A retrospective longitudinal study and comprehensive review of adult patients with glycogen storage disease type III

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ABSTRACT

Introduction: A deficiency of glycogen debrancher enzyme in patients with glycogen storage disease type III (GSD III) manifests with hepatic, cardiac, and muscle involvement in the most common subtype (type a), or with only hepatic involvement in patients with GSD IIIb.

Objective and methods: To describe longitudinal biochemical, radiological, muscle strength and ambulation, liver histopathological findings, and clinical outcomes in adults (≥18 years) with glycogen storage disease type III, by a retrospective review of medical records.

Results: Twenty-one adults with GSD IIIa (14 F & 7 M) and four with GSD IIIb (1 F & 3 M) were included in this natural history study. At the most recent visit, the median (range) age and follow-up time were 36 (19–68) and 16 years (0–41), respectively. For the entire cohort: 40% had documented hypoglycemic episodes in adulthood; hepatomegaly and cirrhosis were the most common radiological findings; and 28% developed decompensated liver disease and portal hypertension, the latter being more prevalent in older patients. In the GSD IIIa group, muscle weakness was a major feature, noted in 89% of the GSD IIIa cohort, a third of whom depended on a wheelchair or an assistive walking device. Older individuals tended to show more severe muscle weakness and mobility limitations, compared with younger adults. Asymptomatic left ventricular hypertrophy (LVH) was the most common cardiac manifestation, present in 43%. Symptomatic cardiomyopathy and reduced ejection fraction was evident in 10%. Finally, a urinary biomarker of glycogen storage (Glc4) was significantly associated with AST, ALT and CK.

Conclusion: GSD III is a multisystem disorder in which a multidisciplinary approach with regular clinical, biochemical, radiological and functional (physical therapy assessment) follow-up is required. Despite dietary modification, hepatic and myopathic disease progression is evident in adults, with muscle weakness as the major cause of morbidity. Consequently, definitive therapies that address the underlying cause of the disease to correct both liver and muscle are needed.

Abbreviations:
GSD, Glycogen storage disease; GDE, Glycogen debrancher enzyme; BMI, Body mass index; DM, Diabetes mellitus; BG, Blood glucose; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; GGT, Gamma glutamyl transferase; Glc4, Glucose tetrasaccharide; CPK, Creatine phosphokinase; TGs, Triglycerides; HDL, High density lipoprotein; LDL, Low density lipoproteins; AFP, Alpha-fetoprotein; CEA, Carcinoembryonic antigen; US, Ultrasound; CT scan, Computed tomography scan; MRI, Magnetic resonance imaging; LT, liver transplantation.

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1. Introduction

GSD III (OMIM 232400) is caused by a deficiency of the glycogen debrancher enzyme (GDE; OMIM 610860), an enzyme with two independent catalytic activities: amylo-1,6-glucosidase (EC 3.2.1.33) and 4-alpha-glucanotransferase (EC 2.4.1.25) [1]. Together with glycogen phosphorylase, GDE degrades glycogen to release glucose and glucose-1-phosphate for use as a source of energy [1]. A deficiency of GDE leads to an accumulation of abnormally structured glycogen, called limit dextrin, which is characterized by short outer chains. This accumulation occurs in different tissues, and especially in the liver, muscle and heart. GSD IIIa is the most common subtype, accounting for 85% of cases, and is characterized by a lack of GDE activity in the liver, muscle and heart. The second subtype, accounting for the remaining 15% of cases, is GSD IIb, with GDE deficiency confined to the liver [2-5].

The predominant biochemical features of GSD III are hypoglycemia with or without ketosis, hyperlipidemia and elevated liver transaminases [6]. Due to intact glucogenesis in these patients, hypoglycemic episodes are usually not as severe as seen in GSD I. Hepatomegaly and growth retardation are present during infancy and early childhood. Hepatic fibrosis can occur at an early age, and is associated with a decrease in liver size and transaminases over time, indicating the progression of liver disease [7]. In addition, hepatic cirrhosis, hepatocellular adenoma and carcinoma are well-recognized late, long-term complications [6,8,9].

In patients with GSD IIIa, the degree of muscle involvement varies significantly [6]. Exercise induced muscle pain and exercise intolerance are common complaints. Proximal and distal muscle weakness, which can be associated with atrophy of the affected muscle groups, is a cause of significant morbidity, limiting movement and daily activities. Although myopathy is reported as more prominent in the 3rd-4th decade of life [6], gross motor delay, muscle weakness and hypotonia are now recognized as part of the clinical spectrum in children with GSD IIIa [6,10,11].

Left ventricular hypertrophy is the most common cardiac manifestation of GSD IIIa, and is often asymptomatic. Although it may manifest in the first decade of life, or even in early infancy, it becomes more prevalent at older ages [6,8,12,13,14]. Symptomatic cardiomyopathy accompanied with heart failure has also been reported [14,15,16]; as a result, the most severe phenotype may need heart transplantation [17]. In addition, GSD IIIa patients are at an increased risk of different types of arrhythmia, such as atrial and ventricular fibrillation [15,18,19]. A number of published case reports revealed an increased risk of sudden death in GSD IIIa patients, which can be secondary to either severe cardiomyopathy or arrhythmia [17,19,20].

Diabetes mellitus type 2 and osteopenia / osteoporosis are among the most common endocrine disorders reported in GSD III patients [21, 22, 23]. Furthermore, females with GSD III are at an increased risk of polycystic ovary disease. Fertility does not seem to be affected as there are reports of successful pregnancies in these women [24,25].

A high protein diet (3-4 g/kg/day in children and 20-30% total calories in adults) with complex carbohydrates (~50% of total calories) is the main treatment for GSD III [6]. Uncooked cornstarch or its extended-release form (Glycosadex®) can be used to prolong fasting tolerance with steady levels of blood glucose [6,26]. A high protein diet promotes gluconeogenesis, and improves blood glucose control, growth parameters, and myopathic symptoms in patients [27,28,29]. Furthermore, in patients with cardiomyopathy and myopathy, there may be potential benefits in using medium chain triglycerides and/or ketogenic supplements, with or without a high protein diet [30,31,32,33]. However, despite achieving normoglycemia by dietary therapy long-term complications still occur.

There are limited studies on the long-term outcomes of adults with GSD III [8,34]. The natural history of adults with GSD III documented in the literature is mainly composed of case reports and studies that have emphasized observation of a single body system, or documented findings after a short follow-up period [9,13–20,21,22,24,25]. Herein, we report the clinical manifestations in combination with the biochemical, radiological, muscle strength and ambulation, and liver histopathological findings in 25 adult patients with GSD III, and review the current management guidelines. We characterize the multi-systemic phenotype and disease course over a period of up to 40 years, to understand the disease progression and its implications on current and future therapy development.

2. Methods

2.1. Subjects & study design

Twenty-five adults (10 M, 15 F, aged >18 years) with GSD III were included in our longitudinal, retrospective natural history study. A diagnosis of GSD III was confirmed by the presence of two pathogenic variants in the AGL gene and/or a deficiency of GDE in the liver or muscle biopsy. All patients were seen at Duke University Health System at least once.

The study was approved by the Duke University Institutional Review Board (IRB). Informed consent or a decedent waiver was obtained from all patients (IRB Pro00013699 and Pro00047556).

Clinical case descriptions of subjects 13, 22, 24, 25, and 39 were published previously [9,17,35].

2.2. Data collection

We reviewed patient charts from January 1979 to September 2020 for the main findings in the history and physical examination, taking into consideration the chronological order of the clinical visits.

2.2.1. Clinical data

General information about sex, age (at diagnosis and at most recent visit), presentation, ethnicity and modality of diagnosis (molecular, liver or muscle biopsy) was collected.

Anthropometric measurements (height and weight) and body mass index (BMI) were calculated and tracked over time.

Based on the extent of hepatic disease, patients were categorized with the input from an experienced hepatologist in GSDs (A. M.) into the following three groups: 1) no detection of cirrhosis on imaging and/or liver biopsy, 2) compensated cirrhosis, and 3) decompensated cirrhosis/portal hypertension. The Model for End-Stage Liver Disease MELD-Na score was calculated for patients with advanced fibrosis/cirrhosis or portal hypertension. The MELD-Na score is used to determine the severity and prognosis of chronic liver disease, as well as to prioritize the reception of a liver transplant in these patients, through the use of serum total bilirubin, the international normalized ratio (INR), serum creatinine, and serum sodium. The MELD-Na score ranges from 6 to 40, with higher scores correlating to a higher risk of liver disease-related 3-month mortality [36].

Patterns of proximal, distal, and generalized muscle weakness, were identified based on clinical symptoms and examination including muscle strength testing. In addition, information was collected on muscle pain/cramping, and exercise intolerance.

Data on the cardiac involvement were monitored and the presence of related symptoms such as palpitations, chest pain, shortness of breath, and symptoms secondary to heart failure, such as orthopnea, were reported.

Charts were also reviewed for other organ system involvement including: renal (creatinine, BUN, GFR, and urinalysis, for evidence of nephropathy or renal stones), endocrine disorders (prevalence of osteopenia/osteoporosis, DM type 2, and polycystic ovary disease), neurological disease (incidence of headaches, migraines, seizures, and results of imaging studies), skin (lipoma) and psychiatric (depression, anxiety, and attention deficit hyperactivity disorder (ADHD)).

Finally, developmental, drug and family history for all patients were
2.2.2. Dietary history

Data related to dietary compliance was collected from the dietary records, including the intake of protein, carbohydrate (CHO), and fat, reported as g/kg and/or as a percentage of total energy intake. A high protein diet was defined as daily ingested protein equivalent to 20–30% of total calories, while a low CHO diet was defined as total CHO consumed (from both diet and cornstarch doses) equivalent to <50% of total calories with limited simple sugars, per the published guidelines [6]. In addition, cornstarch dose and frequency, vitamin D and other supplemenations such as multivitamins and Benepeptin® were collected. Dietary data was reviewed by our metabolic dietitian (S. P.).

2.2.3. Biochemical tests

Trends and correlations of biochemical markers were reviewed and included in the data analysis of this cohort. These included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), urinary glucose tetrasaccharide (Glc-Tetra), total cholesterol, high and low-density lipoproteins (HDL, LDL), liver function testing (albumin, prothrombin time (PTT)), platelet count, bilirubin, glucose, alpha-fetoprotein (AFP), and carcinoembryonic antigen (CEA) levels. Because of reduced fasting tolerance in GSD III, lipid profiles were routinely performed after 3–4 h of fasting.

2.2.4. Radiologic studies

Results of abdominal ultrasound (US), computerized tomography scan (CT) and magnetic resonance imaging (MRI) were recorded longitudinally for changes in liver size, echogenicity, and evidence of fibrosis, cirrhosis or hepatocellular adenoma/carcinoma. The type of imaging modality used was different across patients. Contributory factors to these differences included variability in insurance coverage and in the availability of techniques at the hospitals performing the imaging. Hepatomegaly was defined using as the size of the liver measured at the midclavicular line exceeding 16 cm as stated by Kratzer W, et al. [37]. Cardiac findings were described based on transthoracic echocardiography (ECHO) results.

2.2.5. Muscle strength and ambulation

Data on functional mobility, ambulatory status, and use of assistive devices and home modifications were recorded. Manual muscle testing was performed by physical therapists with extensive experience in metabolic and neuromuscular disorders. Muscle strength was measured using a modified Medical Research Council (mMRC) scale which ranges from 0 (no contraction) to 5 (full strength). Strength testing was performed at the shoulders (flexion, abduction), elbows (flexion, extension), hips (flexion, abduction, adduction, extension), knees (flexion, extension), and ankles (dorsiflexion, plantarflexion). Distal upper extremity strength was assessed by measuring hand grip and lateral pinch strength using Jamar hydraulic dynamometers (Sammons Preston, Bolingbrook, IL, USA) as previously described [38].

2.2.6. Liver histopathology

In our cohort, liver biopsies were performed when clinically indicated. Liver biopsies were reviewed by an experienced pathologist as part of clinical care. To describe the stages of liver fibrosis/cirrhosis, the Batts–Ludwig system was used as described previously [39]. Stage 1 is represented by portal fibrosis, Stage 2 by periportal fibrosis, Stage 3 by bridging fibrosis and stage 4 by cirrhosis.

2.3. Statistical analysis

Continuous variables described by the laboratory numerical values, mean and standard deviation (SD), and median (range). Categorical data was expressed as a proportion. The relationships between continuous measures were examined using generalized estimating equations to account for multiple observations per patient. STATA 15.0 (College Station, TX) was used for the analysis.

3. Results

3.1. Participants

In total, 21 adults with GSD IIIa (14 F, 7 M) and 4 adults with GSD IIIb (1 F, 3 M) met the inclusion criteria for our longitudinal study. The median age recorded at the most recent visit was 36.0 years (range: 18.5–76.7). The median follow-up time for 24 patients was 16.4 years (range: 1.9–41.1). One patient (ID 46) had a single clinic visit record. Table 1 describes the characteristics of 25 patients originating from 23 families. Patients ID 13, 47, and 48 and patients ID 18 and 19 were siblings. Nineteen patients (15 GSD IIIa, 4 GSD IIIb) were non-Hispanic Caucasians, three patients were Hispanic Caucasians, one patient was Asian, and one was African American. Ethnicity was not identified from records for two patients (ID 46 and ID 49).

BMI (Kg/ m$^2$), determined using the WHO international guidelines, was reported for the most recent clinic visit. Ten patients (40%) had a normal BMI (BMI 18.5–24.9), 5 patients (20%) were overweight (BMI $>$ 25 but $<$ 30) and 10 patients (40%) had an obese BMI rating (BMI $>$ 30). The overall mean (SD) of individual patient BMI means was 28.6 ± 7.4 (n = 127 total BMI measurements).

A high protein, low CHO diet was prescribed for all patients. High protein diet was initiated at a later age in the older patients. Periods of non-compliance with the diet after the age of 18 were noted in ten patients (7 GSD IIIa, 3 GSD IIIb) (ID 8, 11, 13, 21, 24, 25, 29, 33, 38, 39). Thirteen patients (11 GSD IIIa, 1 GSD IIIb) used uncooked cornstarch. Additional cohort characteristics are summarized in Table S1.

3.2. Molecular analysis

Two pathogenic variants in AGL were detected in 22 patients (88%). Four patients had homozygous variants, while 18 were found to be compound heterozygous. Deficiency of the GDE enzyme in muscle or liver biopsy was used to confirm the diagnosis in one patient with only a heterozygous inactivating variant identified (ID 49), and two other patients did not have genetic testing performed. Four novel pathogenic variants, not previously reported, were identified (c.1471_1482del(p. Val471Arg494del), c.4259 + 5G $>$ A, c.2039G $>$ A, c.4365del(p. Ile1455fs)). The most commonly observed inactivating variants were nonsense variants (35.6%), followed by splice site intronic, missense, and frameshift deletion or duplication variants (Fig. S1). Table S2 presents the different inactivating variants, their predicted effect and location throughout the AGL gene in 23 patients.

3.3. Genotype-phenotype correlations

All GSD IIIb patients in our cohort had one copy of a GSD IIIb associated variant, c.16C $>$ T(p.Gln6Ter) or c.18_19del(p.Gln6fs) in exon 2, in combination with a nonsense or missense variant in exons 16, 6, and 20 in the second allele.

Severe phenotypes in patients with nonsense variants and intra-familial variability are highlighted in selected cases:

i) Homozygosity for c.3965del(p.Val1322fs) in exon 36 in one GSD IIIa patient (ID 25).

ii) Esophageal varices, portal venous thrombosis, leukopenia and thrombocytopenia as complications of advanced cirrhosis at the age of 23 years was observed in a patient (ID 24) who was compound heterozygous for variants c.118C $>$ T(p.Gln40Ter) and c.2309-1G $>$ A in exon 3 and intron 17.

iii) Compound heterozygosity for the variants c.100C $>$ T(p.
| ID | Sex | Age at diagnosis in months | Age at MRA in years | Genotype | BMI at MRA | Liver Disease | Muscle Disease | Cardiac Disease |
|----|-----|---------------------------|----------------------|----------|------------|---------------|---------------|----------------|
|    |     |                           |                      |          |            | Hypoglycemia  |                  |                |
|    |     |                           |                      |          |            | Cirrhosis     |                  |                |
|    |     |                           |                      |          |            | Decompensated liver disease |                |                |
|    |     |                           |                      |          |            | LT            | Muscle weakness | Assistive device |
|    |     |                           |                      |          |            |               | Generalized     | LVH            | CM             |
|    |     |                           |                      |          |            |               | IL & muscles of the hand | No             | No             |
|    |     |                           |                      |          |            |               | Normal          | No             | No             |
|    |     |                           |                      |          |            |               | No              | Yes            | No             |

**Table 1**

Patient characteristics, and hepatic, muscular, and cardiac findings.

- **GSD IIIa**
- **GSD IIIb**

(continued on next page)
### Hepatic findings

Episodes of hypoglycemia (symptomatic and asymptomatic) were reported in 10 patients (40%) after the age of 18 years, with a median age of 33.8 years (age range 18.6–64.9) (Table 1). The number of episodes was reported in 5 patients, and ranged from 1 to 5 episodes in a time period of 6 months to one year. Median blood glucose during episodes was 60 mg/dl (range 40–69 mg/dl) (desirable blood glucose >70 mg/dl). The most commonly reported hypoglycemic symptoms were irritability, jitteriness, sweating, headache, dizziness, and loss of concentration. One patient presented with a hypoglycemic seizure at the age of 21.5 years. Hypoglycemic episodes were triggered by dietary non-compliance and exercise in most patients, and was related to post-operative management in one patient.

We assessed liver imaging findings of 22 patients (18 GSD IIIa, 4 GSD IIIb) in our cohort (Table 1). Hepatomegaly was found in 11 patients (50%) with a median age of 21.9 years (range 18.3–40.3) at the most recent imaging. Hepatic steatosis was detected in 6 patients (27%), of which 4 were obese and 2 were overweight. Additionally, cirrhosis was detected in 9 patients (40%) with a median age of 40.1 years (range 21.5–54.9) at the time of imaging. Hepatocellular carcinoma was diagnosed in 3 patients (14%), at a median age of 65 years (range 54.9–67.0). Two patients with HCC (ID 22 and ID 39) were previously reported in detail [9].

Seven patients (5 GSD IIIa, 2 GSD IIIb) (28%) developed portal hypertension with ascites or hypersplenism which was secondary to either cirrhosis in 5 of 7 patients (71%) or cirrhosis in combination with HCC in 2 patients (29%). The median age for diagnosis of decompensated liver disease with portal HTN was 39 years (range 21.5–64.8).

MELD-Na scores were calculated in 8 patients with cirrhosis. Five patients had decompensated cirrhosis/portal HTN while the other three patients had compensated cirrhosis. Median MELD-Na was 8.5 (range 7–15).

Two patients in our cohort underwent liver transplantation for decompensated liver disease. The first patient received the transplant at 24 years of age while the second patient underwent combined heart-liver-kidney transplantation at the age of 40 years. Both patients have been previously discussed [17,35].

In our cohort, liver tissue was available for review from 5 patients who had liver biopsies and 2 explanted livers post-transplant, with a median age of 39 years (range 24–67). Changes consistent with fibrosis and/or cirrhosis were seen in six patients. Five patients had cirrhosis (stage 4) on the Batts–Ludwig score while one patient had portal fibrosis (stage 1). The overall prevalence of cirrhosis detected by imaging and/or biopsy was 44% (8 GSD IIIa, 3 GSD IIIb).

### Muscle strength and ambulation findings

Sixteen of 19 GSD IIIa patients (84%) complained of weakness in different muscle groups, with difficulty in performing functional activities, such as buttoning, handwriting, opening jars, picking up objects, pulling out drawers, and walking. Exercise induced muscle pain, stiffness and fatigue were reported in 12 of 14 patients (86%) with available...
data.

Eighteen GSD IIIa patients participated in muscle strength assessment. Muscle weakness was found in 16 of 18 patients (89%). Generalized muscle weakness including proximal and distal muscles of the upper and lower extremities and small muscles of the hand were reported in 12 of 16 patients (75%). Four of 16 patients (25%) had weakness involving the lower limbs and small muscles of the hand while maintaining normal strength in their proximal upper limbs. Muscle weakness limited walking in 6 of 16 patients (37.5%). Three of these patients were wheelchair dependent, while the other three required either assistive devices (cane or walker) or home modifications (ramp) in order to walk safely. Older patients tended to show more severe muscle weakness and walking limitations, compared with younger adults (Table 1).

3.6. Cardiac findings

Cardiovascular system-related symptoms were retrieved for 14 GSD IIIa patients. Half of these patients (7/14) had symptoms. Spontaneous or drug-induced palpitations (3/14), and chest pain at rest or with exertion, and with or without shortness of breath (4/14) were reported. In addition, symptoms secondary to heart failure such as orthopnea was described in one patient (ID 13).

Echocardiographic results were retrieved for 21 GSD IIIa patients (Table 1). LHV was diagnosed in 9/21GSD IIIa patients (42.9%). Seven patients (77.8%) showed concentric LVH, while asymmetric LVH was detected in one patient (11.1%). One patient showed concentric LVH in combination with asymmetric septal hypertrophy. One of the four patients with GSD IIIb (ID11) had mild concentric LVH with normal ejection fraction, which was most likely secondary to longstanding hypertension. Two of 21 GSD IIIa patients (ID 13, 42) (9.5%) presented with symptomatic cardiomyopathy and a reduced ejection fraction, which was treated by heart transplantation at the age of 27 and 40 years respectively. The hepatic, muscular and cardiac findings are summarized in Table 1.

3.7. Biochemical findings

3.7.1. Liver biochemistry

There were 167 readings for each of AST and ALT. Median values and ranges were 81 U/L (28–598) (reference range 15–41 U/L) for AST and 71 U/L (22–249) (reference range 17–63 U/L) for ALT. AST and ALT were not significantly correlated with age (AST regression coefficient 0.30, p 0.68, 95% Conf. Interval – 0.68–1.28; ALT regression coefficient −0.15, p 0.65, 95% Conf. Interval - 0.82–0.51) (Fig. S2a, S2b respectively). ALT and AST were significantly correlated (regression coefficient 0.22, p 0.000, 95% Conf. Interval 0.15–0.29, ALT (regression coefficient 0.34, p 0.000, 95% Conf. Interval 0.22–0.47) (Fig. S4d, S4e respectively), and CK in GSD IIIa patients (see above).

3.7.2. Muscle biochemistry in GSD Illa patients

There were 101 readings for CK in GSD IIIa patients. Median CK value and range were 796 U/L (66–5592) (reference range 55–170 U/L). CK was not significantly correlated with age (CK regression coefficient −2.3, p 0.81, 95% Conf. Interval −21.56–16.9) (Fig. S3a). CK was significantly correlated with urinary Glc4 (regression coefficient 0.00, p 0.000, 95% Conf. Interval 0.00–0.012) (Fig. S3b). There was no statistically significant correlation between CK & AST (regression coefficient 2.02, p 0.13, 95% Conf. Interval −0.60–4.65) (Fig. S3c). Patients with severe muscle disease had higher CK levels of 75.5 IU than the rest of GSD IIIa patients, though this finding is not statistically significant—p value was 0.87.

3.7.3. Urinary Glc4

There were 67 readings for our cohort patients (GSD IIIa & b) with a median value of 6 mmol/mol creatinine (range 0.67–49) (reference range: < 3). Urinary Glc4 was not significantly correlated with age in this adult cohort (regression coefficient 0.18, p 0.42, 95% Conf. Interval – 0.27–0.64) (Fig. S4a). Urinary Glc4 was significantly correlated with AST (regression coefficient 0.22, p 0.000, 95% Conf. Interval 0.15–0.29) (Fig. S4c, S4d respectively), and CK in GSD IIIa patients (see above).

3.7.4. Lipid profile

Twenty-four patients (20 GSD IIIa, 4 GSD IIIb) had lipid profile data available for assessment. Hypercholesterolemia (total cholesterol > 200 mg/dL) was observed in 11 patients (46%) (9 GSD IIIa, 2 GSD IIIb), with a median total cholesterol of 218 mg/dL (range: 200–312). Ten patients (42%) (8 GSD IIIa, 2 GSD IIIb) had elevated triglycerides levels (>150 mg/dL), with a median triglyceride concentration of 190 mg/dL (range 150–372). The total cholesterol and triglyceride median values and ranges for all patients were 185 mg/dL (52–312) (reference range: <200) and 138 mg/dL (46–372) (reference range < 150) respectively.

Median values and ranges for LDL and HDL cholesterol were 113.5 mg/dL (52–297) (reference range < 100) and 48.5 mg/dL (22–93) (reference range: > 60) respectively. There was no statistically significant correlation between age and cholesterol (regression coefficient 0.01, p 0.96, 95% Conf. Interval – 0.67–0.70), triglycerides (regression coefficient 0.27, p 0.50, 95% Conf. Interval – 0.52–1.07), LDL cholesterol (regression coefficient 0.17, p 0.64, 95% Conf. Interval – 0.57–0.91) or HDL cholesterol (regression coefficient – 0.11, p 0.45, 95% Conf. Interval – 0.42–0.18) (Fig. S5a, S5b, S5c, S5d respectively). Cirrhotic patients were found to have cholesterol and triglycerides levels that were on average 9.5 and 33.9 IU higher than non-cirrhotic patients. However, this finding was not statistically significant, with p values of 0.31 and 0.07, respectively.

3.7.5. Other biochemical findings

Hyperuricemia was detected in three patients (2 GSD IIIa, 1 GSD IIIb) and allopurinol was used as a treatment. Protein intake ranged from 20 to 25% of total energy intake in these patients. Biochemical Findings are summarized in Table S3.

3.8. Osteopenia/ osteoporosis

Bone mineral densitometry was performed in 11 patients. Based on the WHO guidelines of diagnosis of osteopenia or osteoporosis, eight patients (6 GSD IIIa, 2 GSD IIIb) (5 F, 3 M) showed osteopenia and two GSD IIIa patients (1 F, 1 M) had osteoporosis. As obesity can predispose patients to osteopenia/ osteoporosis, we examined the BMI in these patients. Of the 10 patients with osteopenia or osteoporosis, obesity was found in 5 patients, while one patient was overweight and the 4 other patients had normal weight. All GSD IIIa patients with reduced bone density had myopathy, seven of them had generalized myopathy and one patient had myopathy involving the muscles of the lower limbs and small muscles of the hand. Wheelchairs and assistive devices like canes or walkers were used for mobility by two patients with osteoporosis and one patient with osteopenia. Treatment with bisphosphonates was prescribed for the two patients with osteoporosis, as well as one patient with osteopenia and a history of a fractured rib after a fall.

Vitamin D insufficiency (25 hydroxy vitamin D level 20 to 29.9 ng/mL) was observed in 10 of 15 patients, while vitamin D deficiency (25 hydroxy vitamin D level < 20 ng/mL) was diagnosed in 6 of 15 patients at different time points of follow up.

3.9. Endocrine findings

Data was retrieved on fourteen females (13 GSD IIIa & 1 GSD IIIb).
Six GSD IIIa patients complained of menstrual problems such as metrorrhagia, menorrhagia and irregular cycles. Three GSD IIIa patients (21%) were diagnosed with polycystic ovary syndrome (PCOS) and were treated with birth control medications. Although the main presenting symptom was the irregular menstrual cycle, hirsutism was present in one patient and increased BMI was noticed in two of the three patients. Two patients were diagnosed in their early twenties and the third patient was 16 years old at time of diagnosis. Bilateral ovarian cysts on pelvic imaging were seen in one GSD IIIb asymptomatic patient who had no menstrual problem or PCOS.

Three patients (2 GSD IIIa & 1 GSD IIIb) (3 M) (ID 22, 38, 47) developed type II diabetes mellitus and were managed by either diet, insulin or oral hypoglycemic medications. High protein diet was used in the two patients who had GSD IIIa. Cornstarch was not prescribed for the 3 diabetic patients. All 3 patients were obese (BMI ≥ 30). The age of DM type II diagnosis was known for two patients (47 and 61 years).

3.10. Renal

Three patients (ID 11, 13, 38) (1 GSD IIIa, 2 GSD IIIb) presented with recurrent kidney stones which needed either surgical excision or lithotripsy. Stones were found to be secondary to hypocitraturia in one patient (ID 11), while hyperuricemia was the cause in another and unknown in the third.

None of our cohort patients had evidence of renal tubular acidosis, proteinuria or hematuria.

One patient (ID 13) had chronic renal failure secondary to heart failure and cardiomyopathy and underwent combined heart-liver- kidney transplantation at the age of 40.3 (see section 3.4).

3.11. Skin

Eight patients (5 GSD IIIa & 3 GSD IIIb) (1 F, 7 M) developed skin lipomas ranging in number from 4 to over 35. Interestingly, three patients (ID 13, 47, 48) were siblings with GSD IIIa. Lipomas were scattered over the upper and lower extremities, abdomen and back of all patients. They were more common in the older group of patients with the median age of diagnosis being 47.3 years (range 35.5–57.4). Surgical excision was indicated in all patients, and confirmation of diagnosis through histopathology in five patients.

3.12. Headache/ migraine

Data on the prevalence of headaches and migraines in our cohort were collected on 20 patients. Five patients (4 GSD IIIa & 1 GSD IIIb) had migraines, while 3 other GSD IIIa patients complained of headaches. Three patients with migraines had brain imaging with normal results. Furthermore, one patient with frequent headaches had a finding of bilateral dense basal ganglia calcifications unrelated to GSD IIIa. Phosphylactic medications were needed for all patients with migraine, although one patient had severe daily migraines which needed different therapeutic options to be used, such as Botox injections and cervical epidural injections of Phenergan.

3.13. Psychiatric disturbances

A total number of seven patients (5 GSD IIIa, 2 GSD IIIb) (4 F, 3 M) out of twenty whose medical records include data on psychiatric disturbances had abnormal findings. Three were diagnosed with combined anxiety, depression and ADHD. In addition, one patient had anxiety and depression. Furthermore, two patients had depression only and one had ADHD. Family history for psychiatric illness was found in one patient. All patients with psychiatric problems received medications to control their symptoms.

3.14. Development and education level

Eighteen patients had data on their career and education level. Thirteen patients finished high school, eleven of them pursued college. Twelve patients were employed in diverse types of jobs requiring different skills, such as communication and executive skills.

3.15. Mortality

Five GSD IIIa patients (5/25) died during the course of follow-up. Median age of death was 36 years (range 20–68). The cause of death was due to liver disease progression in 3/5 GSD IIIa patients. Advanced HCC was the cause of death in two of these patients (ID 22, 39), while one patient (ID 24) died secondary to disseminated infection after receiving liver transplantation, as described previously in detail [17]. The two other patients died unexpectedly at the ages of 20 and 36. The autopsy report of the younger patient (ID 2) showed no clear cause of death, but pointed towards a marked hypertrophic cardiomyopathy induced rhythm disturbance and sudden death. Autopsy of the older patient (ID 25) showed significant glycogen accumulation in the conduction system of the heart, which can predispose the patient to a fatal arrhythmia. Full description of this patient was published formerly, including the autopsy results [17]. The Clinical, Radiological, Functional and Liver Histopathological Findings in our cohort are summarized in Table S4.

4. Discussion

GSD III is a rare disease with limited detailed information in the literature on the clinical, biochemical, radiological, functional, and histopathological aspects of the disease course in adults. Given the recent advances in medical care, the life expectancy of GSD III patients has improved with new manifestations of the disease exhibited in adults. This study describes the comprehensive follow-up of twenty-five adults with GSD III who were monitored for up to 41 years in our center, to add to the growing literature in the field. Of note, complications in older patients in this cohort represent sequelae of the natural history of the disease and could be related to delayed initiation of current practices such as initiation of a high protein diet early in the disease course.

Nevertheless, our study highlights the progression and severity of the disease in adults and clinical features that require close monitoring and a need for definitive treatments. The major clinical findings are as follows:

4.1. Adult patients with GSD III are at risk of hypoglycemia

In past reports, hypoglycemia was considered a problem primarily in early infancy and childhood [40]. Our study showed that adult patients are also at risk of hypoglycemia, with 40% of our cohort experiencing episodes of hypoglycemia. Dietary counseling aimed at improving dietary compliance, glucose monitoring, and nutritional needs during and after exercise may be helpful to prevent the risk of hypoglycemia and improve metabolic liver disease control. Furthermore, special attention is needed on pre-, peri-, and postoperative management with close monitoring of blood glucose levels as emphasized previously in the consensus guidelines for individuals with GSD III [6].

4.2. Cirrhosis and HCC are long term complications of GSD III

The incidence of patients with cirrhosis and HCC in our study (44% and 14% respectively) was higher than that previously published (hepatic cirrhosis, adenomas and/or HCC in 11% of their patients, Senter et al. [8]). Hepatic disease in GSD III patients has been considered to progress slowly from fibrosis to stable cirrhosis. As such, adults with GSD III have a quiescent disease from a perspective of liver enzymes and normal synthetic function, and patients often do not qualify for a liver transplant. Hence, the significance of liver manifestations may be
overlooked. Yet as shown previously in our report of liver manifestations in a pediatric GSD III population [7] and a canine model of GSD III [41], the liver disease can progress to decompensated liver cirrhosis. Thus, patients with GSD III require routine assessment for the development of cirrhosis and portal hypertension. Furthermore, surveillance imaging for hepatocellular carcinoma is required as recommended by the consensus guidelines [6]. The potential development of cirrhosis may play an important role in considering the timing and the inclusion/exclusion criteria of future therapeutic options in these patients.

Follow-up for more than 18 years of one of our GSD IIa patients who underwent combined liver- heart and kidney transplantation showed that liver & heart transplantation corrects the hepatic and cardiac phenotype respectively, but does not prevent the muscle disease progression [42,43]. Liver transplantation can be curative in the GSD IIb subtype [44], nevertheless, it is remains problematic for patients with GSD III due to late listing, paucity of organs, effect of immune suppressants on the heart in patients with GSD IIa, who also have myopathy that continues to progress after liver transplant.

4.3. Myopathy is a significant cause of morbidity in adults with GSD IIIa

The nature of GSD IIa myopathy appears to progress with age with onset increasingly reported in childhood [45]. Generalized muscle weakness was the most common form of myopathy found in our cohort and observed in a majority of patients. However, involvement of the small muscles of the hands and involvement of proximal and distal lower limb musculature were affected more commonly than proximal upper limb muscles. Progressive impairments in ambulation were also noted: individuals with profound weakness were unable to walk and required the full time use of wheelchairs to negotiate their home and community environments. Muscle testing by physical therapy (PT) was sensitive in detecting early signs of muscle weakness in our patients. Consequently, PT assessment is recommended every 6 months or even more frequently as stated in the consensus GSD III guidelines [6].

4.4. Adults with GSD III are at risk for sudden death

It is well known that accumulation of glycogen in different parts of the conduction system may predispose patients with glycogen storage diseases including GSD IIa to arrhythmia [15]. In accordance with previous papers and case reports [15,17,18,19,20], in our study 2 patients likely had sudden death due to arrhythmias. This suggests an increased risk of arrhythmia in these patients. Thus, a regular monitoring by a cardiologist and the use of a more detailed screening test, such as the 24-h Holter monitor, in addition to an electrocardiogram, should be considered in the routine care of patients with GSD III, as stated in consensus guidelines by Khanish et al. [6]. With a growing body of evidence of the cardiac and muscle involvement in these patients, treatments targeting liver disease alone is not sufficient in GSD IIa.

4.5. Endocrine (Osteopenia, osteoporosis, PCOS, and DM), renal stones, and lipomas are comorbidities that may occur in adults with GSD III

As previously reported by Melis D, et al. [23] and Cabrera-Abreu J, et al. [46], our study shows an increased risk of osteopenia and osteoporosis in our patients. Low bone mineral density (BMD) was more common in GSD IIa patients than GSD IIb patients in our cohort. Furthermore, all patients with GSD IIa and osteoporosis/osteopenia showed signs of myopathy. It was previously reported that muscle weakness and decreased mobility in patients with Pompe disease may be considered predictors of bone mineral density [47]. Similarly, the altered bone-muscle interaction (decreased or lack of weight bearing and decreased strength of muscle pull on bone), vitamin D insufficiency or deficiency, decreased mobility, and overweight/obesity are all considered factors affecting bone health in neuromuscular diseases [48]. Nevertheless, the pathogenesis of reduced BMD in GSD III patients is currently not well understood.

While our cohort demonstrates a higher rate of PCOS (21%) in comparison with the estimated prevalence in reproductive-aged women in the United States (6.6%) [49], none of our female patients showed radiological features of PCOS before puberty, as described by Lee PJ, et al. [24].

Three patients in this cohort had type 2 DM and were on different forms of therapy - oral hypoglycemic, insulin or both based on their individual glucose levels and insulin needs. There is limited information in the literature regarding an association between GSD III and DM type II [21,22,50]. Some studies showed that liver cirrhosis and chronic liver disease is associated with the development of DM type II [51], however other factors may be contributory, such as obesity, altered liver metabolism, and family history of DM.

Based on the above, we recommend a routine BMD, pelvic US and glucose tolerance testing if clinically indicated with regular monitoring of serum vitamin D concentrations and related metabolites, for the early detection, diagnosis and treatment of these comorbidities. In addition, a regular follow-up by a metabolic dietitian is an essential component of the multidisciplinary care for these patients, as mentioned in the GSD III management guidelines [6].

In agreement with our study findings, kidney disease was absent in a majority of the patients described by Talente et al. [34], but renal stones were observed in a few. Hyperuricemia was also reported in one of their patients, while two other patients received allopurinol for the same reason. Hyperuricemia in GSD III patients may occur secondary to a high protein diet and regular monitoring of serum uric acid level is advised. Furthermore, the accelerated breakdown of muscle purine nucleotides in myopathic disorders, such as GSD IIa, may also be related to hyperuricemia [52].

To the best of our knowledge, we are the first to report the finding of lipomas in GSD III patients. Formerly, Yi H, et al. described glycogen accumulation in adipocytes of one GSD IIa dog and proposed that this finding could be due to an imbalance between glycogen synthesis and breakdown, secondary to GDE deficiency in the adipose tissue of affected dogs [53]. It is also known that glycogen can be converted to fat in adipose tissue and it has an important role in regulating glucose and lipid metabolism during the fasted to fed status [54]. Despite these theories, we should keep in mind the fact that lipoma formation can be triggered by various factors, such as obesity and diabetes mellitus.

4.6. Neuropsychiatric problems may occur in adults with GSD III, but further studies are needed

Neuropsychiatric and cognitive profiles in GSD IIa patients were previously described in a small cohort [55]. In concordance with Michon et al., four GSD IIa and two GSD IIb patients were diagnosed with either depression, anxiety or both. Additionally, a seventh patient was diagnosed with ADHD. Michon et al. also showed impairment of the cognitive efficiency, executive functions and emotional skills, with sparing of memory in their patients. Formal psychiatric testing was not performed in our cohort; however, a review of our patients’ development revealed that more than 50% attended college and pursued various careers that required different abilities, such as communication and executive skills. We recommend that further studies are needed for a formal neuropsychiatric assessment.

4.7. Biomarker trends in GSD III

Routine serum biomarkers of liver and muscle disease may be challenging to interpret in patients with GSD III. Halaby et al. described a decrease in AST and ALT trends over time in pediatric patients with GSD IIa and b [7]. In our adult cohort, AST and ALT concentrations trended lower than those observed in the pediatric cohort. The finding of lower serum transaminases in the adult cohort is in agreement with trends
observed in a canine model for GSD IIIa, in which these enzymes increased during the first 2 to 3 years of life, and then gradually decreased [41]. This finding supports the previously proposed conclusion that the progression of liver disease (hepatic fibrosis and cirrhosis) is associated with a decrease in liver aminotransferases. In contrast, the trends of CK in the two cohorts were similar, which suggest ongoing muscle injury in the two groups of patients [7].

In pediatric studies, the urinary Glc₄ biomarker was positively correlated with liver transaminases, but not CK [7,56]. Furthermore, Heiner-Fokkema et al. described an association between urinary Glc₄ and serum CK levels, and clinical signs of myopathy in nine adult patients [56]. These observations were explained by the possibility that urinary Glc₄ in pediatric patients largely reflects glycogen accumulation in the liver. In adult patients, an ongoing increase in urinary Glc₄ excretion is most likely related to the progressive muscle disease, and with ongoing liver disease. This is supported by the correlation between urinary Glc₄ and ALT, AST, and CK levels, as noted above.

The prevalence of hypercholesterolemia and hypertriglyceridemia in our patients was 46% and 42%, respectively. In most patients, the findings of hypercholesterolemia and hypertriglyceridemia were not constant, which may reflect non-compliance with diet and poor metabolic control. The evidence in the literature in regards to the clinical significance of hyperlipidemia in GSD III patients remains controversial. For instance, normal lipid profiles and vascular endothelial function (assessed by brachial artery responsivity) described by Hershkovitz et al. in a small group of patients with GSD III suggested that there is no association of GSD III with hyperlipidemia or with a functional measure of vascular reactivity [57]. Alternatively, hyperlipidemia was implicated in a case report of a 24-year old male with GSD IIb. This patient had a history of persistently elevated lipids and presented with cardiac arrest secondary to ventricular fibrillation. He was found to have an 80% mid-left anterior descending artery (LAD) stenosis without occlusion on coronary angiography [58]. To the best of our knowledge, we are not aware of any patient in our cohort who developed complications secondary to hyperlipidemia. However, since many of the adult patients with GSD III are lost to follow up, we suggest that further studies are needed to explore the clinical impact of hyperlipidemia in patients with GSD III.

Finally, we explored genotype-phenotype correlation. As far as we know, the two nonsense mutations (c.100C>T, p.Arg34Ter) in exon 4 and c.2590C>T (p.Arg864Ter) in exon 20) were described separately, in homozygosity with variable clinical phenotypes [59,60,61]. In our cohort, the combination of the two above mentioned mutations was associated with a severe phenotype (hepatic cirrhosis, symptomatic cardiomyopathy, severe myopathy) in one patient, and with a less severe phenotype in the other two younger siblings (no cirrhosis, asymptomatic concentric LVH, myopathy with full independent mobility). This observation endorses the phenotypic intrafamilial heterogeneity of GSD III. Other factors may be contributory to the genotype-phenotype correlation but have not been studied in these patients.

While the results from our study show some similarities with data published previously [8,23,34], there are several differences including highlighting aspects of the disease not previously studied. These may be caused by variability between the cohorts studied, such as age range, genetic background, dietary management, or other environmental factors.

Furthermore, longitudinal data captured in our study facilitates a better understanding of the long-term complications that occur in GSD III, drawing attention to the course and severity of the disease progression among adult patients. This is in contrast to cross-sectional studies that lack longitudinal assessments [8].

The main limitations of this study are the small cohort size, the variability in the length of time that patients were followed, the age when a diagnosis of GSD III was made, and also delays in when treatment was initiated, especially the oldest patients in this cohort. There was also missing data for some of the patients in the study, including incomplete information on dietary therapy and compliance. Although the frequency of the reported complications may be impacted by these limitations and also a bias for patients with more involvement to be seen at our center, we do not believe that it significantly affects the overall results and recommendations. As mentioned earlier, older patients in the study either were diagnosed at a later age, or were not treated early in the course of the disease, thus they may show more severe complications, probably representing the natural history of the disease.

5. Conclusion

GSD III is a multisystemic disorder in which a multi-disciplinary approach with regular clinical, biochemical, radiological and physical therapy follow-up (including strength and functional motor testing) is required. Early recognition of complications of the disease with close monitoring may improve outcomes and quality of life. Although liver disease (fibrosis/ cirrhosis) in these patients is considered a significant cause for morbidity and mortality, myopathy is also a major problem in adults and should not be overlooked. Interventions such as physical therapy and diet modification may delay the progression of muscle weakness, but these interventions are not curative. A growing body of evidence suggests an increased risk of sudden death in adults with GSD III and close monitoring by a cardiologist is needed. Urinary Glc₄ is a promising biomarker in GSD III as it is correlated with serum transaminases and CK levels in adult patients with GSD III and merits further study. Despite dietary modification, complications can still occur. More definitive therapies, such as gene therapy and small molecule therapies that address both the liver and muscle aspects of the disease is needed for patients with GSD III.

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Appendix A. Supplementary data

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