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Identifying SETBP1 haploinsufficiency molecular pathways to improve patient diagnosis using induced pluripotent stem cells and neural disease modelling

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About the Authors:
The latest SETBP1-HD research published from the land down under was spearheaded by a joint effort between The Kids Research Institute of Australia, The University of Western Australia, and Rare Care Centre of Perth Children's Hospital. Together, a skilled team of computational biologists and translational geneticists, including Dr. Lassmann and SETBP1 Quarterly Alliance Members Drs. Shaw and Fear, use CRISPR/Cas9 genome editing technology in pluripotent stem cells to uncover the molecular underpinnings of specific variants in SETBP1-HD.

Background:
While there is a clear link between neurodevelopmental disorders and loss of function mutations in SETBP1, the specific role of SETBP1 in neural development remains to be uncovered. In this article, the researchers use CRISPR/Cas9 gene editing to introduce two pathogenic truncating mutations SETBP1 p.Glu545Ter and p.Tyr1066Ter and a variant of uncertain significance (VUS) into induced pluripotent stem cells (iPSCs). Using a combination of visual and computational methods, the team studied the impact of the variants on molecular and morphological changes as the cells differentiated or changed from unspecialized iPSCs to neural progenitor cells (NPCs).

Main Findings:
Visual investigation of the NPCs revealed reduced SETBP1 staining in pathogenic variant lines compared to the VUS and controls. Looking past morphology (the shape and size of cells), OCT3, PAX6, and NESTIN (markers of immature and mature NPCs, respectively) are all found to be expressed at the expected times in differentiation, confirming that the iPSCs are maturing to NPCs. Biological pathway analysis of differentially expressed genes in all three lines compared to controls revealed changes in cellular pathways relevant to forebrain development, RNA polymerase II (responsible for copying genetic code from DNA to generate a template for protein synthesis), DNA binding, WNT/b-catenin signaling, and a novel relationship between GATA2 and SETBP1 in neurological disorders (which has a previously known role in blood cell

differentiation in other diseases). The three variants, while exhibiting overlapping neural cell phenotypes, also showcased unique differences, underscoring the importance of specific variants potentially driving phenotypic divergence.

What does this mean for SETBP1-HD:
The findings here further support previous research (see Cardo et al. 2023 below) identifying the involvement of the WNT/b-catenin pathway and DNA-binding RNA-polymerase II activity, which is shown to be disrupted in SETBP1-HD neural cells. This is also the first research implicating a role for GATA2 in SETBP1-HD within the context of neurological disorders (a relationship between GATA2 and SETBP1 has been previously revealed in myeloid malignancies with respect to somatic mutations in SETBP1). The research in this study proves that iPSCs can accurately model single nucleotide changes in SETBP1 and provide disease-relevant models for SETBP1-HD. While NPCs are immature cells that are still relevant to the SETBP1-HD phenotype, additional studies using mature neurons would be beneficial for further understanding the mechanisms underlying SETBP1-HD phenotypes. The models created in this work can be induced to form variant-specific brain organoids to study the molecular and cellular mechanisms in neural cell specification. Insights into the molecular pathways altered in SETBP1-HD pave the way for future development of targeted treatment for the community. The research here emphasizes a spectrum of cellular mechanisms that exist within SETBP1-HD that is likely variant-specific. Future research into these three, as well as the impact of additional variants, will continue to aid our molecular understanding of SETBP1-HD.

Accessing the Review article:
The full review article titled “Identifying SETBP1 haploinsufficiency molecular pathways to improve patient diagnosis using induced pluripotent stem cells and neural disease modelling” published in *Molecular Autism* on September 30, 2024, can be accessed here: https://doi.org/10.1186/s13229-024-00625-1

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