ESSAY

NEUROSCIENCE

One brain, many genomes

Single-cell genomic techniques shed light on somatic mutations in brain development and neurologic disorders

By **Gilad D. Evrony**

We each begin life as a single cell harboring a single genome, which—over the course of development—gives rise to the trillions of cells that make up the body. From skin cells to heart cells to neurons of the brain, each bears a copy of the original cell's genome. But as anyone who has used a copy machine or played the childhood game of "telephone" knows, copies are never perfect. Every cell in an individual actually has a unique genome, an imperfect copy of its cellular ancestor differentiated by inevitable somatic mutations arising from errors in DNA replication and other mutagenic forces (*1*). Somatic mutation is the fundamental process leading to all genetic diseases, including cancer; every inherited genetic disease also has its origins in such mutation events that occurred in an ancestor's germline cells. Yet how many and what kinds of somatic mutations accumulate in our cells as we develop and age has long been unknown and a blind spot in our understanding of the origins of genetic disease.

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While in the laboratory of Christopher Walsh at Boston Children's Hospital and Harvard Medical School, I became intrigued by reports of neurologic diseases caused by somatic mutations, including rare cases of epilepsy, neurodegeneration, intellectual disability, brain malformations, and autism spectrum disorder (*2*–*8*). There were also long-standing hypotheses in the field that somatic genetic diversity may be prevalent in the human brain (*9*). Still, the common view was that the brain operates from a unitary genome.

I wondered how much of a genomic patchwork is our brain? What kinds and how many somatic mutations are present, and do they affect brain function? Could somatic mutations underlie some of the neuropsychiatric diseases whose causes remain unknown?

Answering these questions, we recognized, would require development of a technology to sequence the vanishingly small amount of DNA (6 picograms) present in single brain cells. Any individual somatic mutation may be present in only a very small fraction of cells or even just one cell, making it undetectable by standard DNA sequencing, which mixes together DNA from thousands or millions of cells. Together with colleagues, I developed methods to sequence the genomes of single brain cells, allowing detection of even the rarest somatic mutations. Along with novel bioinformatic approaches developed with Eunjung Alice Lee in the lab of Peter Park, this provided the first systematic, genome-wide measurements of somatic mutation in the brain (*10*, *11*). Together with single-cell studies of cancer and sperm (*12*–*14*), this heralded the emergence of the field of single-cell genome sequencing, spurred by the confluence of whole-genome amplification technologies (*15*) and decreasing DNA-sequencing costs.

Our single-neuron genomics studies have identified remarkably diverse somatic mutations that reveal a wide gamut of mutation processes impacting the brain, from small point mutations and microsatellite polymorphisms to larger retrotransposon insertions, copy-number variants, and aneuploidy (*10*, *11*, *16*, *17*) (see the figure). Notably, we are finding that each type of mutation occurs at distinct rates and patterns (*10*, *11*, *16*–*18*). These and single-neuron studies by others provide a proof of principle for the systematic quantification of somatic mutations in any human tissue (*19*, *20*).

During my doctoral work, I was part of a team led by Ann Poduri that identified the first brain-specific somatic mutations causing neurologic disease (*21*). The disease in question was hemimegalencephaly, a rare congenital brain malformation in which one brain hemisphere is severely dysplastic

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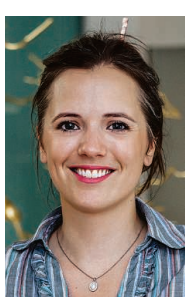
Gilad Evrony

Gilad Evrony received his undergraduate degree from the Massachusetts Institute of Technology. He served in the Intelligence Division of the Israel Defense Forces and completed an M.D. and Ph.D. at Harvard Medical School, with graduate research in the laboratory of Dr. Christopher Walsh at Boston Children's Hospital. Dr. Evrony is currently pursuing clinical training in pediatrics at Mount Sinai Hospital and continuing his research developing novel technologies for studying the brain and neuropsychiatric diseases.



FINALIST:

Anna Beyeler

Anna Beyeler received her undergraduate degree from the University of Bordeaux, in southern France, where she then completed her Ph.D. degree requirements. As a postdoctoral fellow at the Massachusetts Institute of Technology, she has been exploring the neural circuit mechanisms underlying rewarding and aversive memories. Dr. Beyeler is in the midst of establishing an independent research program aimed at identifying neural substrates of anxiety disorders at the University of Lausanne, Switzerland.

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FINALIST:

Arjun Krishnaswamy

Arjun Krishnaswamy received undergraduate and Ph.D. degrees from McGill University. As a postdoctoral fellow at Harvard University, he has been using molecular, electrophysiological, and genetic approaches to learn how developing neurons in the mouse retina choose synaptic targets and establish wiring patterns important for retinal function. Dr. Krishnaswamy will continue this line of research at McGill University.

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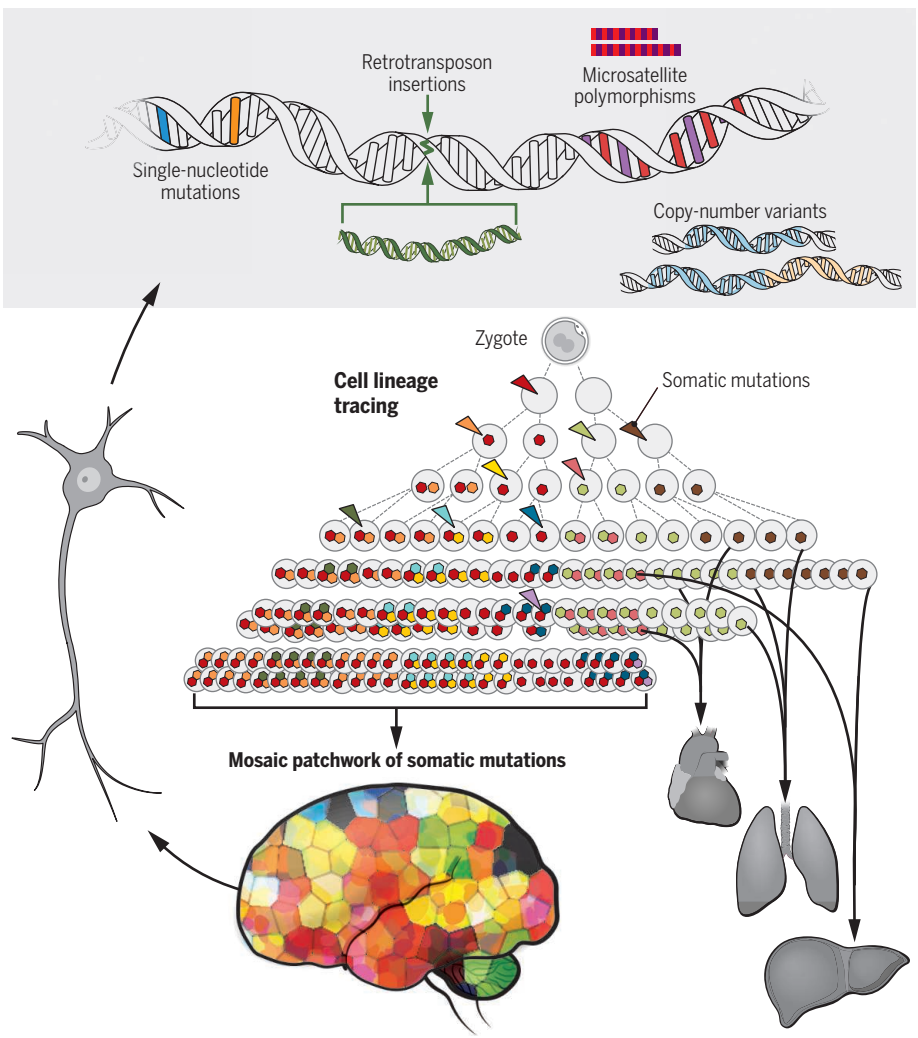
PHOTOS: (LEFT TO RIGHT) JAMES BAREHAM; ELIZABETH CLARK; SIRI POWELL

and grows too large, leading to intractable epilepsy. Using single-cell sequencing, we found that ~20 to 30% of both glial and neuronal cells carry the disease-causing mutations, allowing us to pinpoint neuroglial progenitors of the cortex as the cell type where the mutations occurred (*8*, *10*, *16*). This finding provides insight into the source of hemimegalencephaly and related focal neurologic diseases and may potentially be relevant to other epilepsies of unknown origin. Whereas hemimegalencephaly is visible by imaging, our findings suggest that radiographically invisible somatic mutations— for example, in ion channels or synaptic proteins—may be an occult cause of some neurologic diseases. As a result of these studies, numerous groups have begun systematic investigations of somatic mutations in neuropsychiatric disease.

A key tool in developmental biology is a technique known as lineage tracing, in which the fate of all the offspring of a particular cell (or group of cells) are tracked as the body forms. In animal models, this is achieved by tagging cells with fluorescent proteins or other invasive markers that cannot be used in humans. Somatic mutations, however, occur naturally and, as we were able to demonstrate, can serve as noninvasive lineage markers in humans (*1*, *11*, *17*).

Using somatic mutations identified by our single-neuron sequencing, we have been able to trace cell lineages in the human brain and reconstruct their proliferation and migration during development (*11*, *17*). Clear spatial patterns were revealed, including cell lineages distributed over an entire hemisphere at surprisingly low mosaicism indicating spatial mixing among early brain progenitors, as well as somatic mutations marking focally distributed lineages present in only one small (<1 cm^2) area of the frontal cortex (*11*, *17*). The latter pattern would seem to indicate that every brain is a mosaic patchwork of focal somatic mutations. It is therefore possible that rare, as-yet unrecognized brain disorders may exist in which a focal somatic mutation affects only one small region responsible for a particular cognitive function, while sparing the rest of the brain.

Our studies have generated a number of captivating new questions. Might the brain be particularly susceptible to harmful somatic mutations because of the unique interconnectedness of its cells? Would neurogenetic diseases—for example, genetic cases of autism—manifest differently if the inciting mutation were present in only one hemisphere, one lobe, or just one gyrus of the brain? Single-neuron sequencing may also be useful for measuring the extent to which neurons, whose genomes must function for decades, accumulate mutations with age and determining whether these mutations eventually impair function. We believe that single-cell genomics combined with single-cell epigenomics, transcriptomics, and proteomics will ultimately revolutionize our understanding of brain development and function. ■



Somatic mutation events. Single-cell genomic techniques enable the systematic measurement of somatic mutations that occur throughout normal body and brain development. Each somatic mutation event (triangles) is inherited by all offspring of the cell in which it occurred. Somatic mutations can therefore also be used as spontaneously occurring, endogenous markers for lineage tracing in human tissues, enabling reconstruction of patterns of progenitor proliferation and migration in the brain.

GRAPHIC: ADAPTED FROM G. D. EVRONY BY K. SUTLIFF/*SCIENCE*

REFERENCES AND NOTES

1. E. Shapiro, T. Biezuner, S. Linnarsson, *Nat. Rev. Genet.* **14**, 618 (2013).
2. J. G. Gleeson *et al.*, *Am. J. Hum. Genet.* **67**, 574 (2000).
3. C. Depienne *et al.*, *J. Med. Genet.* **47**, 404 (2010).
4. M. Topçu *et al.*, *Eur. J. Hum. Genet.* **10**, 77 (2002).
5. A. Alzualde *et al.*, *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **153B**, 1283 (2010).
6. P. Castronovo *et al.*, *Clin. Genet.* **78**, 560 (2010).
7. J. A. Beck *et al.*, *Hum. Mol. Genet.* **13**, 1219 (2004).
8. A. Poduri, G. D. Evrony, X. Cai, C. A. Walsh, *Science* **341**, 1237758 (2013).
9. A. R. Muotri, F. H. Gage, *Nature* **441**, 1087 (2006).
10. G. D. Evrony *et al.*, *Cell* **151**, 483 (2012).
11. G. D. Evrony *et al.*, *Neuron* **85**, 49 (2015).
12. N. Navin *et al.*, *Nature* **472**, 90 (2011).
13. Y. Hou *et al.*, *Cell* **148**, 873 (2012).
14. J. Wang, H. C. Fan, B. Behr, S. R. Quake, *Cell* **150**, 402 (2012).
15. F. B. Dean *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 5261 (2002).
16. X. Cai *et al.*, *Cell Rep.* **8**, 1280 (2014).
17. M. A. Lodato *et al.*, *Science* **350**, 94 (2015).
18. G. D. Evrony, E. Lee, P. J. Park, C. A. Walsh, *Elife* **5**, e12966 (2016).
19. M. J. McConnell *et al.*, *Science* **342**, 632 (2013).
20. Y. Wang, N. E. Navin, *Mol. Cell* **58**, 598 (2015).
21. A. Poduri *et al.*, *Neuron* **74**, 41 (2012).

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