Principal Investigator: Marco Trinchera

Grant Title: Novel diseases reveal glycosylations affecting human specific brain functions

Abstract

This project was aimed at: 1) characterizing a novel congenital disorder of glycosylation determining intellectual disability, caused by the mutation of the B4GALT5 gene involved in glycosphingolipid biosynthesis. 2) Unraveling how mutations in a single member of glycosyltransferase gene families are not rescued by the other members in the human brain and specifically affect cognitive processes.

Methods used. We studied:

- the enzymatic properties of the variants as assessed by direct in vitro assay using deuterated GlcCer as acceptor upon transfection in cell models;
- the effect on the sphingolipid profiles of the patients, as determined in their serum and cultured skin fibroblasts by liquid chromatography coupled with mass spectrometry (LC-MS/MS);
- the effect of the variants on the transcription of the gene, and on the expression and subcellular trafficking and localization of the protein, as evaluated though transfection of tagged cDNAs in model cell systems;
- the ability of patient fibroblasts to process exogenous sphingosine for the biosynthesis of complex glycosphingolipids, evaluated by thin layer chromatography;
- the role of other B4GALTs in rescuing B4GALT5, in their functions, as determined by in vitro assays and transfection in model cells.

We have also tried to prepare and analyze various brain cells, including neurons and glia, differentiated from NSCs obtained from fibroblasts of patients or healthy controls.

Results.

We set a novel procedure for in vitro assay of B4GALT5 and other LacCer synthases that uses deuterated GlcCer as substrate and simple UDP-Gal as donor. This procedure allowed us to reduce assay background while improving sensitivity. Using such an approach we demonstrated that the two novel B4GALT5 variants are indeed rid of any activity in vitro. We also found that mouse b4galt6 is kinetically very different from B4GALT5 and human B4GALT6 is substantially unable to use GlcCer in vitro. Coherently, only transfection into HEK-293T cells of a plasmid coding B4GALT5 provided a strong increase of LaCer, but no detectable increase upon transfection of human B4GALT6. Upon growing in the presence of labeled sphingosine, control fibroblasts were found to contain LacCer, Gb3 (globotriaosylceramide), and ganglioside GM3 in addition to GlcCer, while patient fibroblasts lack any of the above glycosphingolipids except GlcCer. Confocal fluorescence microscopy revealed that tagged B4GALT5 variants do not co-localize with Golgi marker, as done by the wild type protein.

We concluded that the reported B4GALT5 variants are pathogenic ad give rise to a novel congenital disorder of glycosylation.