

GSD diagnosis – can liver biopsy be avoided?

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GSD presentation (types I,III,VI,IX)

- Typically presents in first year of life
- Fasting hypoglycaemia
- Hepatomegaly
- Poor growth

Initial investigations

Lactate

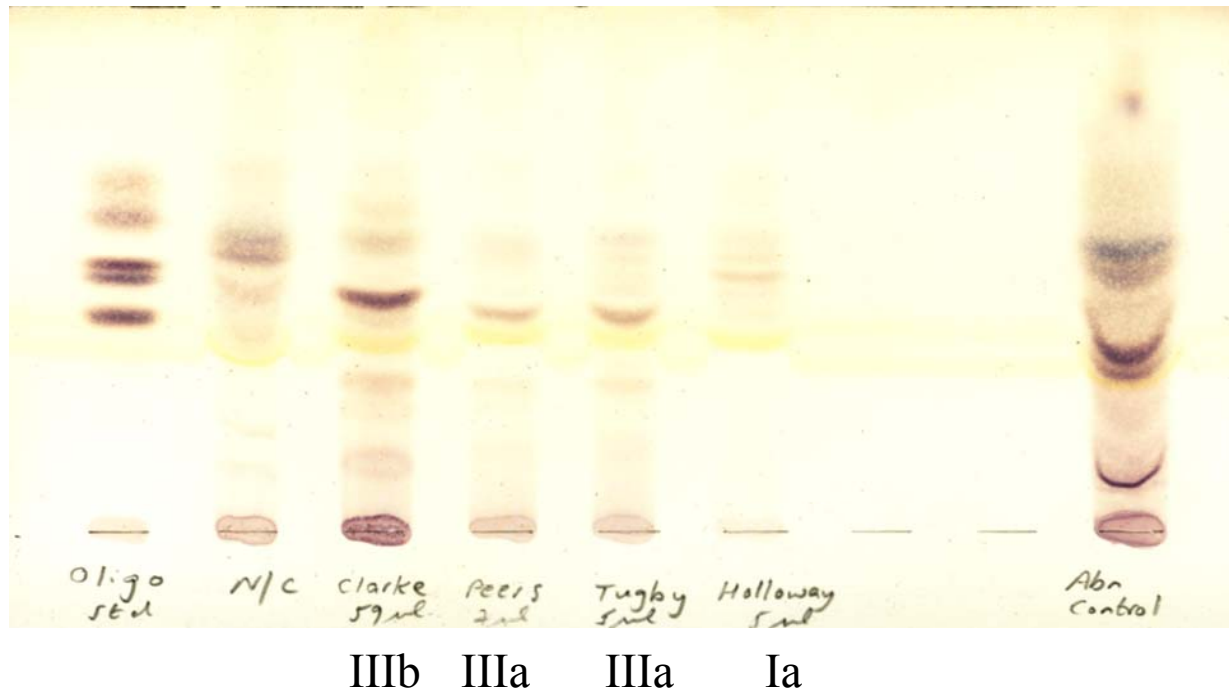
ALT, CK, urate

Cholesterol, triglycerides

FBC (to look for neutropenia)

Urine oligosaccharides

Urine oligosaccharide electrophoresis



Liver biopsy in suspected GSD

Advantages:

- Histology/histochemistry can give rapid result for type Ia
- Evidence of fibrosis/steatosis may help sub-typing
- Definitive diagnosis (no residual risk for Ia)

Disadvantages:

- Invasive procedure
- Enzyme results may take months
- GSD 1 non a difficult assay (may give equivocal result)

GSD diagnosis – histology/histochemistry review

- Historically liver biopsy has been an accurate method of diagnosing GSD (20 out of 20 at BCH)
- By histology/histochemistry/EM, GSD type Ia can be diagnosed.

Beyond this subtyping by histology is not accurate (25% misleading) although the differential can be narrowed.

- The ultimate subtype was mentioned in the initial report in only 50% of cases.

Conclusion

- Aim to reduce number of liver biopsies for ?GSD

GSD - Biochemistry at diagnosis

	Type 1a	Type 1non-a	Type IIIa	Type IIIb	Type VI / IX
Lactate >4 mmol/l	3/3	5/5	1/1	3/4	0/2
ALT >100 IU/l	2/4	2/5	1/1	3/4	0/3
CK >300 IU/l	1/4 (age1 day)	1/4 (v.sick)	3/3	0/4	0/1
urate >400 umol/l	1/4	4/5	0/1	0/3	0/4
triglycerides >5 mmol/l	2/4	3/5		3/4	1/3
neutrophils <0.5 x ⁹ 10 ⁹	0/4	2/5	0/1	0/3	0/2
urine oligosaccharides +ve	0/4	2/5*	2/2	4/4	0/2

* May be due to immaturity in patients <2 years

GSD – Summary of diagnostic biochemistry

- Lactate >4 & ALT >100 – type I and III
- CK >300 - type IIIa (but also increased in very young & very sick)
- Urate >400 – type I
- Triglycerides – variably increased and unhelpful
- Neutrophils - $<0.5 \times 10^9/L$ – type I non-a (but not all)
- Urine oligos – usually abnormal in type 3 (a & b) but may get +ve due to immaturity
- **Much overlap – team discussion for each case**

GSD type IV

- Typically presents in first year of life
- Hepatosplenomegaly, FTT. (Hypoglycaemia is rare)
- Deficiency of branching enzyme

- Very rare, no common mutation
- Branching enzyme activity can be measured in cultured fibroblasts or blood

Urine oligos +ve or CK >300
(Lactate >4, ALT >100)



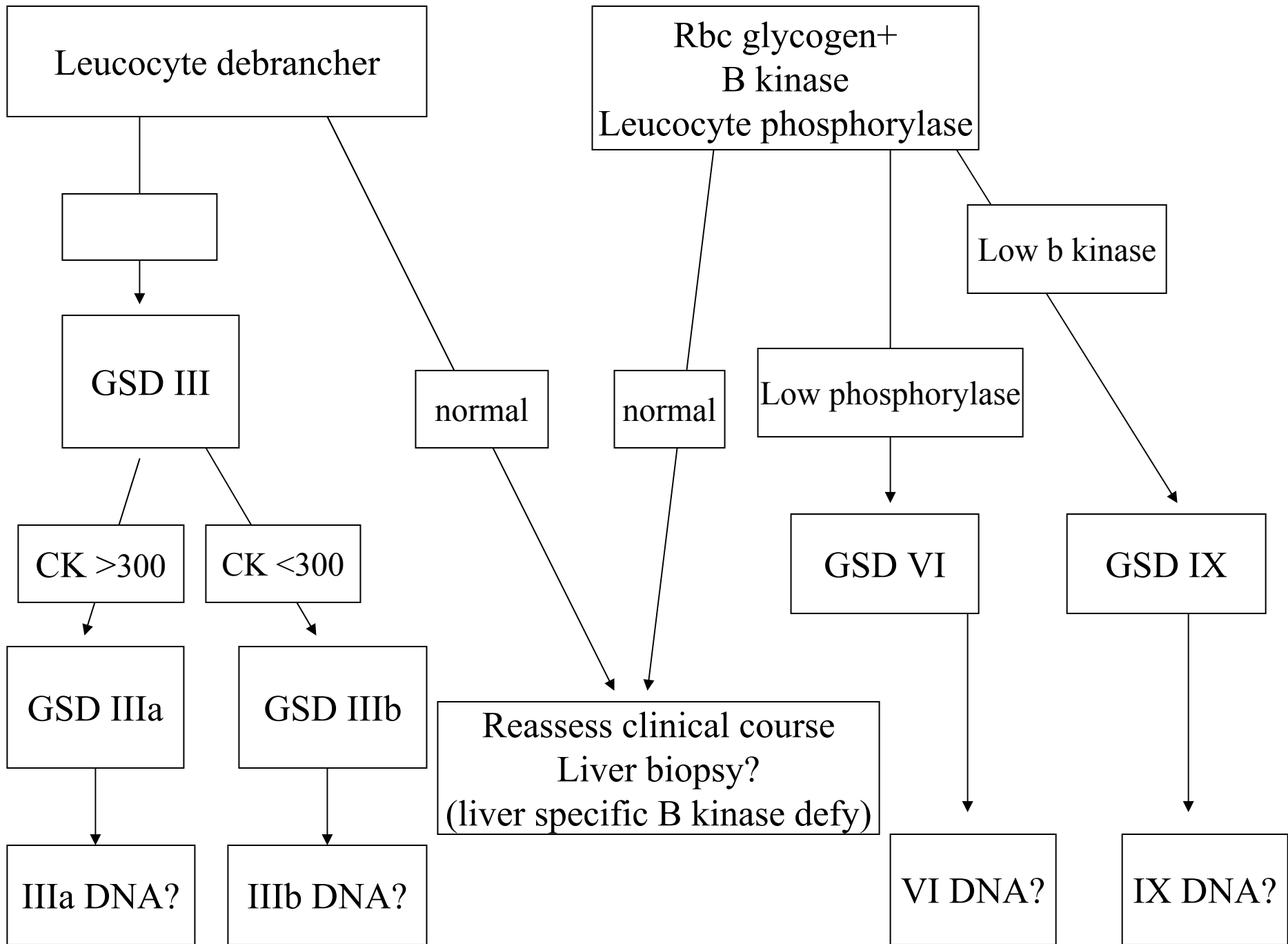
Rbc glycogen
+ leucocyte glycogen debrancher

Urine oligos –ve

Liver function, lactate, CK not grossly abnormal



Rbc glycogen and phosphorylase b kinase
+ Leucocyte phosphorylase



Leucocyte debrancher

GSD III

normal

normal

Low phosphorylase

Low b kinase

CK >300

CK <300

GSD VI

GSD IX

GSD IIIa

GSD IIIb

Reassess clinical course
Liver biopsy?
(liver specific B kinase defy)

IIIa DNA?

IIIb DNA?

VI DNA?

IX DNA?

Rbc glycogen+
B kinase

Leucocyte phosphorylase

DNA analysis in GSD 1a and GSD 1 non-a

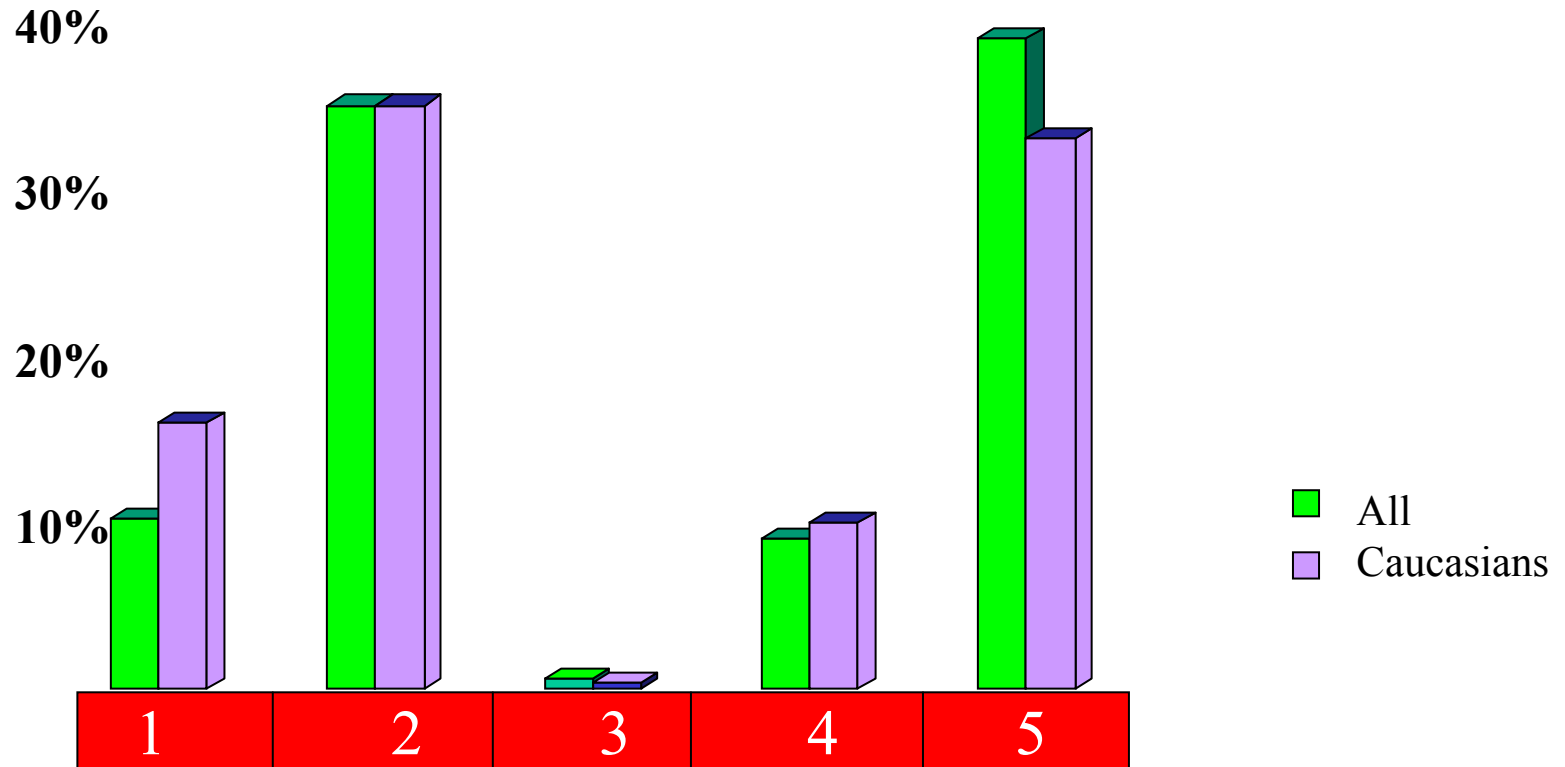
- Mutation analysis has become available
- Common mutations related to ethnic origin identified
- Gene sequencing has become available
- “hot-spot” alleles have been identified

- This has provided an alternative means of diagnosing GSD type without liver bx

Common GSD 1a mutations

Mutation	Exon	% of alleles		
		All (>840)	Caucasian (560)	Pakistan/Indian (12)
247C>T (R83C)	2	26%	32%	0
1039C>T (Q347X)	5	14%	21%	0
648G>T splicing	5	16%	-	0
79delC (35X)	1	3.8%	6%	0
248G>A (R83H)	2	4%	1.3%	0
563G>C (G188R)	4	3%	4%	0
150delGT	2	1%	0	75
Unidentified		5%	6%	0

Frequency of GSD 1a mutations by exon



GSD 1a screening at GOSH

Step 1

Specific mutation test for Q347X and R83C
account for ~62% of N. Europeans.

Cost £100

Step 2

Mutation scan of the gene (SSCP) followed
by sequencing abnormal patterns
Reporting time about 3 months.

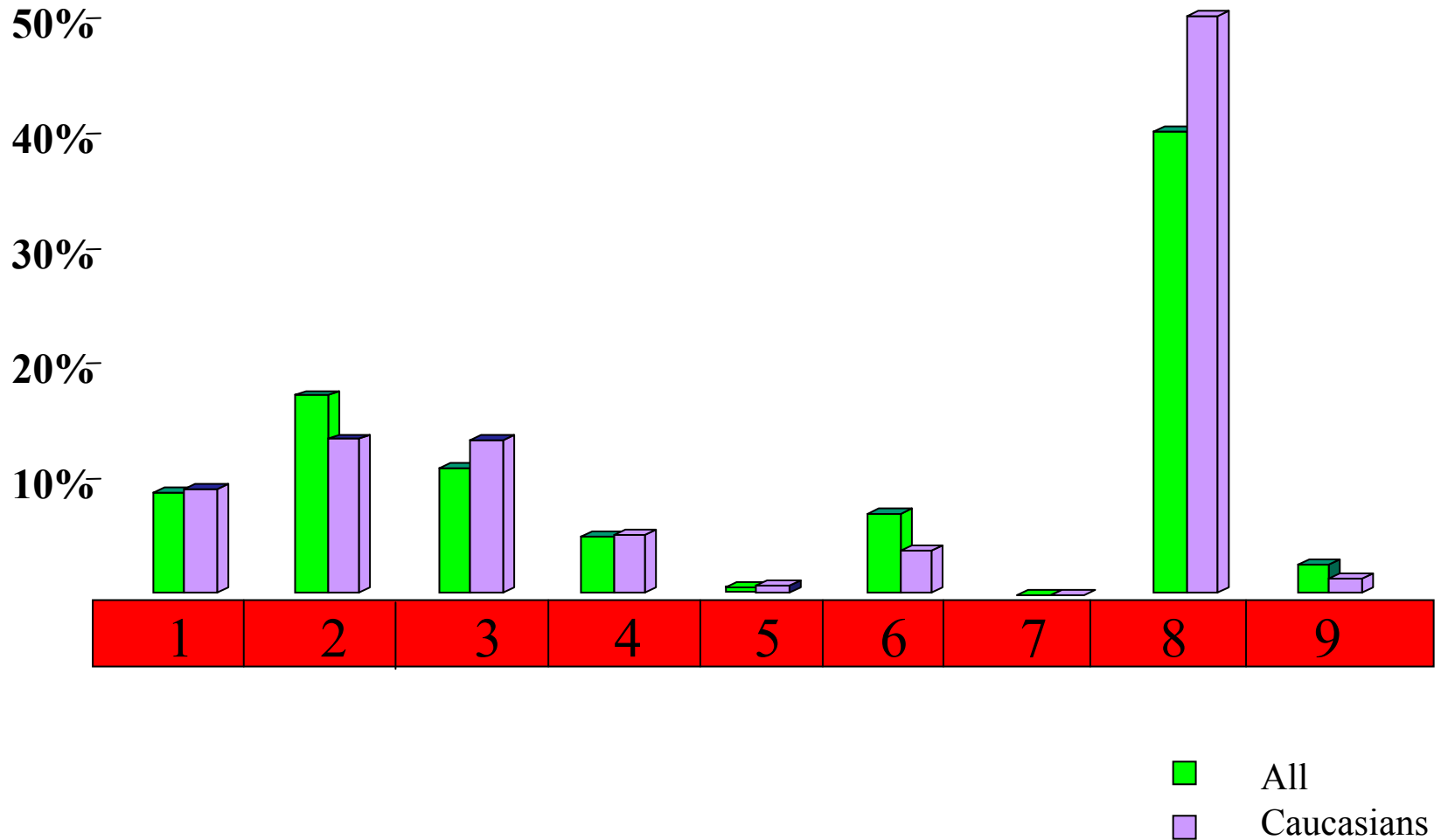
Cost £200-300

Pick up rate is “very high”

Common GSD 1 non a mutations

Mutation	Exon	% of alleles		
		All (>280)	Caucasian (216)	Pakistan/Indian (22)
1042delCT	8	25%	30%	0
1015G>T (G339C)	8	12%	16%	0
359insC	2	2.3%	3.3%	0
352T>C (W118R)	2	4.4%	-	0
169del7	2	2.3%	1.3%	18%
936insA	6	1%	0.6%	55%
IVS8+2del4	8	1.4%	-	18%
Unidentified		2.1%	1%	0

Frequency of GSD 1non a mutations by exon



GSD 1non a screening in Birmingham

Sequence exon 8 in Caucasian patients

Screen for 936insA in Asian patients

If negative DNA sequence remaining exons.

Results to date:

Patients	1 st mutation	2 nd mutation
Caucasian 1	514insG	G339C
Caucasian 2-3	None found by SSCP	None found by SSCP
Pakistan 1-4	1105insA	1105insA
Pakistan 5	F31del	F31del

SUMMARY

GSD 1a

5 exons

>76 mutations reported

Q347X and R83C account for 53-60%

Sequencing exons 2 and 5 will give about 75-80% of mutations

GSD 1non-a

9 exons

>70 mutations reported

Sequencing exon 8 will give about 50% of mutations

1105insA is common in Pakistanis

Lactate >4

ALT >100

CK <300

Urate >400

Urine oligos -ve

Type I

Neutrophils <0.5x10⁹/L

No

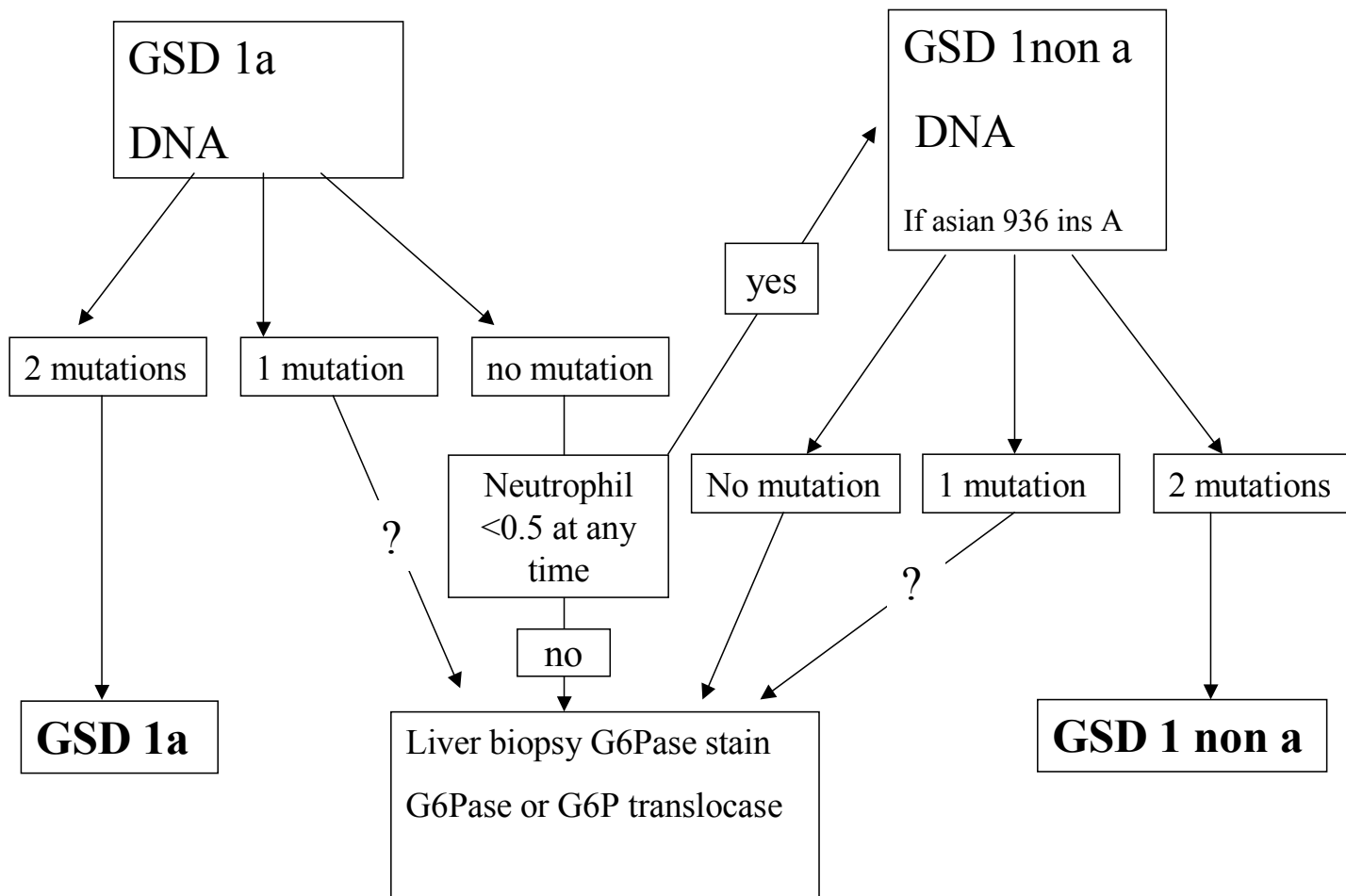
Yes

Type Ia mutation analysis

Type I non a mutation analysis

Residual risk

- GSD Ia approx 20-25%
- GSD I non a approx 50%
- Could sequence other exons (very time consuming and expensive and a residual risk remains)
- At what stage does liver biopsy become the better option?



White cell count should be measured on at least 2 occasions

Abdul

- Presented age 2 months
- Hypoglycaemia
- Hepatomegaly
- Poor weight gain

Lab results : glucose <1.1 mmol/l
 lactate 4.9 mmol/l
 ALT 80, 104 IU/L
 urate 278 umol/l
 triglycerides 2.98 mmol/l
 CK 26 IU/L
 neutrophils 3.2 and 2.5 x10⁹/L
 urine oligos -ve

