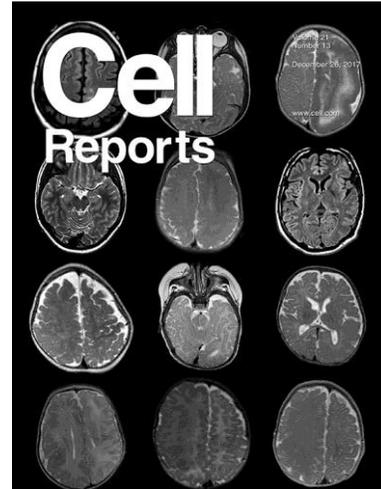


## Somatic mutations cause a variety of human brain diseases

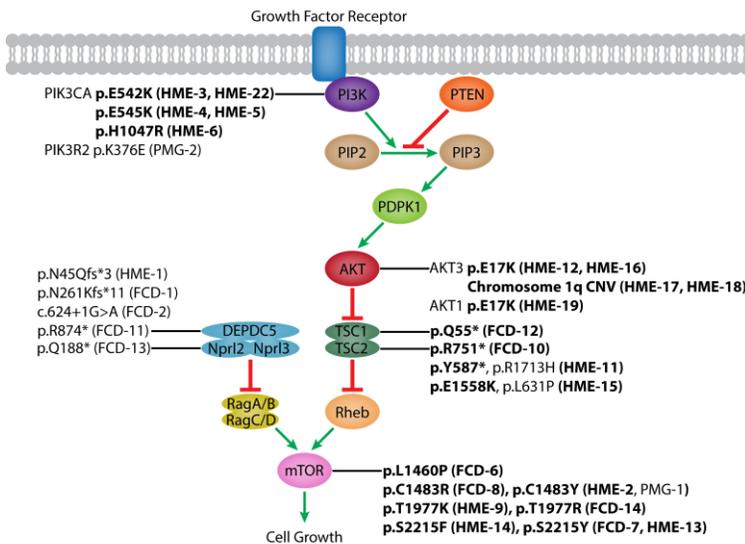
Traditionally, our lab has studied the role of inherited and *de novo* germline mutations, which are present in every cell of an affected individual, in human brain diseases. More recently, we have used next generation sequencing and single cell sequencing to discover the important role of somatic mutations, which occur post-zygotically and are present in only a subset of the cells of an affected individual, in a variety of neurodevelopmental diseases.

### Focal cortical dysplasia and hemimegalencephaly

Focal cortical dysplasia (FCD), characterized by a small region of abnormal cortex, and hemimegalencephaly (HME), characterized by abnormal enlargement of a cerebral hemisphere, are important causes of intractable childhood epilepsy. Given the focal lesions seen on brain imaging, FCD and HME have long been hypothesized to be due to somatic mutations (Poduri et al., 2013). We initially studied resected brain tissue from eight HME cases and reported the first identification of a genetic cause for HME, finding one HME case with a somatic activating *AKT3* point mutation and two HME cases with somatic chromosome 1q copy number increases (which includes the *AKT3* locus) (Poduri et al., 2012). Single cell sequencing showed that at least one of the somatic copy number increases is actually a somatic chromosome 1q tetrasomy (Cai et al., 2014). These somatic mutations lead to abnormal activation of the mTOR pathway, which is critical for regulating protein synthesis and cell growth.



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**Schematic of the mammalian target of rapamycin (mTOR) pathway and pathogenic mutations identified by our lab. Somatic mutations are in boldface. FCD: focal cortical dysplasia; HME: hemimegalencephaly, PMG: polymicrogyria.**

Over the past six years, we have studied over 100 cases of FCD, HME, and related conditions using targeted next generation sequencing of mTOR pathway genes (D'Gama et al., 2015a; D'Gama et al., 2017). Overall, we have identified a genetic cause in 41% of the cases in which resected brain tissue was available and in 0% of the cases in which brain tissue was not available, suggesting that the causative somatic mutations occur relatively late in embryonic development and are “brain only”. The causative mutations include “single hit” somatic activating mutations in positive regulators of the mTOR pathway and germline loss-of-function mutations, in some cases coupled with “second hit”

somatic loss-of-function mutations, in negative regulators of the mTOR pathway. Using single cell sequencing and mouse models, we showed that somatic mutations in neurons, especially excitatory neurons, are critical for FCD and HME pathogenesis. Our studies suggest that FCD and HME are part of a continuum of cortical dysplasias caused by somatic mutations that activate the

mTOR pathway in dorsal telencephalic progenitors, with the lesion size dependent on the progenitor cell and developmental time when the mutation occurs.

### **Autism spectrum disorder**

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder characterized by abnormalities in social interaction and communication and restrictive and repetitive behaviors. ASD is clinically and genetically heterogeneous, and inherited and *de novo* germline mutations have been shown to contribute to ASD risk. We hypothesized that somatic mutations may also contribute to ASD risk, and studied brain tissue and blood from ASD cases to test this hypothesis. Our initial study used targeted next generation sequencing of 78 known ASD candidate genes to analyze 55 postmortem ASD brains (D’Gama et al., 2015b). We identified deleterious somatic mutations in two ASD cases and one Fragile X premutation case, showing that somatic mutations with the potential to contribute to ASD risk occur in ASD brain. We then reanalyzed whole exome sequencing data from almost 6,000 simplex ASD families using optimized calling algorithms to detect somatic mutations (Lim et al., 2017). We showed that postzygotic mutations account for 7.5% of all *de novo* mutations, and that damaging nonsynonymous postzygotic mutations in critical exons of prenatally expressed genes are significantly enriched in ASD cases compared to controls. Interestingly, genes with these mutations were significantly enriched in the amygdala, which is a brain region important for memory and emotions. Thus, in addition to germline mutations, somatic mutations also appear to contribute to ASD risk and may provide insight into the cell types and brain regions critical for ASD pathogenesis.

~ Alissa D’Gama, PhD

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