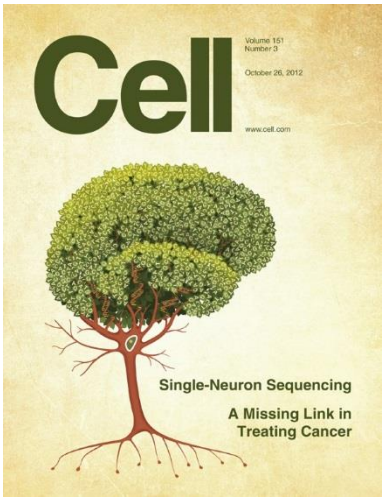
Single cell whole genome sequencing

Building on our longstanding interest in inherited mutations that influence cortical development, our lab has been a pioneer of the use single-cell sequencing to explore how somatic variants, resulting from mutations in cells of the body, shape the genome of neurons in the human brain.

Transposon insertions. We first focused our analysis on somatic retrotransposon insertions, to test the hypothesis that these "jumping genes" might be integrating into and disrupting important genes in human neurons, contributing to neuronal diversity (Evrony et al., 2012). We performed a targeted sequencing experiment specifically for L1 transposon insertion sites across 300 single normal human neurons, identifying insertions present in one or more single neurons but absent in bulk DNA isolated from the heart of the same individual. We found that mosaic transposon insertions did exist in the human brain, but that somatic mobilization was a rare event, with occasional insertions occurring during human brain development resulting in far less than one retrotransposon mutation per neuron. Moving beyond targeted analysis, subsequent single-cell whole-genome sequencing analysis generated in our lab (Evrony et al., 2015) confirmed this estimate. Our independent analysis of a second targeted study of transposon insertions performed by another group on a different set of brains further corroborated our findings (Evrony et al., 2016).



2012 Evrony et al. Cell Cover Photo

Large-scale variation. The loss or gain of entire chromosomes, called aneuploidy, or the deletion or duplication of large regions of chromosome, called copy number variation (CNV), are both potent drivers of human genetic diseases in a variety of contexts, including autism, schizophrenia, and Down's syndrome. In order to test whether mutations at this scale occur in the somatic genome of human neurons, we utilized single-cell sequencing. We found that aneuploidy was very rare in normal human neurons, occurring at a maximum rate of 5% of the cells in the brain, while CNV was more common, with neurons often bearing one or more large (megabase-scale) CNV (Cai et al., 2014).

2015 Lodato, Woodworth, Lee et al. Science Cover Photo

Point mutations. After we defined generally low rates of transposon insertions, aneuploidy, and CNV in human neurons, we were interested in quantifying a potentially more common form of mutation in the brain, point mutations which result in single nucleotide variants (SNVs). In order to characterize somatic SNVs in the human brain, we applied existing bioinformatic tools to single-cell, whole genome sequencing data from 36 single neurons from three normal individuals (Lodato et al., 2015). This initial study provided the first estimate of the somatic SNV rate in normal cells, and identified patterns of mutation reflecting known mutational processes, for example a signature transcriptional damage impacting the neuronal genome. More recently, we used a new, custom algorithm designed specifically for identifying somatic SNVs in single-cell data to analyze somatic mutation accumulation during aging in neurodegenerative disease (Lodato et al., 2017). We showed that neurons of the prefrontal cortex contain hundreds of somatic SNVs per genome at birth, increasing at a rate of ~25 per year per genome, reaching levels in the thousands in old age, a phenomenon that we refer to as "genosenium." Neurons in the dentate

gyrus of the hippocampus accumulate sSNV even faster, ~40 SNV per year, suggesting that temporal lobe neurons may be differentially susceptible to age-related decline. Furthermore, we found that somatic SNVs can be analyzed by patterns of base-pair substitution, analogous to cancer mutations, and this analysis revealed at least three neuronal mutational signatures arising from distinct sources: one being congenital, another age-related, and a third potentially related to oxidative damage.

~Michael Lodato, PhD

References

1. Cai, X., Evrony, G.D., Lehmann, H.S., Elhosary, P.C., Mehta, B.K., Poduri, A., and Walsh, C.A. (2014). Single-Cell, Genome-wide Sequencing Identifies Clonal Somatic Copy-Number Variation in the Human Brain. Cell Rep *8*, 1280-1289.
2. Evrony, G.D., Cai, X., Lee, E., Hills, L.B., Elhosary, P.C., Lehmann, H.S., Parker, J.J., Atabay, K.D., Gilmore, E.C., Poduri, A.*, et al.* (2012). Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain. Cell *151*, 483-496.
3. Evrony, G.D., Lee, E., Mehta, B.K., Benjamini, Y., Johnson, R.M., Cai, X., Yang, L., Haseley, P., Lehmann, H.S., Park, P.J.*, et al.* (2015). Cell lineage analysis in human brain using endogenous retroelements. Neuron *85*, 49-59.
4. Evrony, G.D., Lee, E., Park, P.J., and Walsh, C.A. (2016). Resolving rates of mutation in the brain using single-neuron genomics. Elife *5*.
5. Lodato, M.A., Rodin, R.E., Bohrson, C.L., Coulter, M.E., Barton, A.R., Kwon, M., Sherman, M.A., Vitzthum, C.M., Luquette, L.J., Yandava, C.*, et al.* (2017). Aging and neurodegeneration are associated with increased mutations in single human neurons. Science.
6. Lodato, M.A., Woodworth, M.B., Lee, S., Evrony, G.D., Mehta, B.K., Karger, A., Lee, S., Chittenden, T.W., D'Gama, A.M., Cai, X.*, et al.* (2015). Somatic mutation in single human neurons tracks developmental and transcriptional history. Science *350*, 94-98.