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| GABA/Glutamate neuron differentiation imbalance and increased AKT/mTOR signalling in CNTNAP2 -/- cerebral organoids  **Kleanthi Chalkiadaki** 1, **Elpida Statoulla** 1#, **Maria Zafeiri 1**, **Georgia Voudouri** 1, **Alexandra Typou** 1, **Theoklitos Amvrosiadis** 2, **Niki Theodoridou** 1, **Dimitrios Moschovas** 3, **Apostolos Avgeropoulos** 3, **Martina Samiotaki** 4, **John O. Mason** 2, 5 and **Christos G. Gkogkas** 1\*  1 Biomedical Research Institute, Foundation for Research and Technology-Hellas, University Campus, 45110 Ioannina, Greece  2 Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom  3 Department of Materials Science Engineering, University of Ioannina, 45110, Ioannina, Greece  4 Biomedical Sciences Research Center “Alexander Fleming”, 16672, Vari, Greece  5 Simons Initiative for the Developing Brain, University of Edinburgh, Edinburgh, United Kingdom  # Presenting author: Elpida Statoulla, email: [elpida\_statoulla@bri.forth.gr](mailto:elpida_statoulla@bri.forth.gr" \t "_blank)  \* Corresponding author: Christos Gkogkas, email: <cgkogkas@bri.forth.gr> |

abstract

The polygenic nature of autism spectrum disorder (ASD) requires the identification of converging genetic pathways during early development to elucidate its complexity and varied manifestations. We developed a human cerebral organoid model from induced pluripotent stem cells (iPSCs) with targeted genome editing, to abolish protein expression of the Contactin Associated Protein-like 2 (CNTNAP2) ASD risk gene. CNTNAP2-/- cerebral organoids displayed accelerated cell cycle, ventricular zone disorganisation and increased cortical folding. Proteomic analysis revealed disruptions in Glutamatergic/GABAergic synaptic pathways and neurodevelopment and transcriptomic analysis revealed differentially expressed genes (DEG) belonging to inhibitory neuron-related gene networks. Interestingly, there was a weak correlation between the two datasets, suggesting nuanced translational control mechanisms. Along these lines we found upregulated Protein Kinase B (Akt)/mechanistic target of rapamycin (mTOR) signalling in CNTNAP2-/- organoids. Spatial transcriptomics analysis of CNTNAP2-/- ventricular-like zones demonstrated pervasive changes in gene expression, implicating upregulation of cell cycle regulation pathways, synaptic and Glutamatergic/GABAergic pathways. We noted a significant overlap of all Day 30 (D30) organoids ‘omics datasets DEG from idiopathic ASD (macrocephaly) iPSC-derived telencephalic organoids, where Forkhead box protein G1 (FOXG1) was upregulated. Moreover, we detected increased Glutamate decarboxylase 1 (GAD1) and decreased T-Box Brain Transcription Factor 1 (TBR1) expressing cells, suggesting altered GABAergic/Glutamatergic neuron development. These findings potentially highlight a shared mechanism in the early cortical development of various forms of ASD, further elucidate the role of CNTNAP2 in ASD pathophysiology and cortical development and pave the way for targeted therapies using cerebral organoids as preclinical models.