Variable clinical presentation of glycogen storage disease type IV: from severe hepatosplenomegaly to cardiac insufficiency. Some discrepancies in genetic and biochemical abnormalities

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Glycogen storage disease (GSD) type IV (Andersen's disease, amylopectinosis, polyglucosan body disease) is a rare inherited disorder of carbohydrate metabolism. The disease is caused by autosomal recessive mutations in the *GBE1* gene (OMIM 607839), which leads to glycogen branching enzyme (GBE) deficiency. This is a critical enzyme in the production of both muscle and liver glycogen. Due to its decreased activity, abnormal long branched molecules of low solubility are formed. These deposits lead to glycogen precipitation in the liver, and subsequently build up in the body tissue, especially the heart and liver. The severity of the disease varies according to the amount of enzyme produced.

In adults, the activity of GBE is higher and symptoms do not appear until later in life [1].

Therefore, clinical manifestations of GSD IV consist of different subtypes with variable ages of onset, severity, and clinical features. The fatal perinatal neuromuscular subtype, which presents *in utero* with decreased fetal movements, polyhydramnios, and fetal hydrops, and congenital neuromuscular subtype beginning in the newborn period with profound hypotonia, respiratory distress, and dilated cardiomyopathy, both result in death in the neonatal period. The classic progressive hepatic subtype presents with rapidly developing failure to thrive following birth. Clinical manifestations include hepatomegaly, liver dysfunction,

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and progressive liver cirrhosis, as well as hypotonia, and cardiomyopathy. In this case, death due to liver failure usually occurs by the age of 5 years unless liver transplantation (LTx) is performed. The non-progressive hepatic subtype presents with hepatomegaly, liver dysfunction, myopathy, and hypotonia. However, individuals with this type usually do not show progression of liver disease, and may not even have cardiac, skeletal muscle, or neurologic involvement. The childhood neuromuscular subtype, which is the rarest one, has most variable course. Its onset ranges from the second life decade with a mild disease course to a more severe, progressive course resulting in death in the third decade [2, 3].

The disease diagnosis and management is thus multidisciplinary, and should include specialists in metabolic disorders, hepatology, neurology, and nutrition, as well as in medical or biological genetics.

We report three cases of Polish patients with mutations in the *GBE1* gene with various clinical courses of their disease each.

Patient 1: The girl was born in 2006 as a former 2.4 kg term infant to healthy, non-consanguineous parents of Polish origin. The pregnancy was not complicated, but slow physical development was observed from the 28th gestation week. Labor was complicated with oligoamnios, and pelvic longitudinal lie. Apgar scores were 6–7–8 respectively. Psychomotor development delay and poor body mass gain were observed from her perinatal period. The child presented with mild hypotonia, dysplasia of the hip, and pes equine. From 5 weeks of age elevated liver enzymes, cholestasis (bilirubin: total 32.7 µmol/l, direct 14.3 µmol/l) and increased cholesterol and triglyceride levels were found and persisted. At the age of 9 months the girl remained microsomic (body mass – 2.0 SD, height – 3.48 SD), her psycho-motor development was markedly delayed, and liver and spleen were enlarged. Lysosomal storage diseases suspected due to very high serum chitotriosidase activity (5000 nmol/ml/h, ref. < 150) were excluded by enzymatic testing. During subsequent months portal hypertension, muscle hypotonia of lower limbs, and hypertrophic cardiomyopathy developed. The girl required endoscopic intervention three times due to the massive bleeding from esophagal varices performed at the age of 19 months.
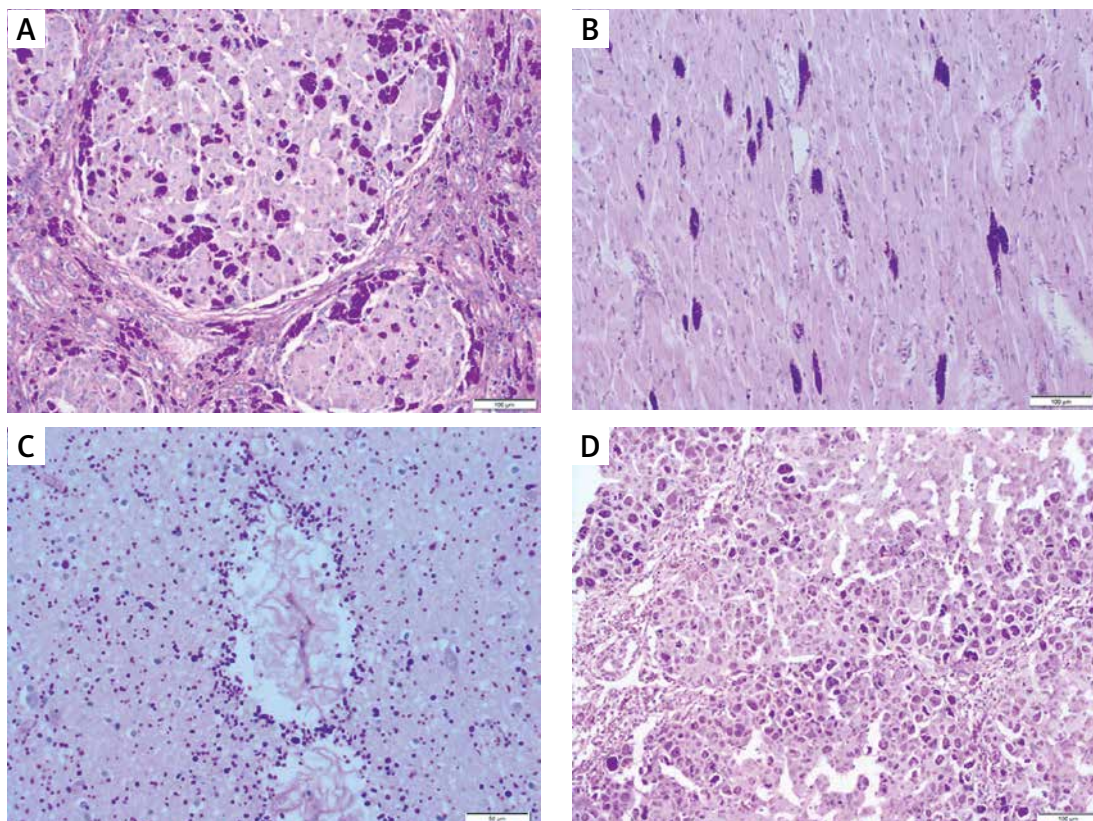

Figure 1. Histological findings in patients 1 and 2. **A** – Patient 1: explanted liver showing cirrhosis and extensive amylopectin deposits. PAS stain. Original magnification 200×. **B** – Patient 1: Autopsy, heart muscle with numerous intrasarcoplasmic PAS-positive deposits. PAS stain. Original magnification 200×. **C** – Patient 1: Autopsy, brain nervous tissue with numerous globular PAS-positive deposits located in the white matter. Original magnification 400×. **D** – Patient 2: Core needle liver biopsy. Intrahepatocytic amylopectin deposits. PAS stain. Original magnification 200×

Liver transplantation was performed at the age of 22 months. Complete micronodular cirrhosis, sparse diffuse lymphocytic infiltration and presence of large or granular PAS-positive intracytoplasmic inclusions located in numerous hepatocytes were found in the liver explant (Figure 1 A). In routine hematoxylin and eosin stain inclusions displayed basophilic or amphophilic staining characteristics. Transmission electron microscopy of the liver revealed aggregates consistent with amylopectin deposition. Enzymatic study showed brancher deficiency both in leukocytes and in the liver but normal activity in fibroblasts (Table I).

After LTx the girl's hepatic function improved, her mental development was good, but her motor skills were still greatly delayed; she was hypotonic, presented with muscle atrophy and did not walk. Her body mass was at the 50th percentile, and height at the 3rd percentile. At 6.5 years of age, the girl was admitted to the hospital due to symptoms of sepsis with pulmonary abscess and severe breathing difficulties. She died after a few weeks of intensive medical care.

Autopsy revealed: (1) heart muscle and pericardial involvement with extensive intracytoplasmic PAS-positive deposits in cardiomyocytes (Figure 1 B) and subpericardial macrophages, and (2) diffuse central nervous system damage caused by widespread deposition of numerous small globular PAS-positive deposits. Their location included the cerebral and cerebellar cortex as well as white matter (Figure 1 C).

Patient 2: The boy was born in 1999 as a former 4.14 kg term infant of a 3rd uncomplicated pregnancy to healthy, non-consanguineous parents of Polish origin. The labor was also not complicated and the Apgar score was 10. His psychomotor development was good. At 9 months of age mild hepatomegaly and hypertransaminasemia (ASP 319 U/l, ALT 263 U/l) were found on a routine health maintenance visit. A complete gastrologic and metabolic work-up was performed at the age of 13 months and revealed deficiency of branching enzyme (Table I), which indicated the GSD type IV. Brancher activity in leukocytes of the patient's parents was at the lower limit of the normal range. Liver core needle biopsy performed at

Table I. Activities of enzymes involved in synthesis and degradation of glycogen and glycogen amount in morphotic elements of the blood in three patients with molecularly confirmed GSD type IV

Activity*	Patient 1	Patient 2	Patient 3
Liver:			
Brancher (ref. 0.3–3.0)	0.0	0.0	–
Debrancher (ref. 0.17–0.72)	–	0.21	–
Fibroblasts:			
Brancher (ref. 0.5–3.0)	1.7 (c 1.73; 2.31)	0.16 (c 1.5; 0.98)	0.30 (c 0.99)
Leukocytes:			
Brancher (ref. 0.22–0.81)	0.11 (M 0.28; F 0.20)	0.04 (M 0.20; F 0.16; B 0.16)	0.20
Debrancher (ref. 26.8–105):			
Phosphorylase a with AMP (ref. 1.0–3.2)	–	1.2	2.1
Phosphorylase a without AMP (ref. 0.46–2.2)	–	–	1.24
	–		
FBP-1,6-ase (ref. 0.89–15.9)	–	4.6	–
Erythrocytes	–		
Glycogen (ref. 5.7–135)	–	16.7	112
Phosphorylase b kinase (ref. 8.6–45.9)			
Activity*	**Patient 1**	**Patient 2**	**Patient 3**
Liver			

*M – mother, F – father, B – brother, c – control, FBP-1,6-ase – fructose-1,6-biphosphatase; *Units brancher – µmol Pi/min/mg protein, debrancher in liver (leukocytes) – µmol glucose/mg protein/hour (nmol glucose/mg protein/h), phosphorylase a (with, without ± AMP) – µmol Pi/h/mg protein, glycogen – µg/g hemoglobin, phosphorylase b kinase – µmol Pi/min/g hemoglobin. FBP-1,6-ase – µmol/min/mg protein. **Normal ranges of brancher in Laboratory in Munich: leukocytes 0.22–0.81; fibroblasts 0.5–3.0; liver 0.3–3.*

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the age 26 months showed moderate fibrosis having no cirrhotic stage of nodular transformation. Intrahepatocytic inclusions had the same morphological and staining characteristics as in patient 1 (Figure 1 D).

Treatment included a high protein diet with restriction of non-utilizable sugars and regular food intake. The boy has been following routine health maintenance visits every 3 months at the beginning, and every 6 months afterwards. Further follow-up revealed mild microalbuminuria of 311 mg/day (ref. < 30 mg/day). Thus, nephroprotective treatment with an ACE inhibitor was commenced. There has been no further progression of renal complication since that time.

The boy is now 17 years old. He has been in good health. His liver is of normal size, and there is no evidence of liver function abnormalities. The patient has experienced normal growth and puberty with normal life activities.

Patient 3: The 24-year old student experienced the first episode of heart failure at the age of 5 months and was then treated with catecholamines. Since then, impaired exercise tolerance, nonspecific chest pain and frequent palpitations on exercise had been observed. At the age of 21 years the patient underwent an electrophysiologic study (due to PR of 70 ms on standard 12-lead electrocardiogram) that ruled out Wolff-Parkinson-White syndrome, and radiofrequency (RF) ablation of frequent ventricular ectopy in the right ventricular outflow tract was performed. At the age of 22 years he was referred to the Institute of Cardiology because of multifocal ventricular arrhythmia to make a diagnosis. He complained of recurrent, longer episodes of palpitations and decreased exercise tolerance. His blood pressure (BP) was of 110/60 mm Hg, heart rate 64/min, weight 104 kg,

192 cm height, and he had no congestive signs. On 12-lead standard electrocardiogram sinus rhythm was present, short PR of 116 ms, left atrial enlargement and ST-T segment changes in lateral leads (Figure 2). Cardiac magnetic resonance imaging (MRI) study confirmed bidimensional Doppler echocardiography findings of the nondilated left ventricle with mildly depressed left ventricle function of 51%. Moderate mitral insufficiency was also present with mitral insufficiency fraction of 23%. Late gadolinium enhancement images showed hyperintense areas in midmyocardial basal and partly medial segments of the interventricular septum (Figure 3). Repeated 24-hour Holter electrocardiogram showed multiform ventricular arrhythmia up to 2500 of single ventricular extrasystoles and complex 10–20 base pairs and infrequent short nsVT episodes. The patient was diagnosed with left ventricular dysfunction associated with mitral insufficiency and complicated with multiform ventricular arrhythmia. Liver enzymes and creatine kinase serum concentrations were within normal limits. The patient was treated pharmacologically (sotalol, candesartan) and did not agree to ICD implantation. During 3 years of follow-up the patient's left ventricular ejection fraction decreased to 35%, and the patient started having paroxysmal atrial fibrillation. Neurological examination was normal. The family history revealed RF ablation of ventricular arrhythmia in the right ventricular outflow tract at the age of 49 years in the mother. At present, aged 50 years she is mildly hypertensive without any symptoms; a 12-lead electrocardiogram revealed sinus rhythm of 61/min, and short PR of 96 ms; 2D Doppler echocardiogram was normal. The proband's father, aged 47 years, is free of symptoms, obese, treated for diabetes type 2
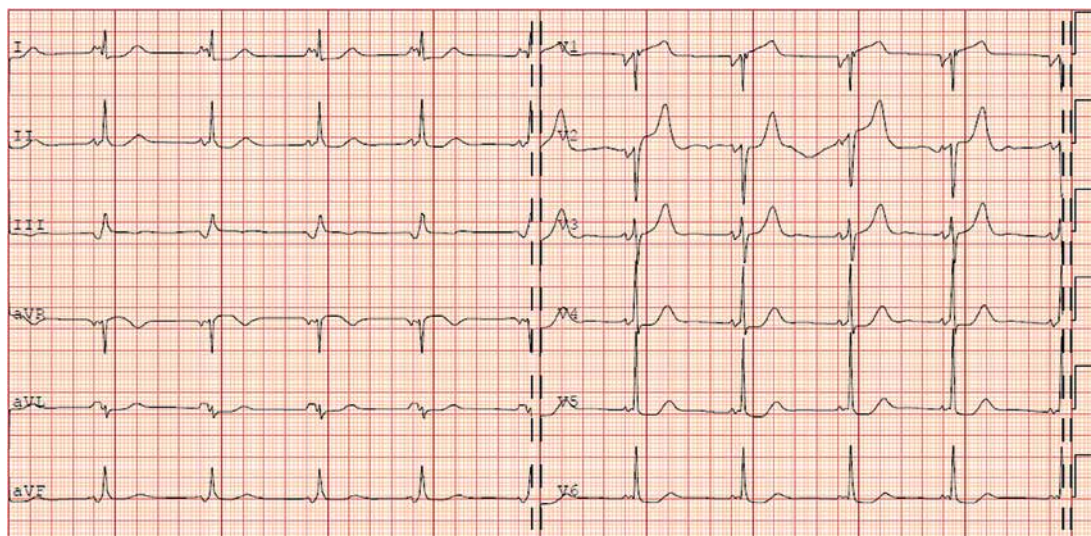

Figure 2. 12-lead ECG of patient 3 which shows sinus rhythm, short PR, left atrial enlargement and ST-T segment changes in lateral leads
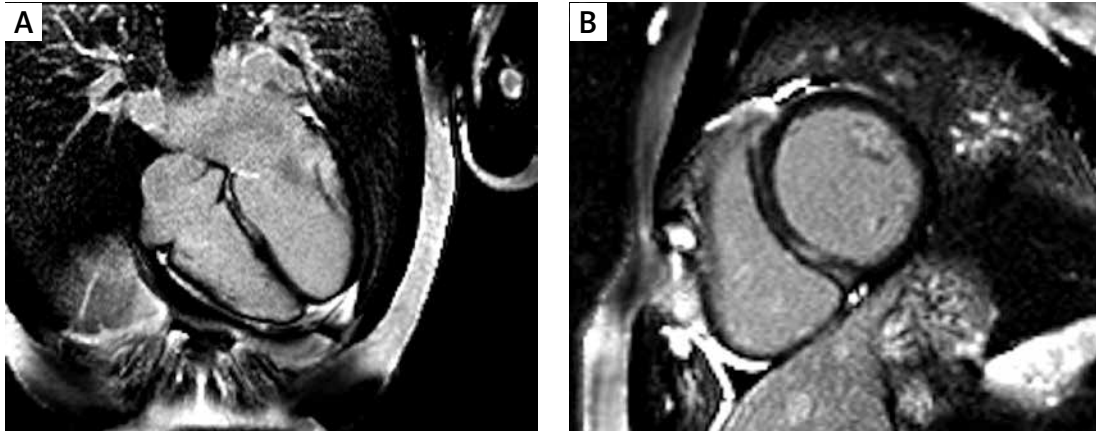
Figure 3. Late gadolinium enhancement images of patient 3 show hyperintense areas in midmyocardial basal (**A**) and partly medial segments of the interventricular septum (**B**)

and hypercholesterolemia, with normal electrocardiographic and echocardiographic study. Whole exome sequencing (WES) was performed and did not reveal any pathogenic/likely pathogenic coding or splicing variants with frequency no greater than 0.01 in ExAC, ESP6500 and POL400 databases in 174 genes from cardiological panel. The patient carried the heterozygous *ACTN2*
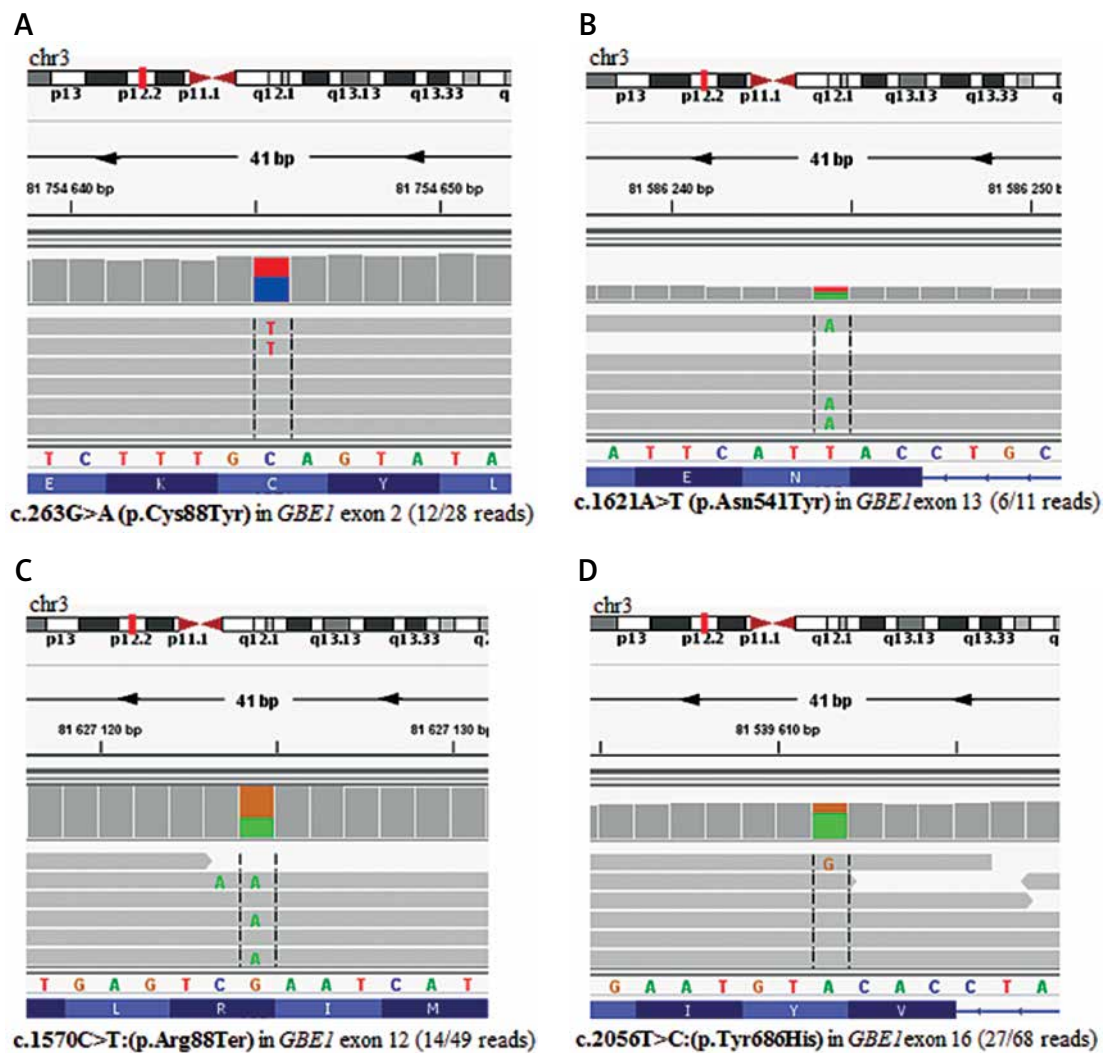

c.263G>T (p.Cys88Tyr) in *GBE1* exon 2 (12/28 reads)

c.1621A>T (p.Asn541Tyr) in *GBE1* exon 13 (6/11 reads)

c.1570C>T:(p.Arg88Ter) in *GBE1* exon 12 (14/49 reads)

c.2056T>C:(p.Tyr686His) in *GBE1* exon 16 (27/68 reads)

Figure 4. Integrative Genomics Viewer view of *GBE1* pathogenic variants in patient 1 (**A**, **B**) and patient 3 (**C**, **D**) by whole-exome sequencing

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NM_001103.3:c.2497G>A: (p.Ala833Thr) variant inherited from his mother classified in ClinVar as a variant of uncertain significance. Additional potentially pathogenic variants were found in the *GBE1* gene in the *trans* position (Figure 4, Table II). Serum branching enzyme activity was below the lower normal range, indicating the diagnosis of GSD type IV (Table I). Chitotriosidase activity in

Table II. Details of molecular variants identified in *GBE1* in three patients with GSD type IV

Variable	Patient 1		Patient 2		Patient 3	
	Mutant allele 1	Mutant allele 2	Mutant allele 1	Mutant allele 2	Mutant allele 1	Mutant allele 2
Chromosome/ rs	3/rs1042498	3/–	3	3	3/ rs137852888	3/ rs754607405
RefSeq cDNA	NM_000158.3	NM_000158.3			NM_000158.3	NM_000158.3
Nucleotide change	c.263G>A	c.1621A>T	IVS5 + 2t>c		c.1570C>T	c.2056T>C
Exon	2	13			12	16
IGV reads	12/28	6/11	ND	ND	14/49	27/68
RefSeq protein	NP_000149	NP_000149			NP_000149	NP_000149
Amino acid change	p.(Cys88Tyr)	p.(Asn541Tyr)		p.(His528Arg)	p.(Arg524*)	p.(Tyr686His)
Mutation:						
Type	Missense	Missense			Nonsense	Missense
Stage	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Status	Novel	Novel			Known (PMID 8613547, 15452297)	Novel
Parental origin	Paternal	Maternal			Maternal	Paternal
Pathogenicity prediction:						
CADD*	1.23	18.49			41.0	21.9
PolyPhen2	Benign	Probably damaging			–	Probably damaging
Mutation Assessor	High (functional)	Neutral (functional)			–	High
LRT	Deleterious	Deleterious			Deleterious	Deleterious
Mutation-Taster	Disease causing	Disease causing			Disease causing automatic	Disease causing
SIFT					Tolerated	Damaging
MAF data**:						
ExAC 60,706	0.000014	0			0.00001685	0.00000817
ESP 6500	0	0			0.00016	0
EUR 1000	0	0			0	0
POL 400	0	0			0	0
Depth of WES	27	11			66	49

*Higher scores are more deleterious, **MAF – minor allele frequency; ExAC – exome aggregation consortium (http://exac.broadinstitute. org); EUR 1000 – 1000 genomes project (http://www.1000genomes.org/); ESP 6500 – exome sequencing project (http://evs.gs.washington. edu/EVS); POL400 – project of 400 exomes from Polish individuals with unrelated diseases (unpublished).CADD – Combined Annotation Dependent Depletion (http://cadd.gs.washington.edu/home); PolyPhen-2 – Polymorphism Phenotyping v2 (http://genetics.bwh.harvard. edu/pph2/index.shtml); MutationAssessor (http://mutationassessor.org); LRT – Likelihood Ratio Test (http://www.genetics.wustl.edu/ jflab/lrt_query.html).

Table III. Clinical and laboratory characteristics at diagnosis of 3 patients with mutations in *GBE1* gene

Patient	Clinical presentation	Laboratory abnormalities
1	Severe course: hepato-splenomegaly, neurological involvement, liver failure with cholestasis	Elevated serum: – Liver enzymes – Chitotriosidase (5000 nmol/ml · h) – Lipids – Lactate cholestasis, coagulative impairment
2	Hepatomegaly without enlarged spleen and no impairment of liver function	Hypertransaminasemia Elevate CK, chitotriosidase (317–613 nmol/ml/h)
3	Cardiological presentation: dilated cardiomyopathy and mild arrhythmia	No specific laboratory abnormalities

serum was assessed and found to be in the normal range (29 nmol/ml/h, ref. < 150). There were neither class I clinical indications for endomyocardial biopsy, nor for liver or muscle biopsy. Branching enzyme activity was assessed in the patient's fibroblasts (Munich, Y. Shin, T. Podskarbi), and it was somewhat lower than that of the control culture (Table II).

Table III presents clinical characteristics and laboratory studies for all 3 patients at diagnosis.

Activity of glycogen-branching enzyme was measured using the spectrophotometric method described by Brown [4, 5]. Briefly, as a result of activity of glycogen-branching enzymes (amylo-(1,4-1,6) transglycosylase; EC 2.4.1.18) and phosphorylase, synthesis of glycogen from glucose-1-phosphate occurs. Resulting from the reaction inorganic phosphate was measured spectrophotometrically (spectrophotometer Cary 1-E, Varian, Mulgrave, Australia). Its concentration is proportional to the enzymatic activity. Enzymatic assays performed in the Laboratory in Munich (Y. Sinn, T. Podskarbi) used C14 UDP glucose instead of phosphorylase [6, 7].

DNA was extracted from the peripheral blood and used for massively parallel exome sequencing. WES Library preparation was performed using Nextera Rapid Capture Exome kits (Illumina). The samples were run on 1/4 lane each on HiSeq 1500 using 2 × 75 bp paired-end reads. Bioinformatics analysis was performed as previously described [8]. The detected variants were annotated using Annovar and converted to Microsoft Access format data for final manual analyses. Alignments were viewed with the Integrative Genomics Viewer.

Variants identified in the *GBE1* gene with next generation sequencing (NGS) were followed up in probands and their parents with Sanger sequencing using a 3500xL Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) and BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) according to the manufacturer's instructions. The results were analyzed with Variant Reporter 1.1 software (Life Technologies).

A list of primers specific to each *GBE1* variant is available on request.

In patients 1 and 3 mutations in *GBE1* were identified by WES and in patient 2 by Sanger sequencing.

The sequencing run for patient 1's sample achieved 52 579 854 reads with a mean value of 32.08. Above 64% and 87% of the target region was covered min. 20 and 10 times, respectively. The sequencing run for patient 3 sample achieved 72 905 031 reads with a mean value of 39.63. Above 73% and 91% of the target region was covered min. 20 and 10 times, respectively.

In patients 1 and 3 two compound heterozygous nucleotide substitutions in the *GBE1* gene (NM_000158.3; NP_000149) inherited in trans were identified by WES in probands and confirmed by bidirectional Sanger sequencing in probands and in their parents (Figure 4, Table II). In patient 1 c.263G>A:(p.Cys88Tyr) of paternal origin and c.1621A>T:(p.Asn541Tyr) of maternal origin and in patient 3 c.1570C>T:(p.Arg524*) of maternal origin and c.2056T>C:(p.Tyr686His) of paternal origin were identified. All variants were heterozygous and inherited from unaffected parents. Both of the variants in patient 1 are apparently novel missense mutations. Cys88Tyr is known as a polymorphism and Tyr686His is predicted to be pathogenic. Substitution c.263G>A (p.Cys88Tyr) affects a weakly conserved amino acid in glycoside hydrolase, family 13, N-terminal carbohydrate-binding module 48 (CBM48), but a large physicochemical difference between Cys and Tyr (Grantham score 194) is observed. Substitution c.1621A>T (p.Asn541Tyr) concerns a highly conserved amino acid in glycosyl hydrolase, family 13, subfamily, catalytic domain and likewise a large physicochemical difference between Asn and Tyr (Grantham score 143).

In addition, the variants in patient 1 were predicted by *in silico* analysis (Table II) as probably damaging enzyme activity, which suggests that they are disease-causing genetic factors. The c.263G>A substitution is referenced on dbSNP (rs1042498) and recorded on ExAC databases (Europeans: 1/37098 alleles (0.0027%); ALL: 1/69262 (0.0014%)). The c.1621A>T substitution is absent on dbSNP, ESP6500 and ExAC databases (as of 14.04.2016).

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In patient 2, sequencing of *GBE1* revealed compound heterozygous IVS5 + 2t>c/H528R genotype, which is deemed to be associated with the mild form of GSD IV [9].

The c.1570C>T:(p.Arg524*) variant, found in patient 3, was previously described in 2 patients. One of those patients had a classic hepatic presentation carrying Arg524* and Phe257Leu *GBE1* variants [PMID 8613547] [9] and the other patient with neuromuscular presentation carried Arg524* and His628Arg variants [15452297] [10]. The second variant in patient 3 (SM) c.2056T>C:(p. Tyr686His) is a novel variant affecting a conserved amino acid in the C-terminal amylase-like barrel domain of GBE1. The c.2056T>C:(p.Tyr686His)

variant was predicted as deleterious by six *in silico* algorithms (CADD, PolyPhen2, Mutation Assessor, LRT, Mutation Taster, SIFT). The c.2056T>C:(p. Tyr686His) variant was reported in the ExAC database (1/120668 [0.000008287]) and is absent in the ESP6500 database.

A large variety of clinical manifestations of GSD type IV have been reported, from early onset hepatic presentation with a probable end result is cirrhosis and death within 5 years, to mild liver disease without progression in people reaching adulthood without liver transplantation [11].

Genotype-phenotype correlations remain unclear but are emerging. Fatal perinatal neuromuscular subtype and congenital neuromuscular subtype of GSD IV are usually caused by 2 null mutations, classic hepatic subtype by compound heterozygous for a null and missense mutation and adult polyglucosan body disease by homozygous or compound heterozygous missense mutations. Milder forms of GSD IV are usually associated with 2 missense mutations [10762170, 15452297] and more severe forms of GSD IV with at least one null mutation or large deletion [17662246, 15520786, 8613547, 15452297, 12913206, 17662246]. However there are exceptions such as – NM_000158.3:c.2003delA found in an APBD patient [23034915] and homozygous missense mutations p.H545R in fatal perinatal cases [15452297] are also observed.

To our best knowledge to date, 63 mutations in the GBE1 gene have been reported. With our 4 novel missense mutations this number increases to 67, of which 34 (51%) are missense mutations, 7 (10%) nonsense, 7 (10%) splice-site mutations, 12 (18%) frameshift mutations, and 6 (9%) large (above > 10 bp) deletions/insertions. Most missense mutations are found in the catalytic domain of GBE, and cluster in exons 5–8 and 12–13 (Figure 5). In some patients only one mutation in GBE1 was identified [8613547, 15452297, 10545044, 20058079]. A recent article by Akman *et al*. showed deep intronic mutation in a repetitive sequence of intron 15 which was originally missed in whole-exome sequence data [25665141].

We report three cases of Polish patients with mutations in the *GBE1* gene, each presenting with a different clinical course of the disease.

Patient 1 was found with elevated chitotriosidase activity, hepato-splenomegaly and liver dysfunction, which led to the initial diagnosis of Niemann-Pick disease type A/B. This is not the first case report of GSD IV being a rare cause of elevated chitotriosidase activity and diagnosed through genetic testing [12]. Since chitotriosidase activity is known to be directly associated with acute or chronic inflammation and macrophage activation [13], its elevated serum activity in patients with
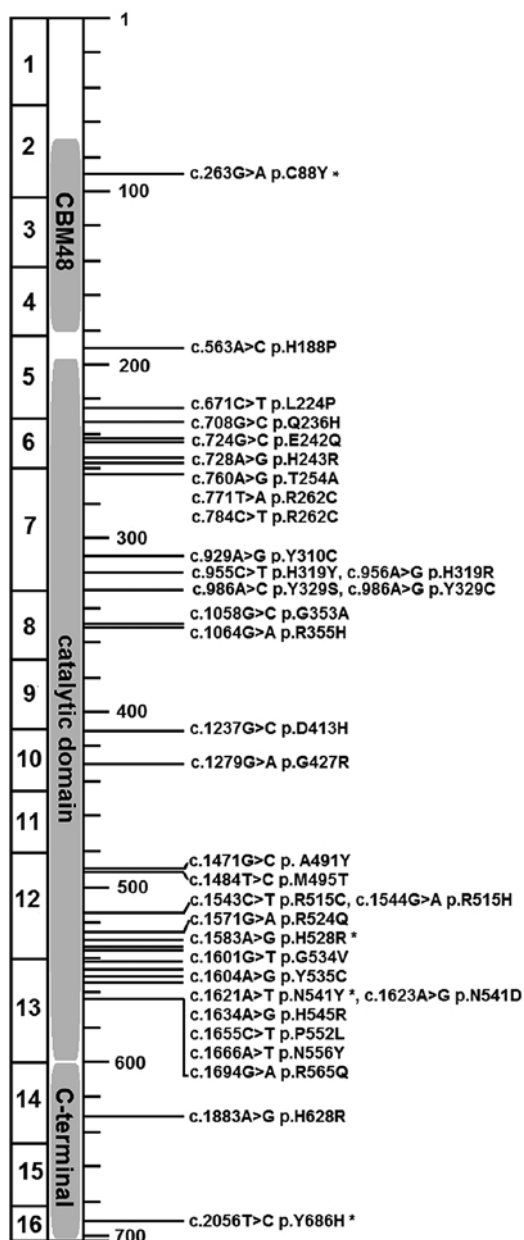

Figure 5. Missense mutations found in catalytic domain of GBE, and clustered in exons 5–8 and 12–13

GSD IV may be due to lipid accumulation, which triggers peroxidation in hepatocytes and activation of liver resident macrophages and Kupffer cells and secretion of proinflammatory cytokines. Therefore, diagnosis of GBE deficiency should be considered in the differential diagnosis in patients with chronic liver disease and elevated chitotriosidase activity, once lysosomal storage diseases have been excluded. The patient required LTx to counteract chronic liver failure, which is a common indication in the classical form of GSD IV, but unfortunately died due to LTx complications. In this case histopathologic examination of the liver and postmortem heart and brain biopsy were crucial for diagnosis. One of the variants found in the *GBE1* gene is only possibly pathogenic but presence of a deletion in *GBE1* on a second allele, unrevealed by WES study, cannot be excluded.

Since GSD IV is a rare disease, the data on its natural history after Ltx are limited. In classical infantile liver form Ltx remains the only effective treatment for progressive liver failure. According to the largest review so far, fewer than 20 patients with this type of GSD have been transplanted [14]. This analysis of 17 patients who had undergone Ltx due to liver cirrhosis by 2008 demonstrated that the majority of patients (12/17) survived, 2 of them required a second LTx, and most complications were related to sepsis or hepatic thrombosis not associated with cardiomyopathy. Skeletal myopathy and cardiomyopathy did not develop after LTx in most cases. A few other single case reports published so far have confirmed the efficacy of LTx for patients with classic hepatic form, but the data on rare, atypical forms are still not available [15–17].

The initial manifestation of GSD IV in patient 2 was slight hepatomegaly with elevated hepatic transaminases and CK activity during the first year of life. In this case, enzyme (brancher) activity in the liver was 0, but in leukocytes was not, which is compatible with the mild variant liver form (Table I). The liver often gives a much lower enzyme activity value, partly due to the bad condition and partly due to the complicated method of Pi production using phosphorylase a.

After the diagnosis was established, proper medical management with a high protein diet and carbohydrate restriction was commenced. Although data supporting such nutritional management are limited, single studies have shown that patients with GSD IV may benefit from a high protein diet similarly to those with type III and IX [18]. This particularly applies to muscular forms (patients with cardiomyopathy) since for infants with classic hepatic form Ltx is the only possible treatment. There are no data on the efficacy of ketogenic diet (KD) in GSD IV, but single case reports of patients with GSD III and cardiomyopathy treated

with KD have demonstrated an improvement of cardiomyopathy in echocardiography [19]. Since the mechanism (storage of pathologic substance in the heart muscle) of heart enlargement in both GSD types is similar, there is a possibility that KD may also have a positive impact on cardiomyopathy and myopathy in GSD IV.

A similar case to our patient was published for the first time in 1988 by Greene *et al.* [20]. Almost 10 years later, in 1996, McConkie-Rosell *et al.* presented the clinical spectrum of non-progressive liver form of GSD IV in 4 patients, and long-term follow-up of the oldest identified patients (ages 13 and 20 years) [21]. These case reports have proved that type IV glycogen storage disease is more heterogeneous and may possibly be more common than previously suspected.

Patient 3 demonstrated the least known form of GSD IV: isolated cardiac involvement, but with progressive myocardial dysfunction. He did not fulfill the criteria of neuromuscular subtype of GSD IV, a "polyglucosan body disease", because he has not presented with either neurological or skeletal muscle symptoms, at least until now. There was no clinical or laboratory evidence clearly pointing to such a diagnosis for years and the diagnosis was established by chance, through application of NGS [22]. Chitotriosidase activity was also within normal values and branching enzyme activity was only slightly decreased. A causal link between the *GBE1* defect and the cardiac involvement in patient 3 seems very probable although not confirmed at the functional level. In the literature Aksu *et al.* reported the first hypertrophic cardiomyopathy case due to type IV GSD in the literature [23]. Based on the phenotype of case 3, a detailed search of desmosomal (*PKP2*, *DSP*, *DSG2*, *DSC2*, *JUP*), and nondesmosomal genes (*TMEM43*, *LMNA*, *PLN*, *CTNNA3*) linked to arrhythmogenic cardiomyopathy (ARC) was performed, yielding no results [24, 25]. Although the majority of cases of ARC are transmitted in an autosomal dominant way, a few of them, such as cardiocutaneous syndromes like Naxos disease (related to *JUP*) and Carvajal disease with dominant left ventricular, related to *DSP*, are transmitted in an autosomal recessive way. Our patient was free of any cutaneous symptoms. Of interest, both the patient and his mother had short PR on 12-lead electrocardiogram. In the literature, short PR/preexcitation is characteristic of glycogenoses, especially in Danon disease and PRKAG2 disease, but also is found in Anderson-Fabry disease, mitochondrial disease and most commonly is associated with hypertrophic cardiomyopathy [15].

In the proband, a compound heterozygote of *GBE1* variant carrier, short PR was associated with myocardial thinning due to fibrosis, and in his mother, a single *GBE1* variant carrier with

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normal myocardial function. Storage/infiltrative disorders with short PR may be transmitted in autosomal dominant, recessive, X-linked and matrilinear fashion [12]. No LT has been necessary so far. However, since long-term prognosis of cardiac involvement in GSD IV (or in the carriers of the *GBE1* mutation) has not been known yet, further studies on its genetic and clinical features should be performed in order to understand the genuine pathogenesis and course of the disease.

These 3 case reports of Polish patients with mutation in the *GBE1* gene and various courses of their disease confirm the clinical heterogeneity of this disorder. Our observation also shows remarkable difficulties in establishing a conclusive diagnostic decision even in the "WES era", as in patient 3. Pathogenicity of his Tyr686His variant is not proven by *in vitro/in vivo* studies and only heterozygous status of GSD type IV cannot be excluded on enzymatic verification. Functional testing of the Tyr686His pathogenicity is not available and probably only a future study on polyglucosan bodies in heart bioptate will appear conclusive.

When it comes to LTx, progression to liver failure in patients with other than classical liver forms is hard to predict. Therefore, close follow-up including both clinical and laboratory (liver enzymes, coagulation and other parameters of liver function) monitoring should be provided. However, the presence of normal liver size and function assessed by laboratory examinations in adults with GSD IV renders progression to liver failure rather unlikely, although periodic assessment including serum liver enzymes and coagulation parameters may be helpful. In children more rigorous follow-up is justified.

Numerous experimental techniques are being considered for GSD IV including inhibitors of glycogen synthase, Myozyme, and rapamycin. However, so far, all these experimental techniques have been used either for GSD II (Pompe disease) or GSD III (rapamycin), not for type IV. Therefore, there are no data to support or to predict their possible impact on the clinical course of our 3 phenotypic variants. However, at Duke University, USA a study to evaluate the use of Myozyme for treatment of glycogen storage disease type III and IV in murine disease models (Roivant Sciences Inc. Sun Boadbong, https://scholars.duke.edu/display/insroivantsciencesinc) is currently being carried out.

We would like to underline that all physicians, not only metabolic pediatricians, but also gastroenterologists and cardiologists should be aware of them, and take GSD type IV into the differential diagnosis in patients at any age with either hepatomegaly and hypertransaminasemia, or cardiomegaly without liver involvement.

Since the natural course of this multi-systemic metabolic disease is still unknown, further studies on this issue are necessary.

In conclusion, GSD IV should be suspected not only in patients with liver cirrhosis and liver failure. The disease should also be considered in the differential diagnosis in individuals with hepato-splenomegaly and elevated chitotriosidase activity once lysosomal storage diseases have been excluded, as well as in patients with isolated and progressive myocardial dysfunction associated with features of preexcitation on ECG. Biochemical, and particularly, histological evaluation plays a crucial role in the diagnosis of GSD IV, especially in cases with unclear molecular findings, since polyglucosan bodies are highly diagnostic, microscopic features for glycogen branching enzyme deficiency.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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