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# Development has the answer: Unraveling psychiatric disorders via thalamocortical organoids

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**Dissecting the role of the thalamus in neuropsychiatric disorders requires new models to analyze complex genetic interactions. In this issue of *Cell Stem Cell*, Shin et al. use patient-derived thalamocortical organoids to investigate 22q11.2 microdeletion impact on thalamic development, revealing significant transcriptional dysregulation linked to psychiatric disorders.**

Mounting evidence underscores the crucial link between developmental processes and psychiatric disorders. Disruptions in neuronal circuit formation during development can lead to significant functional consequences associated with these conditions. While investigations into the molecular and cellular basis of neurodevelopmental psychiatric disorders have traditionally focused on the cerebral cortex, research on subcortical structures remains limited. The thalamus plays a pivotal role as a central hub controlling incoming sensory input to the cortex, regulating outgoing motor signals, and supervising overall cortical activity. Its intricate organization comprises numerous circuits with complex connections to nearly all cortical regions. These connections not only dynamically impact communication between subcortical and cortical regions but also play a crucial role in shaping signaling between different areas of the cortex. As a result, the connectivity between the thalamus and the cerebral cortex significantly influences fundamental processes relevant to the behavioral symptoms observed in neurodevelopmental disorders.[1] In this issue of *Cell Stem Cell*, Shin et al. employ thalamocortical organoids as a tool to unravel the intricate interplay between genetics and thalamus development.[2] The study focuses on subcortical regions by examining the influence of the 22q11.2 microdeletion on the early development of the human thalamus and the formation of the thalamocortical tract. Remarkably, the 22q11.2 microdeletion is linked to an increased risk of various neurodevelopmental psychiatric disorders. In contrast to prior studies primarily focused on cortical structures,[3,4] this research sheds light on subcortical structures often overlooked in the context of 22q11 deletion syndrome (22q11DS).

In their study,[2] the researchers employed cutting-edge single-cell sequencing techniques to meticulously dissect the transcriptional landscape of thalamic organoids derived from both control and individuals with 22q11DS. This approach unveiled striking distinctions, revealing a robust enrichment of genes associated with neuropsychiatric disorders in 22q11DS organoids. Among these genes, the transcription factor *FOXP2* emerged as the most prominently upregulated gene in thalamic glutamatergic neurons of 22q11DS organoids. To further explore the functional implications of elevated FOXP2 expression, the authors conducted a series of innovative experiments. These included FOXP2 gain-of-function experiments using viral vectors in thalamic organoids, as well as co-culture experiments involving both thalamic and cortical organoids. Elevated FOXP2 expression, a direct consequence of the 22q11.2 microdeletion, was identified as a key driver of thalamic axon overgrowth and alterations in thalamocortical connectivity that have been previously implicated in neuropsychiatric disorders.[1,5]

Following this alteration in connectivity, the researchers delved deeper by profiling FOXP2 binding sites. This analysis revealed differential expression in axon guidance cues, notably the downregulation of ROBO2. The role of this guidance cue receptor was explored through additional experiments, wherein the knockdown of ROBO2 in control thalamic organoids mirrored the axon overgrowth observed in 22q11DS organoids. Employing CRISPR technology in organoids and co-culture studies, the researchers demonstrated the interplay between these molecular players. Collectively, these findings offer a compelling narrative: the increase in expression of FOXP2 in 22q11DS thalamic organoids leads to axon overgrowth, a phenotype likely mediated by the downregulation of ROBO2. In the developing thalamocortical tract, the expression of ROBO2 in the thalamus plays a pivotal role in the topographical sorting of growing axons. Beyond ROBO2, the Shin et al. study also identifies additional targets of FOXP2 implicated in axon development, showing distinct expression patterns in 22q11DS thalamic neurons. Notably, the involvement of well-characterized thalamic developmental genes reinforces the link between developmental processes and psychiatric disorders.

This research by Shin et al. was conducted through the integration of human organoid cultures. Three-dimensional cell culture models derived from human cells, such as organoids, continue to provide unique insights into the developing brain and the pathogenesis of diseases. Organoid cultures allow for real-time observation and the creation of patient-specific disease models, providing access to a developmental window that traditional models struggle to capture.[6] Recent pioneering studies have expanded the possibilities of organoid culture by employing regionalized cultures to recapitulate specialized brain areas, with a particular focus on the thalamus. Kiral et al. increased the resolution of regionalization achievable by successfully generating cell types from individual thalamic nuclei and subsequently producing nucleus-specific phenotypes by manipulating disease-associated genes specific

to each nucleus.[7] Furthermore, the assembly of distinct regionalized organoids into complex co-cultures has emerged as a powerful tool to further investigate the developing connectivity between brain regions, neurocircuitry, and diseases. While the applications of assembloid cultures are rapidly expanding, the field is still relatively nascent. Xiang et al. pioneered the modeling of developing thalamocortical circuitry by fusing thalamic and cortical organoid cultures, demonstrating transcriptionally different identities for the thalamus and cortex and successfully establishing reciprocal connections between these two regions.[8] This remarkable capability to explore human-specific aspects of neurodevelopment, particularly connectivity, has led to significant discoveries linking neuropsychiatric disorders with subcortical structures.[9]

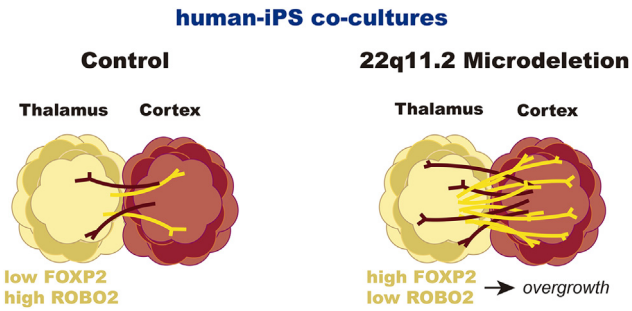


**Figure 1. Organoid co-culture model of 22q11.2 microdeletion**
Co-cultures of human induced pluripotent stem cell (iPSC)-derived thalamocortical organoids with the 22q11.2 microdeletion exhibit thalamus-specific upregulation of FOXP2. This upregulation inhibits ROBO2 expression, leading to the overgrowth of thalamocortical axons and a subsequent increase in corticothalamic projections.

Piece-wise assembly of the neural circuitry has allowed the observation of region-specific contributions to the network. For instance, Miura et al. investigated functional cortico-striatal connectivity in 22q13.3 deletion syndrome using cortico-striatal assembloids derived from patients, revealing altered activity patterns in cortical projection neurons. In a recent preprint, Kim et al. demonstrated functional reciprocal connectivity by employing optogenetic stimulation on thalamocortical assembloid cultures, noting striking differences in thalamocortical circuitry in assembloids generated with CACNA1G pathogenic mutants—a gene associated with intellectual disability and schizophrenia.[10] In this article, Shin et al. distinctively showcase a mechanism by which thalamus-specific FOXP2 overexpression, associated with 22q11DS, leads to increased axon growth, resulting in a consequential increase of corresponding reciprocal cortical projections (Figure 1). Exploring further the molecular pathways responsible for this regional specificity could be a focus of future studies. Similarly, there is a need for further inquiry into additional FOXP2 targets, including several other dysregulated axon guidance molecules, and their potential impact on axonal phenotypes. Lastly, exploring potential defects in thalamic organoid spontaneous neuronal activity is crucial, given the known role of the 22q11.2 microdeletion in inducing excitability changes in cortical organoids.[3]

In conclusion, Shin et al., utilizing state-of-the-art models of developing neurocircuitry, have revealed a mechanism through which phenotypes associated with neuropsychiatric disorders emerge during development. This pioneering study not only delves into the intricacies of neurodevelopmental disorders but also underscores the transformative potential of stem cell research in elucidating the underlying mechanisms of psychiatric conditions. Their meticulous exploration using thalamocortical organoids opens up promising avenues for deciphering the complex genetic and developmental interplay contributing to these disorders. By shedding light on the specific role of the thalamus and its molecular players, this work holds significant promise for future exploration and therapeutic interventions in the complex landscape of neurodevelopmental psychiatric disorders.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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