Human Pluripotent Stem Cell and Mouse Models of PCDH19 Clustering Epilepsy

Protocadherin-19 (*PCDH19)*-clustering epilepsy (PCE) is a severe developmental and epileptic encephalopathy and one of the most common monogenic epilepsies. PCE is characterized by cognitive impairment and intractable seizure clusters with onset in the first few years of life. *PCDH19* is an X-linked gene that encodes a transmembrane cell adhesion molecule critical for cell-cell interactions during brain development. PCE affects females and rare mosaic males, while hemizygous males expressing only mutant *PCDH19* do not develop epilepsy. A leading hypothesis to explain this phenomenon is cellular interference (CI) associated with random X-inactivation (or mosaic mutations), in which cells expressing only wild type and those expressing only mutant *PCDH19* fail to interact properly during brain development. We sought to model CI using human brain organoids and mice. We first generated dorsal (excitatory) brain organoids that were mosaic for *PCDH19* by mixing homozygous *PCDH19* knockout and wildtype human embryonic stem cells and found that this replicated the CI phenotype. Mosaic ventral (inhibitory) brain organoids also displayed CI, although it was less marked than in dorsal organoids. Additional phenotypes of mosaic dorsal organoids included alterations of N-cadherin expression, neural progenitor proliferation and early neurogenesis. To model PCE in mice, we crossed *PCDH19*-null female mice with X-linked GFP reporter males. Female offspring ($PCDH19^{+/-}/X^{GFP}$) express GFP on the wild-type allele and no fluorescent marker on the *PCDH19*-null allele, allowing for easy visualization of wild-type and mutant cell populations. Brains of PCE mice revealed a striking segregation pattern of *PCDH19*+ and null neurons in the cortex, hippocampal CA1 region and in interneuron progenitors in the ganglionic eminences. Prolonged EEG recordings did not reveal spontaneous seizures in PCE or control mice. However, we found a lowered seizure threshold and more severe seizures following hyperthermia exposure in PCE mice compared to age matched controls. We also found a decrease of parvalbumin-expressing hippocampal interneurons in PCE mice. Together, these *in vitro* and *in vivo* PCE models should be useful for exploring disease mechanisms and testing precision therapies.