NEUROLOGY

Neurosurgery elucidates somatic mutations

Surgical innovation is helping to identify roles for somatic mutations in brain disorders

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Somatic variants are genomic alterations present in some cells of the body but not in others. In contrast to inherited germline variants, somatic variants are typically acquired during postzygotic cell divisions or from environmental factors. Somatic variants in a given tissue can also be highly dynamic, such as in the brain, where the number of clonal variants can change during aging and disease (*1*). Neuro-oncology has greatly benefited from the analyses of somatic mutations in tissue obtained from surgical biopsies or resections. Increased understanding of somatic mutations in brain tumors brought a revolution in prognostication and individualized treatments. Somatic mutations are now increasingly being implicated in neurological disorders beyond cancer, particularly in children with congenital brain and cerebrovascular diseases. Teams of neurosurgeons and scientists are performing gene sequencing on surgical brain samples from these patients—paving the way to expand understanding of nononcologic brain disease through the study of somatic mutations.

To date, most studies of somatic mutations in nononcologic neurological disease have relied on post-mortem brain samples or samples from other tissues, such as the blood. This is partly owing to the limited number of medically indicated approaches for obtaining brain tissue while the patient is alive and current clinical practice that frequently discards tissue during surgery because it is thought to be of limited diagnostic value. This lack of fresh brain tissue limits the sensitivity and specificity of somatic mutation detection because DNA and RNA quality can degrade post-mortem, and variants specific to brain cells might not be present in nonbrain tissue. In addition, blood, which is the most readily available tissue for sampling, can accumulate tissue-specific somatic mutations such as variants acquired through clonal hematopoiesis, which complicates the detection of somatic variants present in the nervous system.

Several recent studies have demonstrated the power of exploring somatic mutations directly from tissue collected in the operating room to gain insights into neurological disorders. These disorders include congenital focal cortical dysplastic syndromes such as focal cortical dysplasia (FCD) and hemimegalencephaly (HMG) (*2*–*4*). Clinically, patients with these conditions present with seizures refractory to pharmacological management and are diagnosed by use of brain imaging to detect lesions that disrupt the cortical layering of the brain. Lesion size can vary from subcentimeter in FCD to a whole hemisphere in HMG. The current treatment is radical surgical excision of the lesions, which puts the patient at high risk of morbidity, especially in the case of hemispherectomy to treat HMG.

Genetic causes of these conditions were initially difficult to identify because they only affected a subset of cells. Blood samples obtained from patients with FCD and HMG appear largely normal when analyzed with traditional sequencing approaches that capture germline variants. However, the development of next-generation sequencing technologies and bioinformatic pipelines has made it possible to identify somatic variants from neurosurgically resected samples. Using this approach, somatic mutations in the mechanistic target of rapamycin (*MTOR*) gene that increase mTOR pathway activity were identified as causative of these disorders. The mTOR pathway helps determine cell size and has been implicated in cancer. Larger lesions had a larger proportion of cells affected by the mutations (*4*, *5*). These genetic insights were brought about through collaborations between neurosurgeons and scientists and led to a new clinical classification system for FCD (*6*).

Inhibitors of the mTOR pathway now represent promising therapeutic approaches for patients with FCD and HMG. Small-molecule inhibitors used to treat cancer could in theory be used as monotherapies or adjuncts to surgical resection for the treatment of FCD or HMG. These inhibitors include sirolimus and evorolimus, which target mTOR directly, or duvelisib, and miransertib (ARQ-092), which target proteins that interact with mTOR. Encouragingly, treating patients with tuberous sclerosis and phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome (both neurodevelopmental disorders) with evorolimus resulted in improved clinical outcomes in randomized clinical trials (*7*, *8*). Phase 2 clinical trials on the effect of evorolimus on FCD are currently underway in the United States (NCT02451696) and South Korea (NCT03198949). However, questions remain on the optimal inclusion criteria or treatment combinations for these studies.

More recently, somatic mutations have been implicated in mesial temporal lobe epilepsy, which is one of the most common forms of epilepsy and is often resistant to treatment with antiseizure medication. Whole-exome sequencing of 105 hippocampal samples from patients with this type of epilepsy who underwent surgical treatment revealed pathogenic somatic variants in protein tyrosine phosphatase nonreceptor type 11 (*PTPN11*), son of sevenless homolog 1 (*SOS1*), *KRAS*, *BRAF*, and neurofibromin 1 (*NF1*). These genes are all known or predicted to activate the mitogen-activated protein kinase (MAPK) signaling pathway, which is involved in cancer (*9*). Therefore, established anticancer agents targeting the MAPK pathway could be imagined as neurosurgical adjuncts or even a primary treatment option for this disorder.

Recent studies of somatic mutations in sporadic arteriovenous malformations (AVMs) revealed activating mutations in *KRAS* that can be therapeutically targeted (*10*). AVMs are vascular lesions present in the brain that cause hemorrhagic strokes by forming abnormal connections between arteries and veins, forming a nidus (tangle) of fragile blood vessels. Currently, surgery is the only treatment for this condition, but surgery is highly challenging, and complications such as massive bleeding occur frequently. Consequently, interventions carry high risk of morbidity for the patient and require specialized neurosurgical care, which is not readily available worldwide. In one study, therapeutically

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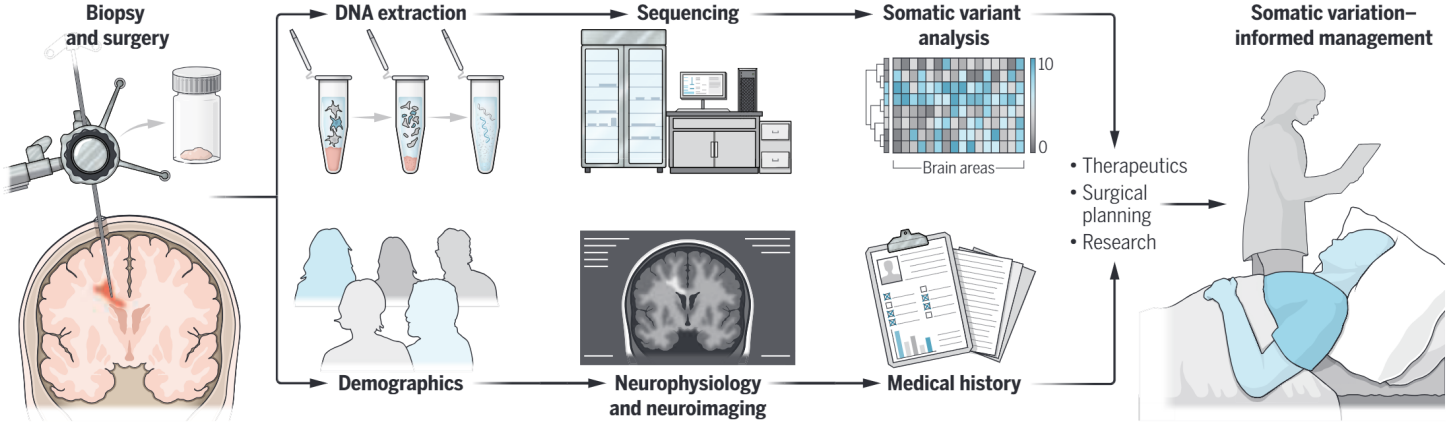
Somatic variation–informed neurosurgery and medical management

In the future, somatic mutation information could be used to direct neurosurgical and medical management of neurological disorders. Biopsies or surgical resection samples could be obtained and processed through next-generation sequencing to identify somatic variants. These variants could then be correlated with clinical data such as demographics, neurophysiology, neuroimaging, and medical history to expand research efforts and identify targeted therapies that could be used as monotherapy or adjuncts to surgery. This information could also be used for surgical planning if necessary.



resected samples from 26 patients with AVMs were obtained, and whole-exome sequencing was used to identify somatic mutations present in at least 1% of cells. Using cell-sorting and digital droplet polymerase chain reaction–based sequencing, the study showed that pathogenic somatic mutations in *KRAS* were enriched in brain endothelial cells. Furthermore, application of MAPK kinase (MEK) and phosphatidylinositol 3-kinase (PI3K) inhibitors to cultured endothelial cells down-regulated the KRAS signaling pathway, demonstrating potential therapeutic efficacy for AVMs.

The full resection of pathological tissues can be difficult or unsafe, particularly in nononcologic disorders such as multifocal epilepsy and neurovascular disease. Therefore, to expand the study of somatic variants in neurological disease, innovative tissue sampling approaches are required. To overcome this challenge, neurosurgeons have started to create methods that permit tissue sampling during common procedures. These sampling approaches could then also be used to inform the pharmacological and surgical approaches that are used to treat the disease before attempting a full resection.

The standard diagnosis of a patient with treatment-resistant epilepsy involves the implantation of stereo-electroencephalography electrodes, which allow intracranial recordings of electrical activity, to identify the source of epileptogenic activity in the brain. In one study, DNA was obtained from the electrodes after they had been removed from the brain. Analysis of this DNA identified pathogenic somatic variants in the FCD-related genes *AKT3* and DEP domain containing 5, GATOR1 subcomplex subunit (*DEPDC5*). The brain regions carrying the highest fraction of cells with somatic mutations in these genes corresponded to the area of largest epileptogenic activity across individuals (*11*). This study highlights the great clinical potential of using electrodes to identify correlations between somatic genomic alterations and functional brain disruption. The approach could be extended to study how somatic variants and epileptic activity disrupt transcription: Highly epileptogenic regions could be compared with those with normal neuronal activity. More specific analyses for detecting somatic variants or designs of electrodes that optimize tissue sampling might provide higher-definition genomic maps of the brain.

Another promising approach is endovascular sampling of blood vessel lumens (*12*), although its application to somatic variant detection is yet to be explored. This approach makes use of diagnostic cerebral angiography, which involves the insertion of a catheter into the patient's vasculature and injection of dye to visualize blood vessel morphology. This procedure is commonly used in the diagnosis and staging of vascular pathologies from strokes to vascular malformations. A guidewire can accurately target a specific region of the lumen for sampling. In a study of patients with AVM, the gene expression profile of the lumen samples not only captured dysregulation in the expected MAPK pathway but also correlated well with the expression profile of the resected AVMs from these patients (*12*). This is a notable finding because this approach could allow for surgical planning based on the molecular profile of the vascular pathology and could be applied to any pathology for which diagnostic cerebral angiopathy is used. However, whether it could yield enough DNA for the detection of somatic variants remains an open question.

In addition to congenital or neurodevelopmental disorders, somatic mutations are being studied in neurodegeneration. A study in Alzheimer's disease performed single-cell whole-genome sequencing on neurons from post-mortem brain samples and found an increased rate of somatic mutations in neurons from individuals with Alzheimer's disease compared with controls. This increase was partly driven by higher amounts of oxidative damage to nucleotides (*13*). Some existing neurosurgical approaches are suited to sample tissue from individuals with neurodegenerative disorders—for example, using the tissue along the path of deep-brain stimulation electrodes in Parkinson's disease, or biopsies of the insertion site when implanting a ventricular shunt in a patient with normal-pressure hydrocephalus. However, these approaches have not yet been used for the detection of somatic mutations. Deep-brain stimulation is beginning to be used for the treatment of neuropsychiatric disorders, and the ability to sample brain tissue directly from this patient population represents an exciting new frontier in somatic mutation research.

To assess the somatic mutation landscape more fully across neurological disorders, it is vital to leverage consortia and biobanks to gather large numbers of surgically resected brain samples. Neurosurgeons and scientists from the Cleveland Clinic in the United States and the European Brain Bank Consortium were recently able to gather 474 neurosurgically resected brain samples from individuals with various forms of drug-resistant epilepsy. They performed

GRAPHIC: A. FISHER/*SCIENCE*

high-depth whole-exome sequencing to identify putative genes associated with epilepsy and to compare somatic mutation rates across disorders (*14*). The use of consortia could also help to obtain control tissue that is typically discarded—for example, intact blood vessels from resected lobectomies or nondiseased tissue from wide resections. Further efforts of consortia to bring neurosurgeons and scientists together have the potential to enhance understanding of these highly complex disorders (*15*).

The basic and translational research of somatic mutations in neurological disorders will require close collaborations across disciplines. From molecular biologists to bioinformaticians and clinicians, it is essential that there is an appreciation of the intricacies of the current treatment of these disorders. However, as the limits of what can be learned from post-mortem tissues are reached, it is critical that neurosurgeons lead innovation in tissue sampling and data analysis to maximize not only how much can be learned from patients but also how these findings can be effectively translated into better outcomes (see the figure). More imperative, training specialized neurosurgeon-scientists able to wield both classical neurosurgical micro-instruments as well as new tools, including "molecular scalpels" (pharmacotherapy or gene therapies) and the computational algorithms required to analyze these samples, will further catalyze the translation of somatic mutation research into benefits for patients. ■

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ACKNOWLEDGMENTS

E.A.M. is supported by the Harvard/MIT MD-PhD program (T32GM007753) and the Ruth L. Kirschstein NRSA F31 Fellowship (F31MH124292). C.A.W. was supported by grants from the NIH (R01-NS035129, R01NS032457, and R01AG070921), the Tan Yang Center for Autism Research, and by the Allen Frontiers Program. C.A.W. is an investigator of the Howard Hughes Medical Institute. K.T.K. was supported by the Yale-Rockefeller Centers for Mendelian Genomics; grants R01-NS109358, R01-NS111029, and R01-NS117609 from the National Institutes of Health; the Simons Foundation; March of Dimes; Hydrocephalus Association; and Rudi Schulte Research Institute. K.T.K. and C.A.W. have a US provisional patent (application no. 63/462,890) for the causal role of Ras/Raf/MAPK pathway overactivation in focal epilepsy pending (planned filing).

10.1126/science.adj2244

PSYCHOLOGY

Increasing policy support for reducing racial health disparities

Perceiving racial health disparities as unjust could catalyze or halt change

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In the United States, Black Americans experience disproportionately negative outcomes in domains as varied as wealth, employment, and health (*1*). However, efforts to obtain support for the policies necessary to tackle these disparities have had limited success, underscoring a critical need for effective interventions to change behavior. On page 1394 of this issue, Brown *et al.* (*2*) report that highlighting racial health disparities was more likely to prompt social media engagement and policy support compared with highlighting disparities in economic measures or belongingness (the feeling of being an accepted member of a group). The authors demonstrated that health disparities are catalyzing because health inequities contravene moral values that should never be violated and, consequently, evoke feelings of injustice. The premise is intriguing and could be used to increase policy support for reducing racial gaps.

Information about racial health disparities results in more social media engagement than information about other types of racial disparities.

Myriad factors contribute to racial disparities in health outcomes at both the interpersonal and structural levels. Interpersonally, clinicians often hold racial biases that influence clinical decision-making (e.g., chemotherapy or screening recommendations) (*3*) and behaviors (e.g., attempts at relationship building) (*4*). Structurally, policies can negatively affect Black Americans' access to and quality of health care (e.g., unequal allocation of resources, such as vaccines, and limited clinic locations and hours) (*5*). Policies that are ostensibly unrelated to health care also have widespread effects on health outcomes. For instance, economic and governmental institutions that restrict wealth for racially minoritized populations (e.g., charging higher interest rates when purchasing a home) can shape Black Americans' access to health care and transportation, insurance costs, and financial resources available for medical treatments (*6*).

Despite the persistence of health inequities, politicians, policy-makers, and the general public in the US have shown little interest in taking action to effectively mitigate these disparities and may actively work to maintain these gaps (e.g., continued efforts to repeal the Affordable Care Act and restrict Medicaid funding) (*7*). In their work, Brown *et al.* randomly assigned Americans to view information (messages and infographics) about racial disparities in health, economic factors, or belongingness in either experimental (online) or social media contexts. They found that information about health disparities increased perceived injustice, which enhanced social media engagement and support for policies to reduce disparities.

Given these findings, one temptation may be to frame all racial disparities in the context of their impact on health. Yet, decades of research, billions of dollars in funding, and

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