

Assessing juvenile batten disease on brain development in *in vitro* 3D organoid models

Introduction

Neuronal ceroid lipofuscinosis (NCL) is a complex, rare, and fatal disease belonging to a larger group of lysosomal storage disorders (LSD) that presents itself with nervous system symptoms including dementia/cognitive decline, intellectual disability, vision impairments, and epilepsy. They are identified into different types typically defined by time of onset, but more recent classification relies on identification of their genetic causes as a predominantly autosomal recessive disease. Batten disease – or *CLN3* disease – is another name given commonly to JNCL, but this terminology has expanded to be inclusive of other forms of NCL in the recent years.¹ Batten disease affects around 1 in 12,500 people in some populations, and albeit rare, childhood-onset NCL is the most common neurodegenerative disorder in childhood. Genetic mutations that cause disruptions to the cell's lysosomal function of breaking down wastes, proteins, lipids, and other molecules for discarding or recycling results in the abnormal accumulation of cellular junk and lipofuscin buildup. These genes involved are designated as *CLN* genes (ex. *CLN1*, *CLN2*, ... *CLN10*), and these different mutations lead to different severity and disease progression rates. Currently, there are no known cures and only limited treatment options looking to manage and reduce symptoms, though an enzyme replacement therapy for *CLN2* disease was approved by the FDA in 2017.²

Dr. Gomez-Giro, et al focuses on understanding the neurodevelopmental process of JNCL in their recent publication that generated and utilized healthy hiPSCs introduced with a disease-causing c.1054C→T pathologic variant into the *CLN3* gene. Leveraging this model, they



generated *in vitro* non-neuronal 2D endothelial and 3D cerebral brain organoid models with the intention to not only recapitulate these neurodevelopment processes in the human context but establish a model that would be reliable and predictive to study JNCL outside animal models. This would help enable further research on this rare disease as it would also help overcome the limitation of accessing patient material and allow for the generation of various cell types to study pathogenicity and cell- and tissue-specific mechanism of the disease. In this highlighted work, their model was used to primarily assess disease-specific phenotypic imaging, as well as whole-transcriptome RNA-seq for differential gene regulatory network (GRN) and expression analysis (DEA), and metabolomics assessment using non-targeted gas chromatography mass spectrometry (GC-MS).³

They proceed to describe the method behind the generation and confirmation of the *CLN3* mutant vs control isogenic pair, which led to the identification of a novel splicing variant resulting in a premature termination codon (PTC) that produces a truncated cDNA amplicon; this phenomenon was confirmed to exist in PBMCs extracted

from a patient sample harboring the same mutant variant. They indicate that this constitutive mutation and truncation could be occurring in other non-sense *CLN3* mutations at exon boundaries and may be a venue for therapeutic targeted strategies. Interestingly, she shared with us her hypothesis behind the potential link this alternative splicing and subsequent exon-skipping event may have in contributing to the severe failure of around half these mutant cerebral organoids to differentiate and develop: these events may be occurring with different probabilities in different cells, and this mosaicism and variability in penetration may be allowing some organoids to become escapers who were able to develop properly while others did not (Figure 1). She noted that additional work would need to be performed to either confirm or disprove this hypothesis.

They also characterize 2D endothelial cells (ECs) derived from these *CLN3*^{Q352X} hiPSC line, which display hallmarks of JNCL disease phenotypes, including increased presence of autophagic vacuoles (AVs) - with an increased size trend -, electron-dense storage material with fingerprint patterned morphology with accompanying higher accounts and co-localization of both subunit c of mitochondrial ATP synthase (SMCAS) and lysosomal-associated membrane protein 1 (LAMP1) immunostaining, broadening and higher degree of ramification of Golgi stacks, and significantly decreased amounts of Ras-related protein Rab-7a (RAB7) positive stained structures; these features indicate likely defects in the maturation/trafficking in the endo-lysosomal transit systems in *CLN3*^{Q352X} ECs (Figure 2).

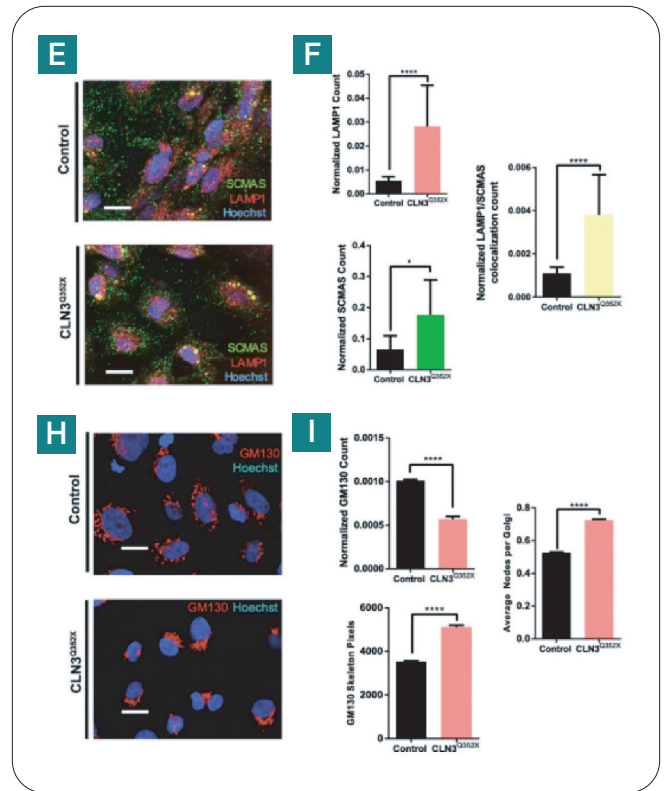


Figure 2: An increase in intracellular deposits of subunit c of the mitochondrial ATPase (SMCAS) and indication of increased presence inside lysosomes via colocalization with lysosomal-associated membrane protein 1 (LAMP1), a pathological JNCL disease hallmarks, in 2D endothelial cells (ECs) derived from *CLN3*^{Q352X} hiPSC lines (e, f). Immunostaining with the cis-Golgi marker GM130 indicated broadening of the Golgi stacks in the mutants with higher degree of ramification and average nodes per Golgi structure despite lower proportions of these structures in the mutants (h, i). Figure taken from Figure 2; additional method details can be found in the method and figure legend of <https://doi.org/10.1186/s40478-019-0871-7>.

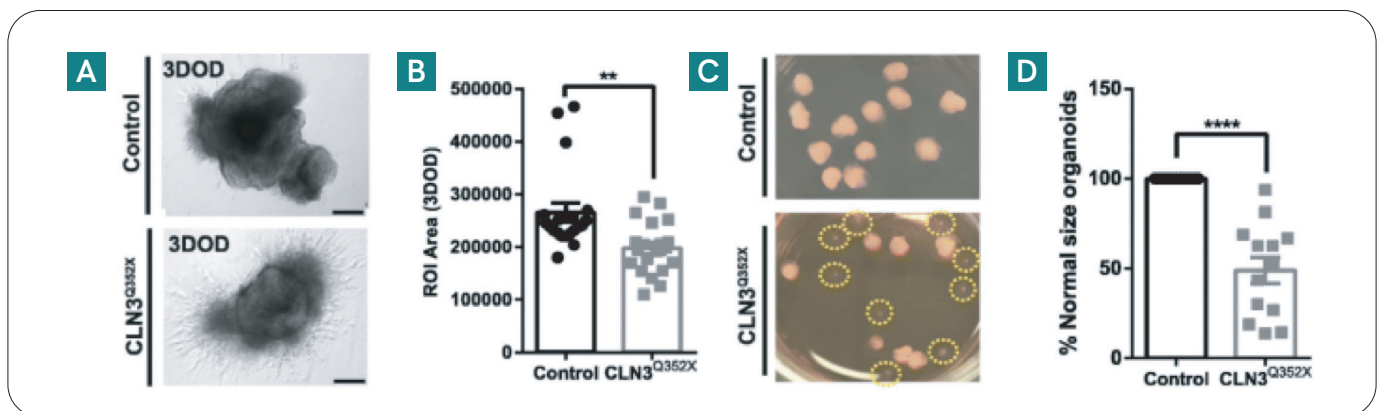


Figure 1: Severe development failure in around half of cerebral organoids derived from *CLN3*^{Q352X} hiPSCs. Upon matrix embedding and start of differentiation (day 11), the average size of mutant organoids was slightly though significantly reduced compared to isogenic controls (a, b). Around half of the organoids (represented in dashed yellow circles) completely failed to develop through to end-point differentiation, which was observable repeatedly across several batches of organoid generation (n = 13 generation events) (c, d). Figure taken from Figure 3; additional method details can be found in the method and figure legend of <https://doi.org/10.1186/s40478-019-0871-7>.

Moving into the phenotypic assessment of *CLN3*^{Q352X} escapers that developed into 3D cerebral organoids, they visualize lysosomal alterations, including increased presence of AVs and depiction of intracytoplasmic and electron-dense storage material with fingerprint morphology and structures resembling curvilinear bodies (CVB); they did not see changes in SCMAS, however, but saw increased protein but not mRNA – levels of TPP1/*CLN2*, known to interact with *CLN3*, and decreased concentration of Cathepsin D (CTSD/*CLN10*). These features indicate potential functional alterations at the lysosomal level in what would be considered an early timepoint in this developmental model. They observe significant increases in GFAP+ cells, indicating an increase in the number of astrocytes compared to control organoids, though MAP 2-positive areas and percentage of apoptotic cells or necrotic cell markers were not significantly different at this timepoint (55 days of differentiation).

Upon subjecting the cerebral organoids to whole-transcriptome RNA-seq and assessing GRN and DEA, they note most up-regulated genes are significantly enriched in cellular processes related to development (tissue, multicellular organism, and extracellular matrix (ECM) organization) while most downregulations target the pathways involved in antigen processing and presentation via MHC1. Pathway enrichment analysis display dysregulation in stem cells and development pathways, particularly those of *TGF-β*, *WNT*, and *BMP* that have been associated with having fundamental roles in embryonic development and homeostasis, as well as downregulation of several transcription factors implicated in CNS development, synaptic proteins, and neurotransmitter receptors such as the γ -Aminobutyric acid (GABA) receptor *GABRA2* and the dopamine receptor *DRD1*.

Continuing their exploration of defects in early synaptic formation and neurotransmitter production in these mutant 3D organoids, they see a significant decrease in pre-synaptic and post-synaptic counts as well as vesicular GABA transporter vGAT immunostaining, which is coupled with a dysregulation (mostly down-regulation) in 66 different metabolites using GC-MS, including amino acids (tryptophan, lysine) and neurotransmitters (GABA).

In summary, the generation and analysis of isogenic hiPSC models speaks to the utility of CRISPR/Cas9-mediated gene editing to introduce mutations such as a c.1054C→T pathologic variant into the *CLN3* gene to isolate the effects of specific mutations without potential variability introduced by patient-specific backgrounds to study rare diseases like JNCL. This is a powerful system as hiPSCs can be differentiated into various cell types to elucidate the effects of said mutation on different cell types, as well as allow for the tailored introduction of mutations for applications in personalized medicine and direct translational research. Similarly, *in vitro* human 3D iPSC-derived organoid systems prove to be reliable models to more easily study disease pathogenicity rooted in dysregulated processes like neurodevelopment occurring in complex organs such as the brain.

About the scientist



Dr. Gemma Gomez-Giro

obtained her Bachelor and Master in Biomedicine in Barcelona, Spain.

After a short research stay in Munich, Germany studying Alzheimer's disease, she worked in Prof. Schöler's lab (Münster, Germany) and Prof. Schwamborn's lab (Luxembourg) to complete her PhD studies, focusing on the development of cellular models for NCL, a neurodegenerative disease affecting children, via genome editing of iPSCs to introduce disease-causing mutations in the *CLN3* gene. With these cell lines, she derived brain organoids to study the disease in the brain *in vitro*. Currently, she is a post-doctoral researcher at CONNECT applying the 3D organoid technology that was developed in the lab to study Parkinson disease with on-chip approaches.

References

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3. Gomez-Giro, G., Arias-Fuenzalida, J., Jarazo, J. et al. (2019) Synapse alterations precede neuronal damage and storage pathology in a human cerebral organoid model of CLN3-juvenile neuronal ceroid lipofuscinosis. *acta neuropathol commun* 7, 222. <https://doi.org/10.1186/s40478-019-0871-7> and references therein.



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