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BRIEF REPORT

Novel mutation of SLC37A4 in a glycogen storage disease type Ib patient with neutropenia, horseshoe kidney, and arteriovenous malformation: a case report

Sepideh Ebrahimi Meimand[1,2] · Gholamreza Azizi[3] · Reza Yazdani[2,4,5] · Negin Sanadgol[2,6] · Nima Rezaei[1,2,7]

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Abstract

Glycogen storage disease type Ib (GSDIb) is an autosomal recessive disorder caused by mutations of *SLC37A4* gene, which encodes glucose 6-phosphate translocase (G6PT). Malfunction of G6PT leads to excessive fat and glycogen in liver, kidney, and intestinal mucosa. The clinical manifestations of GSD1b include hepatomegaly, renomegaly, neutropenia, hypoglycemia, and lactic acidosis. Furthermore, the disorder may result in severe complications in long-term including inflammatory bowel disease (IBD), hepatocellular adenomas (HCA), short stature, and autoimmune disorders, which stem from neutropenia and neutrophil dysfunction. Here, we represent a novel mutation of *SLC37A4* in a 5-month girl who has a history of hospitalizations several times due to recurrent infection and her early presentations were failure to thrive and tachypnea. Further investigations revealed mild atrial septal defect, mild arteriovenous malformation from left lung, esophageal reflux, Horseshoe kidney, and urinary reflux in this patient. Moreover, the lab tests showed neutropenia, immunoglobulin (Ig) G and IgA deficiency, as well as thrombocytosis. Whole exome sequencing revealed c.1245G > A P.W415 homozygous mutation in SLC37A4 gene and c.580G > A p.V1941 heterozygous mutation in PIK3CD gene. This study shows that manifestations of GSD1b may not be limited to what was previously known and it should be considered in a wider range of patients.

Keywords Lymphocyte · Immunodeficiency · Cardiovascular · Gastrointestinal

Introduction

Glycogen storage disease type I (GSDI) is considered an autosomal recessive inherited condition due to glucose-6phospahatase system defect. The incidence of GSDI has been estimated to be around 1 in 100,000 live birth each year. GSDI comprises two main types; glycogen storage disease type Ia (GSDIa) and glycogen storage disease type Ib (GSDIb). GSDIb accounts for approximately 20% of total cases of GSDI and GSDIa 80% of total cases [1]. GSDIa stems from G6PC gene mutations, which results in deficiency of enzyme glucose-6-phosphatase (G6Pase) that turns glycogen into glucose. GSDIb is caused by *SLC37A4* gene mutation, which is located on chromosome 11q23.3 and encodes glucose 6-phosphate translocase [2]. The protein has an important role in glucose homeostasis by transporting glucose 6-phosphate to the endoplasmic reticulum to be broken down and turn into glucose [3]. Disruption of glucose 6-phosphate translocase and G6Pase leads to converting glucose 6-phosphate to fat and glycogen instead of breaking down to glucose [4]. In GSDI, accumulation of

✉ Nima Rezaei
rezaei_nima@tums.ac.ir; rezaei_nima@yahoo.com

1 School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2 Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Dr. Qarib St, Keshavarz Blvd, Tehran 14194, Iran

3 Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran

4 Primary Immunodeficiency Diseases Network (PIDNet), Universal Scientific Education and Research Network (USERN), Tehran, Iran

5 Department of Neurology, Thomas Jefferson University, Philadelphia, PA, USA

6 Student Research Committee, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

7 Network of Immunity in Infection, Malignancy and Autoimmunity, Universal Scientific Education and Research Network, Tehran, Iran

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glycogen and fat in cells happens specifically in liver and kidney, resulting in hepatomegaly and renomegaly and also malfunction of these organs.

Immune responses are considered to be weaker in GSD1b patients than others. Moreover, the number of T cells is lower in GSD1b patients. Neutropenia is also found in 90% of patients and it predisposes them to recurrent infection. There are several mechanisms relating neutropenia to *SLC37A4* mutation. It is previously reported that disruption of G6PT activity may cause oxidative stress and cell apoptosis and finally neutropenia [5]. Moreover, G6PT acts on dephosphorylation of 1,5-anhydroglucitol-6-phosphat. As a result of G6PT deficiency in these patients, the accumulation of 1,5AG6P takes place and inhibits the first step of glycolysis. Neutrophils are dependent on glycolysis for energy metabolism and the lack of glycolysis results in their dysfunction [6]. Regarding the importance of G6PT for energy homeostasis, G6PT defect leads to reduced intracellular levels of G6P, lactate, NADPH, and ATP and also diminished glucose uptake in neutrophils [7]. According to previous studies, prevalence of autoimmune disorders is higher in GSDIb patients, and lack of G6P in endoplasmic reticulum and impairment of Regulatory T cells are the suggested causes [8]. Inflammatory bowel disease (IBD) is also seen in these patients and it seems to be related causally to neutropenia [9].

Delayed puberty, hypoglycemia, hyperlipidemia, lactic acidosis, seizures, anemia, oral health problems, doll-like faces, and short stature are other characteristics of these patients. The manifestations of the disorder usually begin to show up at age 3 to 4 months, but some neonates are presenting [10] (more details of GSDIb characteristics are available in Table 1).

G-CSF therapy has been reported to have some benefits in these patients by increasing bone marrow (BM) cellularity [11]. Furthermore, empagliflozin, an inhibitor of the renal glucose cotransporter sodium glucose cotransporter 2, has been shown to induce rise of neutrophil number and improvement of neutrophil function and regarding clinical symptoms, resolved frequent infections, mucosal lesions, and IBD remission were seen in GSDIb patients who did not respond completely to G-CSF [12, 13].

In the present study, we represent a case of GSDIb with a novel mutation of *SLC37A4*. The presentations included horseshoe kidney, arteriovenous malformation, atrial septal defect (ASD), hepatomegaly, neutropenia, and respiratory distress.

Case presentation

The proband was a 5-month girl born as the first child in a consanguine family after a normal pregnancy through vaginal delivery at 38 weeks with a birth weight of 2400 g. The parents were relatives (first cousins) and they had no history of drug abuse during pregnancy and beforehand. The patient was 3 months and 20 days weighing 4700 g when she was first referred to the immunodeficiency clinic of the children's medical center in Tehran with the symptoms of failure to thrive (FTT) and tachypnea and potential diagnosis of immunodeficiency based on her recurrent respiratory infections and hospital admission due to pneumonia. She expired at 7 months due to acute respiratory distress syndrome (ARDS). Other characteristics of the patient included tachypnea, respiratory distress, sudation during nursing, in the first visit. In the clinical examination, hepatomegaly was beheld, all the other examinations were unremarkable. She had a history of urinary tract infection (UTI) and hypertension. She was hospitalized for 1 week at the age of 5 months in the pulmonary ward for pneumonia. The first cousin (female-father side) of the patient was a case of GSD who died due to pneumonia at the age of three. The diagnosis of GSD in the patient's cousin was based on clinical manifestations, hepatomegaly in ultrasound sonography, liver biopsy, and lab data including hypoglycemia, hypercholesterolemia, and hypertriglyceridemia.

Echography showed mild atrial septal defect (ASD) and the contrast ecography revealed mild arteriovenous malformation (AVM) from the left lung (left upper pulmonary vein). The upper gastrointestinal series showed esophageal

Table 1 Manifestations of GSDIb from previous known cases

Growth	Short stature, Delayed puberty
Head and neck	Doll-like facies, Lipemia Retinalis, Oral ulcers, Xanthelasma
Cardiovascular	Hypertension
Abdomen Gastrointestinal	Protuberant abdomen, Hepatomegaly, Liver adenoma, Hepatocellular carcinoma, Pancreatitis, Chronic inflammatory bowel disease (IBD), Intestinal mucosal ulceration
Kidneys	Reduced creatinine clearance, Focal segmental glomerulosclerosis, Renal stones, Renal enlargement
Skeletal	Osteoporosis, Gouty arthritis
Skin	Xanthoma
Immunology	Neutropenia, Abnormal leukocyte function, Reduced T cells

reflux. Horseshoe kidney and urinary reflux of grade II–III was diagnosed in the first ultrasound sonography after birth.

The lab tests revealed lower levels of polymorphonuclear leukocytes (3.5 cells/cmm^3) and specifically neutropenia (160 cells/cmm^3) when she was a 4-month child. Reduced levels of natural killer cells alongside with IgG and IgA deficiency were also observed. Furthermore, thrombocytosis was detected (more details are available in Table 2). The nitroblue-tetrazolium (NBT) (PMA-stimulated) and dihydrorhodamine (DHR) flow cytometry tests show the functional capacity of neutrophils by measuring their activation after stimulating with phorbol myristate acetate [14]. Despite neutropenia, the indices for neutrophils' activity were normal in the patient.

Whole exome sequencing was conducted at 5 months and revealed homozygous nonsense mutation in *SLC37A4* gene, variant of c.1245G > A p.Trp415Ter located on exon 12 and transcript ID of ENST00000357590.5,NM_001164278, and heterozygous mutation in *PIK3CD* gene, variant of c.580G > A p.Val194Ile located on exon 5 and transcript ID of ENST00000377346.4,NM_005026. No chromosomal rearrangement was distinguished through chromosome karyotype analysis by fluorescence in situ hybridization.

Genomic DNA was extracted from peripheral blood leukocytes after performing the standard phenol/chloroform method. Human whole exome enrichment was conducted utilizing Twist Human Core Exome Kit and sequencing and the library was sequenced on Illumina platform with a raw coverage of 264X and mean on-target coverage of 83X, performed by CeGaT GmbH, Germany. Almost all exons and flanking 10 bp were detected and analyzed. Next-generation sequencing method was used to detect variations that include single point mutations and small indels (within 20 bp). The analytical sensitivity and specificity of next-generation sequencing method are assumed to be higher than 95%. PCR amplification was followed by Sanger sequencing to determine her genotype for c.1245G > A in *SLC37A4* gene; however, it was not conducted for *PIK3CD* gene.

Table 2 Laboratory test results

Measure (unit)	Result	Normal range
WBC (mm^3)	8.54 *10^3	6–17.5 *10^3
Lymphocyte (mm^3)	6.46 *10^3	4–10.5 *10^3
PMN (mm^3)	**3.5**	300–875
Platelet (μL)	**745 *10^3**	250–450 *10^3
Hemoglobin (g/dL)	11.5	11.3–14.1
CD3 (%lymph) Count(mm^3)	65.22% (4.21 *10^3)	30–70
CD4 (%lymph) Count(mm^3)	44.43% (2.87 *10^3)	22–58
CD8 (%lymph) Count(mm^3)	14.15% (0.91 *10^3)	10–37
CD14 (%lymph) Count(mm^3)	9.41% (0.61 *10^3)	4–10
CD16 (%lymph) Count(mm^3)	12.12% (0.78 *10^3)	5–19
CD20 (%lymph) Count(mm^3)	18.92% (1.22 *10^3)	34–37
CD56 (%lymph) Count(mm^3)	**1.43**% (0.09 *10^3)	5–19
IgG (mg/dL)	**371**	800–1600
IgA (mg/dL)	**58**	70–400
IgM (mg/dL)	56	40–230
NBT (PMA-stimulated) (mg/dL)	95%	90–100
Neutrophil Count (cells/cmm^3)	**160**	1700–7000
DHR + PMA (NOI)	215	> 100

WBC, white blood cell; *PMN*, polymorphonuclear leukocytes; *NBT*, Nitroblue tetrazolium test, *DHR*, Dihydrorhodamine; *PMA*, Phorbol-myristate-acetate; *NOI*, neutrophil oxidative index

Footnote: The patient's age was 4 months when the lab tests were performed. Numbers marked by bold font are out of indicated normal range

Discussion

GSDIb is a type of GSD1 with distinctive features of neutropenia and recurrent infection. Here, we represent a novel mutation of *SLC37A4* gene in the case of GSDIb. Henry-Gery Hers reported the first case of GSDIb in 1959 at IX. International Congress of Pediatrics in Montreal, and Lowe et al. published the first report of the combination of glycogen storage disease type I with the normal activity of glucose-6 phosphatase in the frozen liver [15]. In the present case, the consanguinity of parents and history of GSD in the cousin of the patient approves the autosomal recessive pattern of the disorder.

It has been investigated that GSDIb has all the characteristics of GSDIa patients plus neutropenia and failure at maintaining blood glucose level consistent after glycerol intravenous administration. Hepatomegaly, a key finding of GSDIb, was present in this case. Horseshoe kidney is a condition of fusion defect in the kidney, which occurs as a result of disruption in the development and migration of the kidneys. The condition predisposes the patient to infection, ureteropelvic junction obstruction, and kidney stones. Till now, horseshoe kidney has been reported in one case of Glycogen Storage Disease Type 1a, but none in Glycogen Storage Disease Type 1b, which was present in the studied case [16]. The patient had vesicoureteral reflux, which was previously only reported in Glycogen Storage Disease Type 1a patients [17]. The history of recurrent UTI and vesicoureteral reflux in the patient can be explained by the horseshoe kidney. The relationship between horseshoe kidney and the present mutations of the patient is not known to date.

The presence of hypertension in this patient can be explained by pyeloureteral stenosis in horseshoe kidney. The

renin-aldosterone system's activation may result in vasoconstriction of the afferent arteriole which leads to reduction of renal blood flow and arterial hypertension consequently [18]. Pulmonary AVM is a rare condition in which a pulmonary vein is connected to a pulmonary artery through an abnormal vascular structure. Hypoxemia, fatigue, cyanosis, and dyspnea can be the symptoms of this state, and sometimes the patients are asymptomatic [19]. ASD and AVM have never been reported in type b of GSDI patients previously; however, there are some studies indicating presence of pulmonary arterial hypertension and ASD in GSDI patients as a result of abnormal production of vasoconstrictive amines including serotonin [20]. The case of our study had ASD and mild AVM, which is a novel finding in a case of GSDIb.

PIK3CD gene encodes p110 delta (p110δ) protein, a subunit of phosphatidylinositol 3-kinase, which is primarily found in leukocytes. It is vital for activation, migration, maturation, and cytokine production in NK cells. Intermittent neutropenia and recurrent respiratory infections have been reported in patients with heterozygous mutation of *PIK3CD* [21]. Decreased serum levels of IgA and IgG which have been reported in patients with *PIK3CD* [22, 23], predispose the patient to frequent sinopulmonary infections. Thus, it may also have a role in neutropenia and IgA and IgG deficiency of this patient alongside *SLC37A4* gene. Neutropenia and IgA and IgG deficiency can be the underlying cause of recurrent pneumonia in this case.

Neutropenia and recurrent infections need therapy in GSDIb patients, granulocyte colony-stimulating factor (G-CSF) has been used in these patients and has shown promising results with having normal neutrophil count and reducing infections [24]. Liver transplant and allogeneic hematopoietic stem cells are also among the suggested treatments for the disorder [25].

In conclusion, cardiovascular and renal workup can be suggested in GSDIb patients. Moreover, since recurrent infections due to immune deficiency is a major cause of death in these patients, immunotherapy options such as G-CSF and empagliflozin should be considered with more emphasis. Notably, it is vital to keep in mind to investigate other genes related to function of neutrophils including *PIK3CD* in patients with GSDIb.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Irons M, Elias ER. Glycogen Storage Diseases. In: Berul CI, Towbin JA, editors. Molecular genetics of cardiac electrophysiology. US Boston, MA: Springer; 2000. p. 219–37.
2. Narisawa K, Igarashi Y, Otomo H, Tada K. A new variant of glycogen storage disease Type I probably due to a defect in the glucose-6-phosphate transport system. Biochem Biophys Res Commun. 1978;83(4):1360–4.
3. Pan C-J, Chen S-Y, Lee S, Chou JY. Structure-function study of the glucose-6-phosphate transporter, an eukaryotic antiporter deficient in glycogen storage disease type Ib. Mol Genet Metab. 2009;96(1):32–7.
4. Kishnani PS, Koeberl D, Chen Y-T. Glycogen Storage Diseases. In: Scriver CR, Beaudet AL, Sly WS et al., eds. Metabolic and molecular bases of inherited disease. 8th ed. New York: McGraw-Hill, 2009.
5. Chou JY, Jun HS, Mansfield BC. Neutropenia in type Ib glycogen storage disease. Curr Opin Hematol. 2010;17(1):36–42.
6. Veiga-da-Cunha M, Chevalier N, Stephenne X, Defour J-P, Paczia N, Ferster A, et al. Failure to eliminate a phosphorylated glucose analog leads to neutropenia in patients with G6PT and G6PC3 deficiency. Proc Natl Acad Sci. 2019;116(4):1241–50.
7. Jun HS, Weinstein DA, Lee YM, Mansfield BC, Chou JY. Molecular mechanisms of neutrophil dysfunction in glycogen storage disease type Ib. Blood. 2014;123(18):2843–53.
8. Rossi A, Simeoli C, Salerno M, Ferrigno R, Della Casa R, Colao A, et al. Imbalanced cortisol concentrations in glycogen storage disease type I: evidence for a possible link between endocrine regulation and metabolic derangement. Orphanet J Rare Dis. 2020;15(1):99.
9. Visser G, Rake JP, Fernandes J, Labrune P, Leonard JV, Moses S, et al. Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type Ib: results of the European Study on Glycogen Storage Disease type I. J Pediatr. 2000;137(2):187–91.
10. Kishnani PS. Glycogen storage diseases. In: Aminoff MJ, Daroff RB, editors. Encyclopedia of the Neurological Sciences. 2nd ed. Oxford: Academic Press; 2014. p. 454–9.
11. Dror Y, et al. Chapter 29 - Inherited bone marrow failure syndromes. In: Hoffman R, Benz EJ, Silberstein LE, Heslop HE, Weitz JI, Anastasi J, et al., editors. Hematology (Seventh Edition). Elsevier; 2018. p. 350–93.
12. Wortmann SB, Van Hove JLK, Derks TGJ, Chevalier N, Knight V, Koller A, et al. Treating neutropenia and neutrophil dysfunction in glycogen storage disease type Ib with an SGLT2 inhibitor. Blood. 2020;136(9):1033–43.
13. Rossi A, Miele E, Fecarotta S, Veiga-da-Cunha M, Martinelli M, Mollica C, et al. Crohn disease-like enterocolitis remission after empagliflozin treatment in a child with glycogen storage disease type Ib: a case report. Ital J Pediatr. 2021;47(1):149.
14. Bellinati-Pires R, Carneiro-Sampaio MM, Colletto GM. Functional evaluation of human neutrophils. Is the bactericidal activity correlated with nitroblue tetrazolium reduction? J Investig Allergol Clin Immunol. 1992;2(3):146–53.
15. Lowe C, Doray B, Sokal J, Sarcione E. Carbohydrate metabolism in glycogen storage disease and the mode of action of insulin. Mod Probl Paediat. 1959;4:157.
16 Cohn A, Ohri A. Diabetes mellitus in a patient with glycogen storage disease type Ia: a case report. J Med Case Rep. 2017;11(1):319.
17. Saneifard H, Shamsian B, Shakiba M, Karizi Zarea S, Sheikhy A. A rare case of glycogen storage disease type 1a presenting with hemophagocytic lymphohistiocytosis (HLH). Case Reports Pediatr. 2020;2020:8818617.
18. Aguilar-García JJ, Domínguez-Pérez AD, Nacarino-Mejías V, Ruiz-Guerrero CI, Iribarren-Marín MA, Ortega-Seda CJ. Arterial hypertension induced by pyeloureteral stenosis in horseshoe kidney. Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia. 2011;31(3):365–6.
19. Saboo SS, Chamarthy M, Bhalla S, Park H, Sutphin P, Kay F, et al. Pulmonary arteriovenous malformations: diagnosis. Cardiovasc Diagn Ther. 2018;8(3):325–37.

20. Torok RD, Austin SL, Britt LK, Abdenur JE, Kishnani PS, Wechsler SB. Pulmonary rrterial hypertension in glycogen storage disease type I. J. Inborn Errors Metab Screen. 2017:e160060-e160060.
21. Mandola AB, Dadi H, Reid B, Roifman CM. Novel heterozygous PIK3CD mutation presenting with only laboratory markers of combined immunodeficiency. LymphoSign J. 2020;7(2):49–55.
22. Jamee M, Moniri S, Zaki-Dizaji M, Olbrich P, Yazdani R, Jadidi-Niaragh F, et al. Clinical, immunological, and genetic features in patients with activated PI3Kδ syndrome (APDS): a systematic review. Clin Rev Allergy Immunol. 2020;59(3):323–33.
23. Cohen SB, Bainter W, Johnson JL, Lin T-Y, Wong JCY, Wallace JG, et al. Human primary immunodeficiency caused by expression of a kinase-dead p110δ mutant. J Allergy Clin Immunol. 2019;143(2):797-9.e2.
24. Calderwood S, Kilpatrick L, Douglas SD, Freedman M, Smith-Whitley K, Rolland M, et al. Recombinant human granulocyte colony-stimulating factor therapy for patients with neutropenia and/or neutrophil dysfunction secondary to glycogen storage disease type 1b. Blood. 2001;97(2):376–82.
25. Lachaux A, Boillot O, Stamm D, Canterino I, Dumontet C, Regnier F, et al. Treatment with lenograstim (glycosylated recombinant human granulocyte colony-stimulating factor) and orthotopic liver transplantation for glycogen storage disease type Ib. J Pediatr. 1993;123(6):1005–8.