First 3 cases of glycogen storage disease type 1b diagnosed in Morocco: genetic and clinical features

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ABSTRACT: Purpose: Glycogen storage disease type 1b (GSD1b) is a rare autosomal recessive metabolic defect of glycogenolysis and gluconeogenesis which results from a deficiency of the glucose-6-phosphate translocase. GSD 1b is characterized by chubby face, hypoglycemia, hyperlipidemia, hyperuricemia, hepatomegaly, nephromegaly and growth retardation. GSD1b patients also show neutropenia and/or neutrophil dysfunction that cause increased susceptibility to recurrent bacterial infections and inflammatory intestinal diseases. From 2010 to 2014, 3 cases of GSD1b were diagnosed in Morocco. The purpose of this paper is to report the clinical and genetic characteristics of those GSD1b patients.

Methods: We investigated the genetic, immunological and clinical features of 3 Moroccan patients with GSD1b from 3 unrelated kindreds.

Results: All patients experience chubby face, hepatomegaly, hypoglycemia, hypercholesterolemia / hypertriglyceridemia / hyperuricemia and failure to thrive. All cases suffered from recurrent bacterial and/or fungal infections due to neutropenia. The sequencing of SLC37A4 gene showed the same mutation c.1042_1043delCT in the homozygous state. In the absence of treatment with recombinant human granulocyte colony-stimulating factor (G-CSF), the evolution was marked by the death of two cases in an infectious context despite symptomatic and preventive treatment.

Conclusion: Further studies on a large cohort are required to determine the incidence and prevalence of the disease, and to improve the description of the genetic and clinical features of GSD1b patients in Morocco.

KEYWORDS: Glycogen storage disease type 1b, glucose-6-phosphate translocase, sequencing, SLC37A4, mutation.

1 INTRODUCTION

Glycogen storage disease type 1 (GSD1; OMIM 23.2200) is a group of rare autosomal recessive disorders characterized by hypoglycemia, hyperlipidemia, hyperuricemia, hepatomegaly, nephromegaly, hyperlactacidemia and growth retardation [1],[2]. GSD 1 is caused by inherited defects of the glucose-6-phosphatase (G6Pase) complex, which consists of at least 2 membrane proteins, glucose-6-phosphate transporter (G6PT), and glucose-6-phosphatase (G6Pase). This complex has a key
role in both glycogenolysis and gluconeogenesis, converting glucose-6-phosphate (G6P) to glucose [3]. In the liver and kidney - the tissues responsible for maintaining interprandial blood glucose homeostasis - G6P derived from glycogenolysis or gluconeogenesis, is conveyed through the endoplasmic reticulum (ER) membrane by the G6PT [4],[5]. In the ER lumen, G6P is hydrolyzed to glucose and released. Two enzymatically active G6P hydrolases are incorporated in the ER membrane with their active sites facing into the lumen: glucose-6-phosphatase-α (G6Pase-α or G6PC) and G6Pase-β (G6Pc3) [6],[7]. G6Pase-β is expressed ubiquitously [8] while the expression of G6Pase-α is restricted to the liver, kidney and intestine [4],[5].

Deficiencies in G6Pase-α cause GSD1a (GSD1a; OMIM 232200) [4] and deficiencies in G6Pase-β cause a severe congenital neutropenia syndrome of unknown incidence [9].

Glycogen storage disease type 1b (GSD1b; OMIM 232220) is proposed to be caused by a defective G6PT protein (also known as SLC37A4). Neutropenia and/or neutrophil dysfunction is a characteristic hallmark of GSD1b. Patients with GSD1b are thus susceptible to recurrent bacterial infections, commonly involving the perirectal area, ears, skin, and urinary tract, although life-threatening infections, such as septicemia, pneumonia, and meningitis occur less frequently[4],[5],[10]. There are some studies which try to explain the neutropenia as well as the concomitant neutrophil dysfunction, which includes impaired chemotaxis, phagocytosis, and respiratory burst. However, the exact mechanism of immune deficiency in GSD1b remains unknown.

We describe here the genetic, biological and clinical features of the first 3 GSD1b patients diagnosed in Morocco, revealed by neutropenia.

2 PATIENTS AND METHODS

2.1 SUBJECTS

The study was conducted in accordance with the Helsinki Declaration, with informed consent obtained from the patient’s family. Patients were recruited at the Clinical Immunology Unit (CIU) in Casablanca and the Department of Pediatrics of Rabat Children’s Hospital, between 2010 and 2014. The main investigations in biochemistry were: glycemia, choledolishemia, triglyceridemia, uricemia, and ASAT/ALAT. The immune profile included: cell blood count, lymphocyte numeration, quantitative immunoglobulins, and NBT test. The others laboratory analysis were: histological analysis of liver biopsy, blood culture, and urine culture.

The clinical diagnosis of GSD1b was based on chubby face, biological hallmarks (hypoglycemia, hyperlipidemia and hyperuricemia), hepatomegaly, histopathological findings, and the history of recurrent infections due to neutropenia.

2.2 MOLECULAR ANALYSIS

We sequenced the SLC37A4 gene for all patients and their parents to confirm the diagnosis. Genomic DNA was isolated from leukocytes using Maxwell 16 Blood DNA purification kit (Promega, Charbonnières, France), according to the manufacturer’s instructions. All nine coding exons and flanking intron-exon boundaries of SLC37A4 gene (reference sequence NM_001467) were amplified via the polymerase chain reaction (PCR) using the GoTaq G2 Hot Start (Promega). The primers and conditions used for PCR amplification are available upon request. Amplicons were checked by electrophoresis in a 1 % agarose gel and cleaned up using Wizard SV Gel ans PCR Clean-Up system (Promega). PCR products were sequenced by dideoxynucleotide termination, with the BigDye Terminator kit V1.1 (Life Technologies, Courtaboeuf, France) with the same primers. Electrophoresis was performed on the ABIPrism 3130 genetic analyser (Life Technologies). The raw data were then analyzed with SeqScape v2.5 software (Life Technologies) to be compared to the reference sequence.

3 RESULTS

3.1 MOROCCAN GSD1B CASES

GSD1b, which combines a metabolic and immune disorder, belongs to the group of congenital neutropenia (CN) and it is therefore a primary immunodeficiency (PID). From 1998 until December 2014, a total of 502 patients with PIDs were registered, including 59 cases of CN. In our series of CN, 3 cases of GSD1b were diagnosed. The clinical manifestations, biological profile and outcomes of our three cases of GSD1b are presented in table 1.
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Table 1. Summary of clinical and biological findings in the 3 affected children.

<table>
<thead>
<tr>
<th></th>
<th>Patient N°1</th>
<th>Patient N°2</th>
<th>Patient N°3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex/Parental origin</strong></td>
<td>M/Morocco</td>
<td>M/Morocco</td>
<td>M/Morocco</td>
</tr>
<tr>
<td><strong>Consanguinity</strong></td>
<td>3° degree</td>
<td>1° degree</td>
<td>1° degree</td>
</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td>8 Mo</td>
<td>8 Mo</td>
<td>6 Mo</td>
</tr>
<tr>
<td><strong>Age at onset</strong></td>
<td>1.5 Mo</td>
<td>2 Mo</td>
<td>1 Mo</td>
</tr>
<tr>
<td><strong>Chubby face</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Infections</strong></td>
<td>Recurrent respiratory infections</td>
<td>Neonatal sepsis, recurrent respiratory infections, urinary tract infection</td>
<td>Recurrent ear infections, multiple abscesses, urinary tract infection</td>
</tr>
<tr>
<td><strong>Hypoglycemia</strong></td>
<td>+ (0.03 mmol/L)</td>
<td>+ (1.33 mmol/L)</td>
<td>+ (1.67 mmol/L)</td>
</tr>
<tr>
<td><strong>Hypertriglyceridemia/Hypercholesterolemia</strong></td>
<td>+ (8.12 mmol/L) / + (5.76 mmol/L)</td>
<td>+ (13.02 mmol/L) / + (6.54 mmol/L)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Hyperuricemia</strong></td>
<td>ND</td>
<td>+ (571.1 µmol/L)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Neutropenia</strong></td>
<td>+ (0.75-1×10⁹/L)</td>
<td>+ (0.29×10⁹/L)</td>
<td>+ (0.17-0.62×10⁹/L)</td>
</tr>
<tr>
<td><strong>Immune profile</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Deceased, age 10 Mo</td>
<td>Deceased, age 12 Mo</td>
<td>Alive, age 2.5 Y</td>
</tr>
</tbody>
</table>

M male, Mo month, + present, ND not done, GSD glycogen storage disease, Y year

3.2 **Mutation analysis**

The molecular analysis showed a homozygosity for the mutation c.1042_1043delCT in all three cases. The parents of all patients carry the same mutation in the heterozygous state.

4 **Discussion**

GSD1b is an autosomal recessive disorder classified as an inborn error of glycogen metabolism. It occurs because G6PT does not function properly due to a mutation in the gene that encodes it. During periods of hypoglycemia, the liver releases glucose into the blood by breaking-down glycogen. Breakdown of glycogen occurs in steps that require many enzymes and other proteins. One of these steps involves the G6P, a precursor of glucose, to move from the cell’s cytoplasm into the ER. Normally, G6P is transported by the protein G6PT, but since patients with GSD 1b have a defective G6PT, G6P stays in the cell’s cytoplasm and it is not converted into glucose [4],[11]. Hence, many of the body’s processes lack energy and are impaired.

Most patients experience the following dominant symptoms: chubby face, hepatomegaly, hypoglycemia, failure to thrive, hypercholesterolemia / hypertriglyceridemia / hyperuricemia [12],[13], nephrohypertrophy, delayed psychomotor development, osteopenia [14], [15], recurrent infections due to neutropenia and/or neutrophil impairment, and inflammatory bowel disease [16], [17].

Neutropenia and neutrophil dysfunction are found in 95% of cases with GSD1b and can arise late in the evolution [16], [17],[18], unlike our three patients where neutropenia and the recurrent infections were the mode of revelation. This was not described in the literature and represented a factor of delay diagnosis besides the rarity of this pathology making that the pediatricians do not think of it.
GSD1b has an incidence of 1 in 500,000 [4],[5]. Like all PID, GSD1b is underdiagnosed in the world, and especially in low-income countries. In our series, the PID prevalence observed is 0.81/100,000 inhabitants [19], which is largely below prevalence observed in France (5.56/100,000) or Australia (5.6/100,000) [20], [21]. Among 512 cases of PID registered in Morocco since 1988, only 3 patients were diagnosed with GSD1b. The high rate of consanguinity in Morocco, estimated at 15.25% in general population, suggest high incidence of autosomal recessive genetic disorders [22]. In a recent study, we estimated that 27,852 patients should harbor a PID in Morocco in 2011, and around 3,300 new cases should be diagnosed each year [23]. The small number of PID and particularly GSD 1b in Morocco can be explained by the lack of awareness observed in our medical community, the lack of facilities in certain regions, delay in diagnosis, difficulties to access to care and the early death.

In all three cases, we found the same mutation c.1042_1043delCT. This deletion, that affects the exon 8, was firstly described by Veiga-da-Cuncha M et al. in 1998 [24]. Molecular heterogeneity is extreme but several mutations predominate and vary according to the population: c.1042_1043delCT and c.1015G>T (p.Gly339Cys) are the most common in the Caucasian population, where they represent nearly half of the G6PT alleles, and the c.352T>C (p.Try118Arg) mutation is present in nearly 40% of the Japanese G6PT alleles [12].To date, 91 mutations were identified for \textit{SLC37A4} gene (Resource of Asian Primary Immunodeficiency Disease, http://web16.kazusa.or.jp/rapid/mutation?pid_id=AGID_160). No correlation was found between individual mutations and the absence of neutropenia, bacterial infections and systemic complications [25].

The family of the 2\textsuperscript{nd} case benefited from a prenatal genetic diagnosis, done via amniocentesis in the 11\textsuperscript{th} week of amenorrhea. The test showed that the fetus carries the mutation in the heterozygous state.

Since there is no data available concerning GSD1b in Morocco, our goal is also to share our experience with all physicians especially pediatricians. Because of lack of awareness, the first patient died without clinical diagnosis aside from the result of liver biopsy that showed a compatible aspect with glycogen storage disease. The second patient presented also the clinical and biological hallmarks of GSD1b, however the clinical diagnosis was made before the liver biopsy. The third patient was suspected of GSD1b as soon as he was admitted. After biological analysis, we confirmed the diagnosis genetically without using liver biopsy. So, if the pediatrician is sensitized to GSD1b, he could make the diagnosis of GSD1b with basic well-chosen laboratory analysis. The genetic confirmation could also spare GSD1b patients the trauma of liver biopsy.

The main goal of treatment is to maintain appropriate blood glucose levels while still avoiding the excess storage of glycogen. Glucose concentrations can be maintained with a diet based on frequent meals and nocturnal gastric drip-feeding. Biochemical and growth parameters showed significant improvements after the introduction of dietary therapy [26],[27]. Treatment with granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF) increases the level of neutrophils, reduces the incidence of infections and has improved the quality of life of patients with GSD1b. Defects in neutrophil chemotaxis and intracellular bacterial killing have been described and appear to be corrected by the use of G-CSF [28],[29]. In the absence of available G-CSF and GM-CSF for our patients, a continuous anti-infective prophylactic treatment is established with antibiotics accumulated into neutrophils (co-trimoxazole, clindamycin, rifampicin). Nevertheless, secondary infections to neutropenia caused the death of two patients.

5 Conclusion

GSD1b, unknown in our context as all PID, is a rare autosomal recessive metabolic disease. All our patients showed the typical clinical features and biological hallmarks of GSD1b and carry the same deletion c.1042_1043delCT. We also showed a large delay in diagnosis in our series. Further studies on a large cohort are needed to determine the true incidence and prevalence of the disease and find out whether there are more common mutations among Moroccan GSD1b patients.

Acknowledgments

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COMPLIANCE WITH ETHICS GUIDELINES

CONFLICT OF INTEREST:

Ayoub Aglaguel, François Petit, Naima El Hafidi, Asmaa Essouiba, Norddine Habti, Ahmed Aziz Bousfiha, Fatima Ailal, and Houria Abdelghaffar declare that they have no conflict of interest.

INFORMED CONSENT:

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients included in the study.

REFERENCES


