A MULTI-APPROACH CHARACTERIZATION OF GLYCOSPHINGOLIPIDS DISORDERS: THE CASE OF ST3GAL3 VARIANTS

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ST3GAL3 is a member of the sialyltransferase family able to transfer sialic acid to the C-3 position of galactose, terminating the oligosaccharide chain of both glycoproteins and gangliosides. It was supposed to be mainly dedicated to the biosynthesis of histo-blood antigens such as sialylated Lewis a, but the recent discovery of a rare disease caused by inactive variants of the enzyme revealed patients presenting predominantly neurological symptoms and expressing circulating CA19.9. Moreover, enzymatic studies in-vitro showed that ST3GAL3 prefers glycosphingolipid substrates presenting both the Galβ1,3GlcNAc (lacto-series) and the Galβ1,3GalNAc (ganglio-0 series) sequences. These evidences led to the hypothesis that ST3GAL3 may be crucial for the generation of a definite pool of minor brain gangliosides that could be essentials for the function of specific subsets of neurons. To prove this theory, we created a new method to assess enzyme activity using liquid chromatography coupled with mass spectrometry, which allowed us more sensitive detection avoiding the use of radioactive compounds. Then, plasma samples from patients have been analyzed by ultra-sensitive LC-MS/MS to study if and how the global levels of glycosphingolipids produced were affected by the variants. Results obtained for the enzyme activity assay conducted using LC-MS confirmed that most of the known pathogenic variants of ST3GAL3 lack enzymatic activity. The semi-quantitative LC-MS/MS analysis allowed us to detect lower levels of the putative main reaction products, sLc4 (Siaα2,3Galβ1,3GlcNAcβ1,4Galβ1,4Glc-Cer)/GM1b (Siaα2,3Galβ1,3GalNAcβ1,4Galβ1,4Glc-Cer), in plasma patients when compared to controls. Considering the promising results obtained, further studies are required to study the distribution of glycosphingolipid in brain, in order to be able to connect the biochemical changes in patients to the clinical outcome.