A Novel SLC2A2 mutation implicated in Fanconi-Bickel syndrome and dysglycaemia

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Introduction

Fanconi-Bickel syndrome (FBS) is a rare disease but with well characterized phenotypes, inherited in an autosomal recessive manner (1). FBS is due to genetic mutations in the glucose and galactose transporter gene SLC2A2 which encodes for GLUT2 (2). SLC2A2 localizes on human chromosome 3q26.1-q26.3 and consists of 11 exons and 10 introns (3,4). Missense, nonsense, frameshift, and splice site mutations have all been identified in SLC2A2 gene of FBS cases in association with diabetes mellitus (DM). More than 100 FBS cases with 34 variant SLC2A2 mutations were reported (Figure 1). The molecular mechanisms of dysglycaemia in FBS remains to be elucidated.

Objectives

1. To describe the clinical and genetic characteristics of a new case of FBS patient associated with dysglycaemia
2. To understand the molecular basis of DM in Fanconi-Bickel syndrome

Methodology

1. Clinical Approach:
   This study was approved by the Institutional Review Board for the Protection of Human Subject in Sidra Medicine, Qatar. Written informed consent forms were completed from all family members involved in the study.

2. Experimental Approach (CRISPR):

3. 6. T7 endonuclease I assay to detect type of NHEJ repair due to sgRNA/Cas9 mediated cut to DNA ends

4. 1. Co-transfection with the donor template

5. 2. Selection of cells carry specific mutation

4. Experimental analysis:

4.1. Expression exp.: (GLUT2 RNA expression in different cell lines)

4.2. CRISPR: (To introduce GLUT2 mutation in cultured cells)

4.3. Transformation of gRNA/Cas9 plasmid into E. coli

4.4. 3. Extraction of plasmids, and Selection of positives clones by Sanger sequencing

Final conclusions

A novel homozygous nonsense mutation (c.901C>T, R301X) in the SLC2A2 gene was found and confirmed by Sanger sequencing in 2 yrs old FBS patient associated with dysglycaemia. To investigate the impact of this mutation, CRISPR-Cas9 system was used to substitute the nucleotide C by T at position 901. After optimization of the CRISPR-Cas9 gene editing efficiency, different mammalian cell lines will be co-transfected with a plasmid carrying Cas9, the specific gRNA to target GLUT2 and DNA donor template to specifically substitute C by T at the position 901. GLUT2 edited cells carrying the specific mutation will be diluted and cultured at low cell concentrations to isolate single colonies and establish a GLUT2 knock-out cell clone. The GLUT2 knock-out cells will be used to identify the molecular mechanisms underlying DM and develop the targeted precision therapies specifically designed for the molecular changes associated DM and FBS syndrome. Molecular mechanisms of dysfunctional GLUT2 mediated FBS patients can be identified by protein structural modeling, biochemical, physiological and transcriptomic analysis.

References