Generation of a cortico-thalamic human assembloid system for phenotypic validation

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Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene cause CDKL5 Deficiency Disorder (CDD), a rare and severe neurodevelopmental disorder characterized by early-onset, intractable epilepsy, developmental delay, motor dysfunction, and features of autism. Currently, there are no effective treatments for CDD, and existing animal models have not fully replicated key aspects of the disease, especially spontaneous seizures. Therefore, developing advanced human-based models is essential for understanding the molecular basis of CDD and accelerating the discovery of therapies. We previously generated and characterized induced pluripotent stem cells (iPSCs) from CDD patients with distinct CDKL5 loss-of-function mutations, along with matched isogenic controls. Differentiating these lines into cortical neurons and cortical organoids (COs) revealed multiple molecular, cellular, and functional network changes associated with the CDKL5 mutation. In brief, neurons from CDD showed abnormal synaptogenesis, glutamatergic synapses, and dendritic structure. Additionally, electrophysiological analysis of CDD CO uncovered an unusual pattern of early hyperexcitability followed by reduced network activity during development. Interestingly, some of these phenotypes differ from those observed in CDKL5 knockout mice, indicating species-specific disease mechanisms and underscoring the need for human-relevant models of CDD. Given the known role of the cortico-thalamic (CTh) circuit in epilepsy, we hypothesize that impaired axon connectivity between the cortex and thalamus, in the absence of functional CDKL5, contributes to spontaneous seizures. Therefore, we plan to model the CTh circuit by fusing cortical and thalamic organoids (ThO) into CTh assembloids (CThA) and analyze their function in the context of CDD. This innovative human model will offer new insights into the disease's underlying mechanisms and serve as a human-relevant platform for testing therapies.