseases[4-8] associated with histopathological signatures. Somatic variants have emerged as a major cause of focal epilepsies associated with focal cortical dysplasia (FCD). The clinical phenotype of mosaic disorders is determined by the timing of mutation, the level of pathway activation, and the affected cell types, creating a mosaic with variant-positive and nonvariant cells. The level of such mosaicism is defined as variant allele frequency (VAF), which correlates well with the lesion's size and brain regional distribution. Most somatic variants identified in FCDs classified as type 2B show activation of PI3K/Akt/mechanistic target of rapamycin (mTOR) pathway genes.[9] However, somatic variants in non-PI3K/Akt/mTOR genes can also cause focal epilepsies.

In this study by Khoshkhoo et al[6] hippocampus-derived DNA from 105 patients was sequenced via whole-exome sequencing and gene-panel sequencing from fresh frozen specimens, followed by somatic variant calling and annotation using the latest versions of several databases. The histopathologic findings identified in this patient cohort were 91 mesial temporal sclerosis (MTS-only), 4 MTS with low-grade epilepsy-associated tumor (LEATs), 2 MTS with FCD, and 8 non-MTS. A total of 11 candidate variants were validated and 5 cases with 2.5% to 23.6% mean VAFs had the MTS or FCD pathology. In contrast, MTS-only pathology ($n = 6$) had significantly lower mean VAFs, ranging from 0.8% to 3.3%. For the MTS tissue samples, where nonaffected temporal neocortex tissue was available, investigation of similar pathogenic variants indicated that the low VAFs were selectively enriched only in the affected hippocampal tissue, and all but one of these pathogenic variants were predicted to constitutively activate Ras/Raf/mitogen-activated protein kinase (MAPK) signaling pathway.[3] No pathogenic somatic variants were detected in the small non-MTS group. A follow-on retrospective review of the FCD and LEAT literature revealed a significant predilection of enrichment of somatic Ras/Raf/MAPK activating variants in the temporal lobe versus extratemporal enrichment for PI3K/Akt/mTOR activating variants.

The Ras-Raf-MAPK pathway is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell.[10] The signal starts when a signaling molecule binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein and produces some change in the cell, such as cell division. The pathway includes many proteins, such as MAPKs, which act as on or off-switches by phosphorylating the next pathway protein. The signal that starts the MAPK pathway is the binding of extracellular mitogen to a cell surface receptor. This allows a Ras protein (a Small GTPase) to swap a GDP molecule for a GTP molecule, flipping the "on/off switch" of the pathway. The Ras protein can then activate MAP3K (e.g., Raf), which activates MAP2K, which activates MAPK. Given such a fundamental role of the pathway in cell growth and dynamics, it is not difficult to postulate that the phenotypical consequences of germline activating mutations in this pathway would likely not be compatible with life, such that some of these pathogenic mutations could only survive development in a mosaic state and with a limited number of affected cells in the enriched tissues.[4] As discussed by the authors, similar enrichment of other pathway mutations in specific brain regions like FCDs may also define their occurrence and phenotypes as related to seizure onset and severity. Recent research with a similar focus on the well-defined pathology of polymicrogyria,[5] further highlights the point that a wealth of knowledge remains to be explored behind the doors guarding information about somatic mutations for long-identified brain histopathology[4] associated with epilepsy. The origins of these epilepsies remained clouded by multiple possible hypotheses for their causation and clinical phenotypes. Early life excitotoxic insults like ischemia, trauma, febrile seizures, or inflammation when not documented in the patient history were unable to realistically account for most of these cases.

There remains much work to be done to continue somatic gene discovery in epilepsies and learn about the somatic mutation landscape in the brain. However, with insights gained from the results reported in this study and technological advancements in tools available to investigate the hidden causes of epilepsies, the field is poised to make significant advancements. The current need for surgically resected tissue to conduct these studies is prohibitive. A transition of identification of the hidden mutations needs to shift from surgical tissue to fine-needle aspiration cytology, cell-free DNA from cerebrospinal fluid (CSF), or new innovative and less-invasive technologies. Specifically for mTLE which is significantly associated with focal cell death, it will be interesting to ascertain whether cell-free DNA from dying cells is a possibility perhaps following clinically identifiable events such as clustered seizures that could be predicted by the rhythmicity of such events.[11] Histological staining in the MTS-only pathology revealed that hippocampal subregions such as the cornu ammonis with the greatest neuronal loss showed the highest density of pErk1/2 staining (a proxy for Ras/Raf/MAPK pathway activation).[6] Of interest was the finding that the cells with the most intense for pErk1/2 staining showed glial morphology. Dysregulation of

the MAPK pathway has been documented in several cancers for more than a decade but also in glia-induced pathogenesis underlying inflammatory and post-trauma epilepsy and neurodegenerative diseases. This preexisting focus on mutation-specific inhibition of MAPK signaling as a therapeutic target in cancer research can now be harnessed for mTLE as well. Motivated clinician-scientists at the forefront of managing patients with refractory epilepsy, with the awareness of the treasure trove that lies in further linking details of onset and phenotype with allele frequency will help further the goal of improving epilepsy patient management and treatment.

Shilpa D. Kadam, PhD
Axonis Therapeutics

ORCID iD

Shilpa D. Kadam https://orcid.org/0000-0001-5136-9594

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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