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

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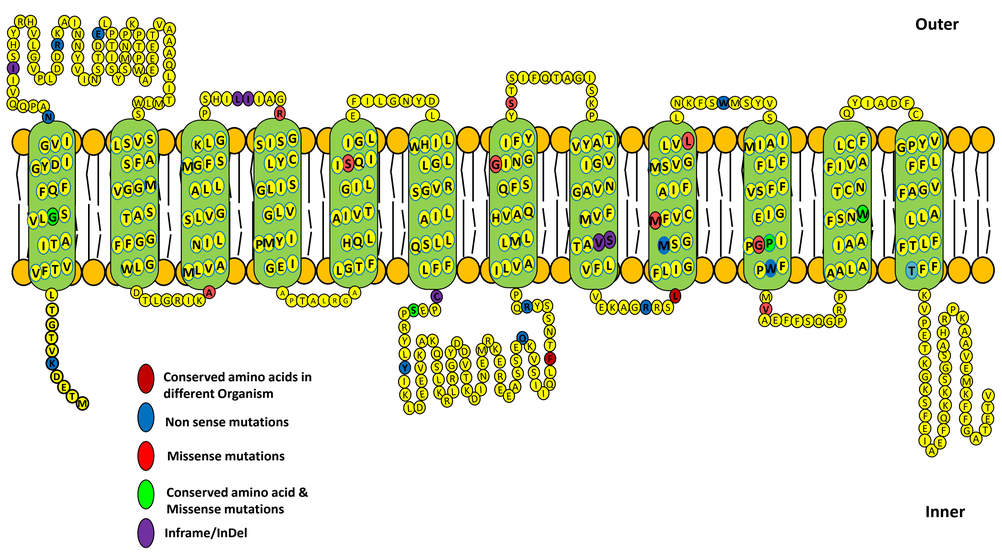
Disclosure-None of the authors have any potential conflict of interest

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 pathogenic variants 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

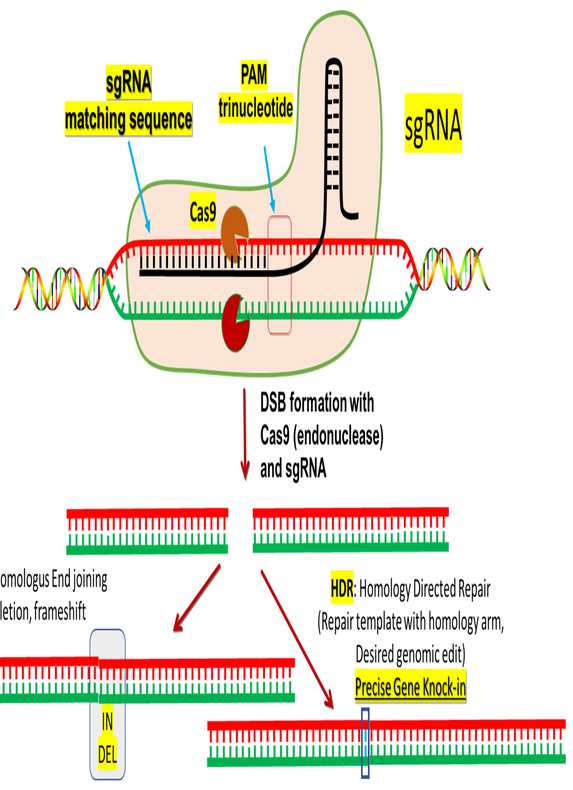
Methodology

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.

Patient's and Parent recruitment (Collecting blood) → **Blood Processing** (DNA, RNA, Serum, Plasma extraction) → **Sequencing** (WES and Sanger)

2. Experimental Approach (CRISPR):

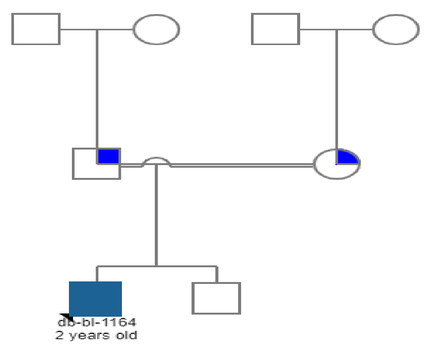


1. Designing different gRNAs specific to the mutation site
2. Transfect gRNAs-Cas9 Plasmid in Hek293 cells
3. Check gRNAs GLUT2 gene editing efficiency
4. Co-transfect the specific gRNA, CAS9 Plasmid, and donor template in HEK293, INS1, and HepG2 cells
5. Molecular analysis to study the mechanisms of dysglyacemia in FBS

Results

1. Clinical Information: (Clinical and radiological feature s of the patient)

➢ **Patient's family pedigree:**

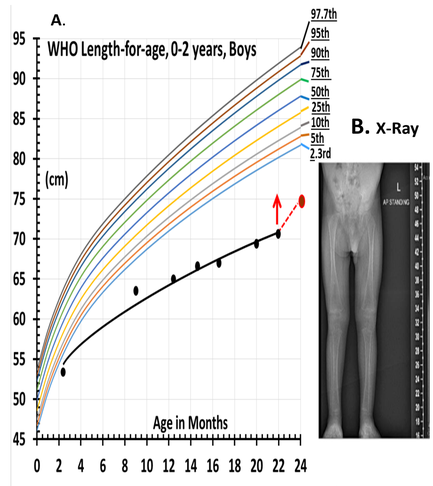


➢ **Patient's clinical presentation:**

1. Severe proximal tubular dysfunction
2. Hepatomegaly with stage 1 fibrosis
3. Rickets, developmental delay
4. Hypotonia
5. Failure to thrive.

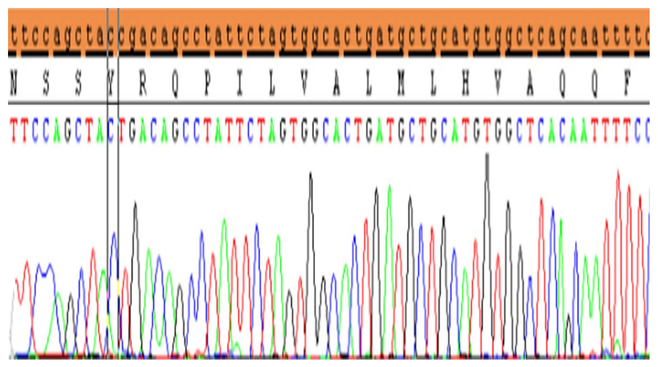
➢ **Summary of biochemical tests for patient**

Investigation	Blood level	Normal range
Alkaline phosphatase (IU/L)	410	48-95
Alanine amino transferase (ALT) (IU/L)	82	8-22
Aspartate transaminase (AST) (IU/L)	110	0-30
Oral Glucose Tolerance Test (GTT 2 hours)		
Glucose random (mmol/l)	5.8	2.1-2.7
2 hours glucose tolerance (mmol/l)	20	7.8-11.1
C-Peptide (ng/ml)	0.33	0.78-5.19
Urinalysis		
Amino Acids	Proteinuria (non nephrotic range), glycosuria, phosphaturia	

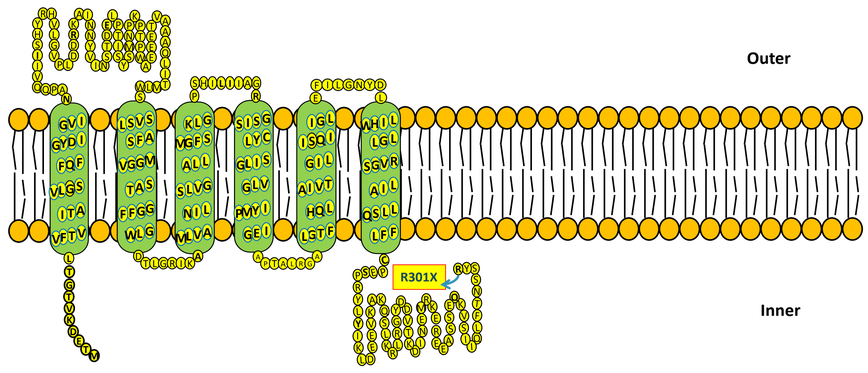


➢ **A. WHO growth chart** for age 0-2 years, boys, displays stature (cm). Red arrow and dot mark indicate measurement following growth hormone treatment. **B. X-ray** for lower limb. Diffuse osteopenia and metaphyseal flaring seen. Transverse acetabular noted.

2. Genetic Analysis: (Sanger sequencing of Patient's cDNA to confirm the GLUT2 mutation c. 901 C>T)

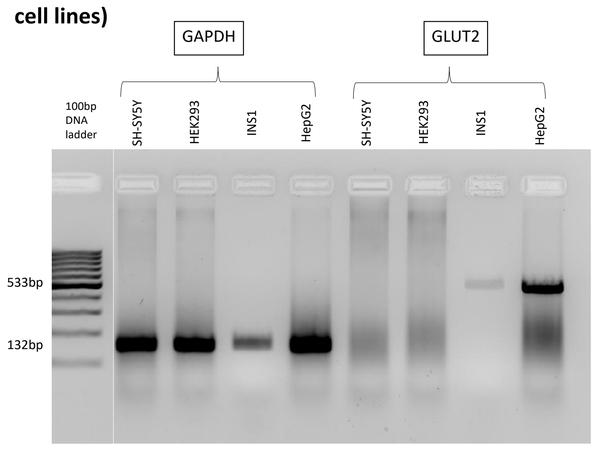


3. Topology: (GLUT2 mutation R301X)



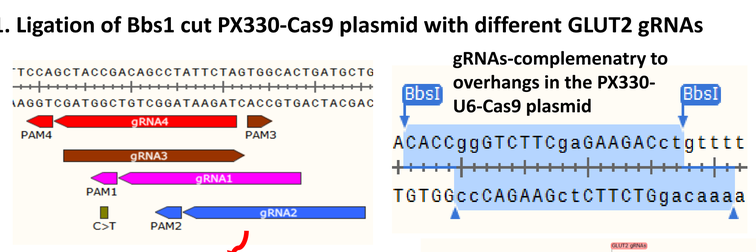
4. Experimental analysis:

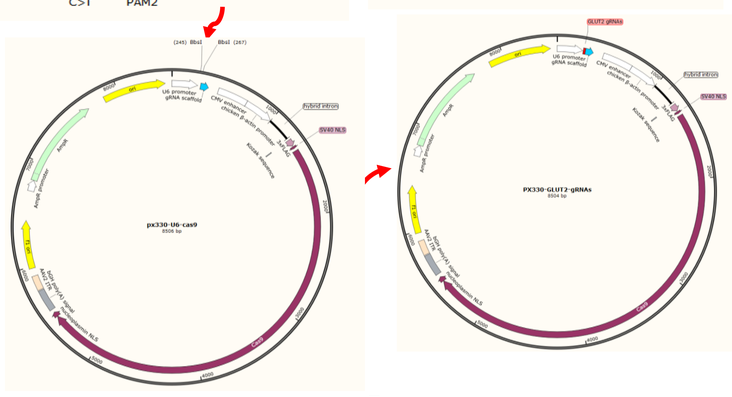
➢ **Expression exp:** (GLUT2 RNA expression in different cell lines)



➢ **CRISPR :** (To introduce GLUT2 mutation in cultured cells)

1. Ligation of Bbs1 cut PX330-Cas9 plasmid with different GLUT2 gRNAs





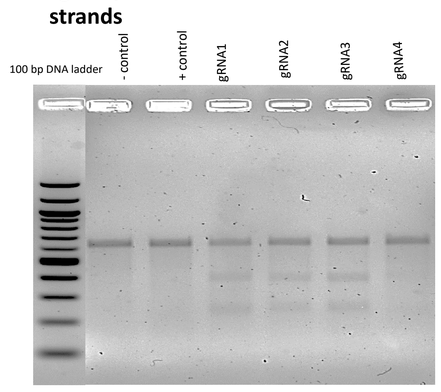
2. Transformation of gRNAs-Cas9 plasmid into E. coli

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

4. Amplify the plasmid and DNA extraction using Maxiprep (Qiagen)

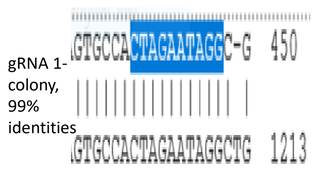
5. Transfection in Hek293 cells

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



* Size of gRNAs and fragments of gRNA1, gRNA2, gRNA3, and gRNA4 respectively: 654 bp, 259-395, 266-388, 247-407, and 252-402

7. Topo Cloning - Sanger seq to check gene editing efficiency for each gRNA



1. Co-Transfection with the donor template
2. Selection of cells carry specific mutation

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 and 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.

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58ESPE P1-189
Diabetes and insulin
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Poster presented at: ESPE 2019 VIENNA
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