Principal Investigator: Luis M Bredeston
Grant Title: Role of SLC35A3 Transporter on Congenital Disorders of Glycosylation

Abstract

1. Objectives: Congenital disorders of glycosylation (CDG) are inherited diseases caused by alterations in the synthesis of glycoconjugates, mainly in the Golgi apparatus and the endoplasmic reticulum. The identification of more than 160 genes associated with CDG and the characterization of the biochemical phenotypes of patients have been driven, in recent years, by advances in the use of massive sequencing techniques and mass spectrometry. Some of these disorders are related to mutations in genes of the SLC35 family, encoding proteins known to have a role in the transport of nucleotide sugars to the Golgi lumen and endoplasmic reticulum. The long-term goal of this project is to reveal the role of the transporter SLC35A3 in the Golgi apparatus in congenital disorders of glycosylation. Two specific objectives were proposed in the research plan: Goal 1. Study the structure-function relationship of the human SLC35A3 and its pathogenic variants. Goal 2. Generate an animal model to study SLC35A3 deficiency. **2. Methods**: We performed a comprehensive characterization of human SLC35A3 function, and the pathogenic variants identified in patients with SLC35A3-CDG. This included the production of SLC35A3 variants in yeast, followed by solubilization with detergent, and their reconstitution in liposomes to evaluate their transport activity with radiolabeled nucleotide sugars. A homology and docking modeling approach was also used to identify potentially relevant residues involved in the interaction of SLC35A3 with substrates during the transport cycle. The *in vitro* activity of alanine substituted variants of the selected residues was tested. To generate an animal model with SLC35A3 deficiency, we injected morpholinos designed to knockdown the mRNA corresponding to the genes ZfSLC35A3 a and b in zebrafish and subsequently performed phenotype analysis of embryonic development. **3. Results**: The SLC35A3 transporter, purified and reconstituted in liposomes, was found to import other nucleotides in addition to UDP-GlcNAc. This newly identified transport function helps explain abnormal proteoglycan synthesis in SLC35A3-CDG patients and mouse models, expanding previous understandings limited to GlcNAc-related deficiencies. The countertransport mechanism appears flexible, as UDP-GlcNAc uptake was activated not only by UMP, suggesting broad specificity across the SLC35A family members. Homology modeling identified 15 critical residues involved in substrate interaction, of which alanine substitutions in several variants impaired transport activity, highlighting their functional role. SLC35A3-CDG variants displayed significantly reduced or undetectable UDP-GlcNAc import, correlating with severe patient phenotypes. To further investigate SLC35A3 deficiency, zebrafish models were developed through morpholino-induced knockdown. Injection of antisense morpholinos against the ZfSLC35A3a and b genes into zebrafish eggs produced a slight but significant decrease in larvae length 5 days after injection. Additionally, several craniofacial parameters, such as ceratohyal angle (Ch-a), ceratohyal length (Ch-l), palatoquadrate length (Pq-l), head length (HL), anterior head width (AHW) and posterior head width (PHW) were measured to evaluate the phenotype developed after gene blockade. We observed a slight but significant increase in Pq-l, Ch-l, HL, AHW and PHW in morpholino-treated larvae.