Molecular Study of GLUT1 Deficiency Syndrome: Functional Characterization of Causative Mutations and New Genotype-Phenotype Correlations

GLUT1 DS (OMIM 606777, GLUT1 deficiency syndrome, GLUT1 DS) is a metabolic brain disorder with great clinical heterogeneity caused by different types of SLC2A1 mutations, thereby making clinical and genetic diagnosis challenging in some cases. The pathogenic effects of coding altering sequence mutations is usually evaluated using deleteriousness scoring systems, but an effective approach to study dynamic effects of mutations on molecular mechanism of GLUT1 is highly required. Moreover, 10% of patients with signs suggestive of GLUT1 DS, are actually negative to SLC2A1 mutations. On this regard, the occurrence of pathogenic mutations in promoter and intronic regions of SLC2A1 has been recently reported (1;2).

For patients with GLUT1 defects, the ketogenic diet, which produces ketone bodies as an alternative energy source for brain metabolism, is administered. The purpose of our study is to bring new insights into the GLUT1 DS molecular pathology to clarify genotype-phenotype correlations and improve the diagnostic yield of genetic test, as establishing the diagnosis is crucial for the ketogenic diet effectiveness.

GLUT1 is found primarily in the cell membrane and on the cell surface and is located at the blood-brain barrier where transports glucose into the brain. Thus, first aim of the study is to explore the functional impact of SCL2A1 pathogenic missense mutations on GLUT1 sub-cellular localization using cellular and molecular biology approaches. To this aim we generated a plasmid system to express and visualize wt and mutated GLUT1 in fusion with GFP protein. Through this system we will be also able to assess the effects of mutations on cell glucose uptake.

Additionally, we hypothesize that at least a part of patients, negative for SLC2A1 mutations, could harbor mutations in regions not routinely screened in standard analyses. To this aim we are implementing sequencing data analysis workflow specifically designed to evaluate also non-coding and regulatory sequences and large deletions. Finally, phenotype heterogeneity and occurrence of mutations in regulatory regions suggest that factors that modulate gene expression could potentially contribute to pathophysiology mechanisms.

On this regard, analysis of the genome region of SLC2A1 disclosed an annotated transcript that could potentially act as a natural antisense transcript. In order to characterize the functional role of this transcript we verified its expression in different tissues and cell lines. Moreover, we assessed its subcellular distribution. We will characterize non-coding antisense RNA structure and expression in cell lines and patients. Additionally, experiments of non-coding antisense over-expression and silencing will be performed to assess the effects on SLC2A1 expression levels. The knowledge on this non-coding RNA functional roles could shed light on molecular pathogenesis and clarify genotype-phenotype correlations. Moreover, it could be used for therapies development as modulation of gene expression levels has been suggested as a potential therapeutic approach.

References:

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