Generation of iPSC-derived human neuronal progenitors for the study of GM1 gangliosidosis.

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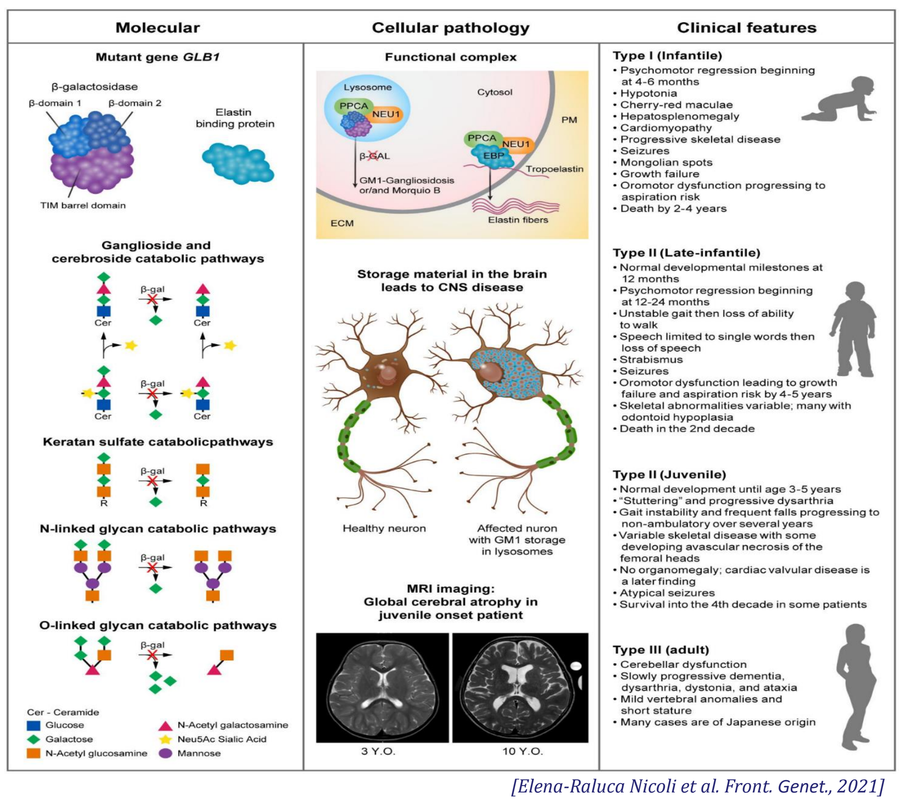
BACKGROUND

GM1 gangliosidosis is a progressive, neurosomatic, lysosomal storage disorder caused by mutations in the *GLB1* gene encoding the enzyme β-galactosidase (β-gal). Absent or reduced β-gal activity leads to the accumulation of β-linked galactose-containing glycoconjugates including the glycosphingolipid (GSL) GM1-ganglioside in neuronal tissue.

The pathogenic pathway by which GM1 storage leads to cell death involves both the accumulation of toxic products causing an inflammatory response and of aberrant mitochondria, a pathogenic pathway common to many neurodegenerative diseases (*Ballabio et al. 2009*).

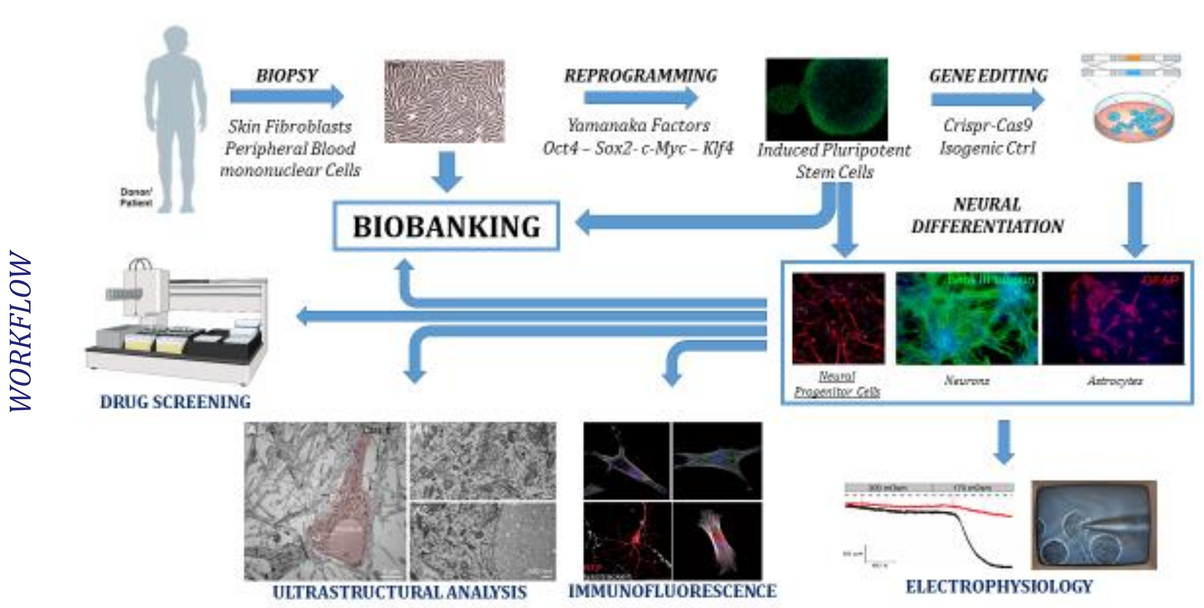


[Elena-Raluca Nicoli et al. Front. Genet., 2021]

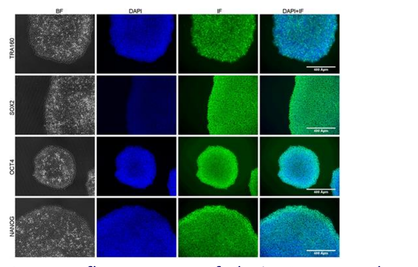
AIMS

To generate a specific and complex cellular model of GM1 Gangliosidosis disease, in order to conduct immunofluorescence and ultrastructural tests to highlight the typical cellular phenotypes of the disease, and subsequently to perform drug screening tests and develop validated biomarkers for simplifying diagnosis and monitoring disease progression.

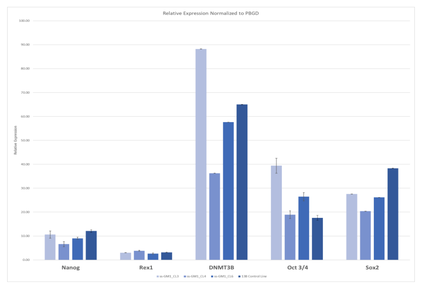
METHODS



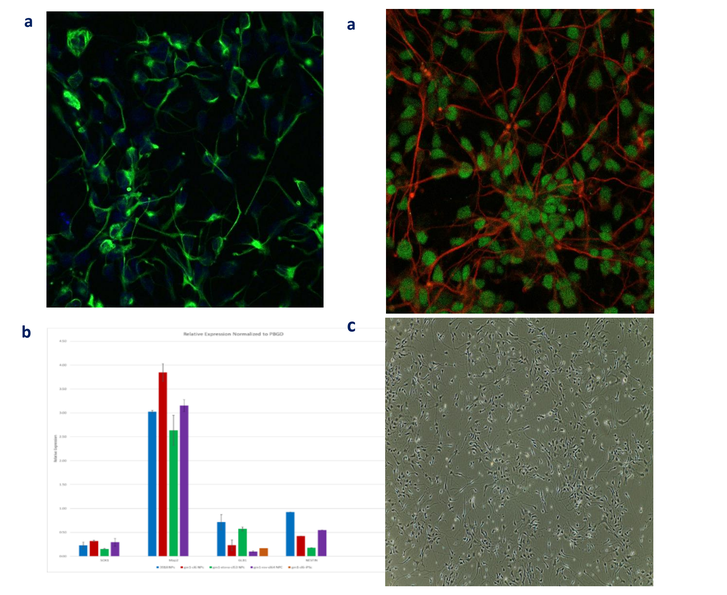
Skin biopsy and primary culture of fibroblasts from a patient affected by GM1 Gangliosidosis. Reprogramming in Pluripotent Stem Cells (iPSCs) by Sendai Virus and differentiation into Neuronal Progenitors (GM1-NPc). The isogenic control was generated by CRISPR-CAS 9 method.



Immunofluorescence of pluripotency markers



Real time PCR for quantification of expression of endogenous pluripotency markers (SOX2, OCT4, NANOG, TRA-1-60).



Neural Precursor Cell labeled with anti-nestin/DAPI (A), with anti-sox1 (green), betaIII-tubulin (red) /DAPI (B). Quantification of expression of Neural precursor markers (SOX1-MAP2-Nestin); NPC in bright field image (C).

RESULTS

- The NPC of the GM1 type I patient shows an increased accumulation of GM1 Ganglioside and a plasma membrane rupture that could be caused by alteration of the plasma membrane Ca^{2+}-ATPase activity *(Figure 1a).*
- In many neurodegenerative diseases, lack of autophagosomal-lysosomal fusion is believed to be the main cause of autophagic failure and apoptosis *(Figure 1b-1c).*
- Immunofluorescence and electron microscopy highlighted the accumulation of GM1 gangliosidosis in the NPC patient in comparison to the control cell line and to the patient's reverted isogenic line which allowed a clear genotype-phenotype correlation *(Figure 2).*

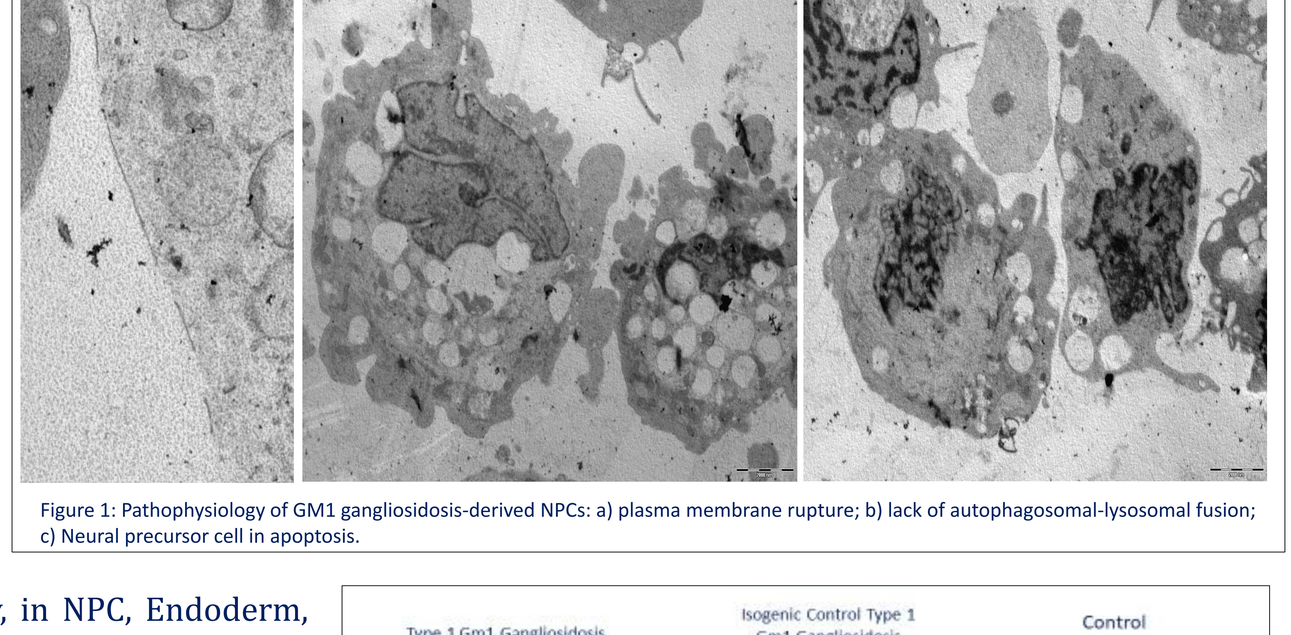


Figure 1: Pathophysiology of GM1 gangliosidosis-derived NPCs: a) plasma membrane rupture; b) lack of autophagosomal-lysosomal fusion; c) Neural precursor cell in apoptosis.

- For the validation of the different GM1 type I models, β-gal enzyme activity, in NPC, Endoderm, Mesoderm and Ectoderm cells was measured (*Figure 3*).
- The NPC of the GM1 gangliosidosis type I patient showed a significant reduction of GM1 ganglioside levels (25%) after administration of 0.125 mM of the substrate inhibitor Miglustat. A comparison of MFI values for treated and untreated cells is shown in *Figure 4*.

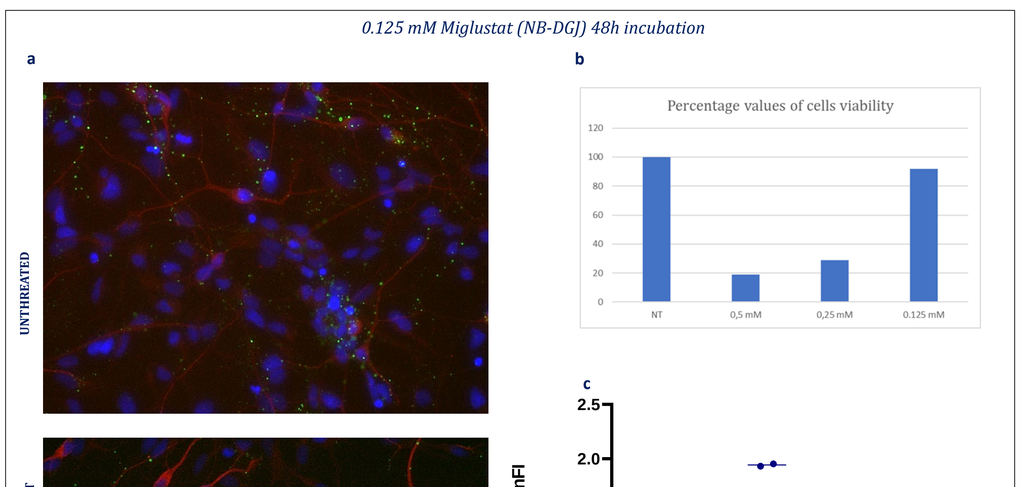


Figure 4: a) Neural Progenitor Cell labeled with DAPI (Blue), with CTXβ (green), β III-tubulin (red). b) Viability test of NPC after Miglustat treatment. c) Miglustat reduces the amount of GM1 ganglioside in neural precursor cells from GM1 gangliosidosis patients.

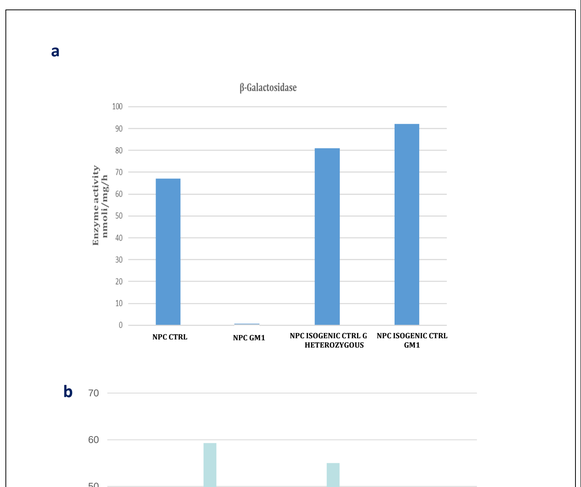


Figure 3: a) GM1 gangliosidosis-derived NSCs showed markedly lower β-gal activity compared with control NPCs. b) β-Galactosidase activity and control enzyme measured in three germ layers lines.

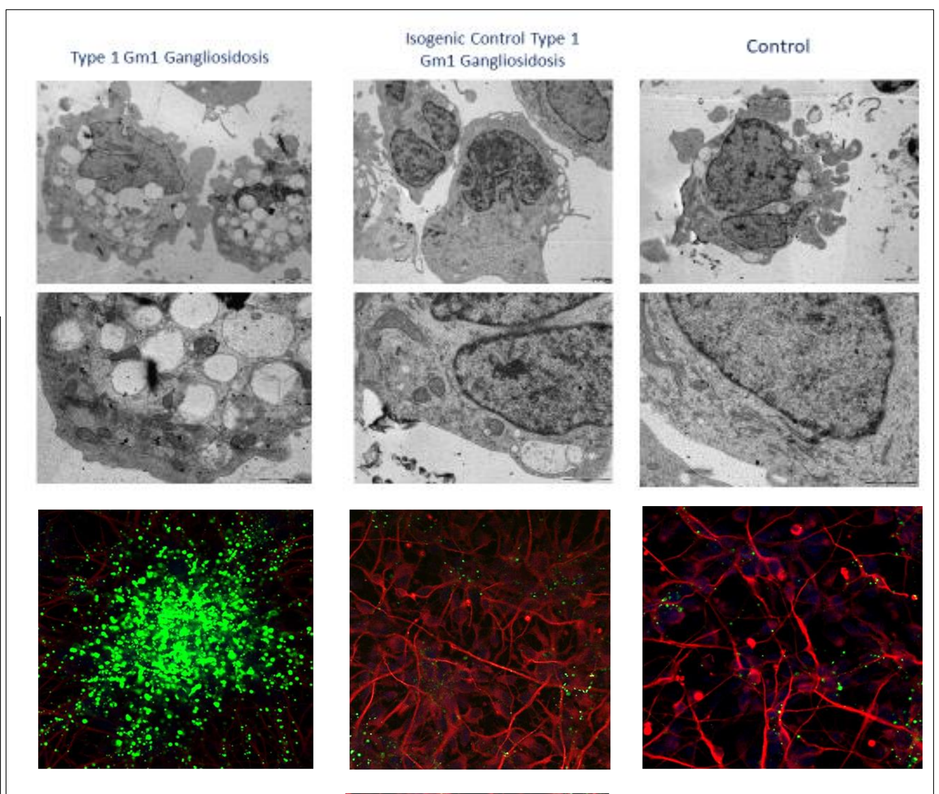


Figure 2: Immunofluorescence and ultrastructural analyzes of NPc from a patient affected by GM1 gangliosidosis vs control/reverted isogenic line

CONCLUSIONS

GM1-NPCs are an important *in vitro* cellular model of pathological neural dysfunction in GM1 gangliosidosis.

This model appears to be essential for the genotype-phenotype correlation of novel identified *GLB1* gene variants, especially for those of uncertain significance (VUS).

GM1-NPCs offers a fundamental contribution to developing, screening and validating new drugs for the treatment of GM1 Gangliosidosis

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CONFLICT OF INTEREST: nothing to declare.