Diagnsosis of Glycogen Storage Diseases

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Introduction

The glycogen storage diseases (GSDs) are a group of inherited metabolic disorders that result from a defect in any one of several enzymes required for either glycogen synthesis or glycogen degradation

Classification of GSDs

The GSDs can be classified on the basis of organ affected and the enzyme deficiency involved. On the basis of target organ involved GSDs are comprised of three broad groups including; hepatic type of GSDs, muscles type of GSDs and the group affecting both liver and muscles. Each group has several different types; caused by defect of a different enzyme or a transporter, which is encoded by a different gene

GSDs with Hepatic involvement

Types	Enzymes/Transport Defect	Genes
GSD 0	Glycogen Synthase	GYS2
GSD Ia	Glucose-6-phosphotase	G6PC
GSD Ib	Glucose-6-phosphotase transporter	SLC37A4
GSD VI	Glycogen phosphorylase (liver)	PYGL
GSD IXa	Phosphorylase kinase (α subunit)	PHKA2
GSD IXb	Phosphorylase kinase (β subunit)	PHKB
GSD IXc	Phosphorylase kinase (γ subunit)	PHKG2
GSD XI	Glucose transporter-2	SLC2A2

GSDs with Neuromuscular involvement

Types	*Enzymes/Transport Defect*	*Genes*
GSD IIa	α-1,4 glucosidase	GAA
GSD IIb	LAMP-2 protein	LAMP2
GSD V	Glycogen phosphorylase (muscle)	PYGM
GSD VII	Phosphofructokinase	PFKM
GSD IXd	Phosphorylase kinase (δ subunit)	CLAM1

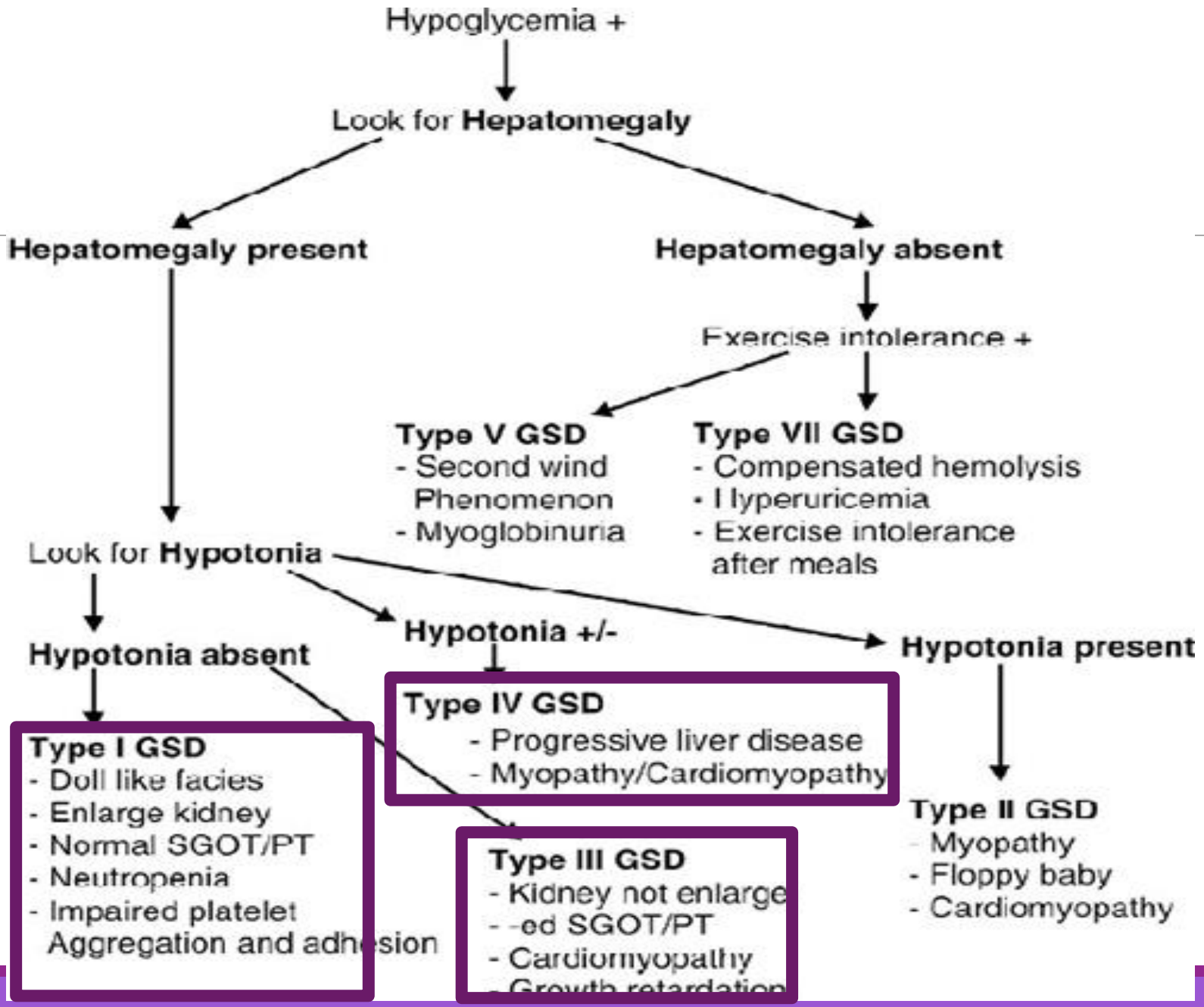
GSDs with both Hepatic & Neuromuscular involvement

Types	*Enzymes/Transport Defect*	*Genes*
GSD III	Amylo-1,6-glucosidase	AGL
GSD IV	Amylo-1,4 ◊1,6 transglucosylase	GBE1

Clinical Diagnosis of GSDs

From a clinician's perspective the hepatic group of GSDs often has an easily recognizable clinical phenotype usually apparent as doll-like facies, failure to thrive/short stature, hepatomegaly and the biochemical features of fasting ketotic hypoglycemia, lactic academia, raised alanine transaminase with or without hypertriglyceridemia and hyper-uricemia.

The muscular group on the other end presents with neuromuscular symptoms, weakness and cardiomyopathy.


Hypoglycemia +
Look for Hepatomegaly
Hepatomegaly present
Hepatomegaly absent
Exercise intolerance +
Type V GSD
- Second wind Phenomenon
- Myoglobinuria
Type VII GSD
- Compensated hemolysis
- Hyperuricemia
- Exercise intolerance after meals
Look for Hypotonia
Hypotonia absent
Hypotonia +/-
Hypotonia present
Type I GSD
- Doll like facies
- Enlarge kidney
- Normal SGOT/PT
- Neutropenia
- Impaired platelet Aggregation and adhesion
Type IV GSD
- Progressive liver disease
- Myopathy/Cardiomyopathy
Type III GSD
- Kidney not enlarge
- -ed SGOT/PT
- Cardiomyopathy
Type II GSD
- Myopathy
- Floppy baby
- Cardiomyopathy

Diagnostic Approach

In Iran, the conventional diagnostic approach towards hepatic types of GSDs includes recognition of clinical symptoms followed by biochemical work up and ultimately the more invasive liver biopsy to reveal features consistent with fatty change, nuclear hyper-glycogenation and fibrosis.

Additional electron microscopy and enzyme studies on the liver tissue are required to specify the type of GSDs. Neither the electron microscopy nor the enzyme assays for GSDs are locally available, which compromises the diagnostic yield of liver biopsy for diagnosing GSD in local setting.

Glycogen Storage Diseases

Predominately Hepatic GSDs:

GSD I – glucose-6-phosphatase or transport systems in ER

GSD III – debranching enzyme

GSD IV – branching enzyme

GSD VI – liver phosphorylase

GSD IX – liver phosphorylase b kinase

GSD 0 – glycogen synthase

Predominately Muscle GSDs:

GSD II – acid a-glucosidase

GSD V – muscle phosphorylase

GSD VII - muscle phosphofructokinase

GSD	Hepato-megaly	Muscle symptoms	Glucose homeostasis	Other Biochemistry
GSD 0	No	None	Fasting ketotic hypoglycaemia	
GSD I	Yes	None	Severe (ketotic) hypoglycaemia	Raised lipids, urate, lactate, AST/ALT, Abnormal renal biochemistry including proteinuria
GSD II	No	Truncal & proximal muscle weakness. More severe infantile form.	No overt effect	Raised CK,vacuolated lymphocytes
GSD III	Yes	Myopathy can occur	Fasting ketotic hypoglycaemia	Raised lipids, AST/ALT, CK may be raised
GSD IV Hepatic	Yes	Myopathy can occur	Normal until end stage liver disease	Raised AST/ALT, CK can be raised
GSD V	No	Exertional muscle weakness with risk of rhabdomyolysis	No effect	Raised CK
GSD VI	Yes	None	Fasting ketotic hypoglycaemia	Raised AST/ALT
GSD VII	No	Exertional muscle weakness with risk of rhabdomyolysis	No effect	Raised CK
GSD IX liver form	Yes	Myopathy can occur	Fasting ketotic hypoglycaemia can occur	CK can be raised
GSD XI	Yes	None	Ketotic hypoglycaemia	Raise AST/ALT, Abnormal renal biochemistry including tubular markers.

Type	Enzyme Deficiency	Fasting Blood Glucose	Blood Lactate	Blood Lipids	Blood Uric Acid	Liver Function Tests
0	Glycogen synthetase	↓	Normal	Normal	Normal	Normal
I	Glucose-6-phosphate	↓	↑	↑	↑	±
II	Acid maltase	Normal	Normal	Normal	Normal	Normal
III	Amylo 1,6-glucosidase (debrancher)	Normal or sl. [a] ↓	Normal	Normal or sl. [b] ↑	±	±
IV	Amylo 1,4-1, 6-trans-glucosidase (brancher)	Normal or sl. ↓	Normal	Normal	Normal	Abnorma
V	Muscle phosphorylase	Normal or sl. ↓	Normal	Mild ↑	Normal	Abnorma after exercise
VI	Liver phosphorylase	Mild-moderate ↓	Mild ↑	Mild ↑	Normal	Normal
VII	Phosphofructokinase	Normal	Normal	Normal	Normal	Normal

a. sl. ↓ – slightly reduced
b. sl. ↑ – slightly elevated

Type (Eponym)	Enzyme deficiency (Gene[3])	Incidence (births)	Hypo-glycemia?	Hepato-megaly?	Hyperlip-idemia?	Muscle symptoms	Development/ prognosis	Other symptoms
GSD 0	Glycogen synthase (GYS2)	?	Yes	No	No	Occasional muscle cramping	Growth failure in some cases	
GSD I / GSD 1 (von Gierke's disease)	Glucose-6-phosphatase (G6PC / SLC37A4)	1 in 50,000 – 100,000[4][5][6]	Yes	Yes	Yes	None	Growth failure	Lactic acidosis, hyperuricemia
GSD II / GSD 2 (Pompe's disease)	Acid alpha-glucosidase (GAA)	1 in 40,000 – 50,000[7][8]	No	Yes	No	Muscle weakness	*Death by age ~2 years (infantile variant)	Heart failure
GSD III / GSD 3 (Cori's disease or Forbes' disease)	Glycogen debranching enzyme (AGLdf)	1 in 100,000	Yes	Yes	Yes	Myopathy		
GSD IV / GSD 4 (Andersen disease)	Glycogen branching enzyme (GBE1)	1 in 500,000[9]	No	Yes, also cirrhosis	No	Myopathy and dilated cardiomyopathy	Failure to thrive, death at age ~5 years	
GSD V / GSD 5 (McArdle disease)	Muscle glycogen phosphorylase (PYGM)	1 in 100,000 – 500,000[9][5]	No	No	No	Exercise-induced cramps. Rhabdomyolysis		Renal failure by myoglobinuria, second wind phenomenon
GSD VI / GSD 6 (Hers' disease)	Liver glycogen phosphorylase (PYGL) Muscle phosphoglycerate mutase (PGAM2)	1 in 65,000 – 85,000[10]	Yes	Yes	Yes [11]	None	initially benign, developmental delay follows	
GSD VII / GSD 7 (Tarui's disease)	Muscle phosphofructokinase (PKFM)	1 in 1,000,000[12]	No	No	No	Exercise-induced muscle cramps and weakness	developmental delay	In some haemolytic anaemia
GSD IX / GSD 9	Phosphorylase kinase (PHKA2 / PHKB / PHKG2 / PHKA1)	?	Yes	Yes	Yes	None	Delayed motor development. Developmental delay	
GSD X / GSD 10	Phosphoglycerate mutase (PGAM2d)	?	?	?	?	Exercise-induced muscle cramps and weakness		Myoglobinuria[13]
GSD XI / GSD 11	Muscle lactate dehydrogenase (LDHA)	?	?	?	?			
Fanconi-Bickel syndrome formerly GSD XI / GSD 11, no longer considered a GSD	Glucose transporter (GLUT2)	?	Yes	Yes	No	None		
GSD XII / GSD 12 (Aldolase A deficiency)	Aldolase A (ALDOA)	?	No	In some	No	Exercise intolerance, cramps. In some Rhabdomyolysis		Hemolytic anemia and other symptoms
GSD XIII / GSD 13	β-enolase (ENO3)	?	No	?	No	Exercise intolerance, cramps	Increasing intensity of myalgias over decades[14]	Serum CK: Episodic elevations. Reduced with rest[14]
GSD XV / GSD 15	Glycogenin-1 (GYG1)	Rare[15]	No	No	No	Muscle atropy	Slowly progressive weakness over decades	None

Type	Enzyme	Gene locus	Enzymatic test	Liver abnormalities
0	Glycogen synthase	12p12.2	Liver	Steatosis
Ia	Glucose-6-phosphatase	17q21	Liver	Steatosis and GHD Adenoma, later HCC
Ib (non-a)	Glucose-6-phosphate translocase	11q23	Freshly removed liver	Steatosis and GHD Adenoma, later HCC
II	Lysosomal-γ1-4 and-γ1-6-glucosidase	17q25	Leukocytes, liver, muscle, amniocytes	Cytoplasmic vacuoles Lysosomal monoparticulate glycogen in EM
IIIa/b	Amylo-1-6 glycosidase (debrancher)	1p21	a. Liver, muscle, heart b. Liver	GHD and steatosis, fibrosis, rarely cirrhosis
IV	Amylo-1-4 glycan6-glycosyltransferase	3p12	Leukocytes, liver, amniocytes	Ground-glass, diastase resistant inclusions Non-membrane bound fibrillar material on EM
VI	Liver phosphorylase E	14q21-22	Liver	GHD, steatosis, fibrosis, rarely cirrhosis
IX	Liver phosphorylase kinase	Xp22.1-22.2 16q12-13 16p11.2-12.1	Liver, muscle, erythrocytes, leukocytes	Non-uniform GHD, steatosis
XI	GLUT2 transporter	3q26.1-q26.3	Liver	GHD

GHD, glycogenic hepatocyte distension; EM, electron microscopy; HCC, hepatocellular carcinoma.

Limitations of liver biopsy

Controversial diagnostic accuracy for GSDs with lack of specificity based on light microscopy only.

• The procedure is **invasive** with serious complications including **pain, hemorrhage, bile peritonitis, penetration of abdominal viscera, pneumothorax, and even death.**

• The high cost including multiple clinic visits, anesthesia reviews and procedure cost.

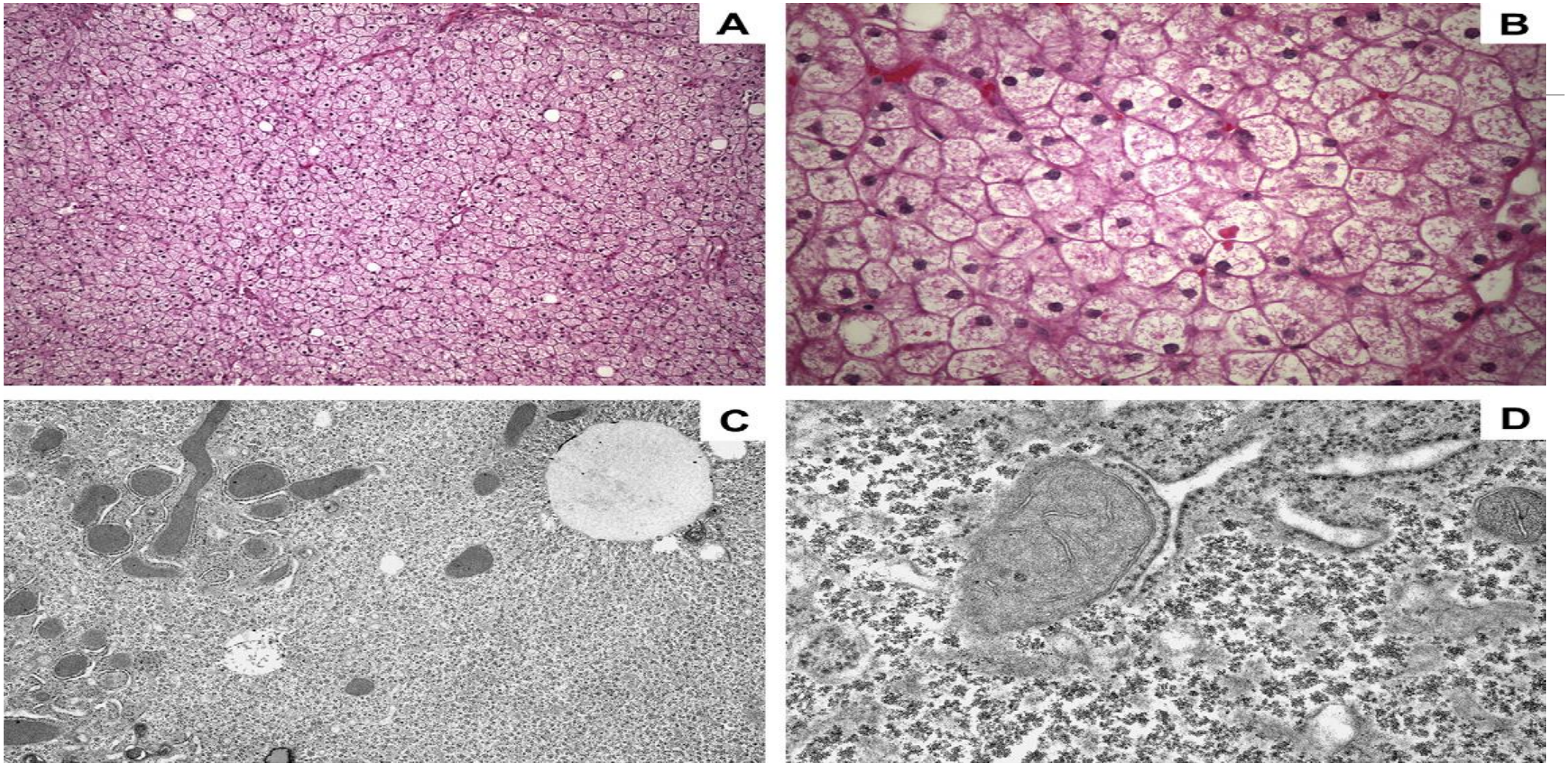
• Usually 30–40 mg of tissue or four cores of hepatic tissue including about 15 mg of snap-frozen hepatic tissue in liquid nitrogen is required for all the studies necessary including light microscopy, electron microscopy and enzymes analysis to make a definitive diagnosis.

• Specialized laboratories offering enzyme studies for hepatic GSDs are not available in Iran thus liver tissues are needed to be out-sourced to overseas laboratories in completely frozen condition with very vigilant temperature control, which is extremely challenging and chances of losing the precious hepatic tissue collected after an invasive procedure are very high.

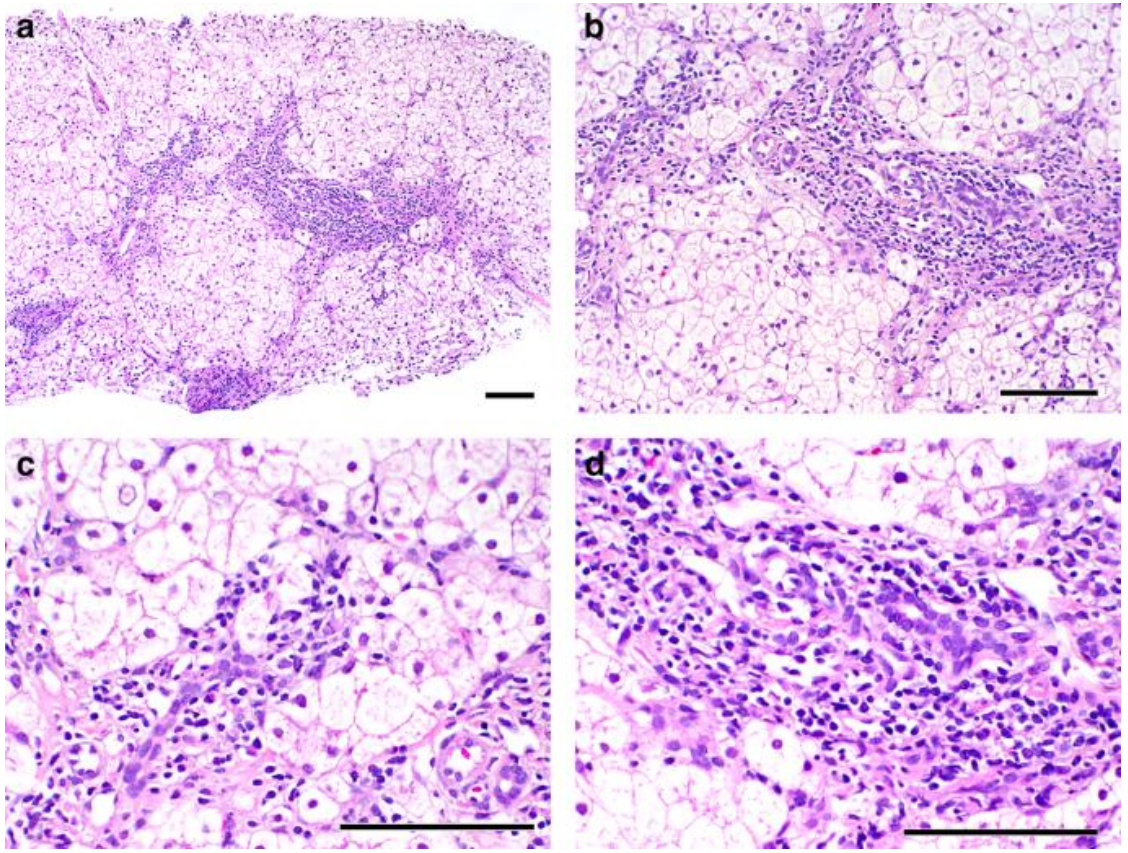
• Electron microscopy on the hepatic tissue, which is required for the diagnosis of GSDs is not readily available at most diagnostic centers in the country owing to its high cost.

• The treatment is aimed at specific type of GSDs, which is not possible based on only histo-pathological findings of hepatic tissue on light microscopy without the enzyme testing, which is not available in Iran.

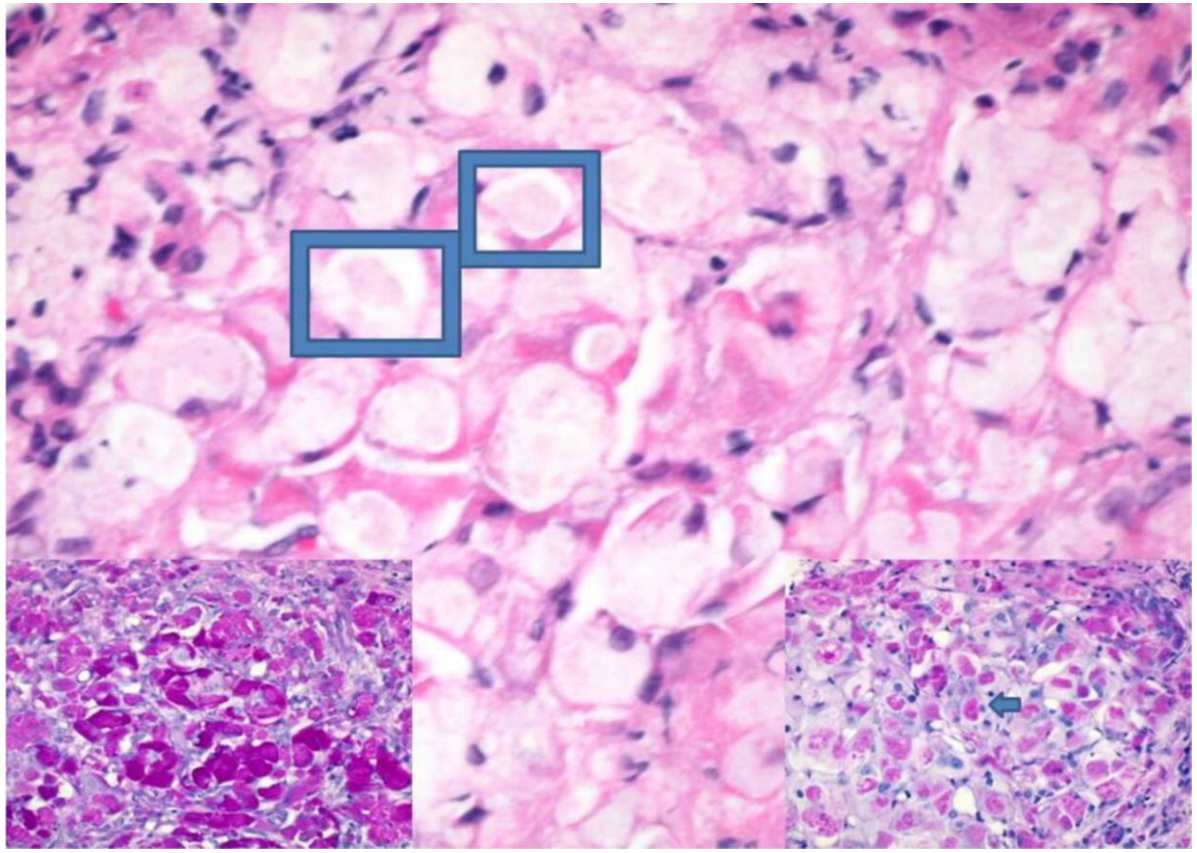
GSD type I



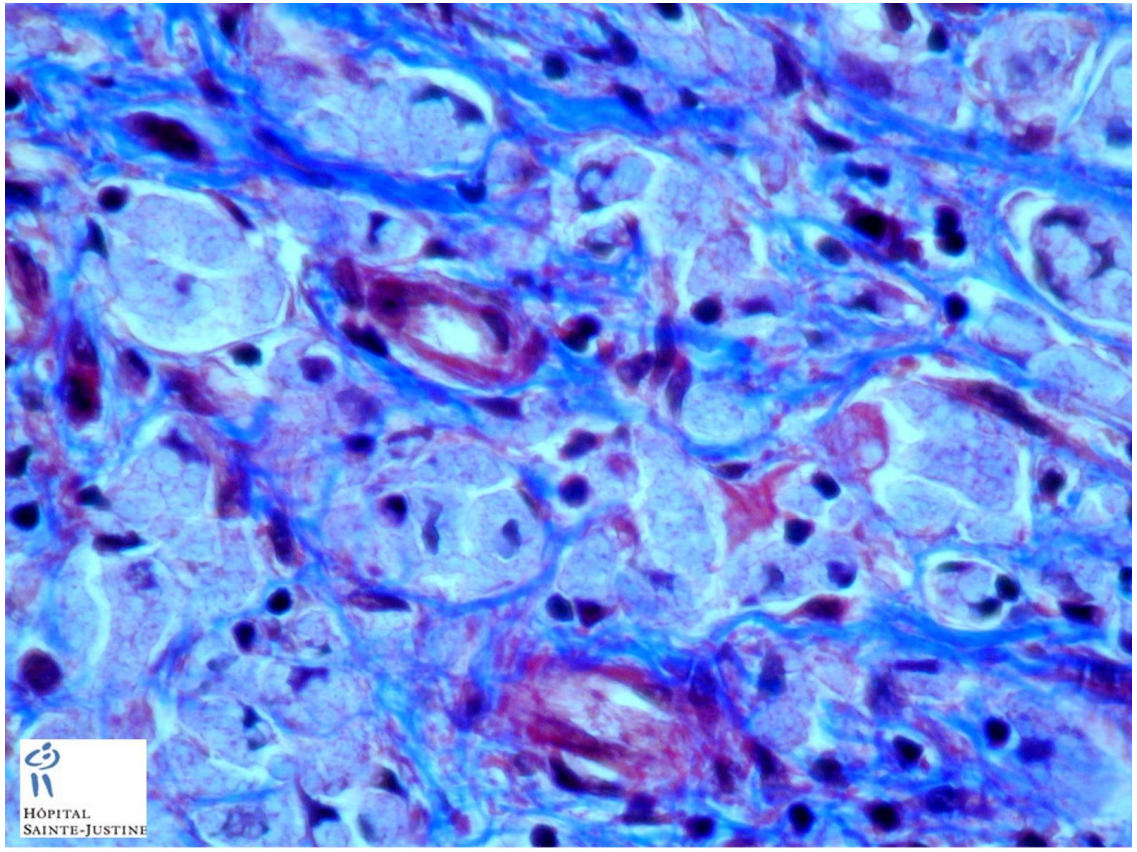
GSD type III

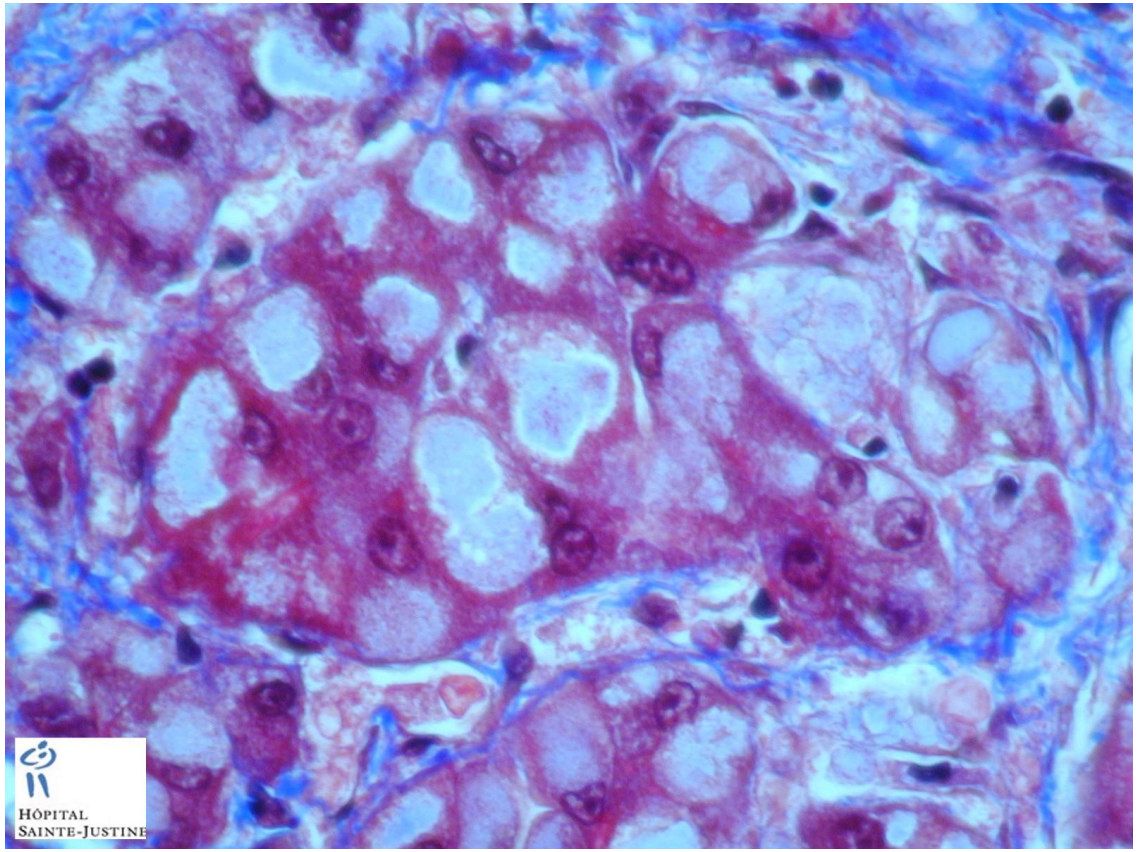


GSD type IV

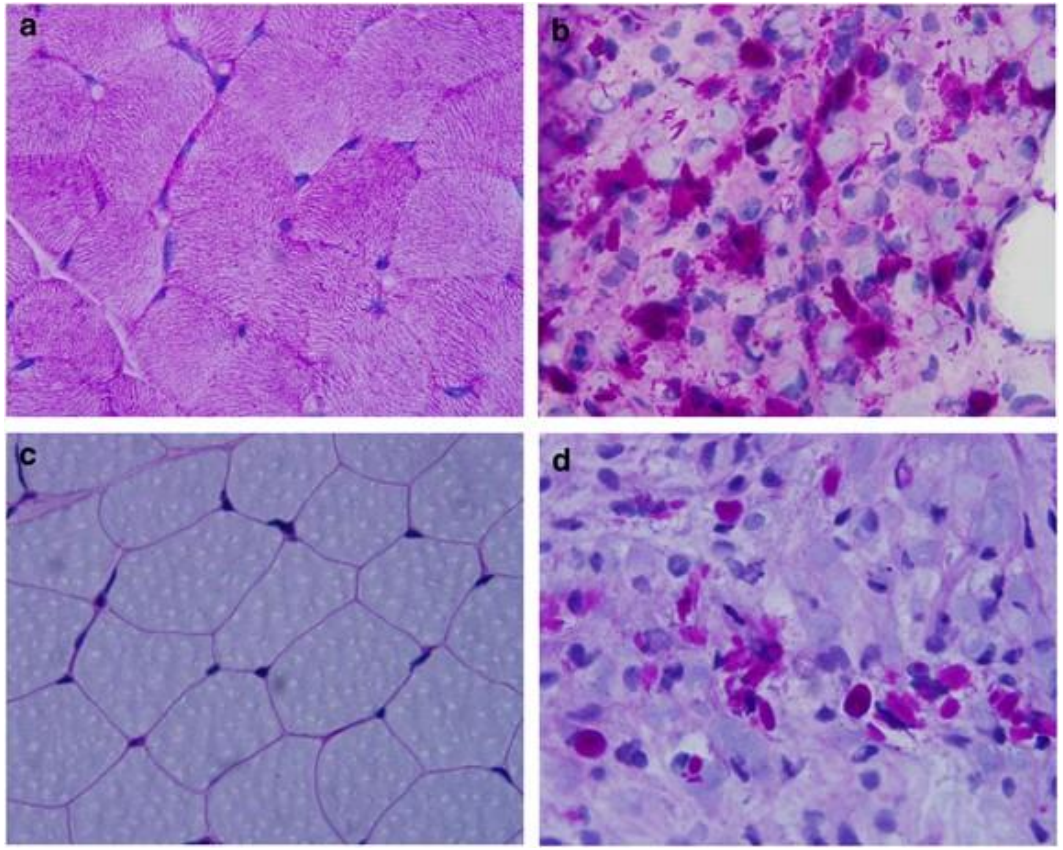


GSD type IV





GSD (Muscle)



Molecular Tests

- Non-invasive compared to the liver biopsy.
- High diagnostic accuracy as molecular methods minimize false positive test results by targeting the specific gene of interest.
- Molecular testing clearly differentiates between different types of GSDs allowing physicians to initiate specific type-based treatment of GSDs and organizing the specific type-based surveillance plan, which varies significantly for various GSDs.
- Ease of sample transportation to the laboratory without vigilant temperature control.
- Automated analyzers are available, and a single trained pathologist can report numerous samples.
- Short turnaround times, which is 48 hours.
- Cost-effective, as the cost of next-generation sequencing (NGS) allowing analysis of multiple genes It is roughly half the cost of a liver biopsy procedure followed by light microscopy, electron microscopy and enzyme testing.
- Provision of reliable prenatal diagnosis and carrier testing by offering targeted familial variant testing to at-risk couples and carrier testing for family members

Diagnosis and Treatment

- Early diagnosis of GSDs is imperative for initiation of appropriate treatment and achieving better prognosis.
- Owing to the nonspecific clinical presentation of GSDs and the lack of specific biomarkers to differentiate various types of GSD, NGS has become the first line diagnostic tool for the evaluation of GSD.
- NGS depends on massive molecular parallelization and allows analyzing **multiple genes at the same time in a single DNA sample**, it is a rapid and a much cost-effective way of not only diagnosing GSD but also differentiate different types of GSDs in absence of the invasive procedure of liver biopsy

Next Generation Sequencing

- In context of NGS instrumentation availability, a wide variety exists e.g. MiniSeq and Miseq from Illumina, Ion Torrent from Life Technologies Thermo Fischer Scientific and MinION from Oxford Nanopore etc.
- These differ primarily in terms of cost, capacity, principal chemistry and read length, DNA library preparation and run time.
- On the basis of feasibility, the Illumina MiSeq is the most popular sequencer due to its relatively low cost, significantly low error rate and capacity to deliver the moderate throughput required by most centers.

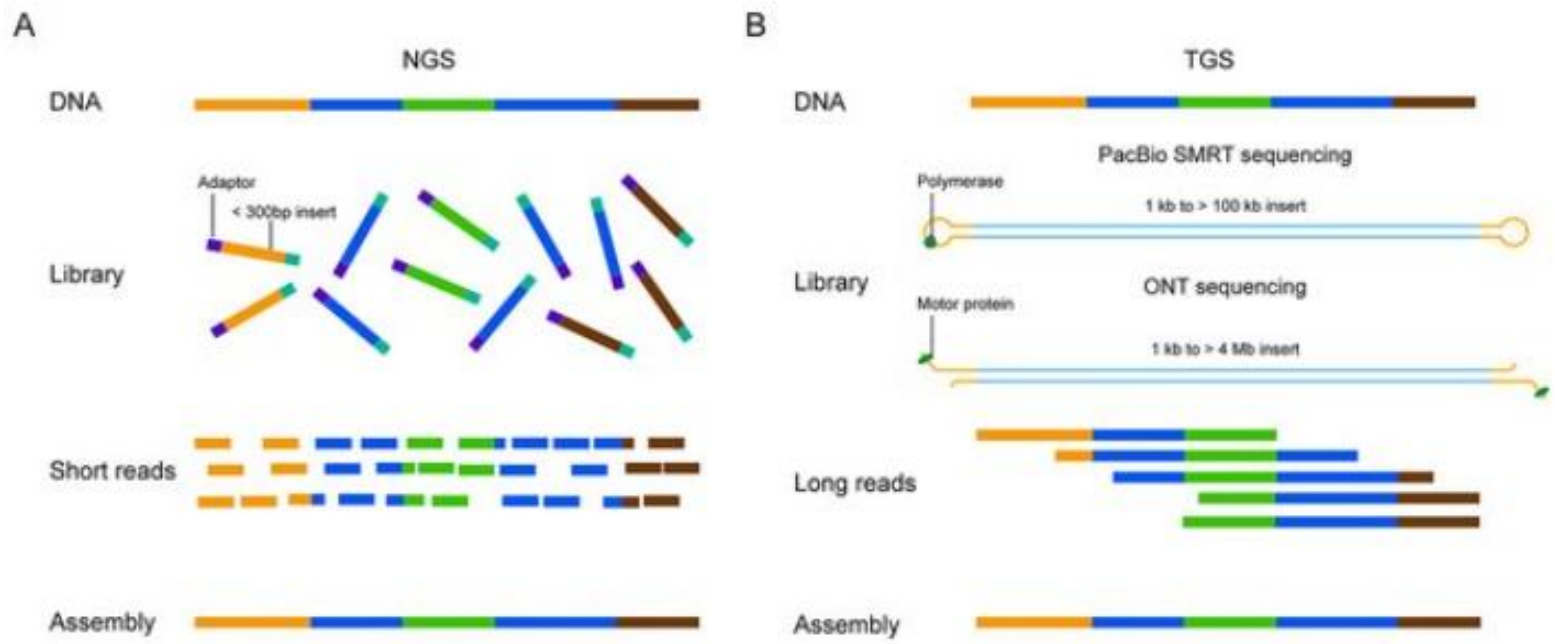
NGS advantages

- From a local perspective, measurement of enzyme activity suffers from logistic issues and is technically more challenging alongside a dearth of laboratory expertise.
- Furthermore, enzyme analysis is usually not reliable in detecting heterozygous carriers of a disease.
- These testing modalities are often very laborious, time consuming and require a pre-selection by clinical phenotype for targeting the specific enzyme.
- On the contrary, NGS has now become the gold standard to confirm a suspected diagnosis of GSD owing to its comparatively low cost, rapid analysis time and availability of less technically demanding automated platforms.
- Enzyme analysis on hepatic tissue is only needed in certain cases when there are unclear molecular results like variants of uncertain significance found on gene testing

NGS

- NGS has been applied in clinical diagnostics for a diversity of symptoms to characterize the inherent genetic cause of diseases. Although single-gene testing and gene panels for specific disorders are still being used, NGS is progressively being utilized in diagnostic evaluation, especially for disorders that are genetically heterogeneous, such as GSDs .
- Currently, targeted gene sequencing (TGS) panels have gained popularity for heterogeneous genetic anomalies in monogenic disorders (MDs) because of their time- and cost-effectiveness as well as their ability in simultaneous detection of common and rare genetic variations

- In line with the best practices, the undertaking of molecular testing for suspected cases with GSDs, by outsourcing the samples abroad to accredited laboratories has been a standard practice.
- For this purpose, the extracted DNA samples from peripheral (whole) blood specimen is outsourced for NGS based GSDs panels, with ease of transportation at room temperature


A
NGS
DNA
Adaptor
< 300bp insert
Library
Short reads
Assembly
B
TGS
DNA
PacBio SMRT sequencing
Polymerase
1 kb to > 100 kb insert
Library
ONT sequencing
Motor protein
1 kb to > 4 Mb insert
Long reads
Assembly

https://doi.org/10.1186/s13023-020-01573-8

Orphanet Journal of Rare Diseases

REVIEW Open Access

Diagnosis of hepatic glycogen storage disease patients with overlapping clinical symptoms by massively parallel sequencing: a systematic review of literature

Zahra Beyzaei[1], Bita Geramizadeh[1,2*] and Sara Karimzadeh[3]

According to our results, TGS analysis can considered as the first-line diagnostic method with valuable results and ES can be used to diagnose be complex cases of GSD with liver involvement. Overall, these molecular methods are considered as accurate diagnostic tools, which expedite correct diagnosis and treatment with significant cost-effectiveness by reducing unnecessary and inaccurate tests

scientific reports

OPEN

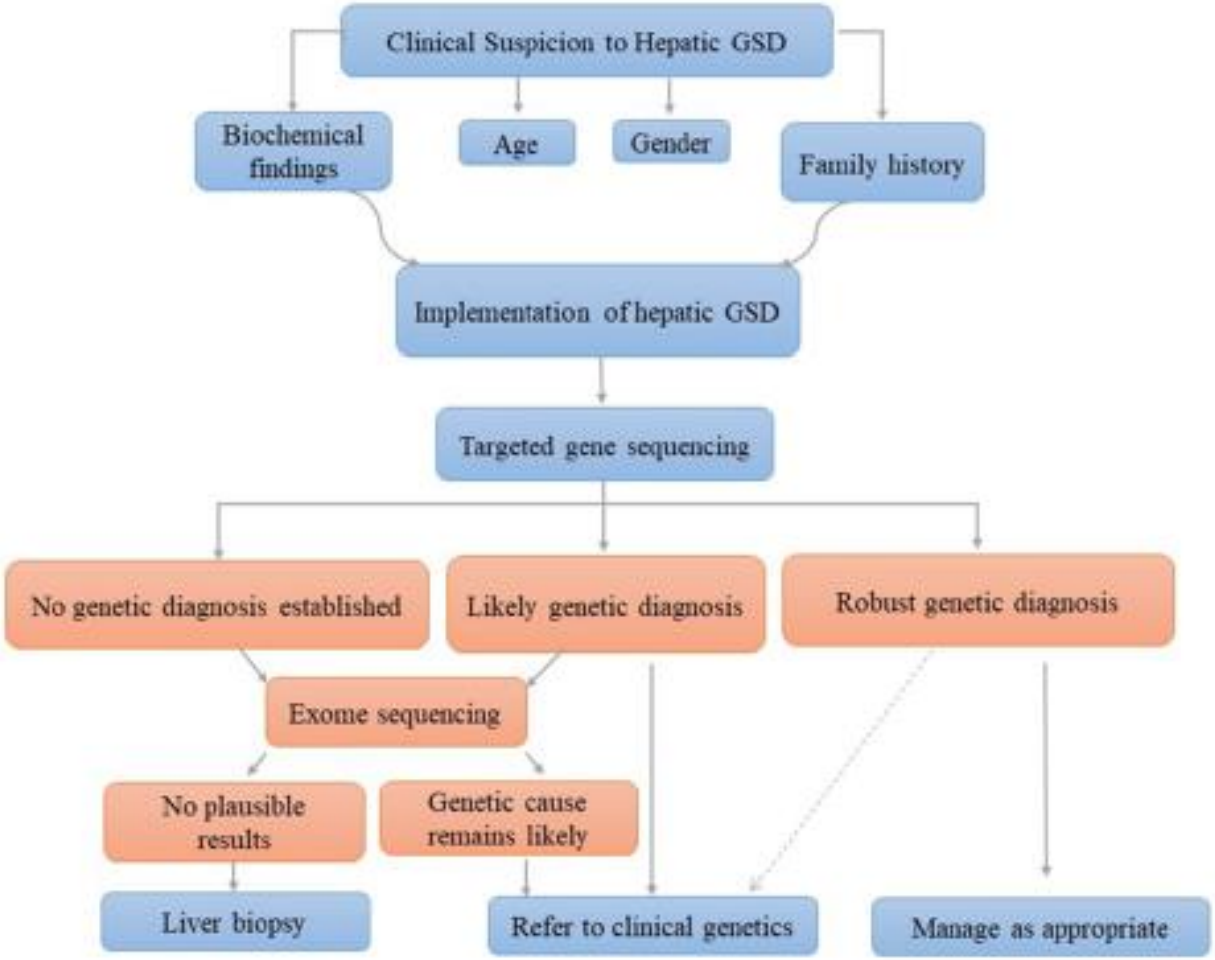
Clinical and genetic spectrum of glycogen storage disease in Iranian population using targeted gene sequencing

Zahra Beyzaei[1], Fatih Ezgu[2], Bita Geramizadeh[1,3]✉, Mohammad Hadi Imanieh[4], Mahmood Haghighat[4], Seyed Mohsen Dehghani[4], Naser Honar[4], Mojgan Zahmatkeshan[4,5], Amirreza Jassbi[6], Marjan Mahboubifar[6] & Alireza Alborzi[7]

Patients / gender/GSD type	Age at genetic diagnosis	Gene/ inheritance pattern[a]	Chr: loc (hg19)	Nucleotide change	Predicted protein change	Variant type	Zygosity	Feature of liver histopathology	Previous definition and pathogenicity	Iranome database
P1/F/Ib	14 mo	*SLC37A4*/AR	11: 118900056	**c.24T > G**	**p.Tyr8Ter**	Nonsense	Homozy-gous	GSD I with severe fibrosis, cirrhosis	Not defined, Pathogenic	NA
P2/F/III	41mo	*AGL*/AR	1: 100336041	c.753_756delCAGA	p.Asp-251Gluf-sTer23	In-frame deletion	Homozy-gous	GSD I or III with early septal cirrhosis	Defined in HGMD, patho-genic	NA
P3/M/III	29 mo	*AGL*/AR	1: 100336041	c.753_756delCAGA	p.Asp-251Gluf-sTer23	In-frame deletion	Homozy-gous	GSD I or III with mild portal fibrosis	Defined in HGMD, patho-genic	NA
P4/F/III	47 mo	*AGL*/AR	1: 100342081	**c.1351_1355delAAAGC**	**p. Lys451Leuf-sTer14**	Frame shift	Homozy-gous	GSD I or III with severe fibrosis	Not defined, pathogenic	NA
P5/M/III	36 mo	*AGL*/AR	1: 100379113	c.3980G > A	p. Trp1327Ter	Nonsense	Homozy-gous	GSD I or III with cirrhosis	Defined in HGMD, patho-genic	NA
P6/M/IV	51 mo	*GBE1*/AR	3: 81643169 3: 81754616	c.998A > T **c.292G > C**	p.Glu333Val **p.Val98Leu**	Missense Missense	Homozy-gous Homozy-gous	GSD IV with cirrhosis	Defined in HGMD, patho-genic Not defined, uncertain significance	NA NA
P7/F/VI	48 mo	*PYGL*/AR	14: 51378453	**c.1964A > G**	**p.Glu655Gly**	Missense	Homozy-gous	Unclassified GSD with marked fibrosis	Not defined, uncertain significance	NA
P8/M/VI	19 mo	*PYGL*/AR	14: 51410891	c.229_231delGAC	p.Asp77del	Deletion	Homozy-gous	GSD I or III with fibrosis	Defined in HGMD, patho-genic	NA
P9/F/IXc	28 mo	*PHKG2*/AR	16: 30762461	c.130C > T	p.Arg44Ter	Nonsense	Homozy-gous	Unclassified GSD with fibrosis	Defined in HGMD, patho-genic	NA
P10/M/IXb	36 mo	*PHKB*/AR	16: 47531367	**c.134T > A**	**p.Leu45His**	Missense	Heterozy-gous	Unclassified GSD with bridg-ing fibrosis	Not defined, uncertain significance	0.0025
P11/F/Ib, IXb	41 mo	*SLC37A4*/AR *PHKB*/AR *PHKB*/AR	11: 118898407 16: 47628046 16: 47727384	**c.337C > T** **c.1127-2A > G** **c.2840A > G**	**p. Leu113Phe** **p.?** **p. Gln947Arg**	Missense Potential splice site Missense	Heterozy-gous Homozy-gous Homozy-gous	Unclassified GSD with mod-erate periportal fibrosis	Not defined, uncertain significance Not defined, likely patho-genic Not defined, uncertain significance	0.003125 NA NA
P12/M/X	29 mo	*PGAM2*/AR	7: 44105115	**c.14G > A**	**p.Arg5His**	Missense	Heterozy-gous	Unclassified GSD with early septal cirrhosis	Not defined, uncertain significance	NA
P13/F/ GSD of heart, lethal congenital	29 mo	*PRKAG2*/AD	7: 151329185	**c.592A > T**	**p. Met198Leu**	Missense	Heterozy-gous	Unclassified GSD with cir-rhosis	Not defined, uncertain significance	NA
P14/F/NA	28 mo	NA	–	None in GSD or similar phenotype genes	–	–	–	Unclassified GSD or lipid storage disease with mild portal fibrosis	–	–

Our Experience with molecular testing

- A total of the 14 pediatric patients were admitted to our hospital and referred for molecular
- Genetic testing using TGS. Seven genes namely SLC37A4, AGL, GBE1, PYGL, PHKB, PGAM2, and
- PRKAG2 were detected to be responsible for the onset of the clinical symptoms.
- A total number of 15 variants were identified i.e. mostly loss-of-function (LoF) variants, of which 10 variants were novel.
- Finally, diagnosis of GSD types Ib, III, IV, VI, IXb, IXc, X, and GSD of the heart, lethal congenital was made in 13 out of the 14 patients.
- Notably, GSD-IX and GSD of the heart-lethal congenital (i.e. PRKAG2 deficiency) patients have been reported in Iran for the first time which shown the development of liver cirrhosis with novel variants.
- These results showed that TGS, in combination with clinical, biochemical, and pathological hallmarks, could provide accurate and high-throughput results for diagnosing and sub-typing GSD and related diseases


Clinical Suspicion to Hepatic GSD
Biochemical findings
Age
Gender
Family history
Implementation of hepatic GSD
Targeted gene sequencing
No genetic diagnosis established
Likely genetic diagnosis
Robust genetic diagnosis
Exome sequencing
No plausible results
Genetic cause remains likely
Liver biopsy
Refer to clinical genetics
Manage as appropriate

Beyzaei *et al. BMC Med Genomics* (2021) 14:33
https://doi.org/10.1186/s12920-021-00879-1

BMC Medical Genomics

CASE REPORT Open Access

Novel *PRKAG2* variant presenting as liver cirrhosis: report of a family with 2 cases and review of literature

Zahra Beyzaei[1], Fatih Ezgu[2], Bita Geramizadeh[1,3*], Alireza Alborzi[4] and Alireza Shojazadeh[4]

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Identification of a novel mutation in the PHKA2 gene in a child with liver cirrhosis

Zahra Beyzaei, Fatih Ezgu, Mohammad Hadi Imanieh and Bita Geramizadeh

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