**Presentation title:** Ketogenic diet therapies for drug-resistant epilepsy might affect ion channels activity through the combination of both epigenetic changes and splicing events

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**Abstract (250-300 words):**

Background

Diet is among the most relevant epigenetic modulators, and increasing findings suggest that epigenetic changes (DNA methylation, histone acetylation and chromatin remodeling) are also associated with ketogenic diet (KD) therapies. These are considered an effective approach for the management of drug-resistant epilepsy, such as GLUT1 deficiency syndrome (GLUT1-DS; #606777), whereas its underlying mechanisms remain elusive.

After the confirmation of the metabolic status, we aimed to explore the transcriptional dysregulations of two SLC2A1-mutated pediatric patients before and 6/8 months after the treatment with KD and to also assess alterations in the methylome profiling.

Methods

FIA-MS/MS platform was carried out for the analysis of 11 amino acids and 31 acyl carnitines. Bulk RNA sequencing was performed using Illumina Stranded Total RNA Pre kit and sequenced on NS500. Differential expression analysis was conducted using R Package DESeq.2 and gene set enrichment analysis (GSEA) was performed using Cluster Profiler. Analysis of alternative splicing and isoform switches was implemented using IsoformSwitchAnalyzeR. Methylation sequencing was carried out on PromethION 2 Solo platform and data analysis was showed using Remora algorithm.

Results

Metabolite analysis has confirmed the ketosis status in two patients in diet (increase of C2 and C4OH). RNA-seq data highlighted a significant splicing change in GLUT1-DS children under KD treatment identifying the snRNA U1 up-regulated before the therapy. In addition to amino acids metabolism, translation and mitochondrial activity deregulated, when performing GSEA with Reactome a strong epigenetic signature was also observed: histones deacetylation and DNA methylation resulted down-regulated. Molecular function analysis showed a down-regulation in voltage-gated ion channel and passive transmembrane transporter activities. Moreover, analysis for 5mC and 5hmC profiles identified differentially methylated CpG sites before and after KD.

Conclusions

The observed epigenetic changes related to KD treatment might influence the alternative RNA processing of voltage-gated ion channels also by epigenetic splicing code.

**Biography (150-200 words):**

I am a determined women at third and final year of Ph.D. in Nutrition Sciences at the University of Milan (Italy) with a great desire to grow professionally and personally. My studies are oriented towards genomics and epigenomics, with a deep interest in epigenetic regulation (specifically, RNA sequencing and DNA Methylation sequencing are the two approaches I am currently focusing on) in rare pediatric genetic diseases.