Glycogen Storage Disease 1b: Diagnosis and Workup of a Novel Mutation

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Abstract

Glycogen Storage diseases (Glycogenoses) are a diverse group of disorders, numbering more than 12, resulting from deficiencies of various enzymes & transport proteins in the pathways of glycogen metabolism. GSD 1 is caused by absence or deficiency of glucose-6-phosphatase activity in the liver, kidney or intestinal mucosa. In GSD 1(b), the enzyme which transports Glucose-6-Phosphate across the microsomal membrane is defective, thereby resulting in accumulation of Glycogen. The clinical features of 1a & 1b are similar with fasting hypoglycaemia, hepatomegaly, growth retardation and metabolic abnormalities except for the presence of neutropenia with recurrent gingivitis in GSD 1b. A genetic diagnosis solves this conundrum with the added benefit of antenatal diagnosis of future pregnancies & identification of carrier state in patients. We report the work up of an infant with suspected GSD where a novel mutation with heterozygous carrier state in the parents was diagnosed by genetic testing.

Introduction

Glycogen Storage Disease (GSD) Type Ib is an autosomal recessive disease in which glucose 6-phosphate, a product of metabolic cleavage of glycogen, cannot be transported to the inner surface of the microsome due to a deficiency of glucose 6-phosphate translocase[1]. Therefore, glucose 6-phosphate cannot be metabolised to glucose even though microsomal glucose 6-phosphatase activity is normal.

We report a case in which patient's DNA was used to perform PCR and DNA sequencing tests to look for the presence of mutation in the gene SLC37A4 which causes GSD 1b.

The Case

A 10 month old male child born of second degree consanguineous marriage was evaluated for progressive abdominal distension, failure to thrive and seizures. He was born full-term with a birth weight of 3600 gm. He had no history of perinatal insult. He had past history of four attacks of seizures with first seizure on 5th day of life.

On examination baby had a round cherubic face. His weight was 7.2 kg and length was 69 cm, both were less than 3rd percentile for his age. There was pallor but no icterus. He had hepatomegaly with liver span of 12 cm, but no splenomegaly or ascites. Gross motor developmental milestones were delayed. On admission, baby had a seizure with documented hypoglycaemia (blood sugar 30mg/dl), in the morning. However, no ketone bodies were seen in urine. Hemogram showed normal neutrophil count. Serum concentrations of transaminase and CPK were normal. Triglycerides, cholesterol, uric acid and liver transaminase were mildly elevated. A repeat overnight fasting resulted in blood sugar of 25 mg/dl but after feeds, blood sugar was 84 mg/dl and 104 mg/dl after half an hour and 1 hour respectively. After overnight fasting, lactate level were also high and serial lactate readings after feeds continued to be high but fell steadily. Hepatitis B and C serology was normal. ECHO, x-ray chest and fundoscopy examination were also normal.

The child was suspected to have glycogen storage disorder based on gradual abdominal distension, history of convulsions, massive hepatomegaly, hypoglycemia, hypertriglyceridemia and hypercholesterolemia. Since GSD 1a being the commonest, we sent for mutation testing of GSD 1a which was negative. Then, we sent patient's blood for PCR and DNA sequencing tests to look for the mutations in the coding sequence of the GSD1b, SLC37A4 gene. There was a homozygous deletion of 3 base pairs in Exon 2 of the coding region of the SLC37A4 gene which confirmed the diagnosis of GSD 1b in our patient. DNA PCR sequencing of both father and mother revealed identical heterozygous deletions of 3 bases TCA c.152-154 in the SLC37A4 coding region. This indicates a carrier status for GSD1b in both the parents.

Discussion

Two major types of the GSD-1 have been reported: GSD-1a which is caused by a deficiency of microsomal Glucose6Phosphatase, and GSD-1b which is caused by a glucose -6-phosphate translocase (G6PT) deficiency[2,3]. A patient with GSD-1 presents with severe hypoglycemia, truncal obesity, a rounded doll-like face,

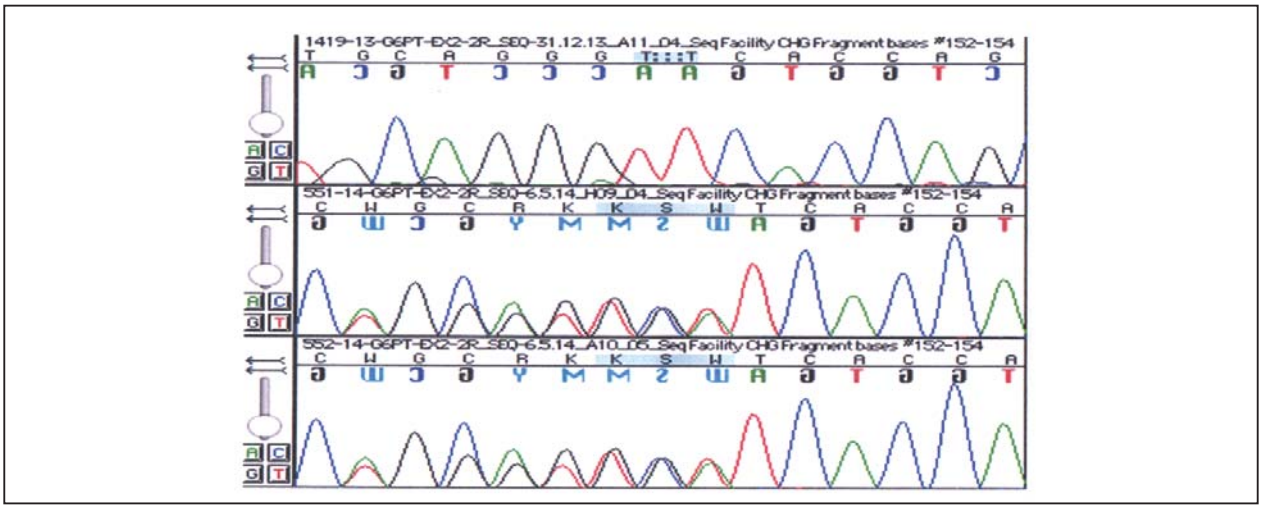

Fig 1: Sequencing chromatogram showing the sequence traces of the proband, father and mother. The three base pairs deleted in the proband are seen as missing (:::) and the parents show a heterozygous deletion state for the same

PROVEAN Human Protein Batch Result (Download)

VARIATION		PROTEIN SEQUENCE CHANGE				PROVEAN PREDICTION				SIFT PREDICTION	
ROW_NO.	INPUT	PROTEIN_ID	POSITION	RESIDUE_REF	RESIDUE_ALT	SCORE	PREDICTION (cutoff=-2.5)	#SEQ	#CLUSTER	SCORE	PREDICTION (cutoff=0.05)
1	NP_001157749.1,52,I,.	NP_001157749.1	52	I	.	-11.115	Deleterious	205	30	NA	NA

Fig 2: A query of the wild type peptide sequence along with the mutation TCA c.152-154 harbored in the proband was analysed using PROVEAN (Protein Variation Effect Analyser, a bioinformatics tool to predict the impact of an amino acid deletion or substitution on the protein's biological function), gave a score of -11.115 and predicted a deleterious effect (variants with a score equal to or below -2.5 are considered 'deleterious').

wasted muscles, hepatomegaly & kidney enlargement[4]. GSD-1b also shows features of neutropenia and neutrophil dysfunction[5]. Major progress has been made in patient survival and the prevention of neurological sequelae secondary to hypoglycemia in affected children with early diagnosis and meticulous treatment.

The advocated dietary treatment for GSD type 1b comprises of frequent high protein and carbohydrate feedings and a high protein snack at night[6,7]. Gastric drip feeding at night may be introduced in the infant if hypoglycemia is a problem. Uncooked corn starch therapy can be useful in the older child[7,8,9]. The parents were explained to give every two hours feeds of corn starch, soya milk, rice and lentils in between the meals.

He was followed up regularly. He has gained height and weight though his liver size has not regressed and serum transaminase levels have been normal. Neutropenia is noted in GSD Ib patients, after the 1st 2-3 yrs of life only and that may be the reason why we did not find neutropenia or fulminant infections in our patient. Gene-based mutation analysis provides a noninvasive way of diagnosis for most patients with type Ib disease.

The current treatment for GSD-1b consisting of dietary therapy and granulocyte colony-stimulating factor (G-CSF) therapy may restore metabolic & myeloid functions with improvement in prognosis. Long-term complications, such as renal calculi, inflammatory bowel disease, hepatic adenomas & splenomegaly, develop in a significant proportion of adult patients. Poor compliance frequently mars the efficacy of dietary treatment. The patients with GSD-1b may require liver transplantation for refractory hypoglycemia & to prevent malignant transformation of hepatic adenomas . Although hypoglycemia improves after liver transplantation, neutropenia generally continues to be present.

Additionally In our patient, sequencing results revealed that the patient has a homozygous deletion of 3 bases TCA or ATC c.152-154 in the SLC37A4 coding region.[10] This was the result of both parents being carriers and their sequencing correspondingly showed heterozygosity for the mutation. This is a novel mutation and would be indicative of GSD1b as it would cause a change in the amino acid sequence and hence the protein as shown by protein analysis using PROVEAN[11].

Conclusion

Prudent use of genetic testing in GSD1b helps define the illness & facilitates appropriate counselling of parents in the context of patient care as well as antenatal diagnosis in affected families.

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