OXFORD Molecular Diagnostics Centre

Oxford University Hospitals NHS NHS Trust

Epsilon-gamma-delta-beta thalassaemia:- a rare cause of severe anaemia in newborns

Dr Shirley Henderson

Red Cell SIG

24th September

BBTS Annual Conference

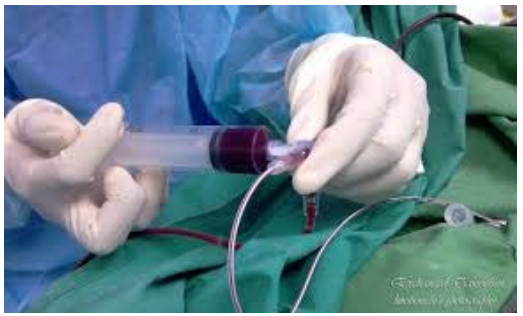
Harrogate 2014

Oxford Biomedical Research Centre
Enabling translational research through partnership

NHS National Institute for Health Research

Haemolytic Disease of the New born

- Rh D allo-immunisation
- ABO incompatiblity
- Other RBC allo-immunisation
- RBC membrane disorders:- HS, HPP
- RBC enzyme defects:-G6PD, PK, others
- Haemoglobinopathies:- alpha thalassaemia major, unstable structural variants, εγδβ thalassaemia

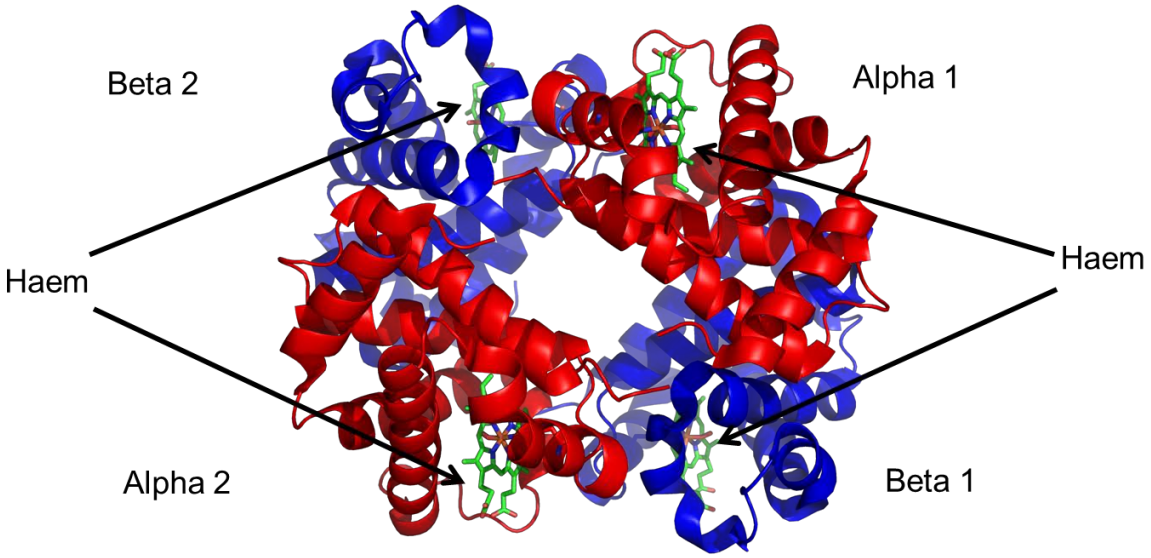


Normal Haemoglobins

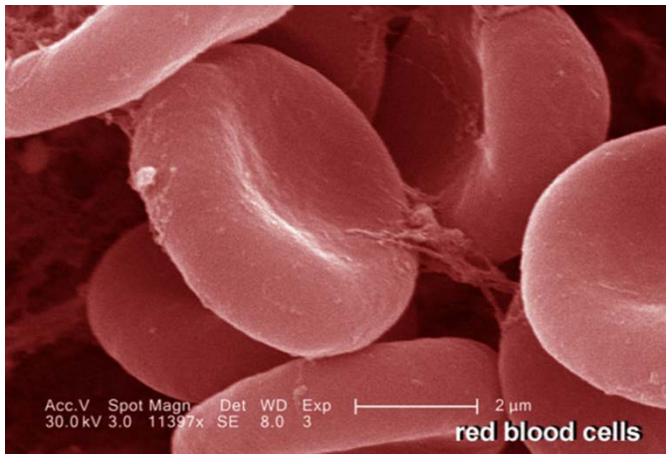
Hb A ($\alpha_2 \beta_2$) 95% of total

Hb A_2 ($\alpha_2 \delta_2$) <3.2% of total

Hb F ($\alpha_2 \gamma_2$) Predominant Hb in babies <1.0% in adults

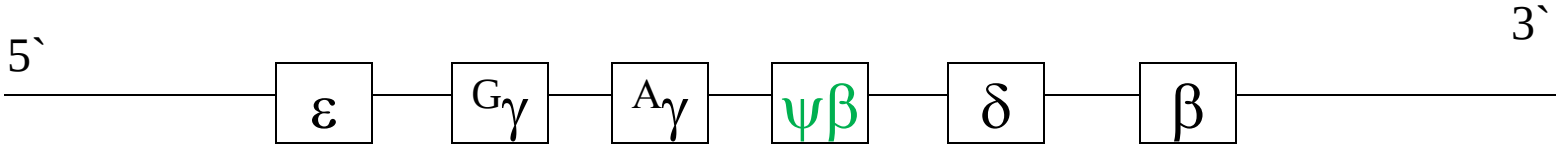


Haemoglobin A molecule

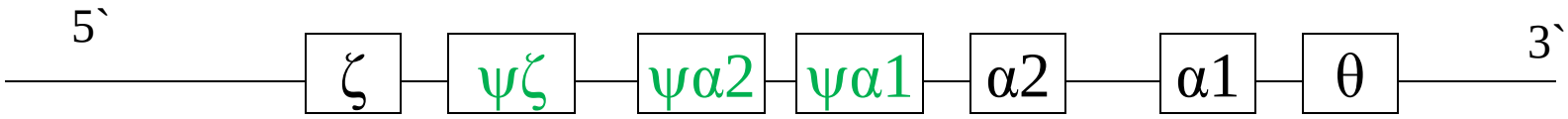


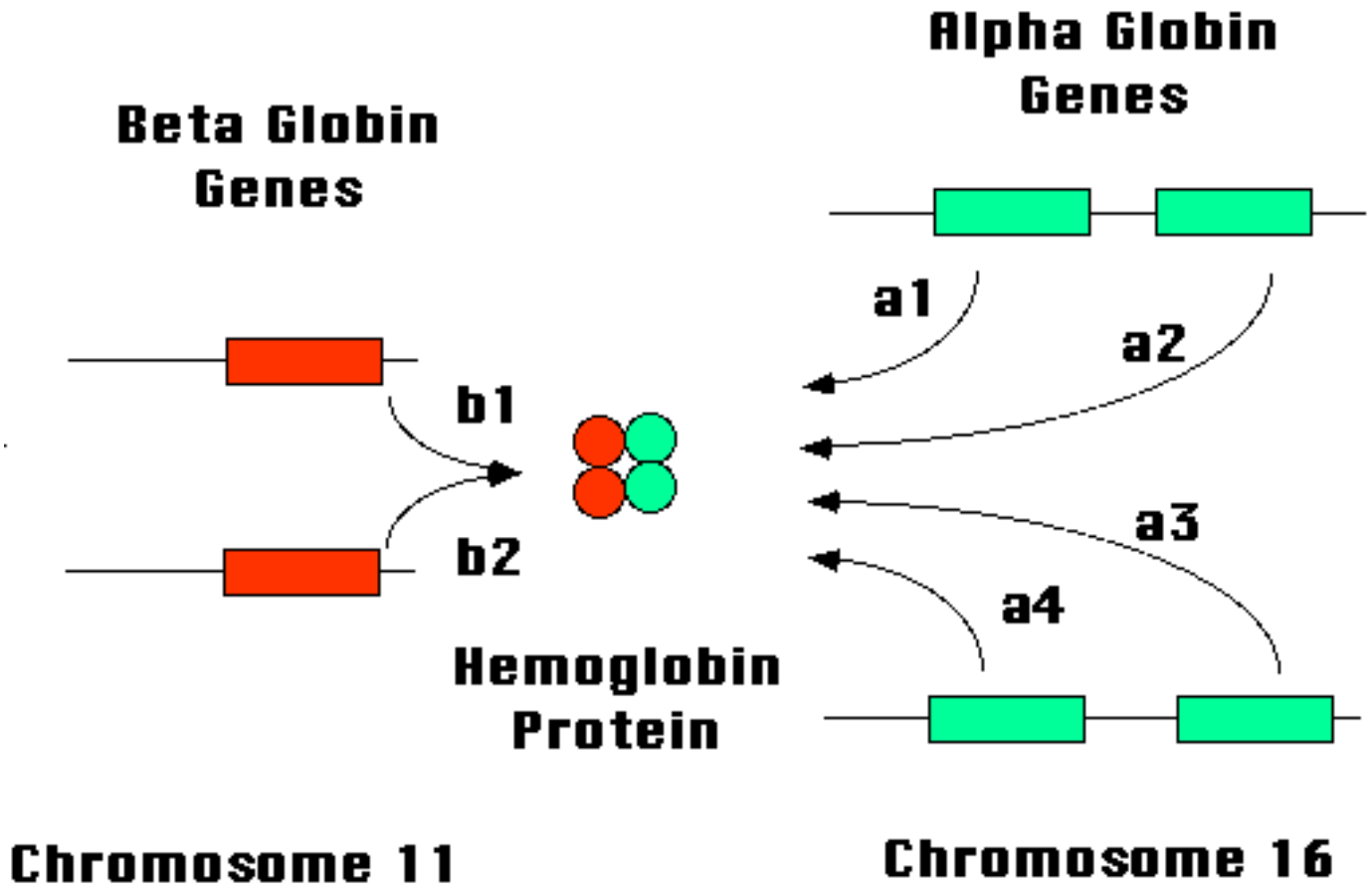
Genetics of haemoglobin synthesis

Chromosome 11



Chromosome 16



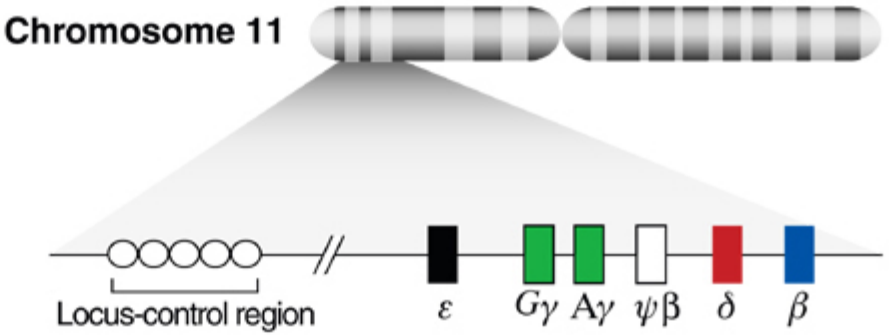

Alpha Globin Genes
Beta Globin Genes
a1
a2
a3
a4
b1
b2
Hemoglobin Protein
Chromosome 11
Chromosome 16

Haemoglobin switching

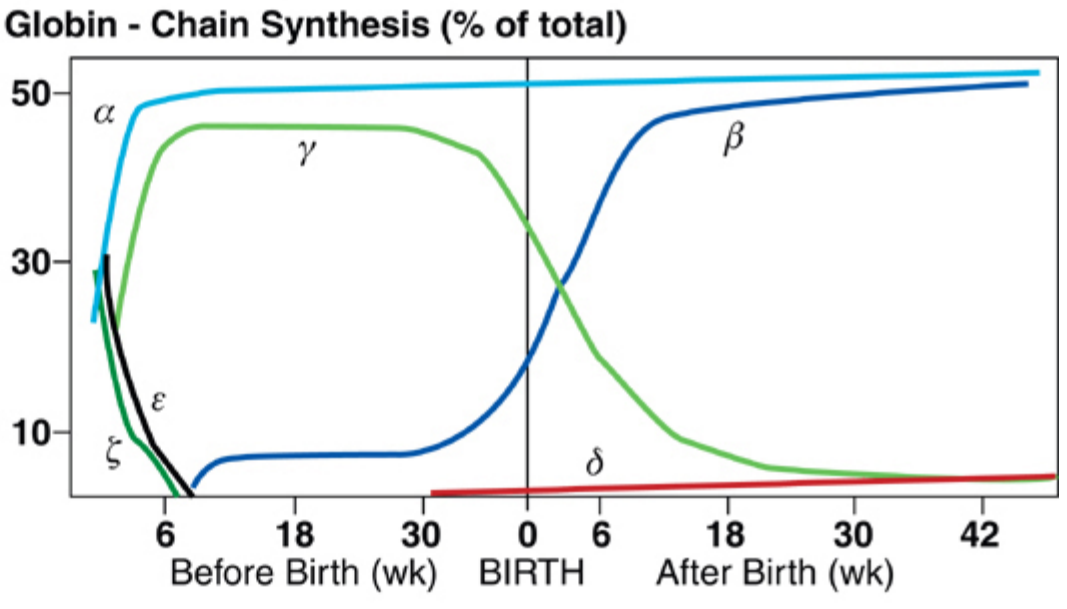
HEMOGLOBIN:

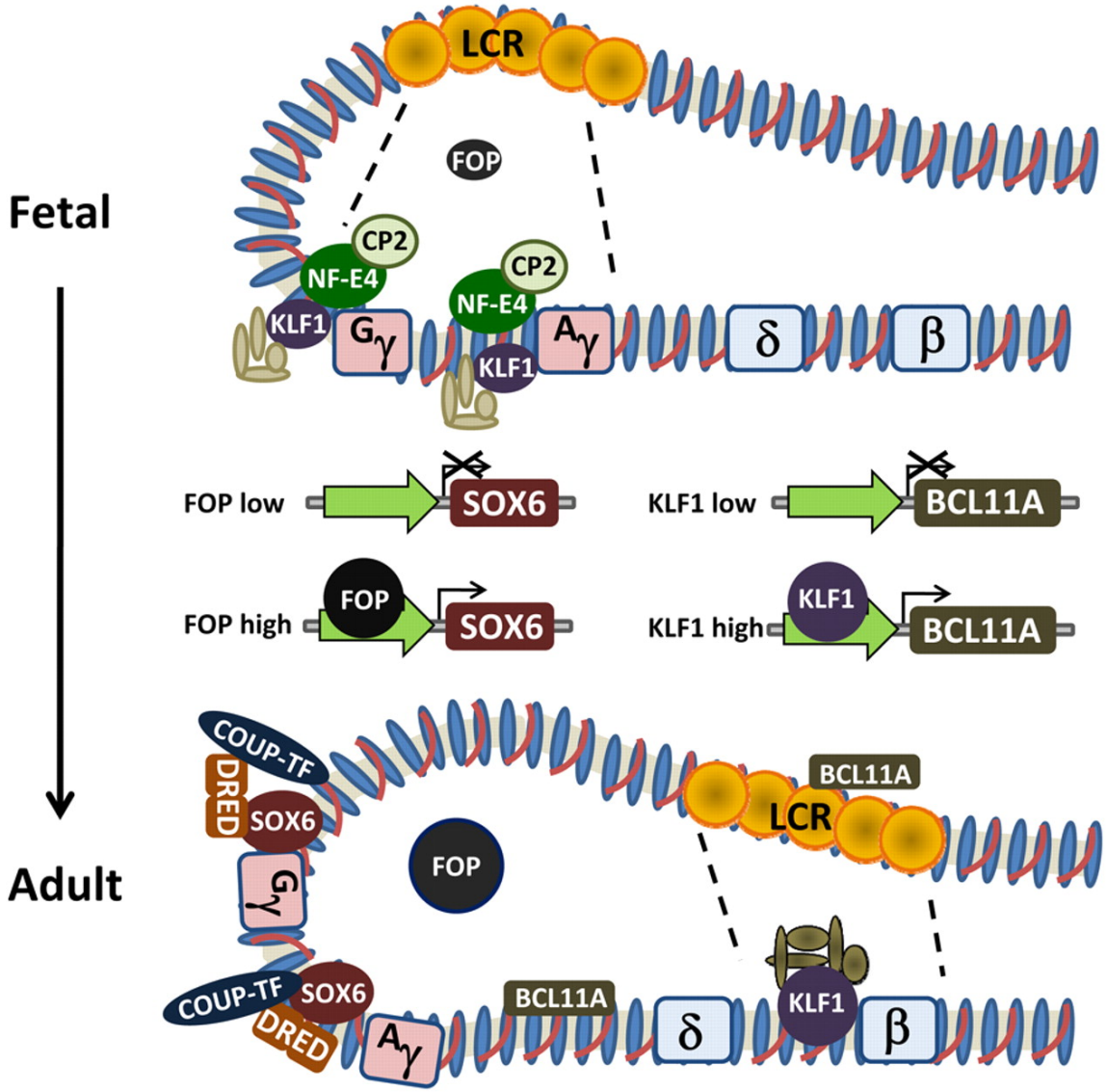
-genes
-Chain Synthesis

A



B




Fetal
LCR
FOP
CP2
NF-E4
KLF1
Gγ
Aγ
δ
β
FOP low
SOX6
KLF1 low
BCL11A
FOP high
FOP
SOX6
KLF1 high
KLF1
BCL11A
Adult
COUP-TF
DRED
SOX6
BCL11A
LCR
FOP
Gγ
KLF1
Aγ

The Haemoglobinopathies

1) Structural variants

mutation changes an amino acid in globin chain

2) Thalassaemias

α-thalassaemia – mostly deletions

- α^{+}-thalassaemia
- α^{0}-thalassaemia

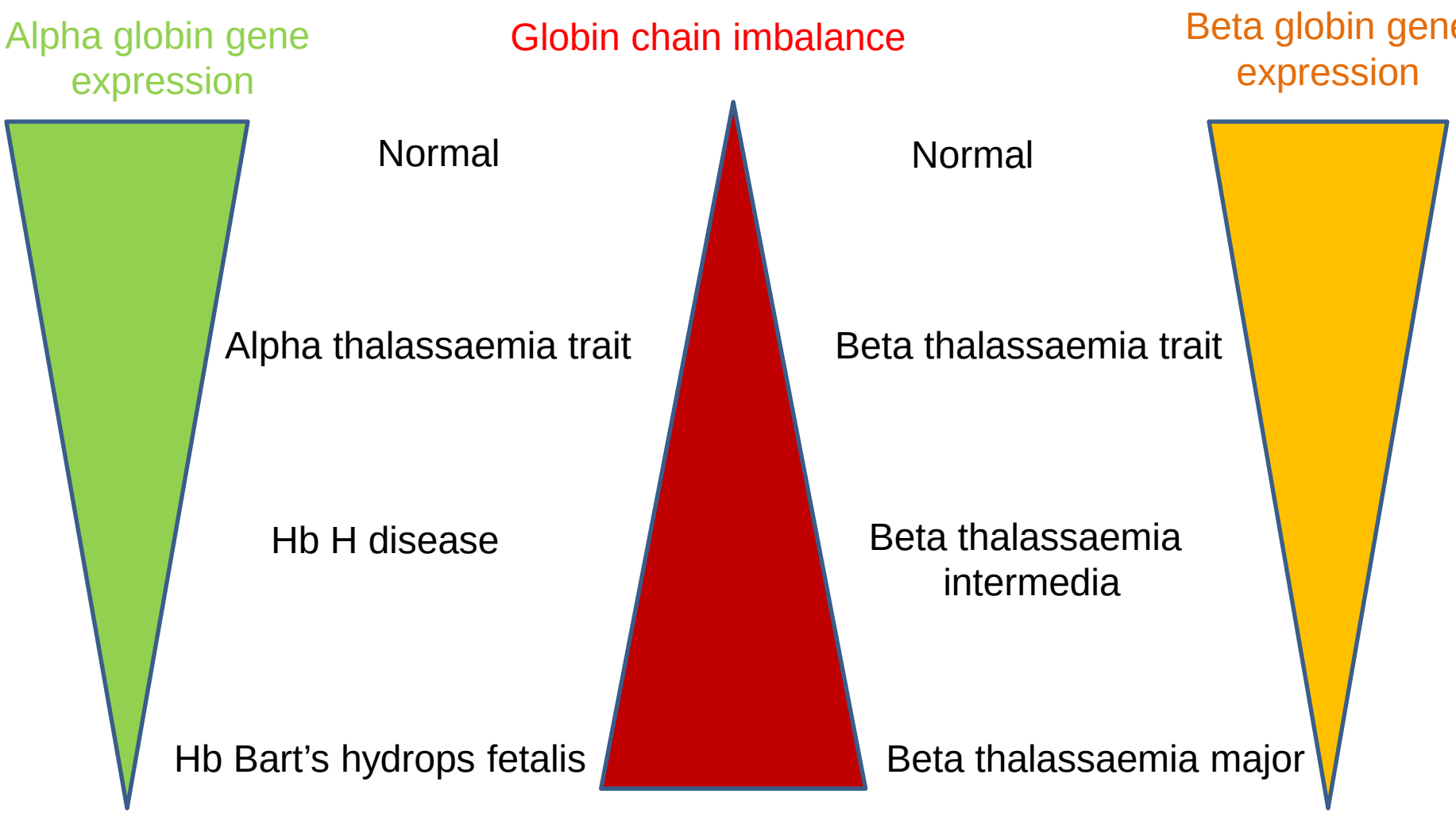
β--thalassaemia – mostly point mutations

- β^{+}-thalassaemia
- β^{0}-thalassaemia

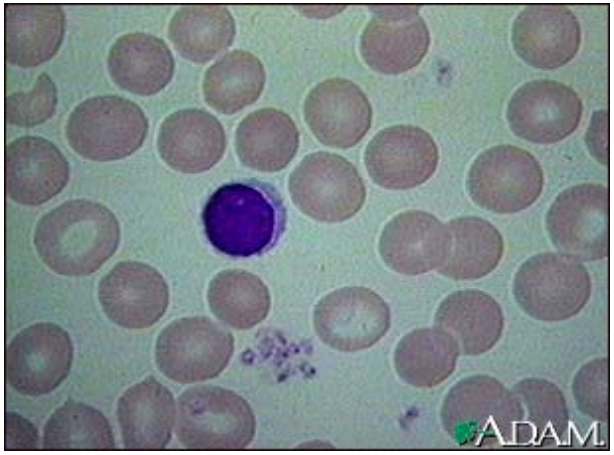
Pathophysiology of thalassaemia

Clinical syndromes of alpha thalassaemia

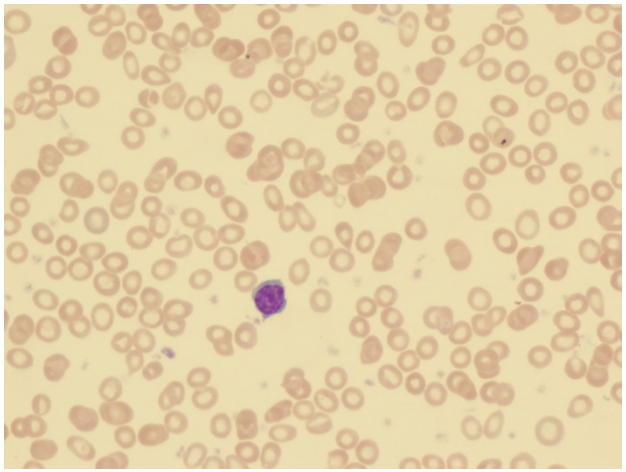
Clinical syndromes of beta thalassaemia



Thalassaemia trait



Normal red blood cells



Microcytic/hypochromic red blood cells

NR	α-^{0} thalassaemia trait	β-^{0} thalassaemia trait
Hb 12-17 g/dl	11.0	12.6
RBC 3.8-5.5X10^{12}/l	6.8	6.3
MCV 83-101 fl	65	67
MCH 27-32pg	20.7	22.7
Hb A2 2.0-3.3%	2.3	6.5
Hb F <1.0%	0.5	1.9

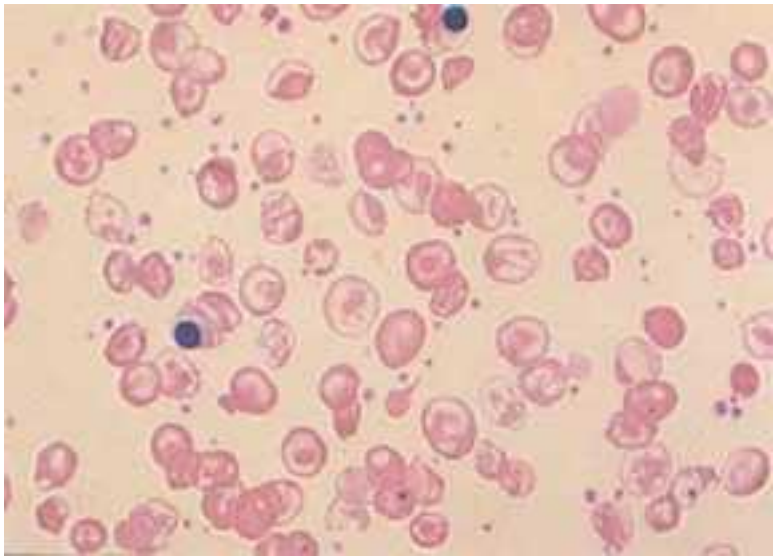
β-thalassaemia major

Red Cell indices

Hb 3-7g/dl

MCV 50-60fl

MCH 12-18pg



HPLC/electrophoresis: Only Hb F and Hb A2 present

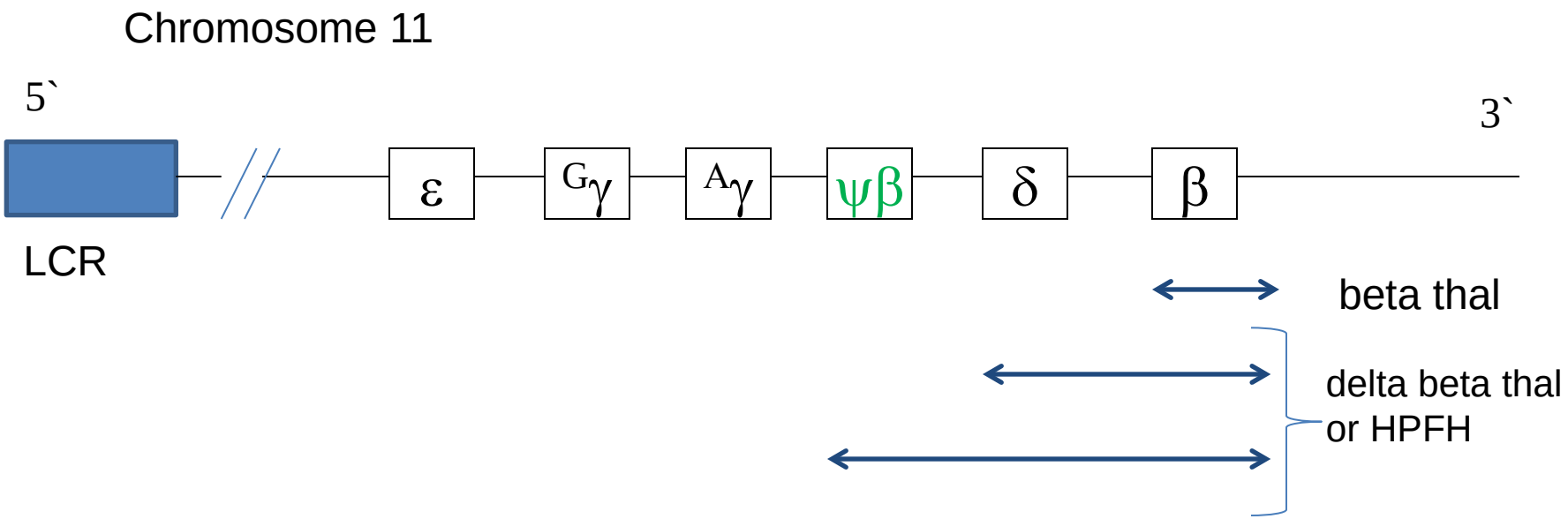
HPFH & Delta beta thalassaemia

- Complete switching doesn't occur, gamma globin production remains at significant levels.

- HPFH:- Elevated levels of fetal haemoglobin with normal red cell indices
  - Point mutations e.g gamma gene promoter mutations
  - Deletion mutations

- Delta-beta thalassaemia:- Elevated levels of fetal haemoglobin with reduced red cell indices
  - Deletion mutations

Deletional beta thalassaemia/HPFH



HPFH deletions:- lack of beta globin completely compensated for by increased gamma production – clinically benign

Delta-Beta thalassaemia deletions:- some but not complete compensation for lack beta globin production – beta thal phenotype with low/normal Hb A2

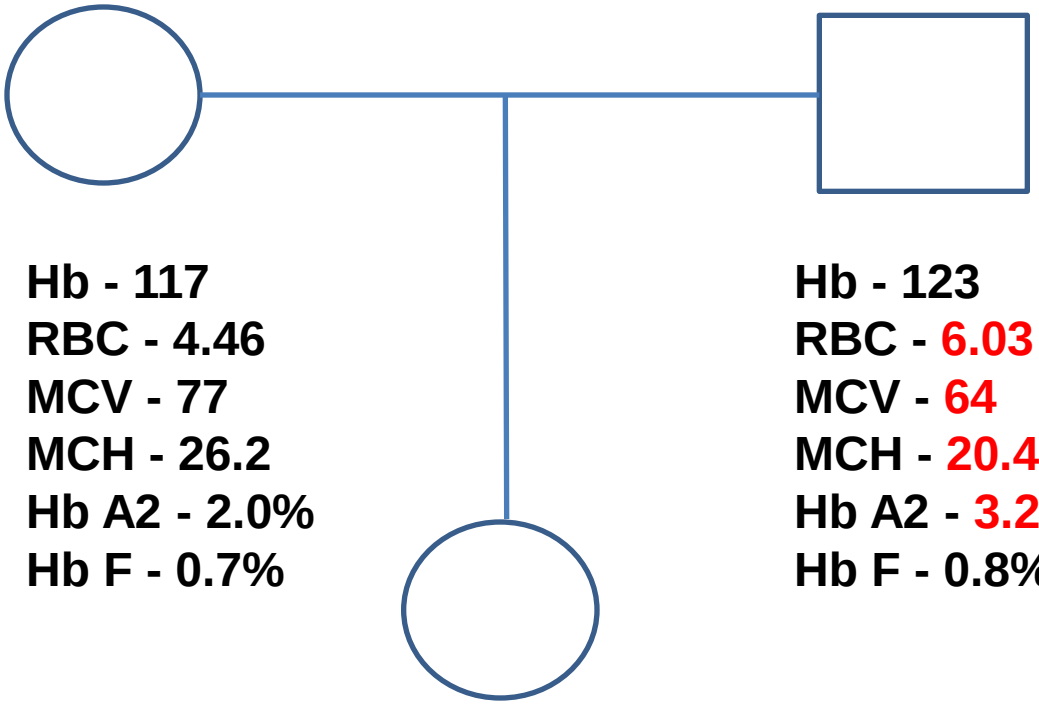
Case study

- 4 day old baby, family origins Pakistan
- ? Hydropic, Hepatosplenomegally, severe anaemia requiring transfusion
- Morphology:- erythroblasts
- Retics :- 8.3%
- ? Non-deletional alpha thalassaemia ? Poly A mutations

Pre transfusion results

HB – 64g, RCC – 2.41, MCV – 92, MCH – 26.6, Hb F – 67.5%

Baby and parents



Hb - 117
RBC - 4.46
MCV - 77
MCH - 26.2
Hb A2 - 2.0%
Hb F - 0.7%

Hb - 123
RBC - 6.03
MCV - 64
MCH - 20.4
Hb A2 - 3.2%
Hb F - 0.8%

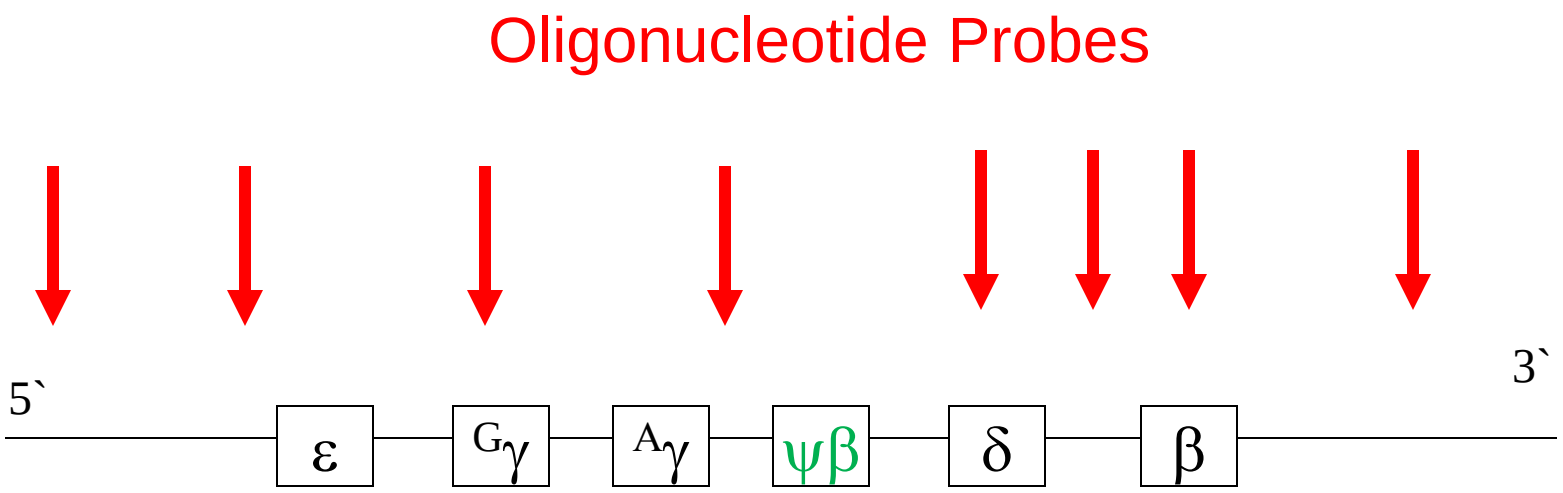
HB - 64
RCC - 2.41
MCV - 92
MCH - 26.6,
Hb F - 67.5

All 3:-

- Normal alpha and beta globin gene sequencing
- Negative results for alpha thalassaemia deletion mutations

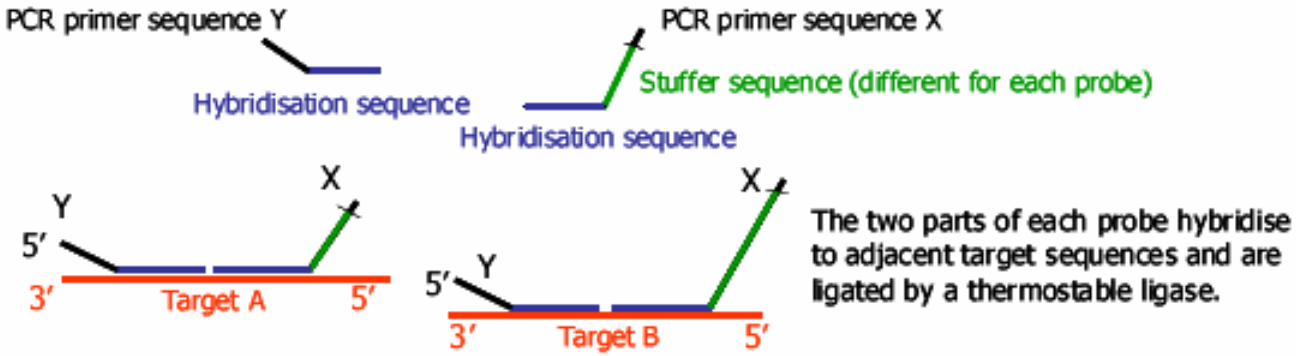
MLPA

(Multiple Ligase dependent Probe Amplification)



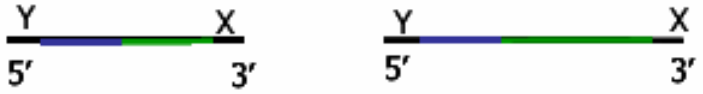
MLPA: Multiplex Ligation-dependent Probe Amplification

- Denatured genomic DNA is hybridized with a mixture of ~ 40 probes.
- Each MLPA probe consists of two oligonucleotides, one synthetic and one M13-derived.



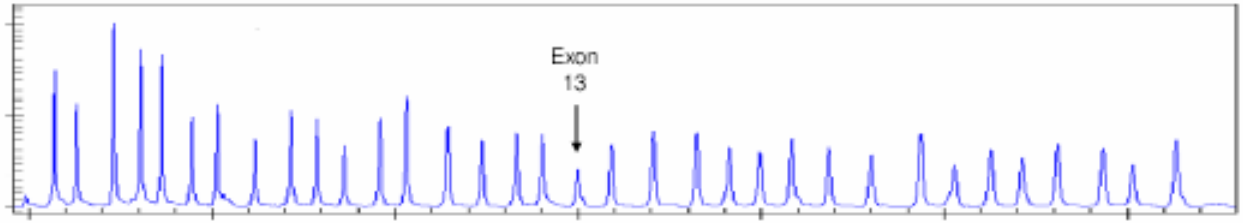
The two parts of each probe hybridise to adjacent target sequences and are ligated by a thermostable ligase.

All probe ligation products are amplified by PCR using only one primer pair.



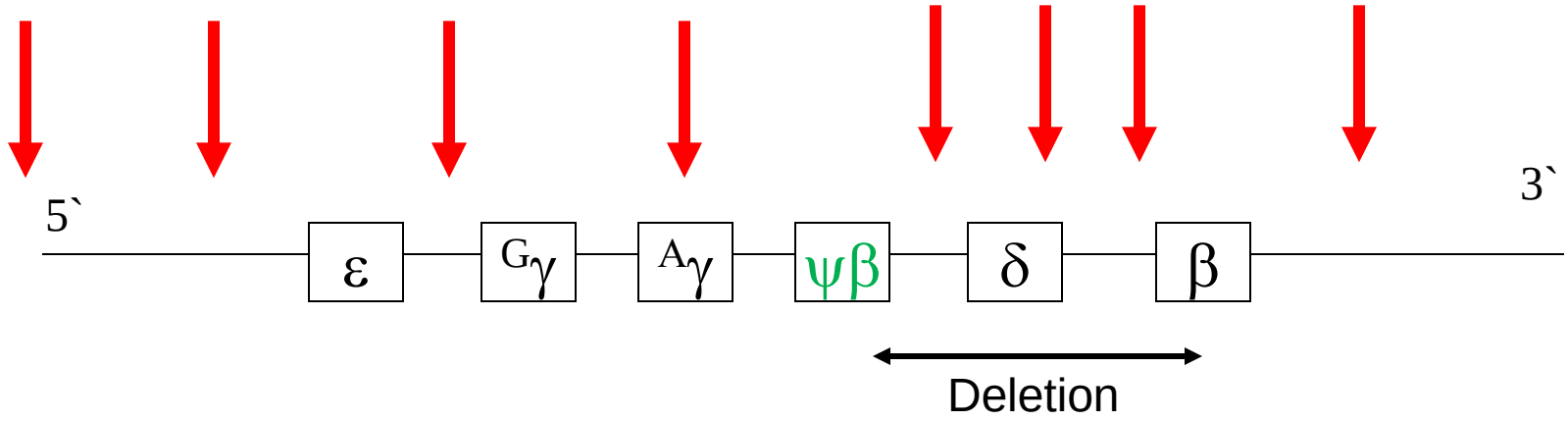
The amplification product of each probe has a unique length (130-480 bp).

Amplification products are separated by electrophoresis. Relative amounts of probe amplification products, as compared to a control DNA sample, reflect the relative copy number of target sequences.

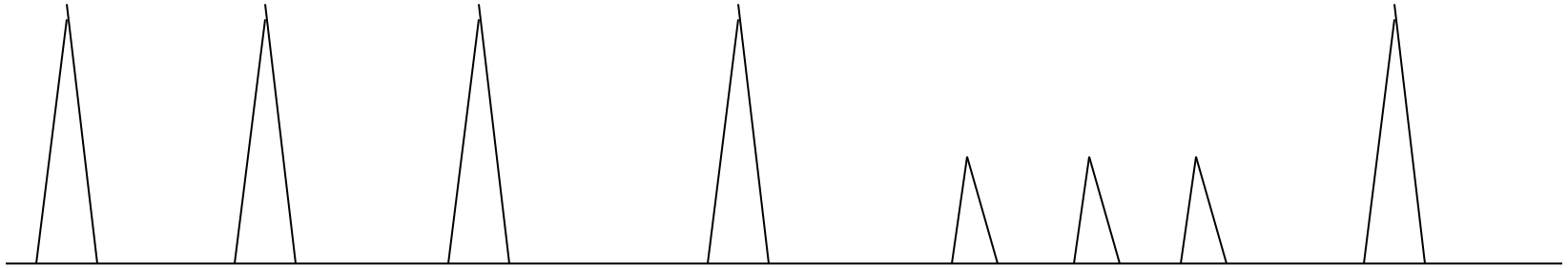


Schouten, J.P. et al. Nucl. Acid Res. 30, e57.

MLPA

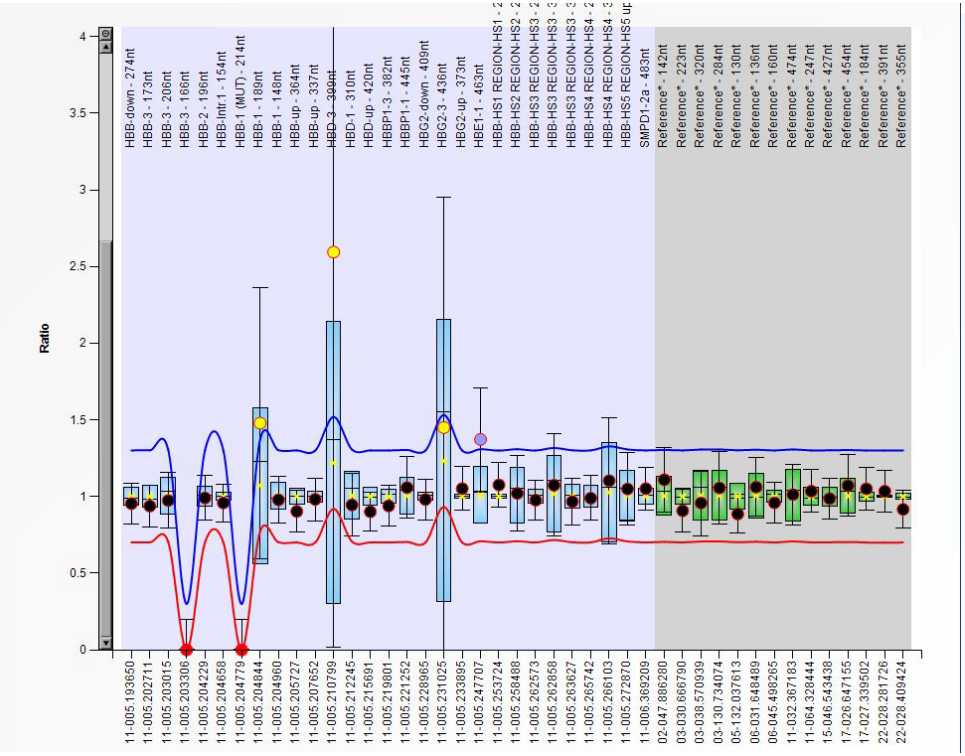


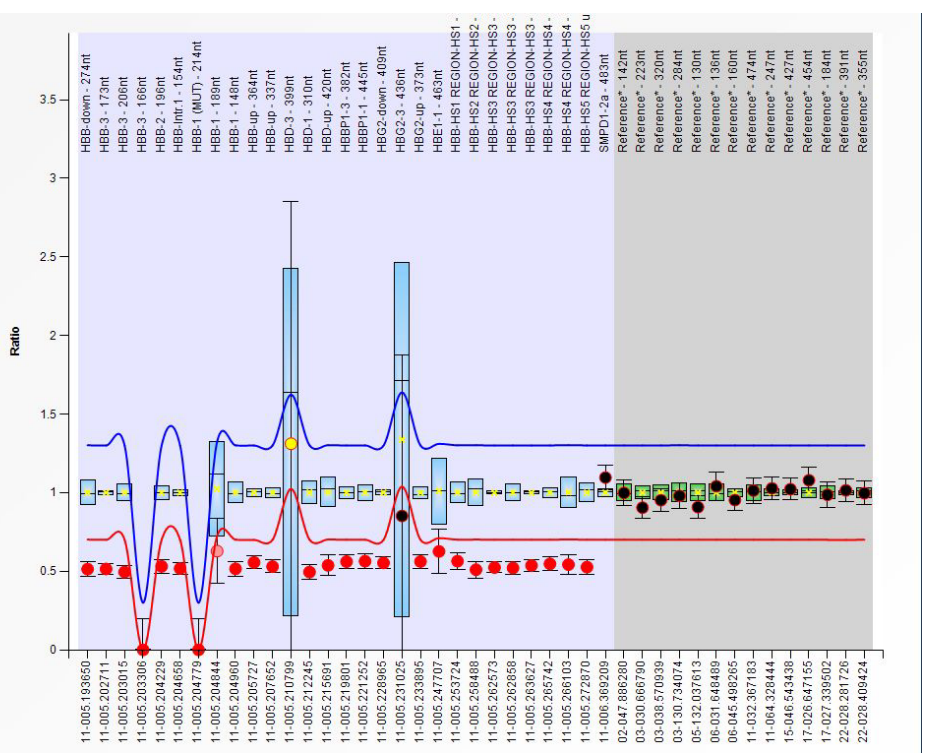
Peak heights on capillary electrophoresis

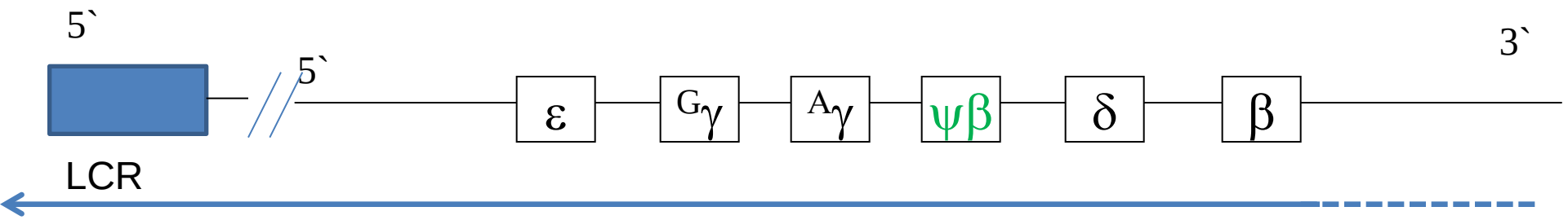


Mother

Father and new born

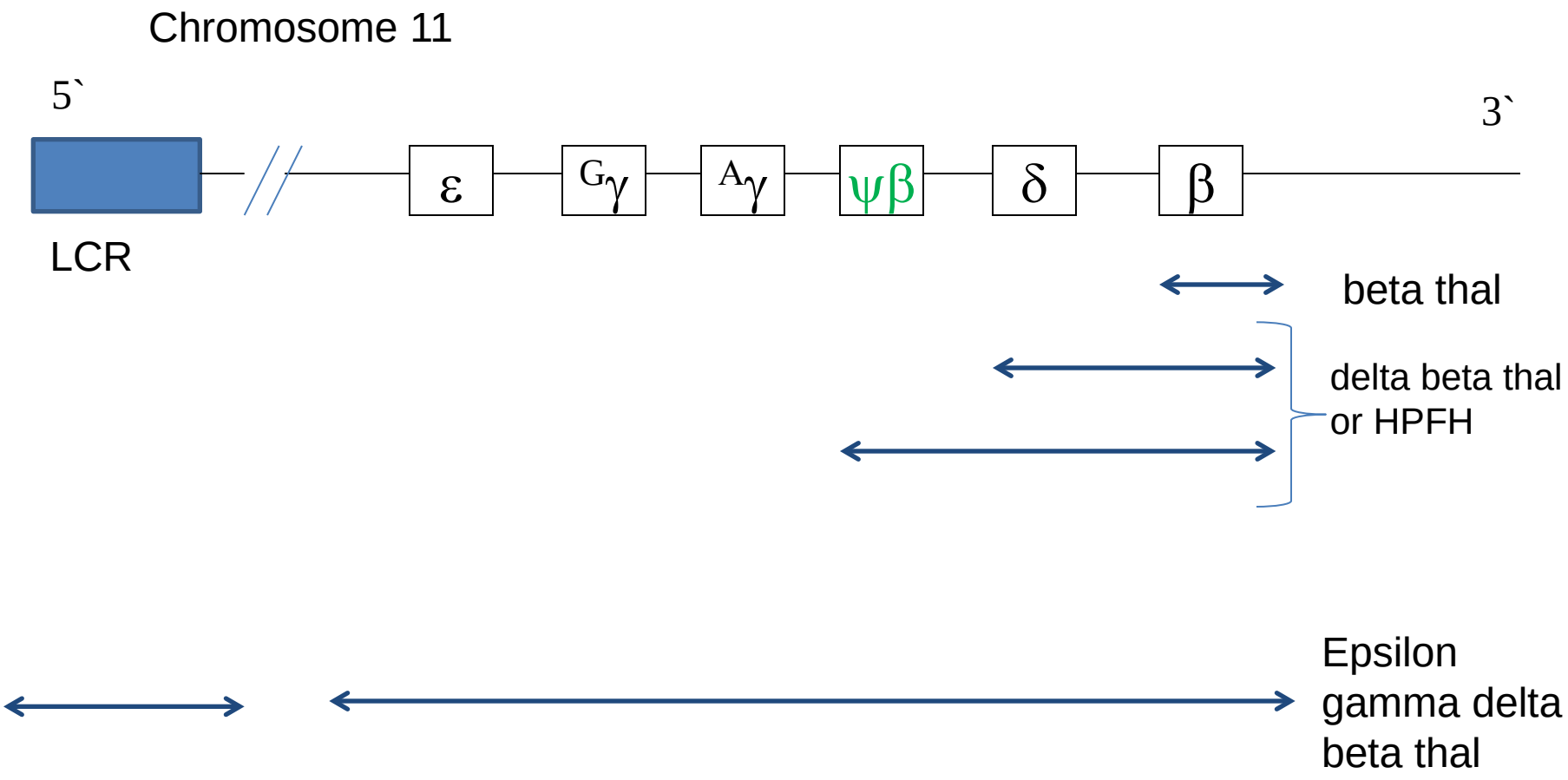


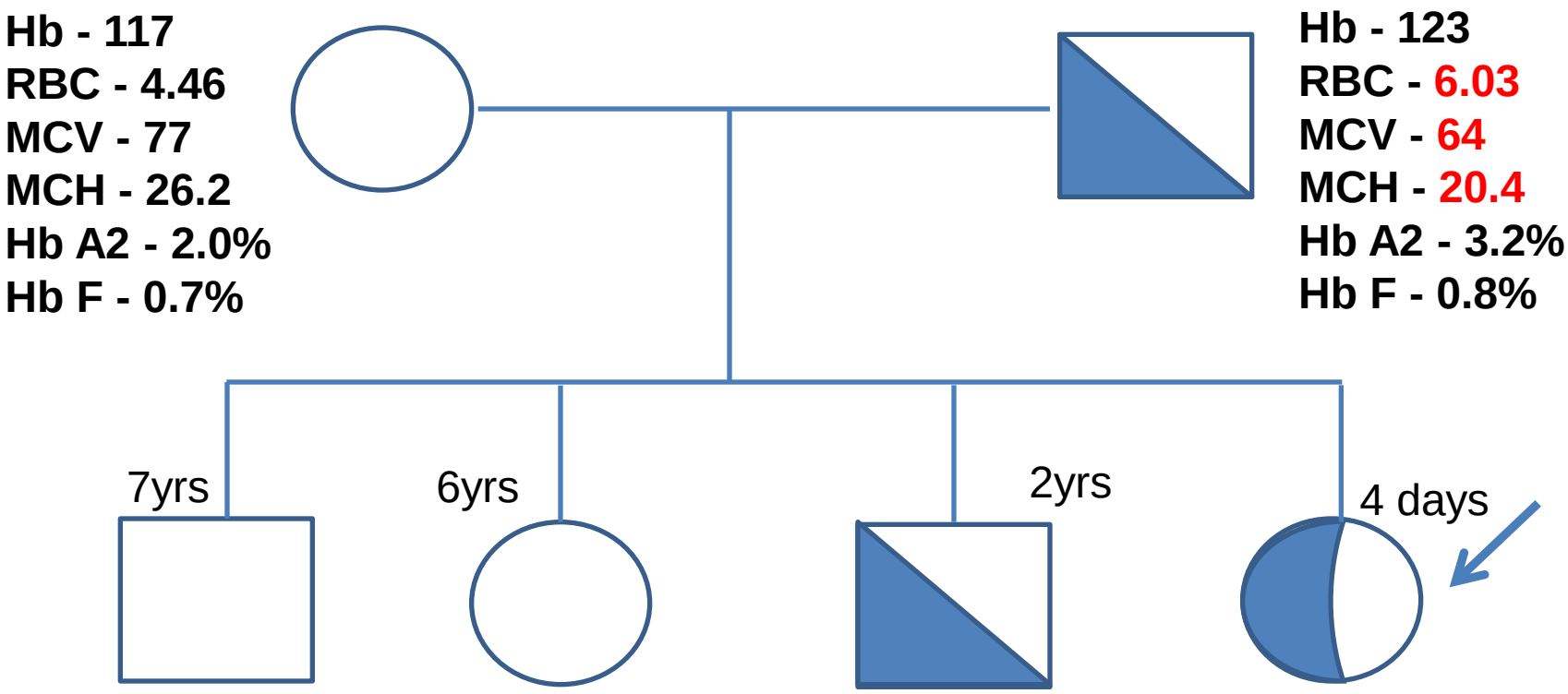




εγδβ – thalassaemia deletion

Deletional beta thalassaemia/HPFH




Hb - 117
RBC - 4.46
MCV - 77
MCH - 26.2
Hb A2 - 2.0%
Hb F - 0.7%
Hb - 123
RBC - 6.03
MCV - 64
MCH - 20.4
Hb A2 - 3.2%
Hb F - 0.8%
7yrs
6yrs
2yrs
4 days
Hb - 112
RBC - 4.90
MCV – 91.2
MCH – 24.5
Hb A2 - 2.6%
Hb F - 0.5%
Hb - 112
RBC - 4.63
MCV – 96.3
MCH – 25.9
Hb A2 - 3.0%
Hb F - 0.5%
Hb - 108
RBC – 6.16
MCV - 55
MCH – 17.5
Hb A2 – 3.1%
Hb F – 2.6%
HB - 64
RCC - 2.41
MCV - 92
MCH - 26.6,
Hb F - 67.5

Epsilon-gamma-delta-beta thalassaemia

- Presents as severe neonatal anaemia
- Transfusions often required
- Still birth and death shortly after birth have been described
- Resolves during first months of life (as haemoglobin switching occurs)
- Adult phenotype is similar to beta thalassaemia trait but with near normal A2 levels.
- ? Why so much more severe that beta thalassaemia trait
- Homozygous state has never been observed (most likely lethal)

Table 1 Origin, length, and presentation of previously described *εγδβ*-thalassemia deletions

Origin	Length (Kb)	Deletion group	Intrauterine presentation	LBW	Anemia and transfusion
Chilean	153	I	Intrauterine transfusion	NA	+
Croatian	>148	I		–	–
English III	114	I	–	–	+
English III	114	I	–	–	+
English IV	439	I	–	–	+
Canadian	>185	I	–	NA	+
Scottish Irish	205	I	–	–	+
Scottish Irish	100	I	–	–	+
Scottish Irish	198	I	Intrauterine transfusion	+	+
Mexican-American	>105	I	–	NA	+
Irish	>205	I	–	+	+
Japanese	1400	I	–	–	–
France	100	I	Intrauterine transfusion		+
Pakistani	506	I	–	–	+
Anglo-Saxon	95.5	II	–	+	+
Dutch III	112	II	–	NA	+
English I	110	II	–	–	–
English II	98	II	–	–	–
Dutch	99	II	Still birth	NA	+
Hispanic	30	II	–	–	+
Dutch	>200	II	–	–	–
Norwegian	130	II	Intrauterine transfusion	+	+

European Journal of Haematology

European Journal of Haematology 90 (127–133)

A novel epsilon gamma delta beta thalassemia presenting with pregnancy complications and severe neonatal anemia

Hanna Shalev[1], Daniela Landau[1], Serge Pissard[2], Tanya Krasnov[3], Joseph Kapelushnik[1], Oded Gilad[4], Arnon Broides[1], Orly Dgany[2], Hannah Tamary[5]

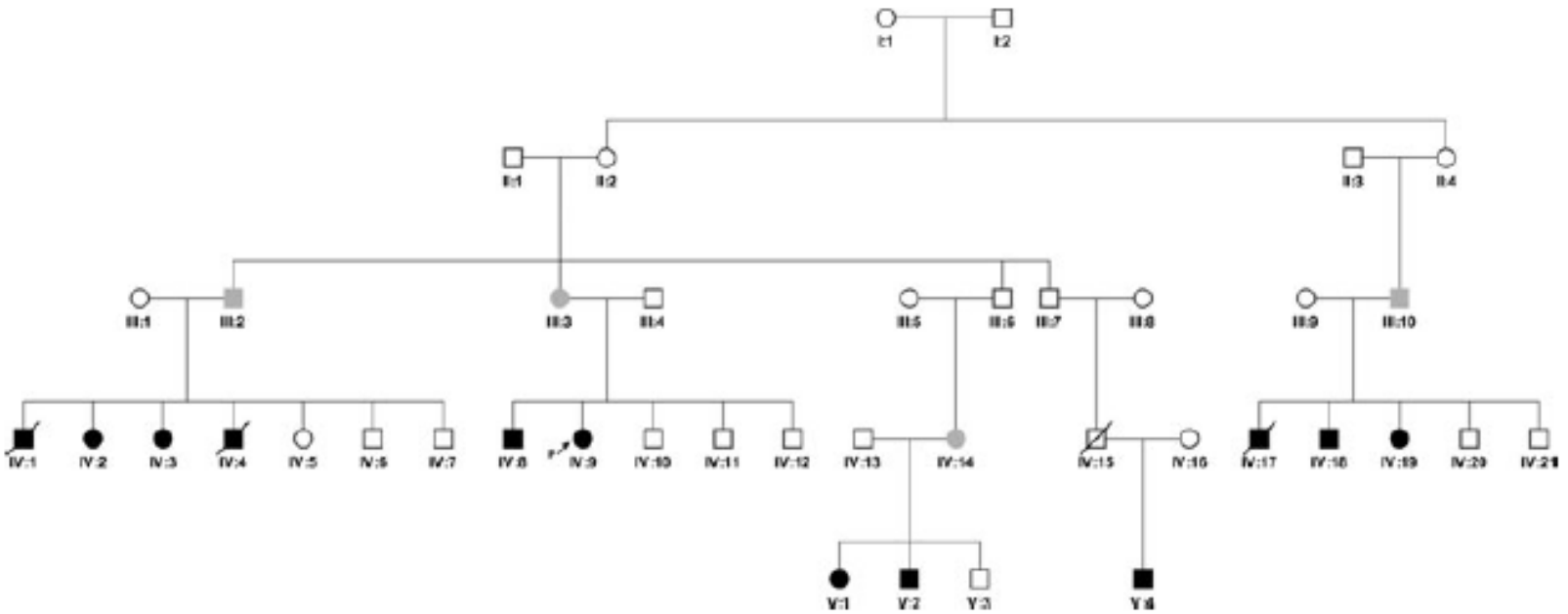


Figure 1 Pedigree of Bedouin family with *εγδβ*-thalassemia. Affected family members are denoted by blackened symbols: males are denoted by squares, and females by circles. In all patients, excluding the three who were born dead or died immediately after birth, diagnosis was confirmed by DNA analysis. Patients known to be carriers by DNA analysis but with no detailed clinical picture available are denoted by gray symbols. The arrow marks the propositus.

Table 2 Prenatal and neonatal manifestations of heterozygotes for $\varepsilon\gamma\beta\delta$-thalassemia

Patient	Gender	Gestational age (wk)	IUGR	Oligohydramnios	Hb (gr/dL)	Blood transfusions (No.)	PPHN	Outcome
IV-1	M	34	+	+	ND	ND	+	Died immediately
IV-2	F	30	+	+	6	8–10	–	Mild anemia
IV-3	F	32	+	+	6.2	6–8	–	Mild anemia
IV-4	M	35	ND	+	ND	ND	ND	Stillborn
IV-9	F	Term	–	–	10	6–8	+	Mild anemia
V-1	F	Term	–	–	5.6	2	+	Mild anemia
IV-15	M	Term	ND	ND	10	No	–	Birth asphyxia died
IV-16	M	29	+	+	5	8–10	–	Brain hypoxia
IV-17	F	36	+	+	8.5	8–10	+	Mild anemia
V-4	F	Term	+	+	8.6	2	+	Mild anemia
Summary/mean			6/8	7/9	7.5 ± 2		5/9	

Hb, hemoglobin; PPHN, persistent pulmonary hypertension of the neonate; IUGR, intrauterine growth restriction; ND, no data.

- 12 affected infants
- 10 had prenatal and post natal complications :- all severely affected
- 2 (siblings) were asymptomatic at birth

A novel (εγδβ)°-thalassemia deletion associated with an α globin gene triplication leading to a severe transfusion dependant fetal thalassemic syndrome

haematologica | 2009; 94(4)

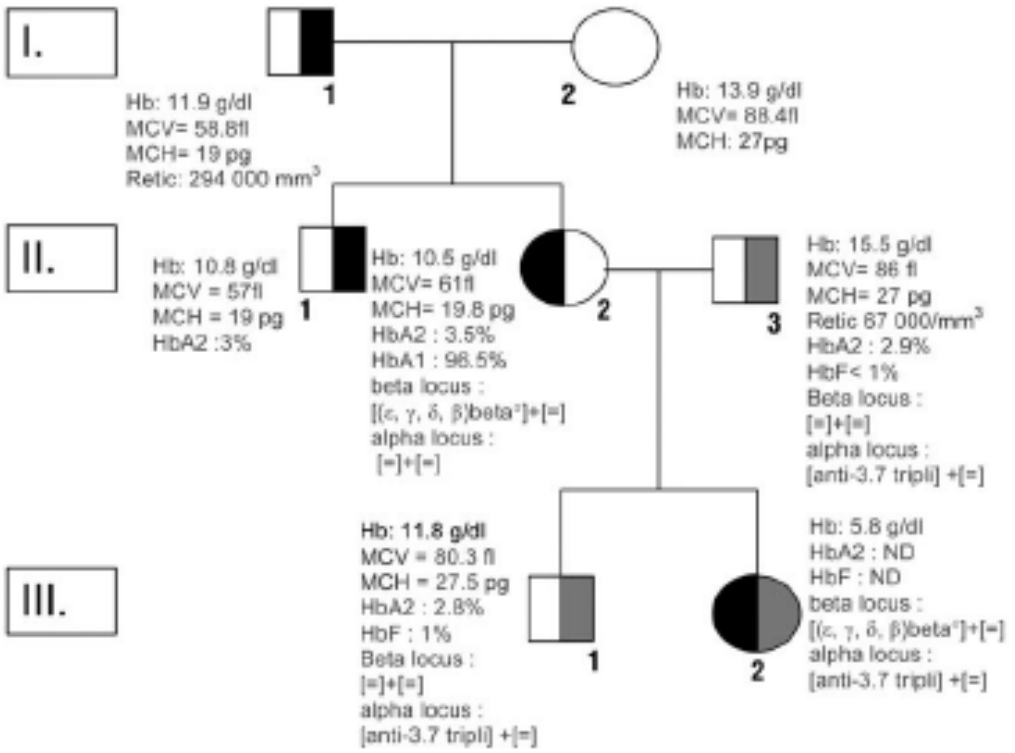
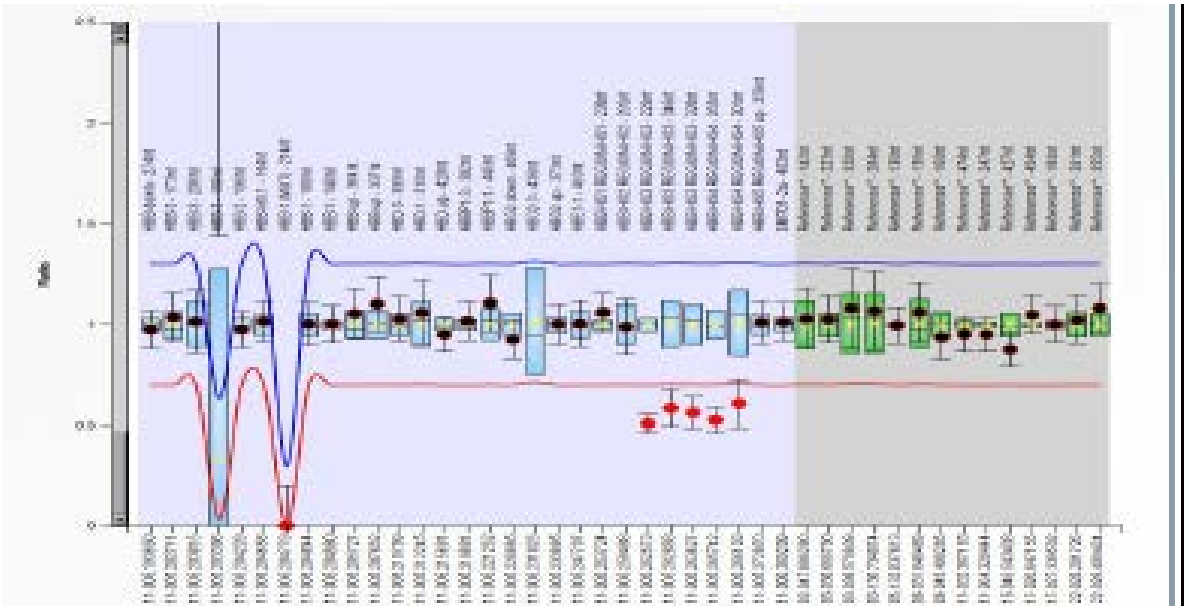


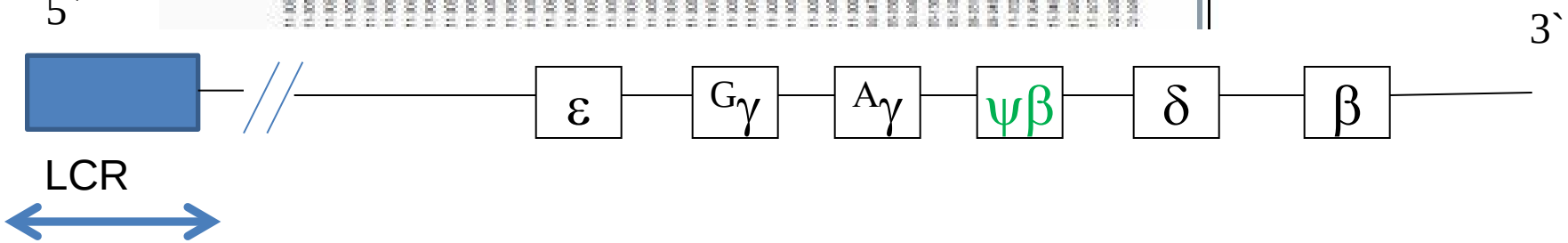
Figure 1. Family pedigree and hematologic data. Individuals indicated by half-black shaded symbol presented neonatal hemolytic anemia and microcytosis and those with a half grey shade symbol presented anti-3.7 triplication α gene. Erythrocyte parameters of patient III2 were determined during fetal life.

- Triplicated alpha gene increases severity:- fetal hydrops
- Mother required transfusions during pregnancy – erythropoietic stress
- Found in all ethnic groups

Case Study

- 15 year old boy (mixed family origins)
- Hb 129, MCV 66.6, MCH 21.4., A2 2.9%, Hb F 0.4%
- Deletion of beta globin locus LCR
- No reported problems in the newborn period
- Mother “known thal carrier” repeated episodes of anaemia sometimes requiring transfusion





Summary

- Consider εγδβ thalassaemia in unexplained HDN
- Occurs in all ethnic groups
- Could also be a cause of hydrops and IUGR
- Co-existing increased alpha globin gene copy number will increase severity - ? Screen affected families for alpha CNVs
- Consider possibility of adult carriers having symptoms during period of erythropoietic stress (e.g. pregnancy)
- Considerable unexplained phenotypic variation

OXFORD Molecular Diagnostics Centre

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NHS Trust

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Oxford Biomedical Research Centre
Enabling translational research through partnership

NHS
National Institute for Health Research