Investigating the Impact of a Novel Mutation in DNA Topoisomerase Beta on Early Neural Brain Development

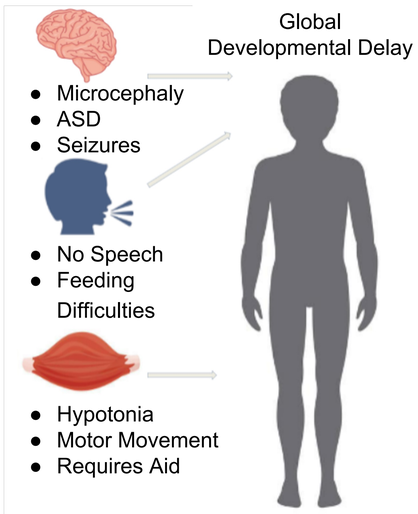
Skyla Witt, Glen Sequiera, Oguz Ozgoren, Costanza Bardile, Millan Patel, Mahmoud A. Pouladi

Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, British Columbia Children's Hospital Research Institute, University of British Columbia

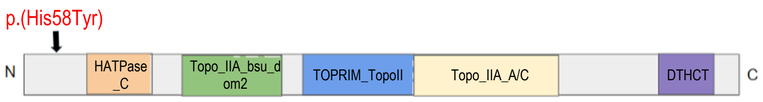
Background

A *de novo* variant in ***TOP2B***, encoding type II topoisomerase beta, has been identified in several patients with intellectual disability, **autism spectrum disorder**, **seizures** and **microcephaly**.

TOP2B plays a key role in regulating transcription of many genes important for nervous system development and is seen to affect neurite length and differentiation when differentially expressed.



*How this mutation, which results in a histone-to-tyrosine substitution **(p.His58Tyr)**, causes disease is currently unknown.*



Multiple sequence alignment shows the mutated residue to be highly conserved across species.

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TOP2B_H58Y_HUMAN RVYQKKTQLEYILLRPDTYIGS
TOP2B_HUMAN      RVYQKKTQLEHILLRPDTYIGS
TOP2B_MOUSE      RVYQKKTQLEHILLRPDTYIGS
Q14TE9_RAT       RVYQKKTQLEHILLRPDTYIGS
F1RS45_PIG       RVYQKKTQLEHILLRPDTYIGS
Q1LUT2_DANRE     RIYQKKTQLEHILLRPDTYIGS
TOP2_YEAST       DKYQKISQLEHILKRPDTYIGS
TOP2_DROME       QMYQKKSQLEHILLRPDSYIGS
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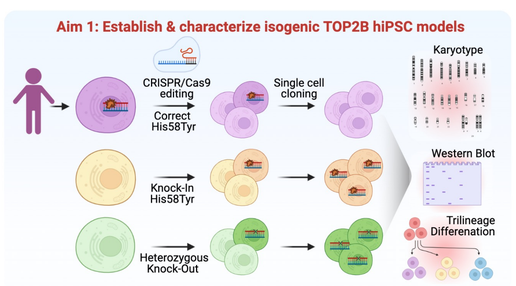
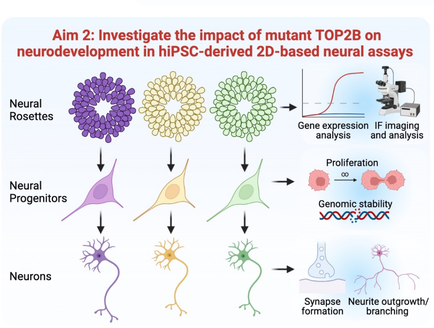
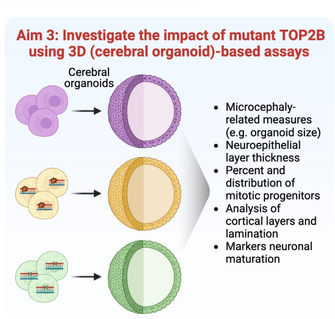
The **objective** of this study is to

Investigate the functional role of TOP2B p.His58Tyr substitution and its effect on neural development

Experimental approach



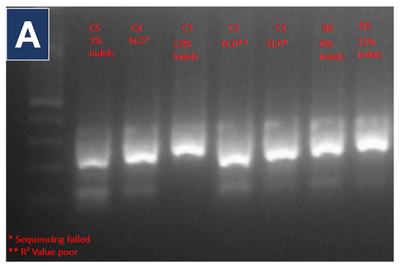




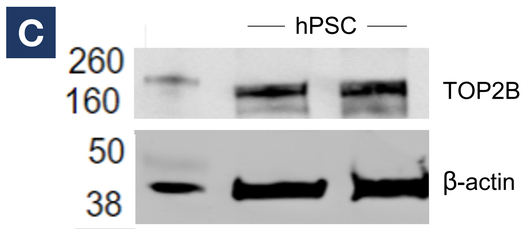
Results

Heterozygous TOP2B mutant hPSC lines established using CRISPR/Cas9

- Multiple cell lines have been established **(A)** with several indels **(B)** within the promoter / exon 1 of *TOP2B*, which are predicted to knockout the gene within one allele.

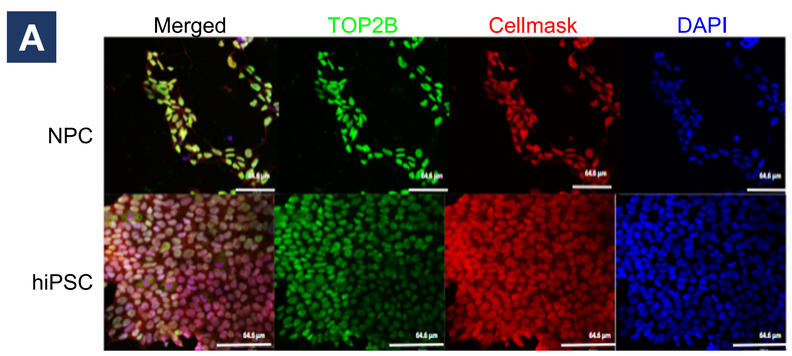


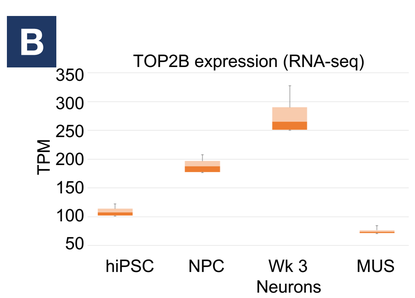
- Once subcloning is completed, TOP2B protein levels will be evaluated using Western Blot analysis **(C)**.

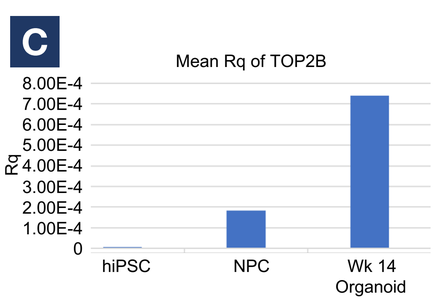


TOP2B is expressed in hPSCs and derived neural cells

- TOP2B is seen to have an increased expression later in Neural Development within mouse and human fetal samples.
- Consistent with this observation, RNA Seq **(B)**, RT-qPCR **(C)** shows TOP2B has an increased protein expression in neural tissue. This expression can also be seen with immunohistochemistry (**A**).



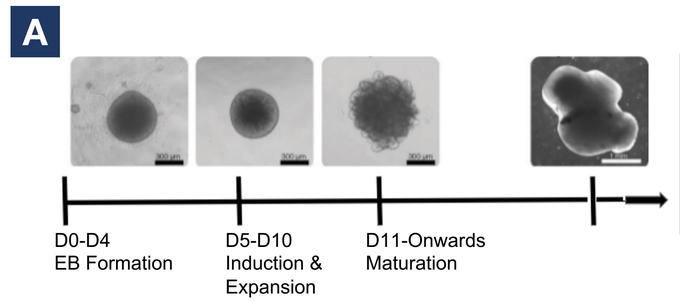


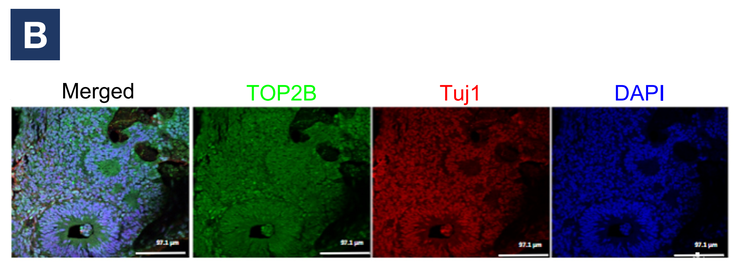


- Cell lines made in Aim 1 will be differentiated into neural tissue to determine the effect the TOP2B variants have on neurodevelopment

Cerebral Organoids Will Model The Microcephaly Phenotype

- Cerebral organoids **(A)** have been created with the control lines and have shown the presence of TOP2B protein expression and radial glial formation **(B).**





- Cerebral organoids will be used to model the microcephaly phenotype and to investigate the impact of the TOP2B H58Y and heterozygous knockout mutations on cortical layering and neuronal lamination.

Conclusion

The creation of isogenic TOP2B H58Y and heterozygous knockout hPSC lines provides an opportunity to investigate the effects of *TOP2B* during neural development using monolayer and 3D neural models. We anticipate that the study will provide insights into the functional effects of the *TOP2B* variant p.His58Tyr of relevance to patients with neurodevelopmental abnormalities harboring mutations in *TOP2B*.

Acknowledgments

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