DNA Analysis in Glycogen storage disease

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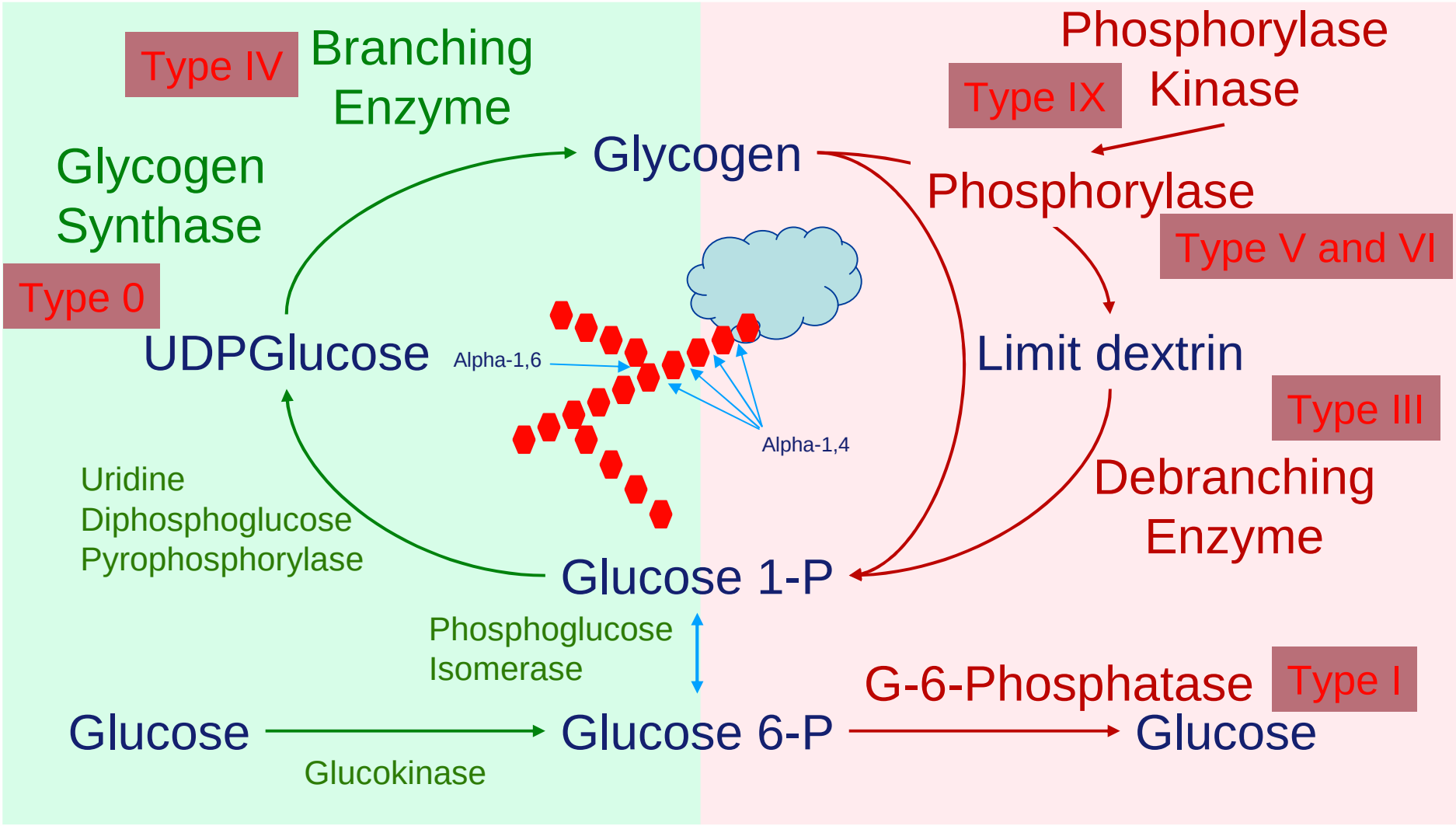
Glycogen Synthesis and Breakdown



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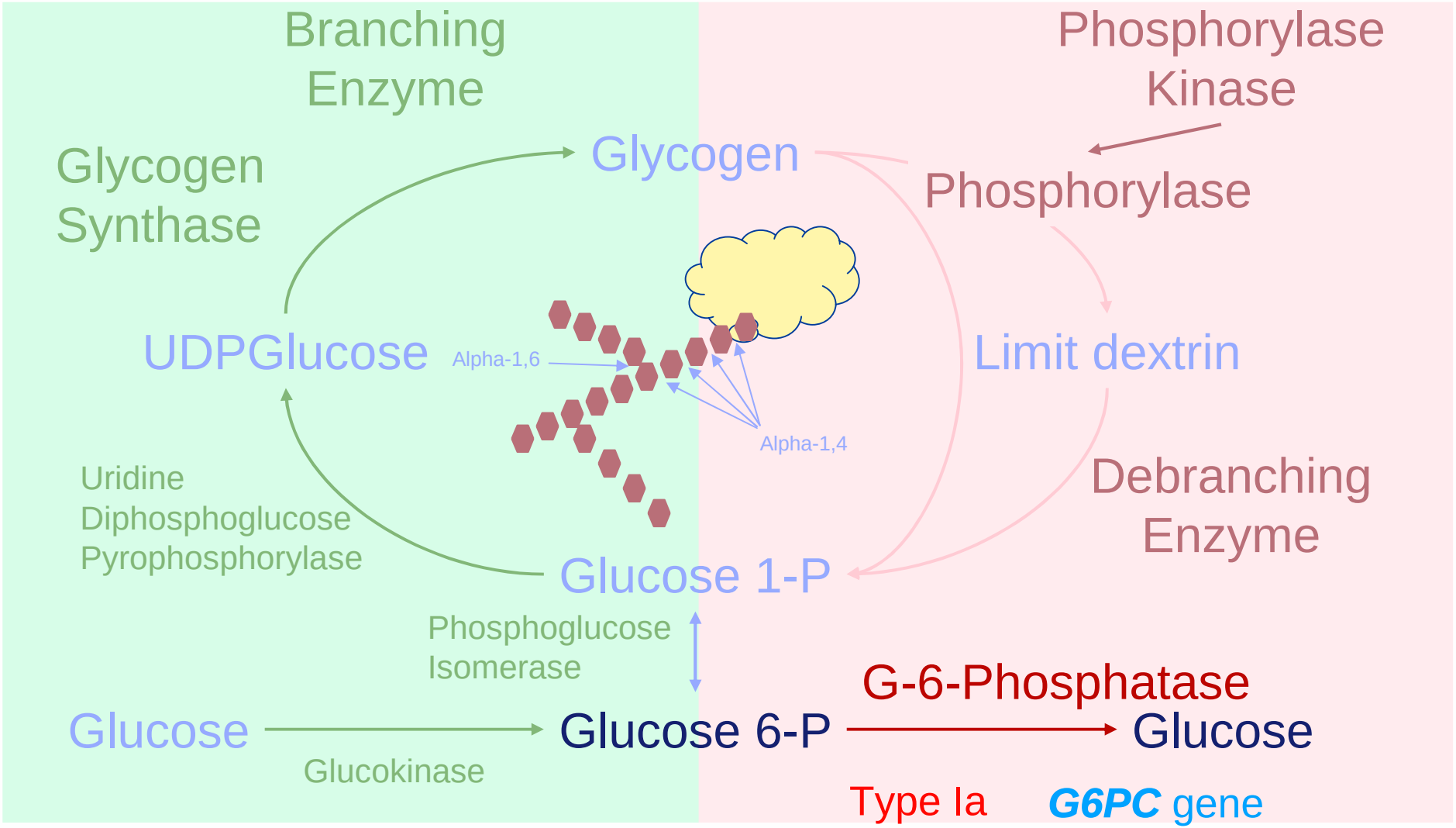
Glycogen Synthesis and Breakdown



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GSD Type I

- First described by von Gierke in 1929
- Approx 1 in 58,000 newborns affected
- Autosomal recessive
- Classification:
  - Ia: Deficiency of glucose-6-phosphatase enzyme
  - Ib/Inon-a: Deficiency of glucose-6-phosphate transporter
- Approx 10% of Type 1 cases are Ib

GSD Type I

- Type Ia
  - ***G6PC*** gene
    - 5 exons, 13 kb on Chr 17q21
  - >80 mutations reported
  - Common changes:
    - p.Arg83Cys - 33%
    - p.Gln347X - 18%
- Type Ib
  - ***SLC37A4*** gene
    - 9 exons, 6 kb on Chr 11q23.3
  - >65 mutations reported
  - Common changes:
    - p.Leu348fs - 28%
    - p.Gly339Cys - 19%
- Genetic analysis:
  - Avoidance of liver biopsy
  - Confirms diagnosis - type Ia versus Ib
  - Phenotypic heterogeneity
  - No clear genotype/phenotype correlation

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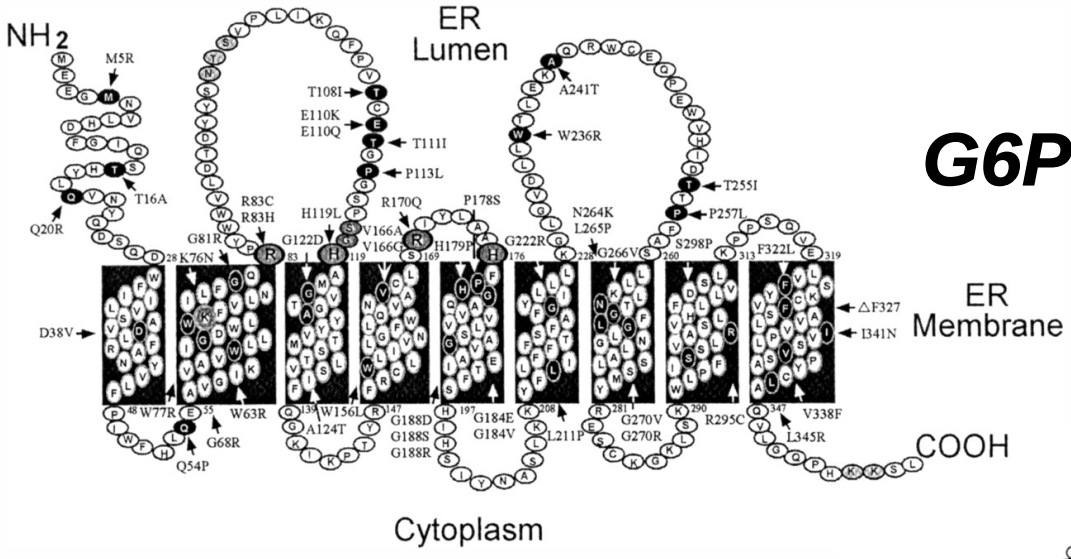
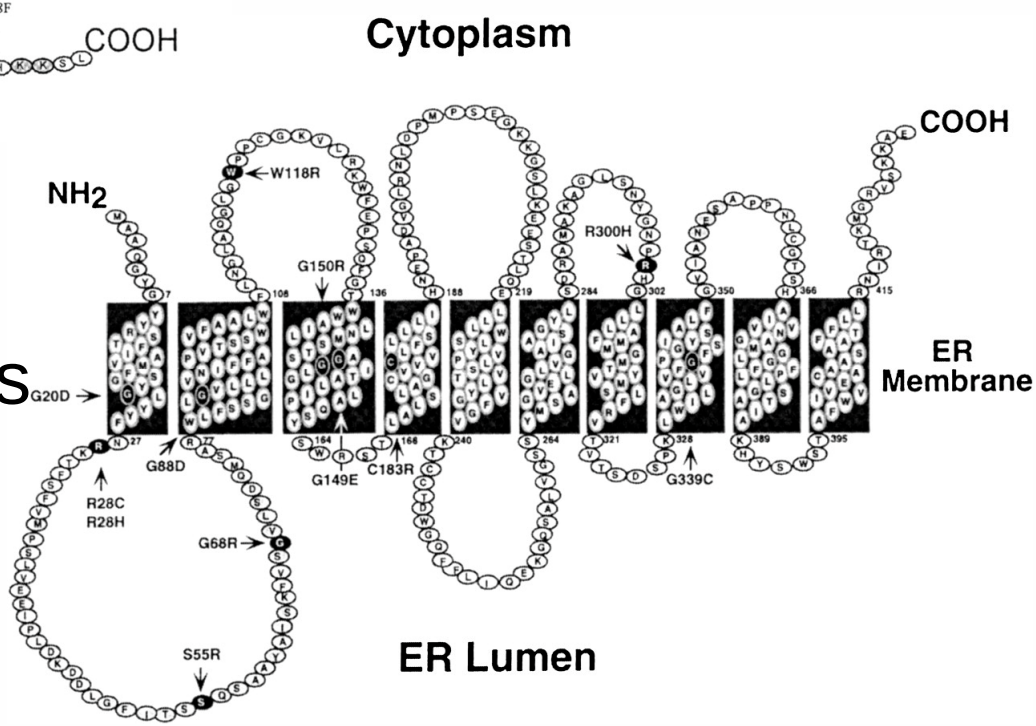


G6PC gene mutations

SLC37A4 gene mutations



From Foster and Nordlie 2002. Exp Biol Med 227: 601-608.

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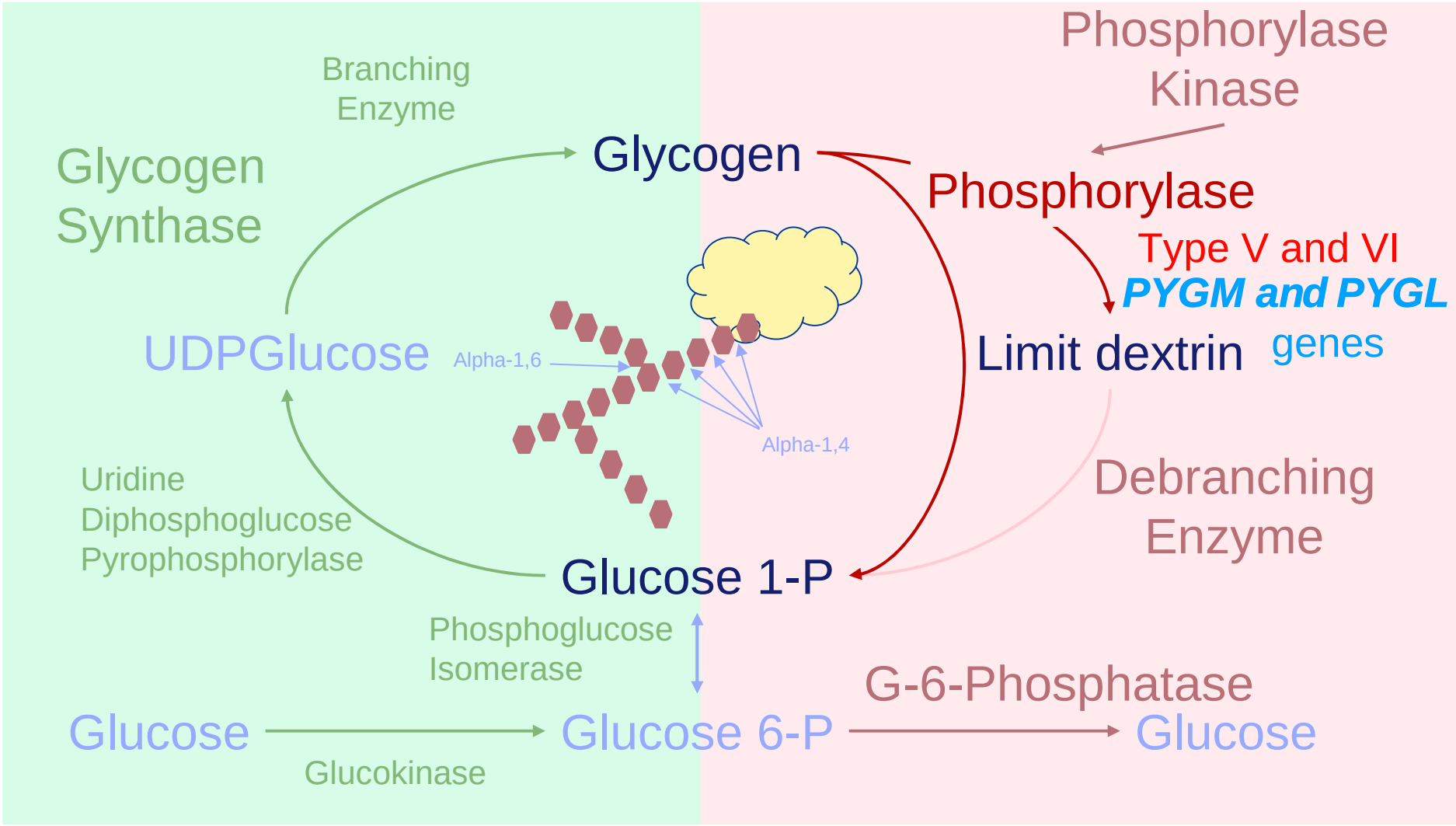
Glycogen Synthesis and Breakdown



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GSD Type V

- Also known as McArdle Disease
- Deficiency of muscle glycogen phosphorylase
  - Autosomal recessive
  - ***PYGM*** gene
    - 20 exons, 40kb on Chr 11q12-q13.2
- 2.5% of GSDs

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Mutations of the ***PYGM*** gene

- Common mutations:
  - p.Arg50X - 32% - 81% of alleles
  - p.Gly205Ser - 0% - 10% of alleles
- Other mutations
  - >85 rare mutations
- Non-sense mediated mRNA decay

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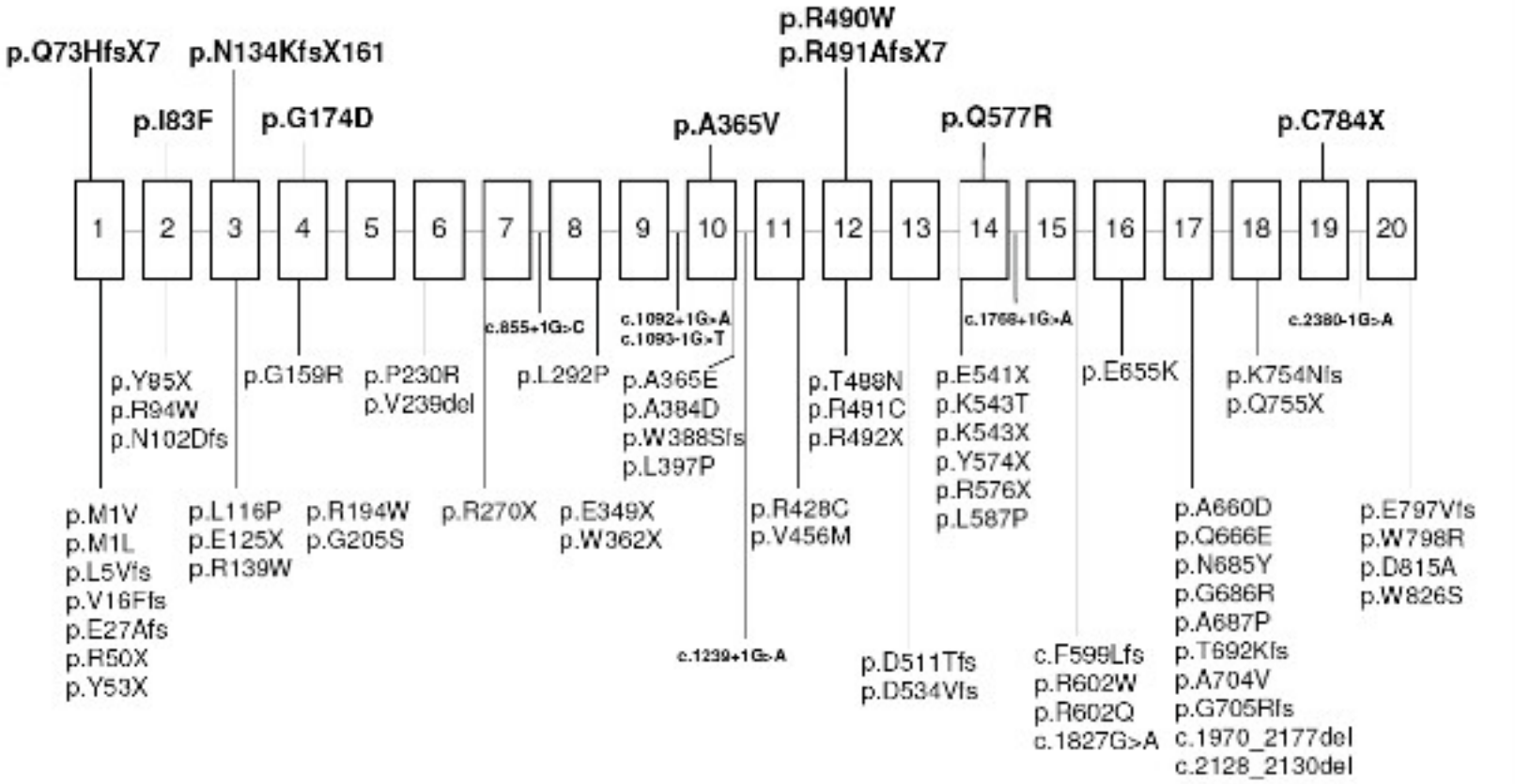
Mutations of the ***PYGM*** gene



From: Rubio JC ***et al 2007*** Hum Mutat. 28(2): 203-4.

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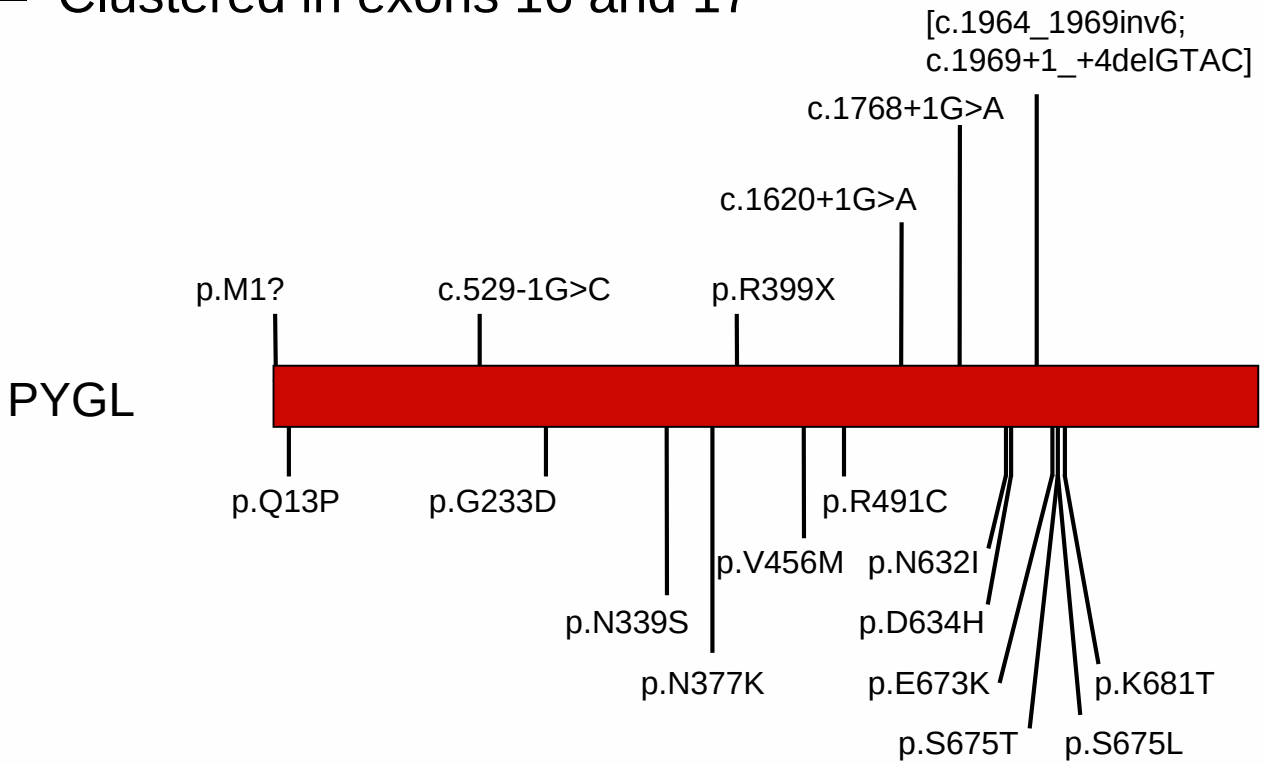
GSD Type VI

- Also known as Hers Disease
- Deficiency of liver glycogen phosphorylase
  - Autosomal recessive
  - ***PYGL*** gene
    - 20 exons, 40kb on Chr 14q21-q22
- Rare

GSD Type VI - Reported Patients

- 11 patients published with 17 mutations
- Majority are missense mutations
  - Clustered in exons 16 and 17



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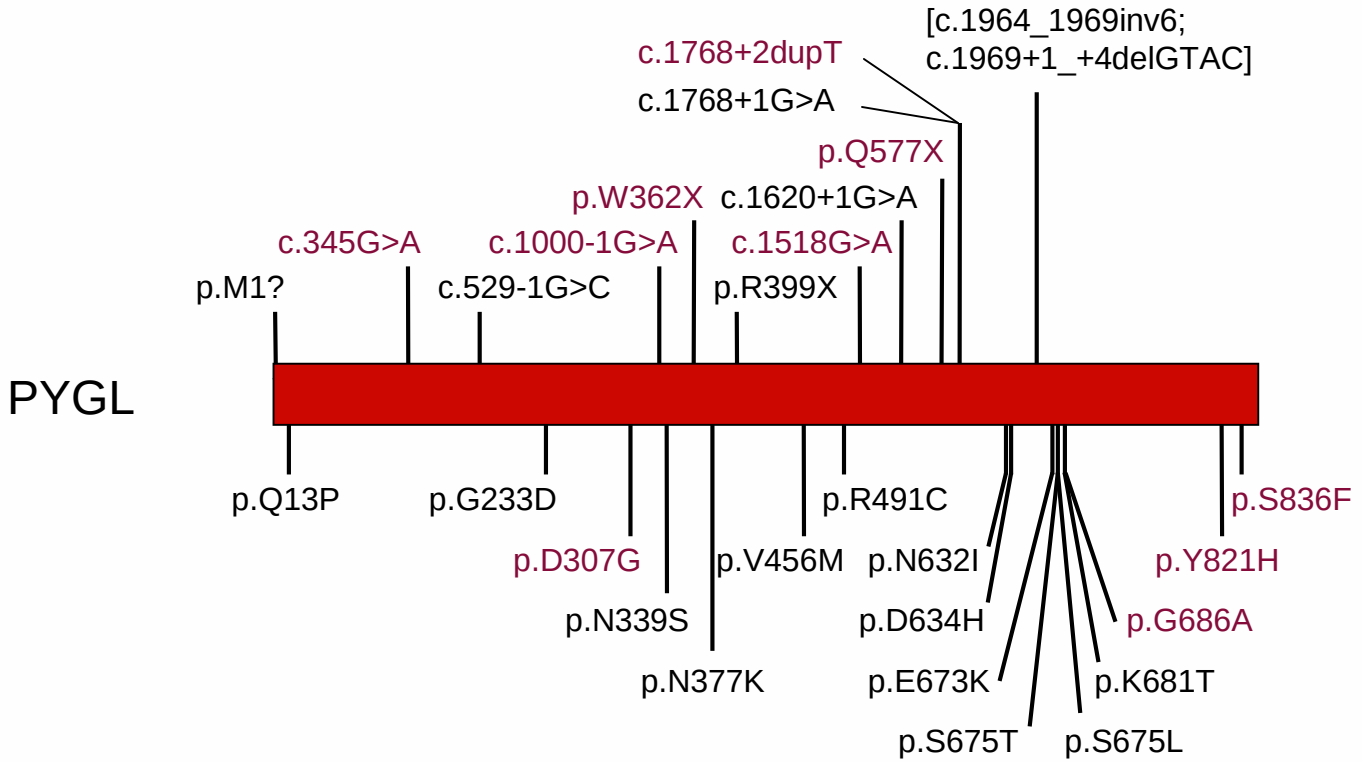
GSD Type VI - Screened Patients

- All published patients and 17 patients from clinical service



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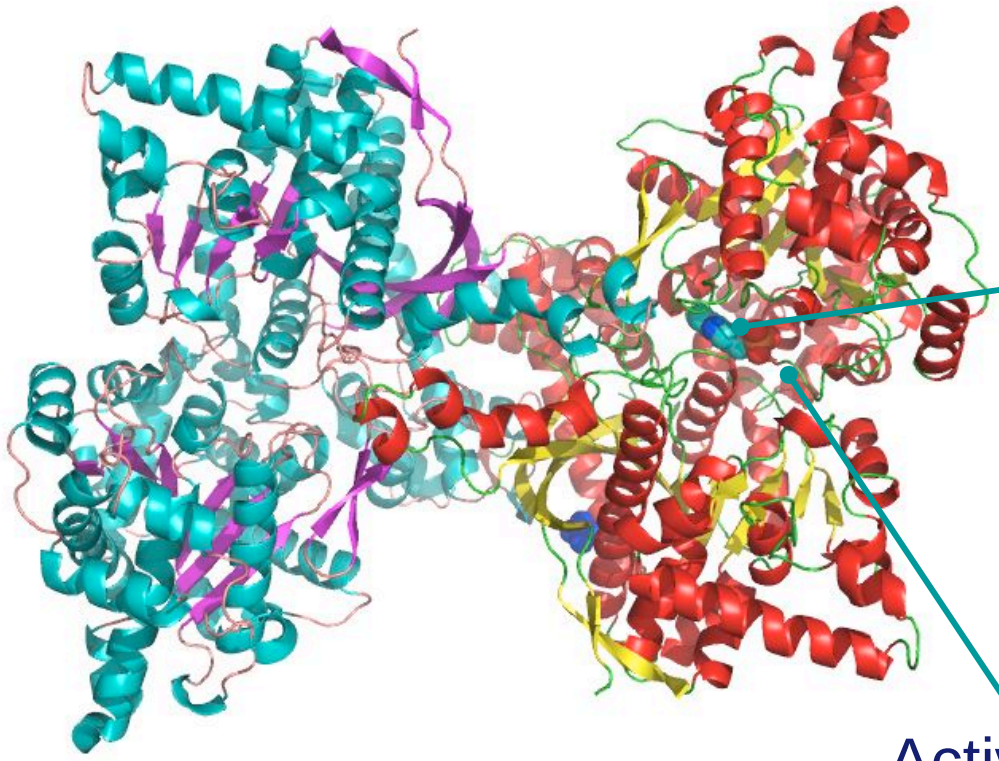
Glycogen Phosphorylase



Pyridoxal Phosphate

Active Site

Glycogen + P_i ⟶ Glycogen + Glucose-1-P

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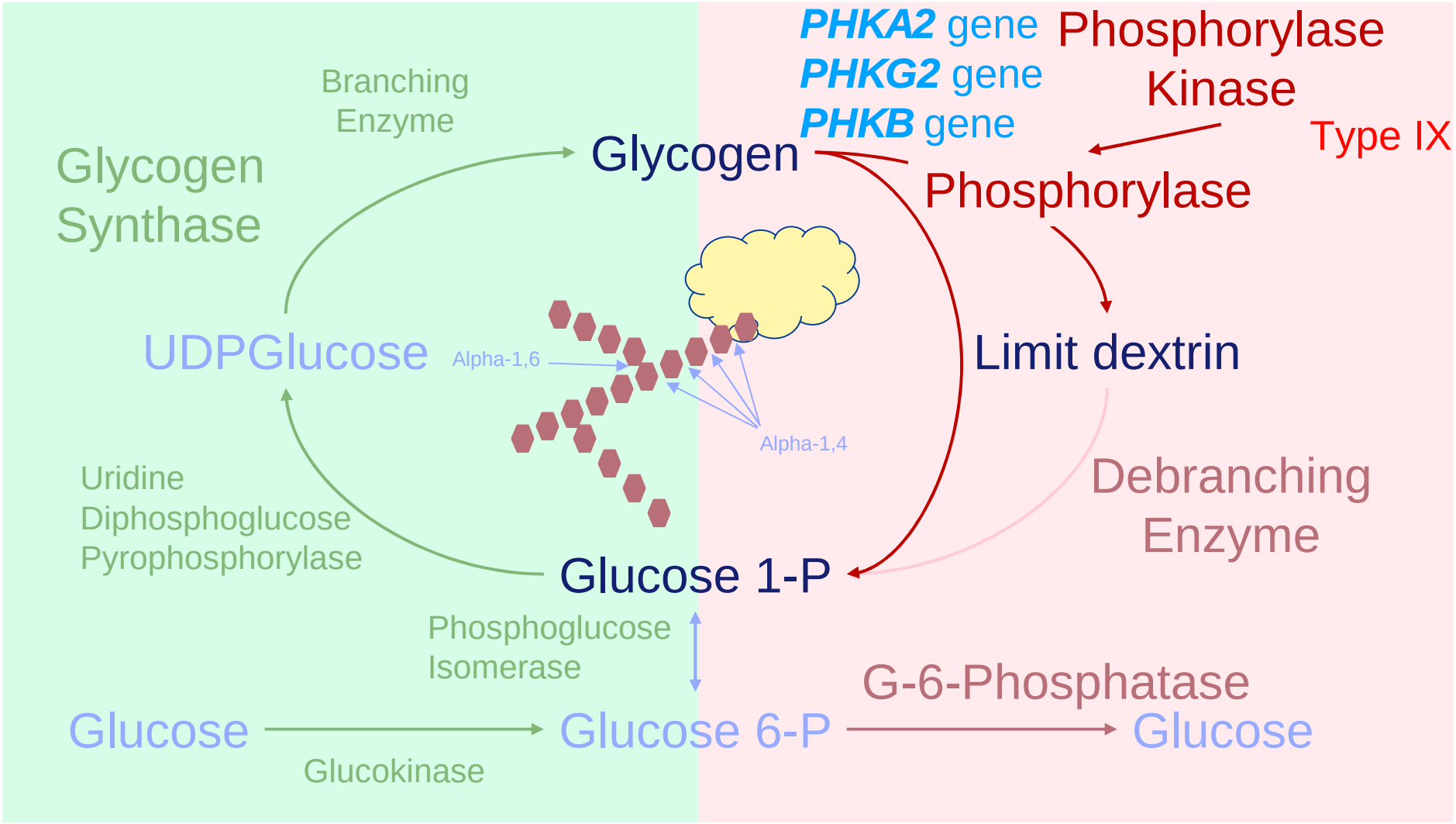
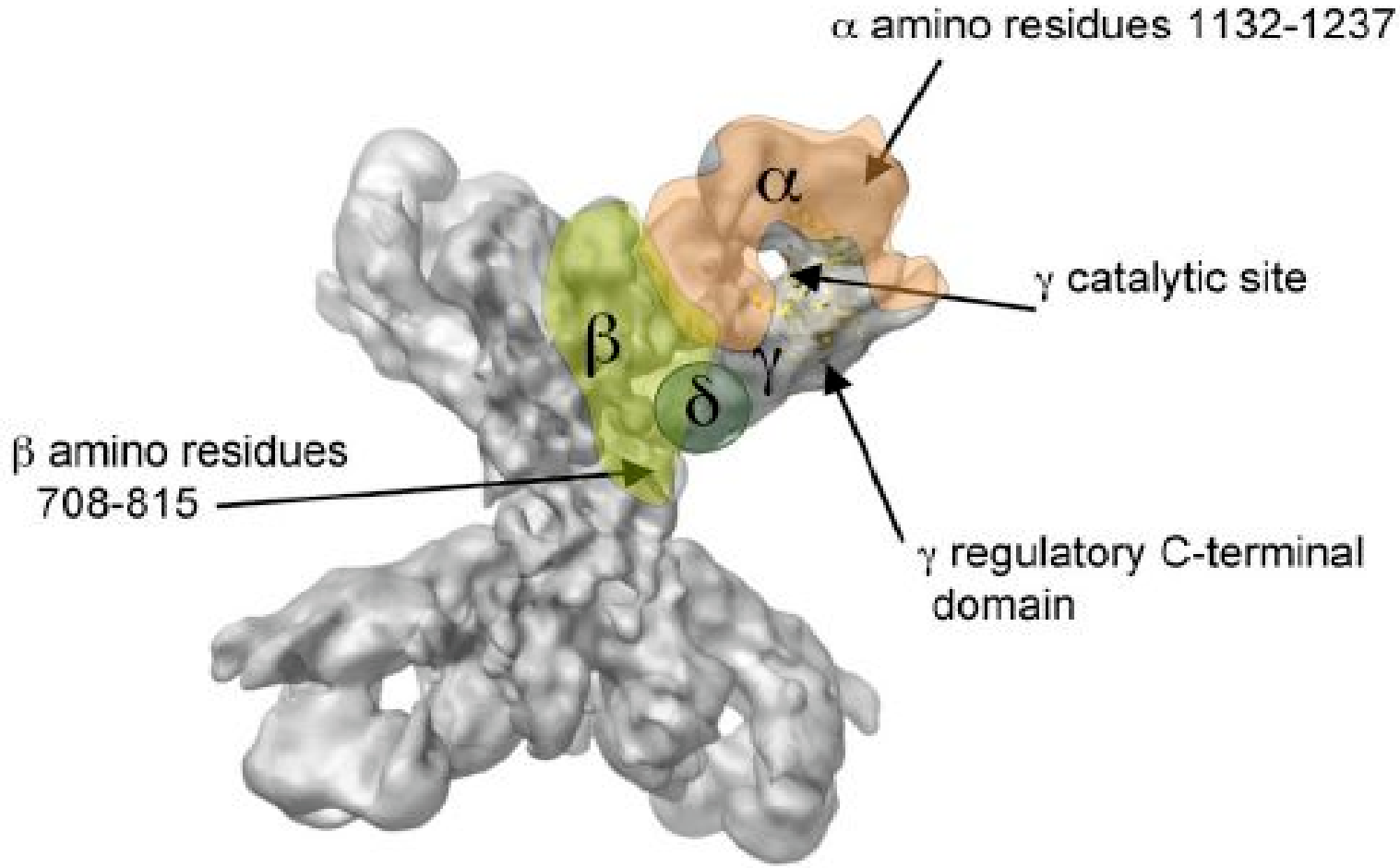


Phosphorylase kinase

- Four copies of each of α, β, γ, δ subunits



From: Vénien-Bryan ***et al*** 2009 Structure 17: 117–127.

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X-linked GSD Type IX

- Deficiency of liver α subunit (***PHKA2*** gene)
  - (Muscle subunit - ***PHKA1*** gene)
- Mutations in ***PHKA2*** gene
  - 33 exons, 92 kb on Chr Xp22.2-p22.1
  - wide range of mutations described

- X-Linked Glycogenosis type 1 (XLG1)
  - Reduced PHK activity in RBC and liver
- X-Linked Glycogenosis type 2 (XLG2)
  - Reduced PHK activity in liver only

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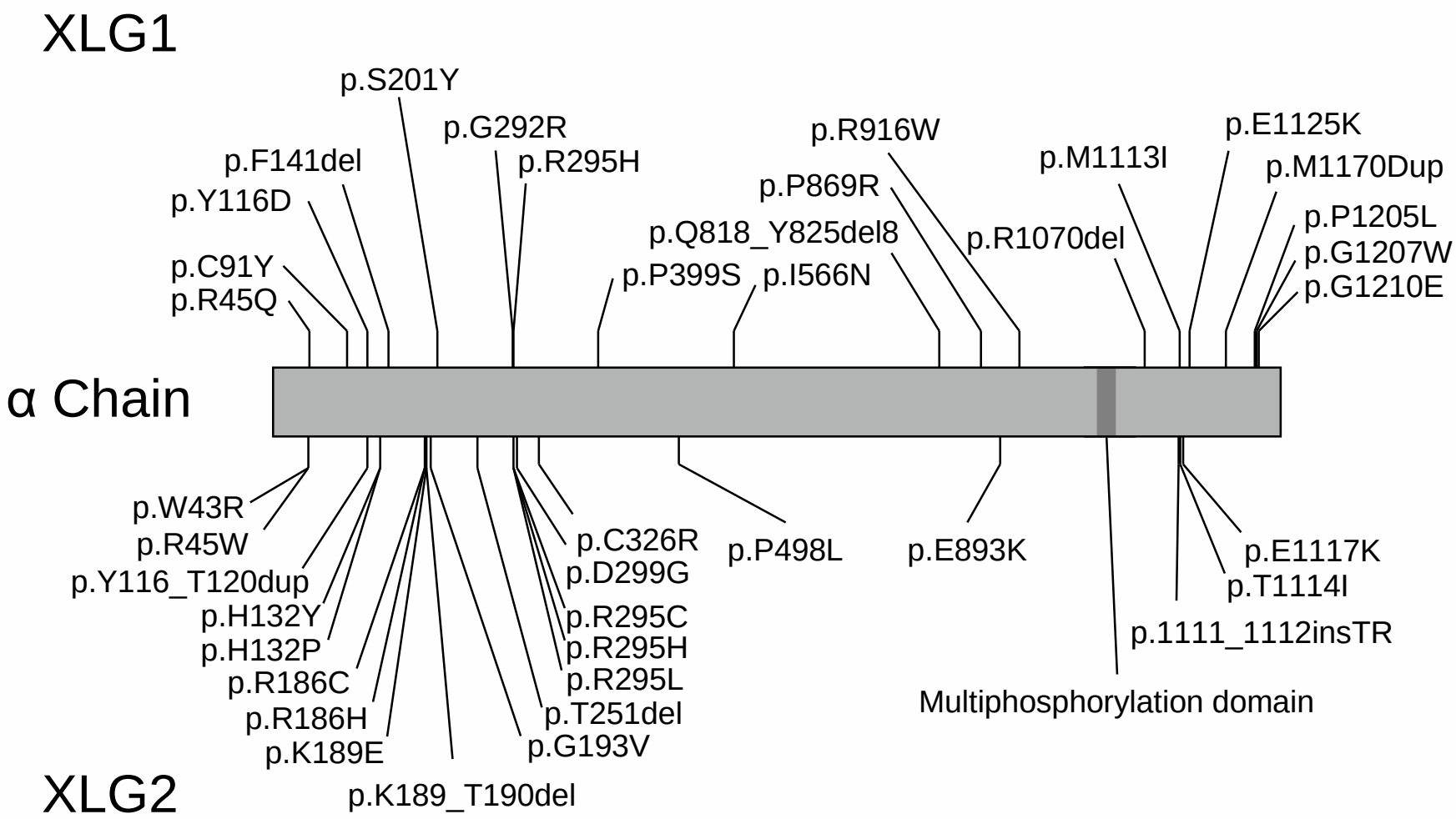

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Case 1

- Symptoms present at 1 year, diagnosed type VI at 7 years
  - Hepatomegaly
  - Normal fasting
  - Raised transaminases
  - Growth retardation
  - WBC Total GP: 1.4 (NR: 1.0-3.2 µmol Pi/mg alb/h)
  - WBC Activated GP: 0.3 (NR: 0.5-2.2 µmol Pi/mg alb/h)
  - RBC PHK: 21.8 (NR: 8.6-45 µmol Pi/min/g Hb)
- No mutation identified in ***PYGL*** gene – Not GSD type VI
  - Analysis of ***PHKA2***: p.Arg182Cys – X-linked GSD Type IX

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Analysis of GSD type VI

- 17 of 42 (40%) patients initially suspected of having GSD type VI shown to have GSD type IX
- 15 (35%) have mutations in ***PHKA2***
  - Majority previously described XLG2 mutations
- Two have ***PHKB*** mutations
  - c.1207+1G>T and p.Q657X
  - p.R429X and p.Q516X

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Autosomal GSD Type IX

- Autosomal recessive
- Rarer than X-linked form
- Deficiency of either β or liver γ subunit
- Mutations in ***PHKB*** gene
  - 33 exons, 238kb on Chr 16q12-q13
  - Majority result in null alleles
  - Mild symptoms compared to defects in ***PHKA2*** gene
- or ***PHKG2*** gene
  - 10 exons, 9kb on Chr 16 p12-p13
  - Missense mutations and null alleles
  - Severe symptoms compared to defects in ***PHKA2*** gene

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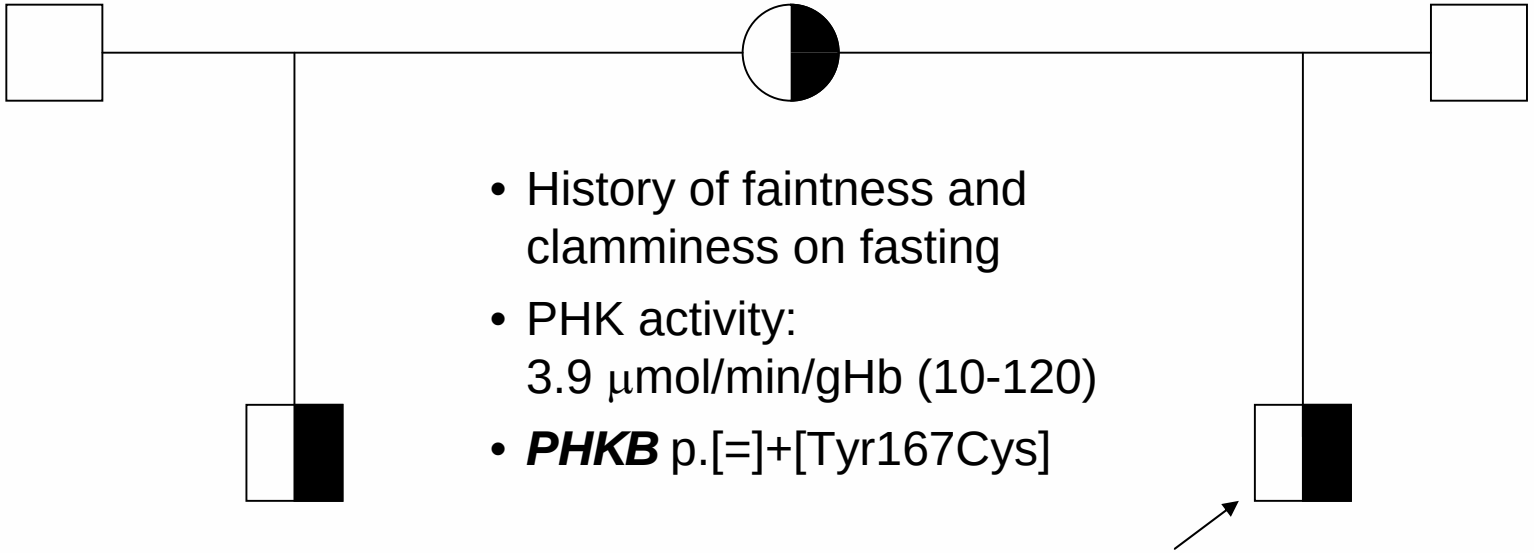
PHKB mutations

- ***PHKB*** missense mutations found in heterozygous isolation:
  - p.Gln657Lys
    (Burwinkel B ***et al*** 1997 Hum Genet 101: 170-174.)
  - p.Ala118Pro
    (Burwinkel B ***et al*** 2003 Eur J Hum Genet 11 : 516-526.)
  - p.Met185Ile
    (Beauchamp NJ ***et al*** 2007 Mol. Genet. Metab. 92: 88-99.)
  - p.Tyr167Cys
    (Unpublished)

Case 2



- History of faintness and clamminess on fasting
- PHK activity: 3.9 μmol/min/gHb (10-120)
- ***PHKB*** p.[=]+[Tyr167Cys]

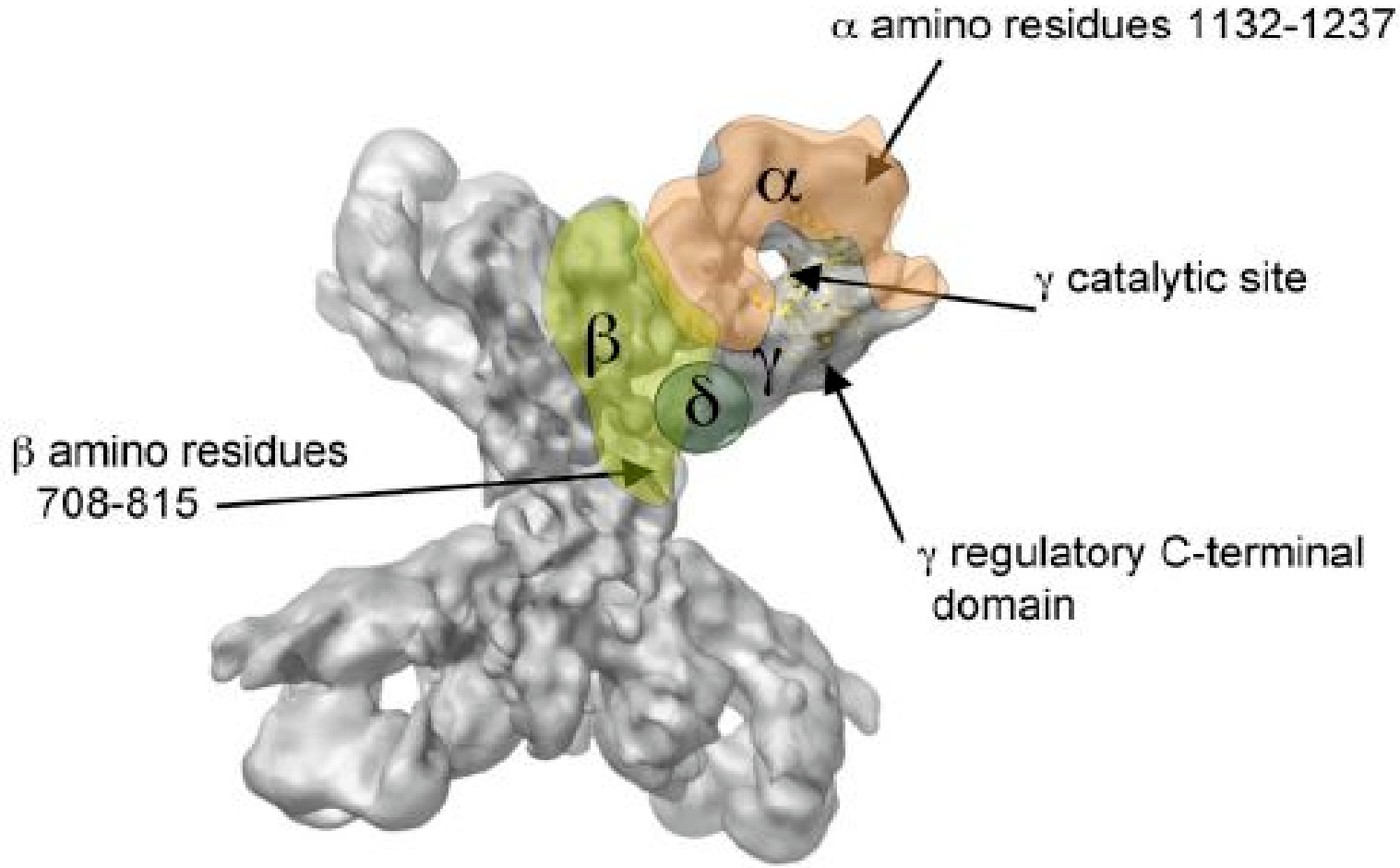
- 6 year old ADHD
- Sleepy and sweaty if fasted, better with sugary drinks
- PHK activity: 0 μmol/min/gHb (10-120)
- ***PHKB*** p.[=]+[Tyr167Cys]

- 2 year old
- Recurrent hypoglycaemia with seizures
- PHK activity: 0.8 μmol/min/gHb (10-120)
- ***PHKB*** p.[=]+[Tyr167Cys]

Conclusion: Dominant negative mutations in PHKB result in phosphorylase kinase deficiency.

Phosphorylase kinase

- Four copies of each of α, β, γ, δ subunits



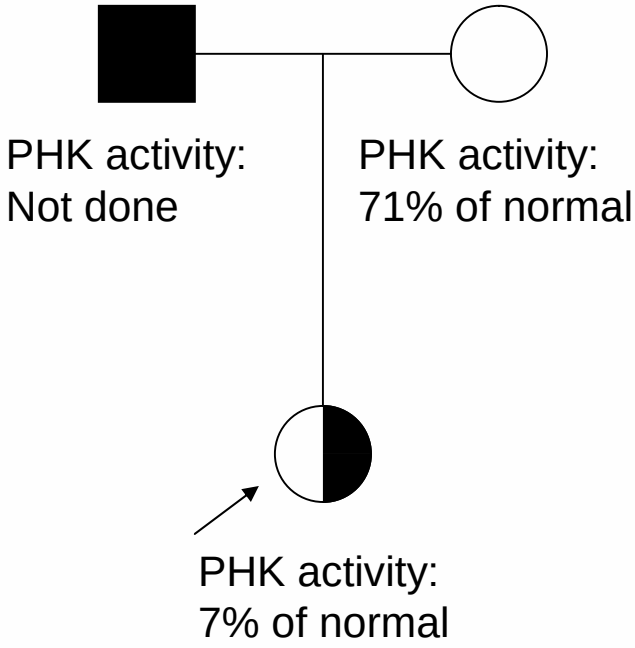
From: Vénien-Bryan ***et al*** 2009 Structure 17: 117–127.

Case 3

Female X-Linked GSD type IX

- Index case is heterozygous for ***PHKA2*** p.Pro1205Leu

- Possibilities
  - Additional defects in ***PHKB*** or ***PHKG2*** genes
  - Additional ***PHKA2*** gene mutation
  - Skewed X-inactivation
  - Monosomy X (Turner syndrome)



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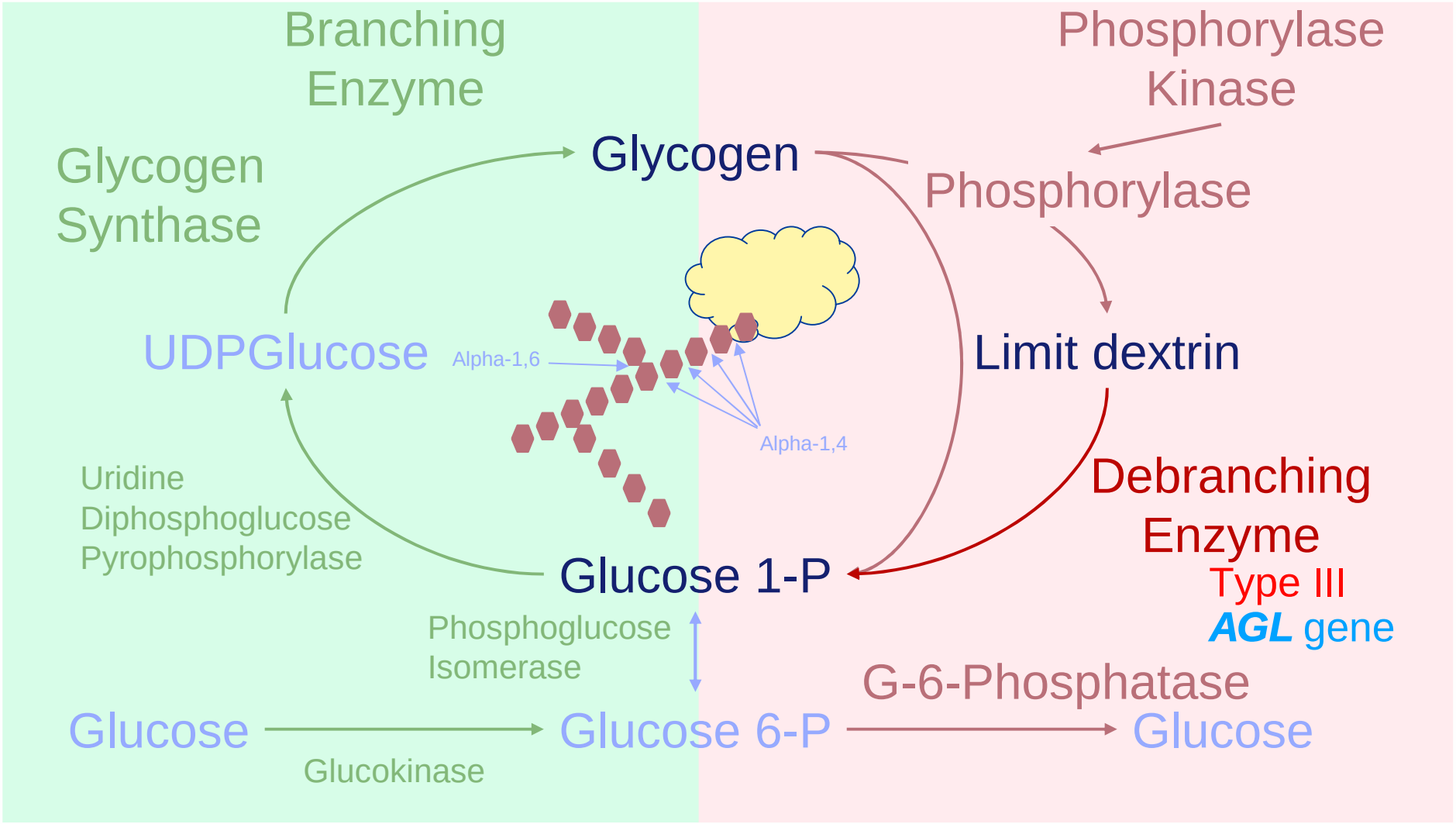

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GSD Type III

- Also known as Cori or Forbes Disease
- Deficiency of glycogen debrancher enzyme
- Autosomal recessive
- Four subtypes:
  - Type IIIa (~85% of patients)
    - Enzyme deficient in both liver and muscle
  - Type IIIb (~15% of patients)
    - Enzyme deficient in liver
  - Type IIIc
    - Loss of glucosidase activity
  - Type IIId
    - Loss of transferase activity

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GSD Type III

- ***AGL*** gene
  - 35 exons, 85 kb on Chr 1p21
- Type IIIa
  - Majority (65%) of mutations are nonsense, frameshift or splice site mutations
  - Common changes
    - p.Arg408X, c.4260-12A>G, p.Arg864X
  - Rare changes
    - 118 mutations reported
- Type IIIb
  - Exon 3 nonsense mutations
    - c.16C>T, p.Gln6X,
    - c.18_19delGA, p.Gln6HisfsX20
    - c.22C>T, p.Arg8X

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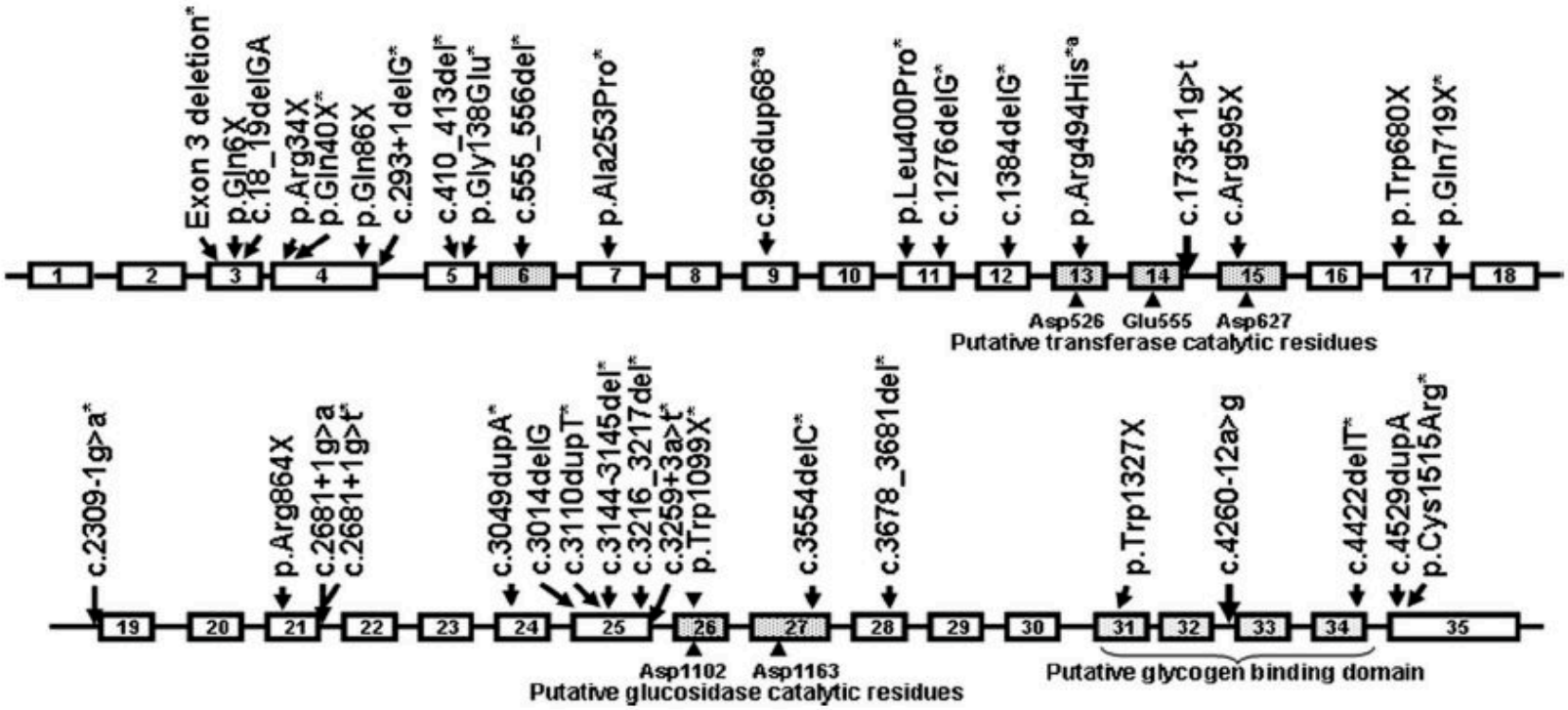
GSD Type III – ***AGL*** mutations



From: JL Goldstein ***et al*** 2010. ***Genet Med*** 12:424–430

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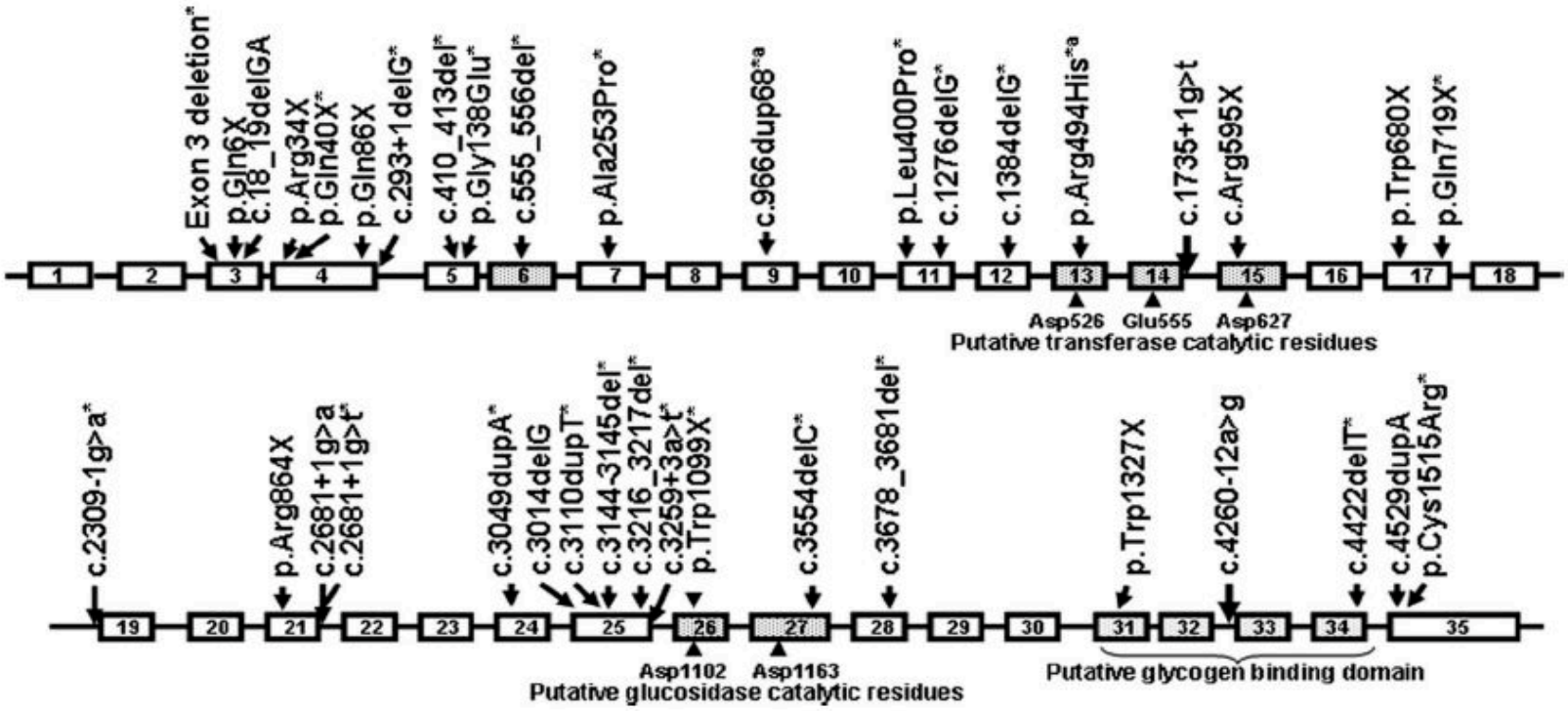
GSD Type III – ***AGL*** mutations



Deletion of exons 29-31

From: JL Goldstein ***et al*** 2010. ***Genet Med*** 12:424–430

Cases 4 and 5

- Patient, aged 5 years
- Permanent hepatomegaly
- Fasting <3 hours
- Permanent CK elevation
- Cardiomyopathy
- Fatigue not observed
- Leuk Debrancher: 5.7
  Normal range: 26.8-105 nmol glu/mg protein/hour
- RBC Glycogen: 565
  Normal range: 5.7-135 μg/g Hb
- p.Arg408X homozygote

- Patient , aged 20 years
- Hepatomegaly till 16 years
- Normal feeding
- Normal CK
- No Cardiomyopathy
- Periodic fatigue
- Leuk Debrancher: 0.69
  Normal range: 26.8-105 nmol glu/mg protein/hour
- RBC Glycogen: 726
  Normal range: 5.7-135 μg/g Hb
- p.Arg8X and c.4260-12A>G

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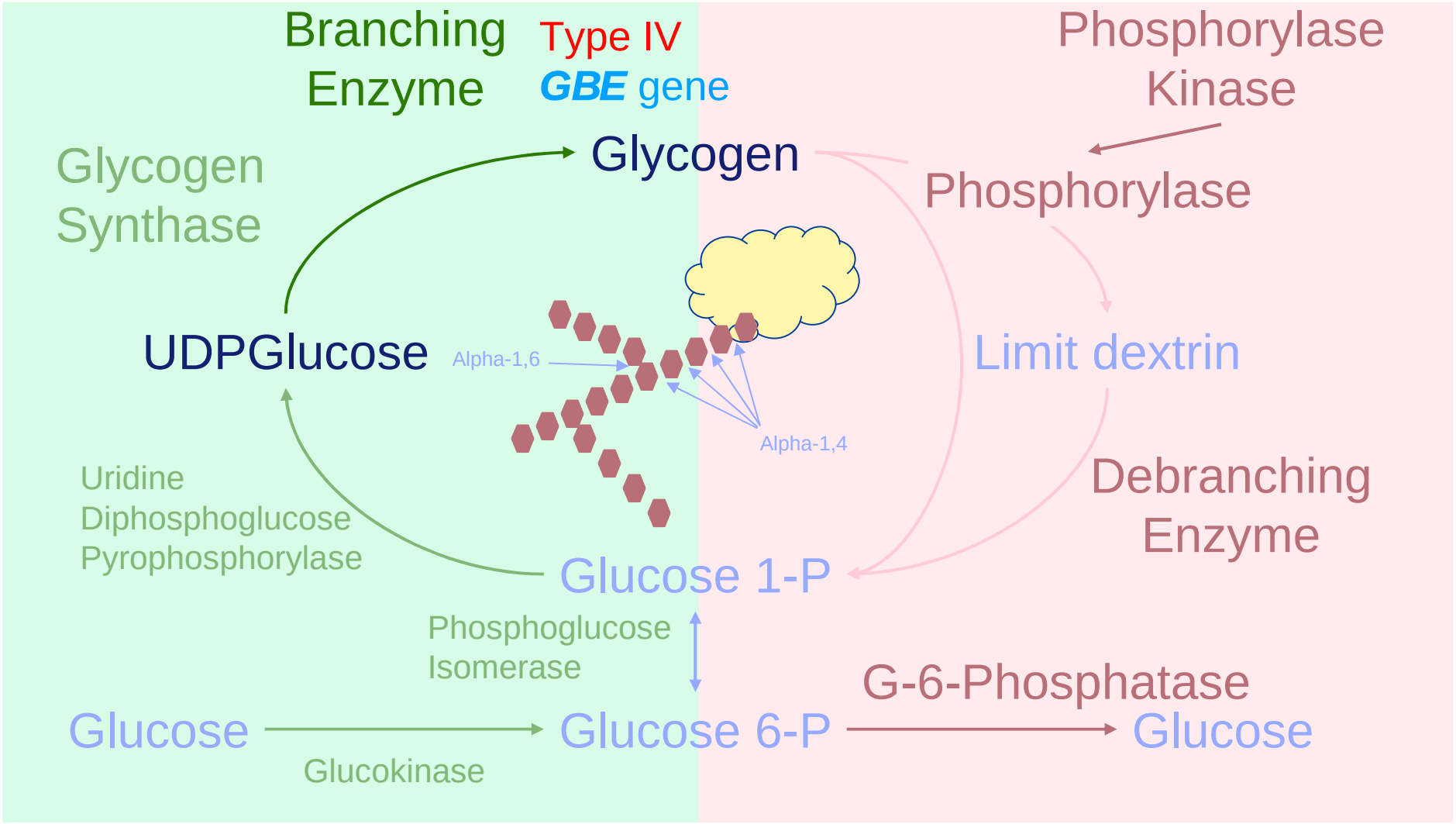

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GSD Type IV

- Also known as Anderson Disease
- Deficiency of glycogen branching enzyme
- Autosomal recessive
  - ***GBE1*** gene
  - 16 exons, 262 kb on Chr 3p14
- Rare
- 20 Patients reported
  - 37 unique mutations
  - Some phenotype/genotype correlation
    - Wide range of phenotypes
    - Congenital presentation to polyglucosan body disease
- Genetic analysis allows prenatal diagnosis

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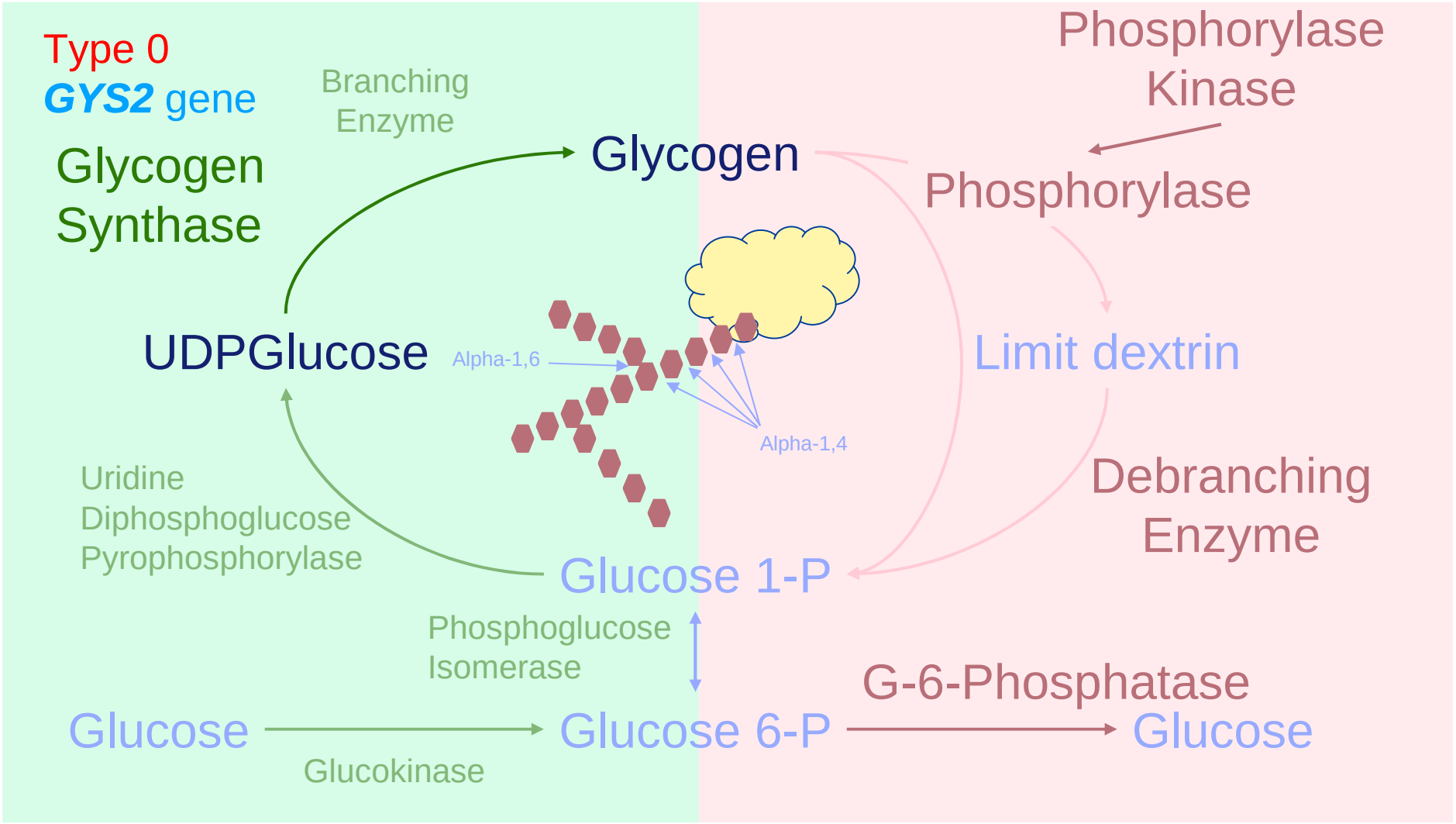

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GSD Type 0

- Deficiency of glycogen synthase
- Clinical symptoms
  - Ketotic hypoglycaemia
  - Post-prandial hyperglycaemia and hyperlactataemia
  - Low activity in liver biopsy
- Autosomal recessive
  - ***GYS2*** gene
    - 16 exons, 69kb on Chr 12p12.2
    - 18 mutations described
- Rare - 3 of 59 (6%) patients with suspected GSD type 0 have mutations
- Molecular analysis avoids biopsy

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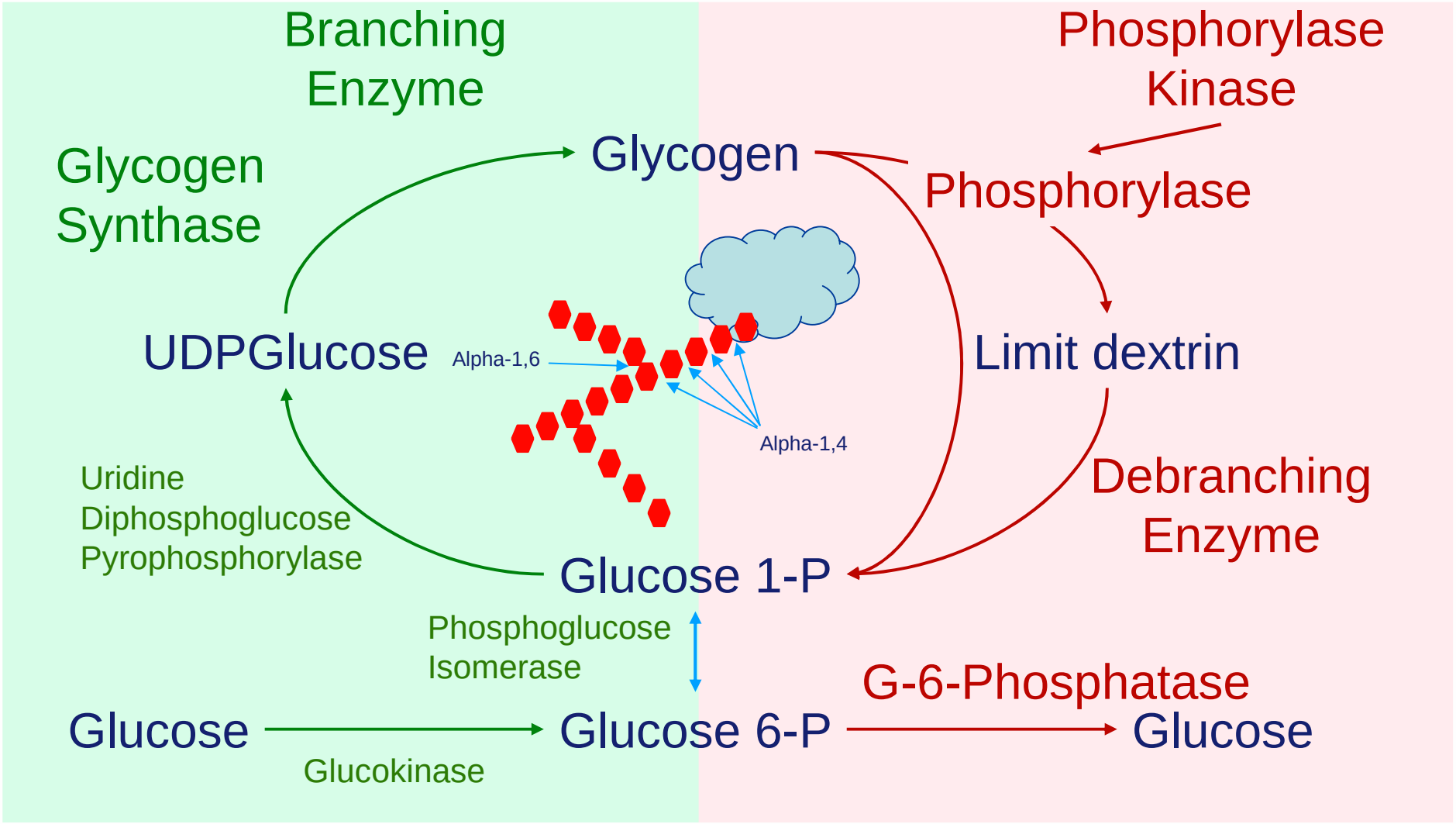
Glycogen Synthesis and Breakdown



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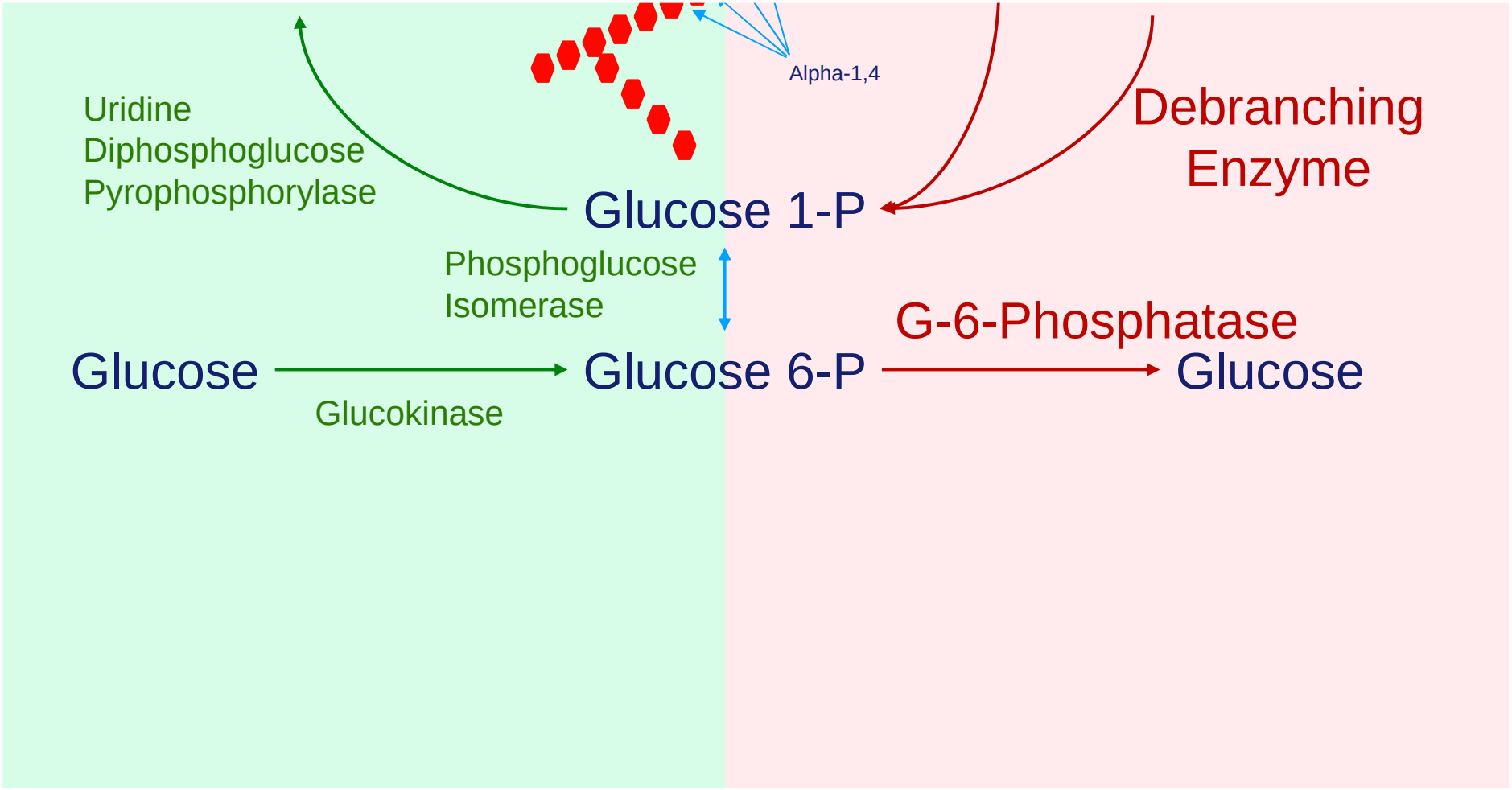
Glycogen Synthesis and Breakdown



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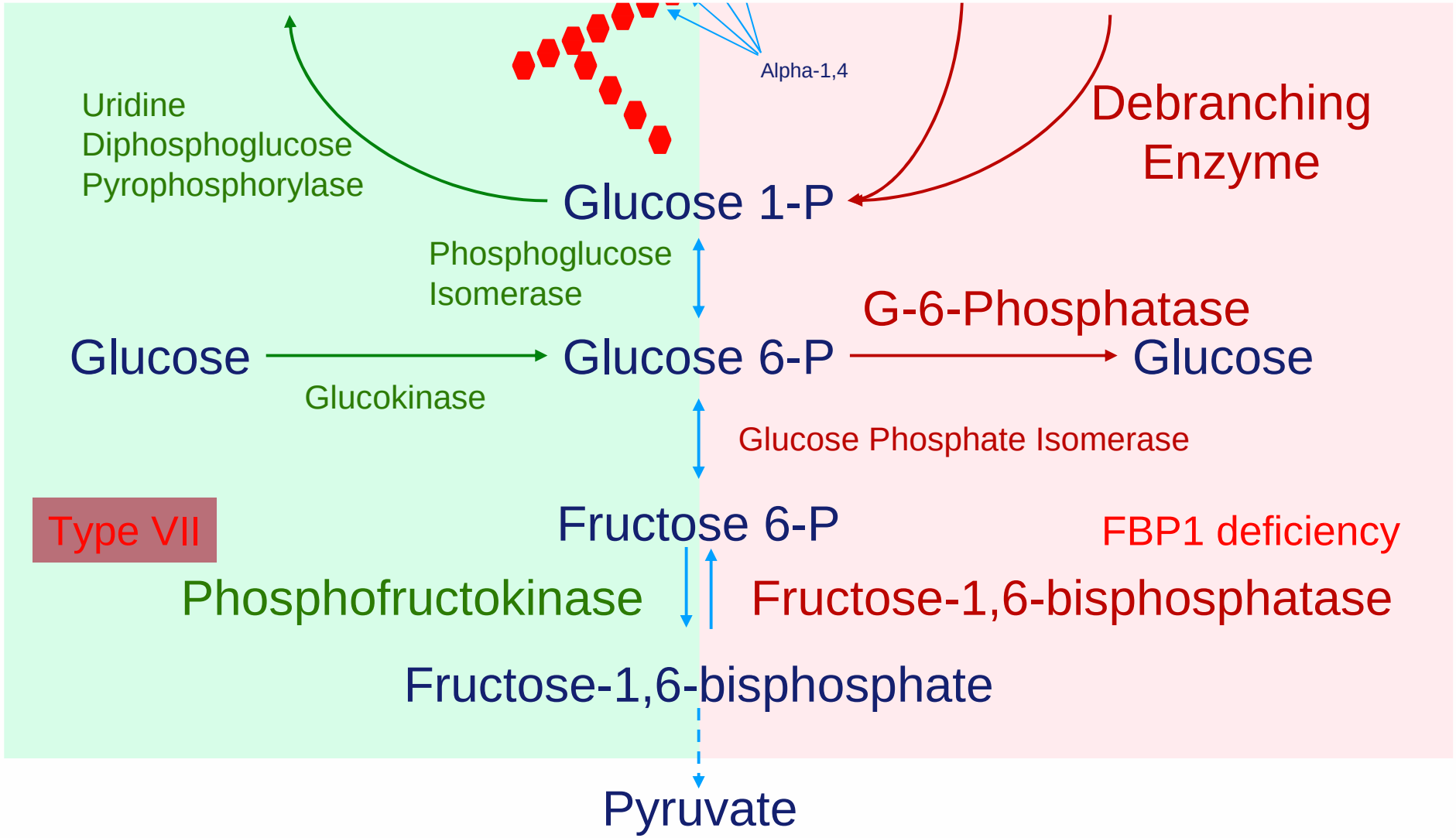

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GSD Type VII

- Also known as Tarui disease
- First identified in 1965
- Deficiency of muscle phosphofructokinase
  - Three isoforms muscle, liver and platelet types
- Autosomal recessive inheritance
  - ***PFKM*** gene
    - Located on 12q13
    - 24 exons covering 30kb (differential splicing of 5' region)
- Rare - 15 mutations reported -
  - No genotype/phenotype correlation described
- Genetic analysis
  - ? avoid muscle biopsy and allows prenatal diagnosis

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GSD Type II

- Also known as Pompe Disease,
  acid α-glucosidase deficiency or acid maltase deficiency
- Autosomal recessive
- Lysomal storage disease - accumulation of glycogen in all tissues
- 1 in 40,000 live births
- GAA gene on Chr 17q25.2-q25.3
  - Common mutations:
    - c.-45T>G - Mild phenotype
    - c.525del - severe phenotype
  - >150 listed on mutation database at: www.pompecenter.nl
- Part of Newborn Screening in USA

Analysis of liver type GSD Patients in Sheffield

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Type	0	Ia	Ib	III	IV	VI	IXauto	IXx	Totals
Total Samples	59	45	26	81	32	42	38	99	422
Confirmed Diagnosis	3 (6%)	27 (60%)	15 (58%)	56 (69%)	7 (22%)	12 (29%)	14 (37%)	61 (62%)	195 (46%)
Investigated Further	5	10	5	10	1	23	9	29	92
Diagnosis Changed	0 (0%)	4 (9%)	4 (15%)	3 (4%)	1 (5%)	18 (43%)	2 (5%)	12 (12%)	44 (10%)
0								1	1
Ia			2						2
Ib		4							4
III						1			1
VI				1			2	2	5
IX X-linked			2	2		15			19
IX Autosomal					1 PHKG2	2 PHKB		6 PHKG2 3 PHKB	7 PHKG2 5 PHKB

Analysis of muscle type GSD Patients in Sheffield

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Type	II	V	VII	IXx Muscle	Total
Total Samples	37	67	9	4	117
Confirmed Diagnosis	25 (68%)	28 (42%)	1 (11%)	0 (0%)	54 (46%)
Investigated Further	1	3	1	3	8
Diagnosis Changed	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Grand Total
539
249 (46%)
102
44 (8%)

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Conclusions

- Genetics analysis can:
  - Provide a definitive diagnosis
  - Avoids liver or muscle biopsy
  - Allow carrier testing and prenatal diagnosis
  - Change diagnosis and inheritance patterns
  - In some cases indicate prognosis