



Haemoglobinopathy In Ireland

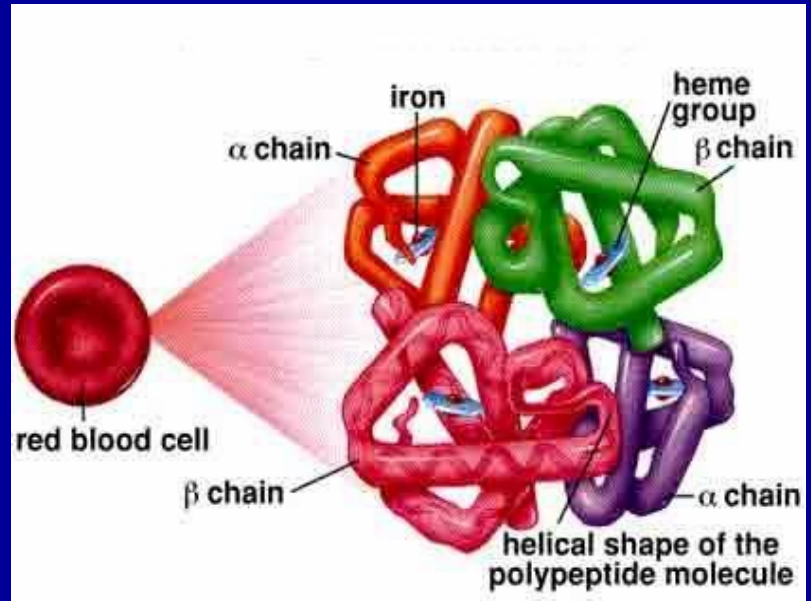
More than simply a case of haemoglobin S
detection!

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Haemoglobin Molecule

- Composed of two pairs of polypeptide (globin) chains
- Each globin chain encloses an iron-containing haem group
- Haem group is essential for oxygen transport



Normal Globin chain synthesis

- Hb structure changes during embryonic, foetal and adult life
- All Hb's composed of two α -like (α , ζ) and two non- α chains (ε , γ , β , δ)
- Three embryonic Hb's:
 - Hb Portland ($\zeta_2 \gamma_2$)
 - Hb Gower 1 ($\zeta_2 \varepsilon_2$) and Gower 2 ($\alpha_2 \varepsilon_2$)

Normal Globin chain synthesis (cont'd)

- Embryonic Hb production continues to the yolk-sac stage of development – then replaced by Hb F ($\alpha_2 \gamma_2$)
- Shortly before birth switch from foetal to adult Hb takes place
- At this stage, production of Hb's A and A₂ replace Hb F production

Normal Adult Haemoglobins

- Haemoglobin A: $\alpha_2\beta_2$ >95%
- Haemoglobin A₂: $\alpha_2\delta_2$ 2 - 3.5%
- Haemoglobin F: $\alpha_2\gamma_2$ 0 - 1.5%

Genetics of Haemoglobin synthesis

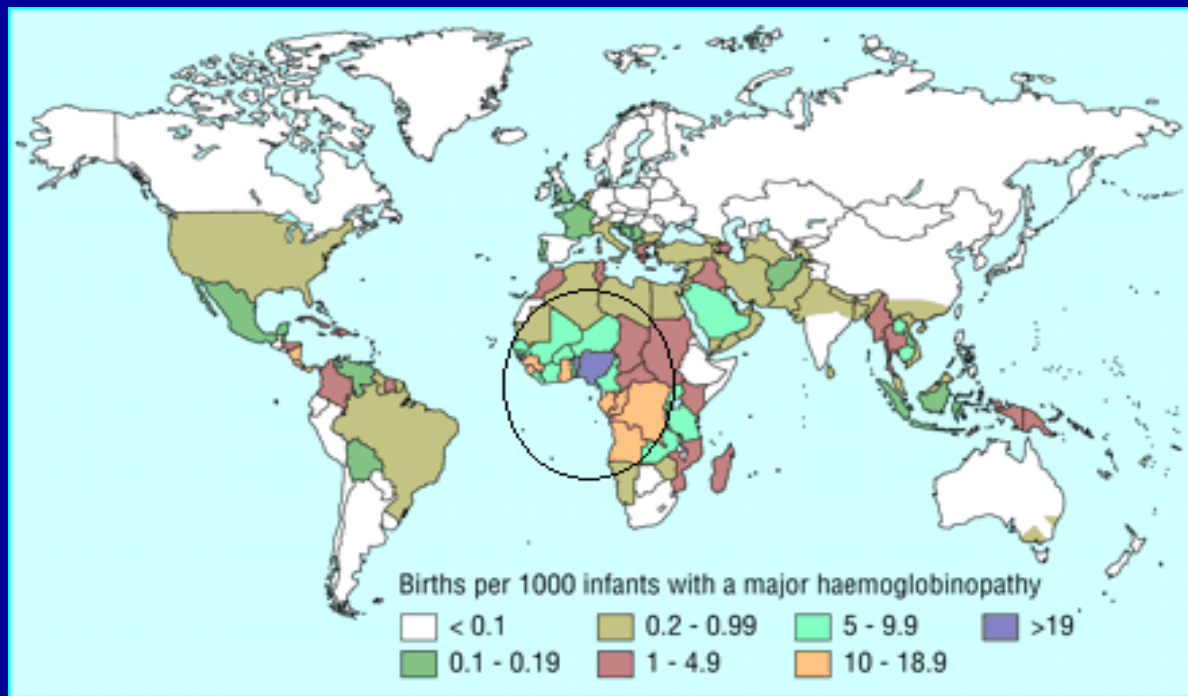
- Genes for globin chain synthesis organised in clusters on Chr.11 and Chr.16
- α -like genes (α , ζ) located on short arm of Chr.16. α -gene is duplicated and both α genes are normally active
- β -like genes (ϵ , γ , β , δ) located on short arm of Chr.11
 - Diploid cell has four functional α -genes and two functional β -genes

Haemoglobinopathies

- Inherited disorders of haemoglobin synthesis
 - Abnormal Haemoglobins: production of a structural haemoglobin variant
 - The Thalassaemia syndromes: reduced rate of synthesis of normal globin chain

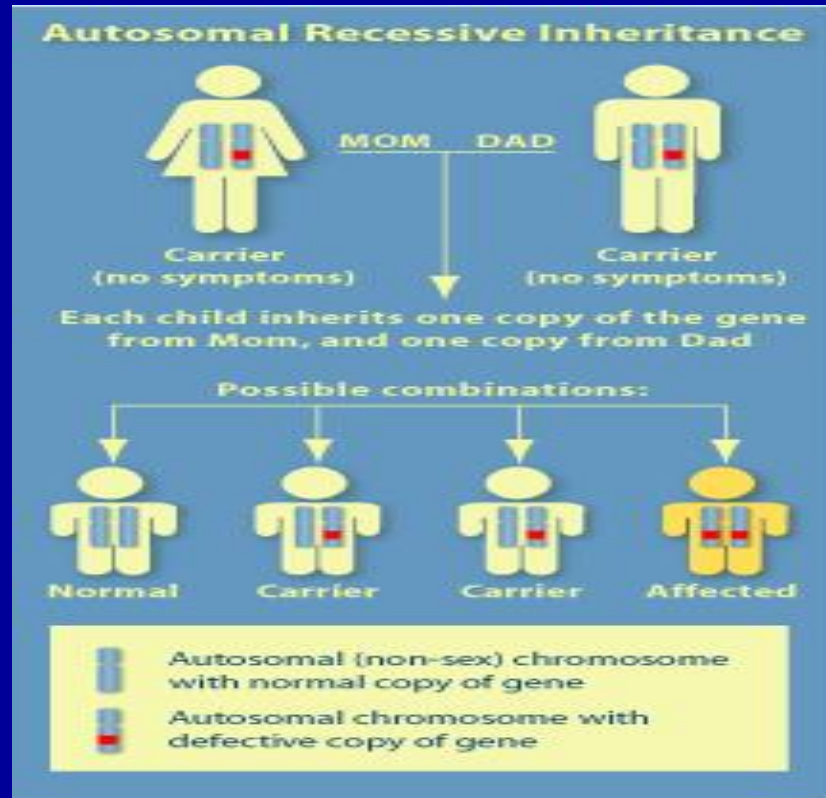
Most common monogenic disorders - approx 7% population heterozygotes (Weatherall D. 2000)

Geographical distribution of Hb disorders



(Christianson et al BMJ 2004;329:1115-7)

Inheritance of haemoglobin disorders



The Structural Haemoglobin variants

- In excess of 1000 described
- Most arise from single nucleotide polymorphisms in genes encoding globin chain synthesis
- Hb altered in structure (usually single amino acid substitution)
- Few of clinical significance
- Small number of significant variants exist (Hb S, C, E, D-Punjab, O-Arab, Lepore)

The Thalassaemia syndromes

- Inherited disorders of Haemoglobin synthesis
- Most common Monogenic diseases worldwide
- Reduced rate of production of one or more normal globin chains of haemoglobin

The Thalassaemia syndromes

- Alpha Thalassaemia
 - Normal α globin genotype: $\alpha\alpha/\alpha\alpha$
 - α Thal genotypes
 - Heterozygous Alpha⁺ Thal trait ($-\alpha/\alpha\alpha$)
 - Homozygous Alpha⁺ Thal trait ($-\alpha/-\alpha$)
 - Heterozygous Alpha⁰ Thal trait ($--/\alpha\alpha$)
 - Haemoglobin H disease ($--/-\alpha$)
 - Haemoglobin Barts Hydrops Foetalis ($--/--$)

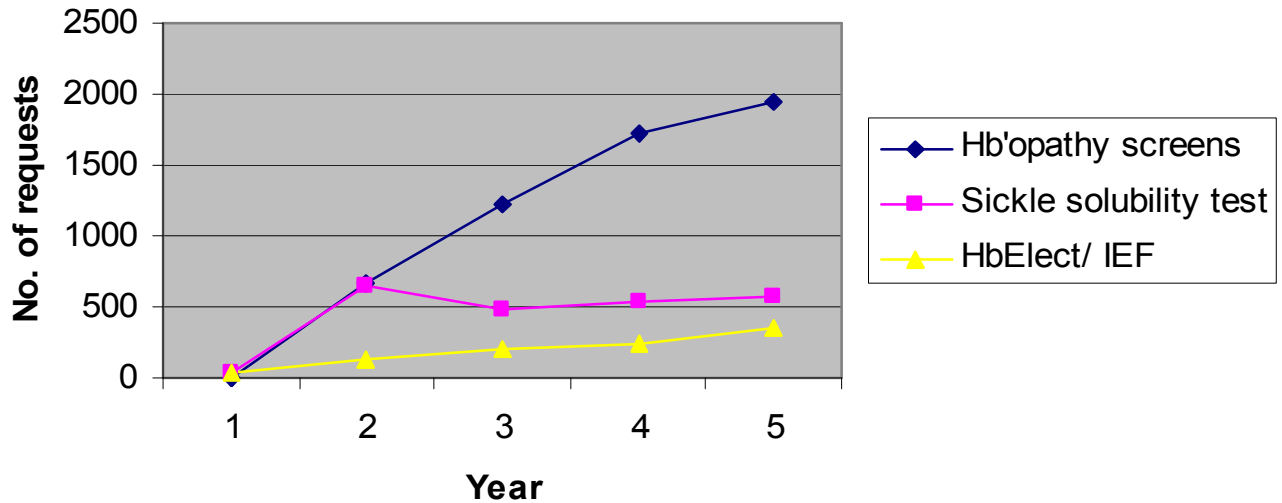
The Thalassaemia syndromes

- Beta Thalassaemia
 - Normal β -globin genotype β/β
 - β Thal Genotypes
 - Beta⁺ Thal trait (reduced beta globin output)
 - Beta⁰ Thal trait (no beta globin output)
 - Beta Thal Intermedia/ Major (β^+/β^+ , β^+/β^0 , β^0/β^0)

An Irish perspective



Haemoglobinopathy Test requests OLCHC 1998-2006



A Changing service



1998

Sickle solubility test

If pos – Hb Elect

2007

HPLC

Hb Elect

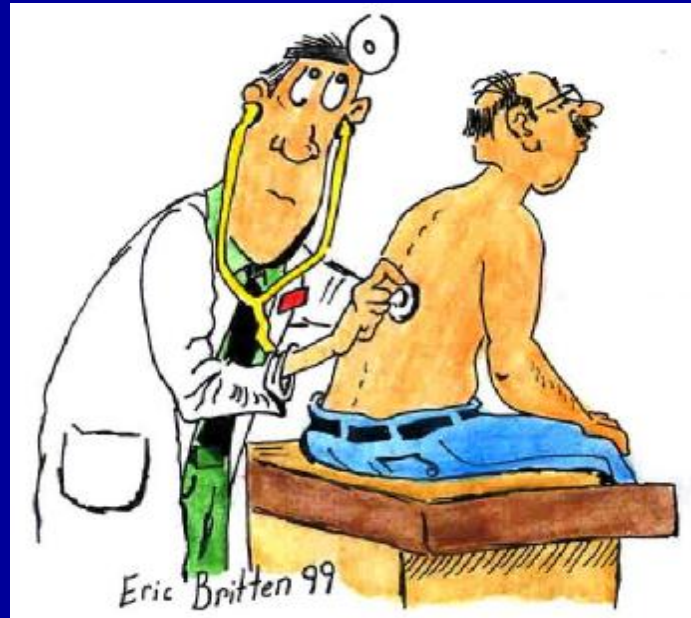
IEF

Solubility Test

HbH/ Heinz body stain

Molecular Diagnostics

Cases from OLCHC



Case Study 1:

- 3 month old girl referred for screening from CWH
- Mother Hb A/S, Father not available for testing
- Baby well clinically

FBC results:

- Haemoglobin: L 11.4 g/dL (9.0-14.0)
- WBC: 9.4 x 10⁹/L (6.0-18.0)
- Plt: H 462 x 10⁹/L (150-400)
- RBC: 4.40 x 10¹²/L (3.1-4.5)
- MCV: L 75.0 fL (77-115)
- MCH: L 25.9 pg (26-34)
- MCHC: 344 g/L (300-330)

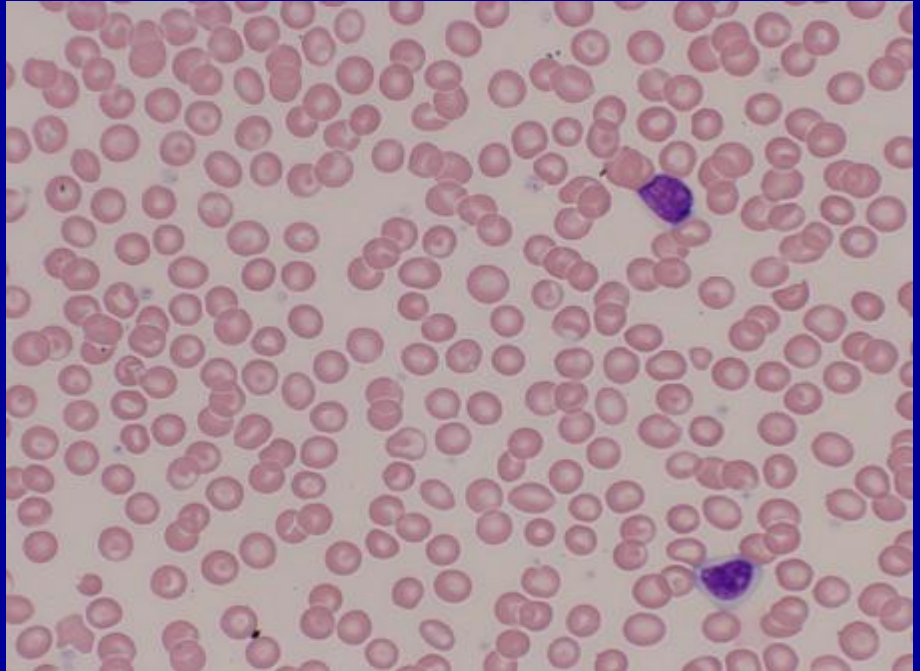
Morphology

Slight increase in
target cells

Slight
microcytosis

No sickle cells

Unremarkable
RBC morphology



Haemoglobinopathy screen:

- HPLC

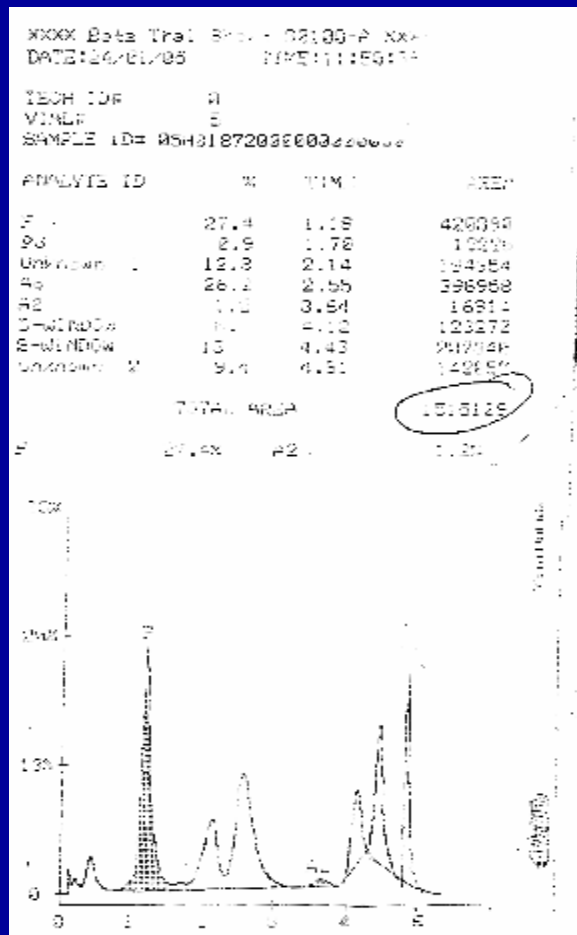
- 6 Peaks!

- Hb F, A, S-Window

- D-window and 2 unknown Fractions

? Alpha Chain variant

Hb G-Philadelphia



Haemoglobin Electrophoresis

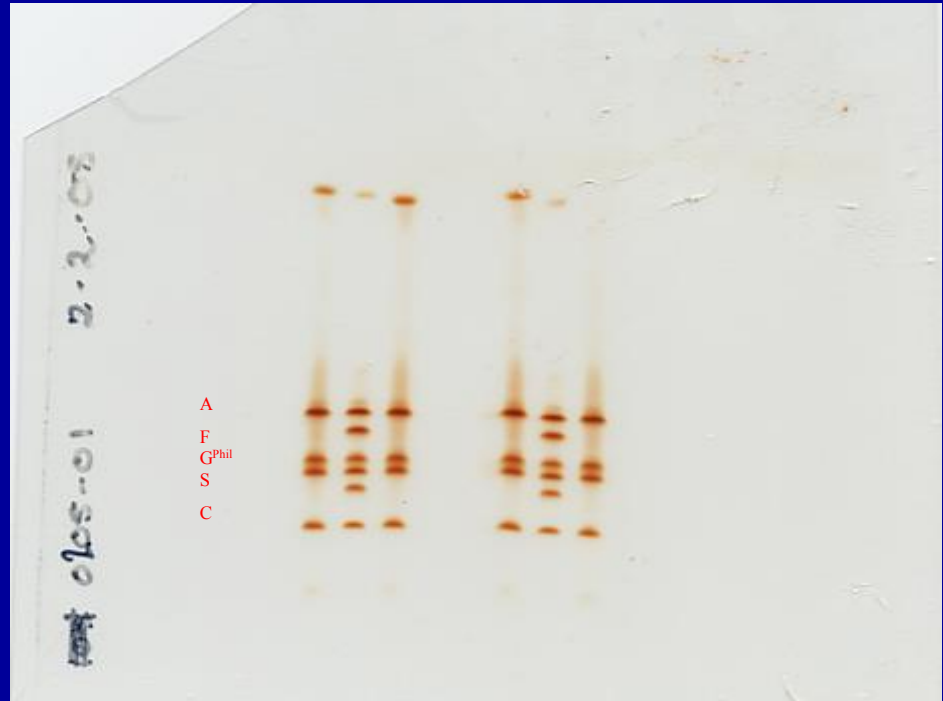
- Alkaline Gel: Bands with the mobilities of Hb's A, F, S, and two unknown bands.
- Acid Gel: Three bands with mobilities of Hb's A, S and F

Iso-Electric Focusing

Lane 1: A/S/Gphil
ctl

Lane 2: Patient

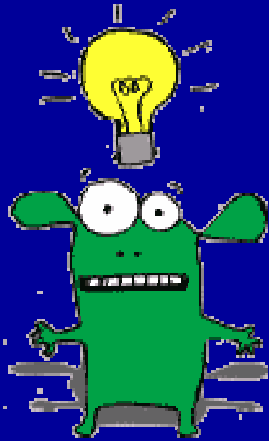
Lane 3: A/S/Gphil
ctl



What do we know??

- Hb S confirmed – Three methods
 - Hb A present
 - Beta chains accounted for
 - Hb F present
 - Alpha Chain variant present
 - Hb G-Philadelphia on HPLC/IEF
 - But!
- 6 bands on IEF, 6peaks on HPLC





6 Peaks explained!!

- 1: Hb F ($\alpha_2\gamma_2$)
- 2: Hb A ($\alpha_2\beta_2$)
- 3: Hb S ($\alpha_2\beta^S_2$)
- 4: Hb G-Philadelphia ($\alpha^{G-Phil}_2\beta_2$)
- 5: S-G^{Phil} Hybrid Hb ($\alpha^{G-Phil}_2\beta^S_2$)
- 6: γ -G^{phil} Hybrid Hb ($\alpha^{G-Phil}_2\gamma_2$)

Diagnosis:

- Double heterozygosity for a β -chain variant (Hb S) and an α -chain variant (Hb G^{Phil})
- Hb G^{Phil} in Black Africans is present on a Chr with the 3.7kb deletion (α^+ Thal : - $\alpha^{\text{GPhil}}/\alpha\alpha$)
- Homozygosity for Hb G-Phil in this population results in 100% Hb G-Phil ($-\alpha^{\text{GPhil}}/-\alpha^{\text{GPhil}}$) with distinct microcytosis (α^+ Thal homozygotes)
- Hb G^{Phil} inherited with α^0 Thal results in HbH disease (very rare) ($-\alpha^{\text{GPhil}}/--$) and 100% Hb G^{Phil}

Case study 2:

- 17month old Thai boy pres in A/E dept
- Vomiting and diarrhoea
- No rash, alert, active
- No jaundice or Hepatosplenomegaly
- Gastroenteritis ? Anaemia
- FBC/ U+E, Ca, Mg ,P

FBC results:

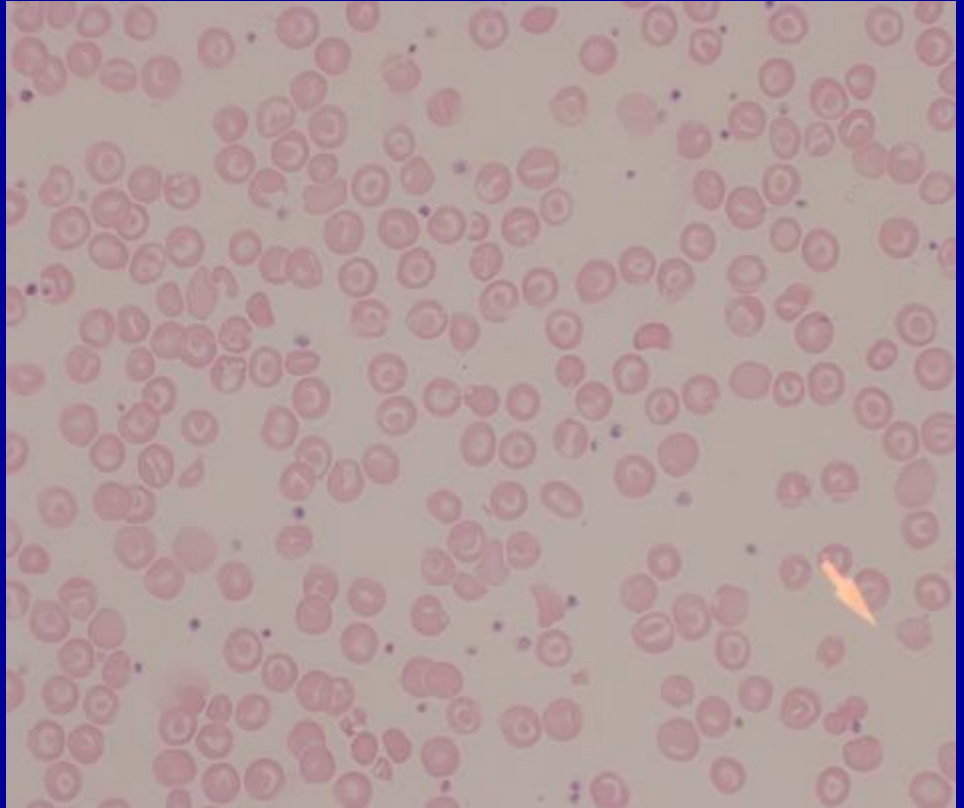
- Haemoglobin: 10.7 g/dL (10.5-13.5)
- WBC: H 20.4 x 10⁹/L (6.0-18.0)
- Plt: 257 x 10⁹/L (150-400)
- RBC: H 5.93 x 10¹²/L (3.7-5.3)
- MCV: L 55.3 fL (70-86)
- MCH: L 18.0 pg (23-31)
- MCHC: 326 g/L (300-330)

Investigation of microcytic hypochromic FBC:

- Morphology
 - Examination of blood film to assess RBC morphology
- Haematinic assessment
 - Serum ferritin
 - Vitamin B12
 - Serum folate
- Haemoglobinopathy screen
 - HPLC analysis

Morphology:

- Target cells
- Uniform size
- No pencil cells



Haematinics:

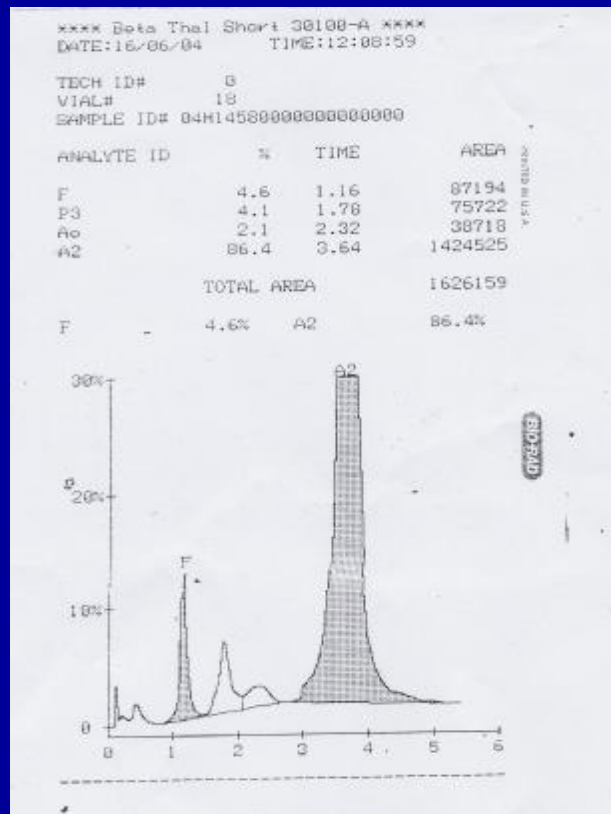
- Serum ferritin: 15.7 ug/L
- Serum Vitamin B12: 359.0 pg/mL
- Serum Folate: 8.6 ug/L

Normal Haematinic profile

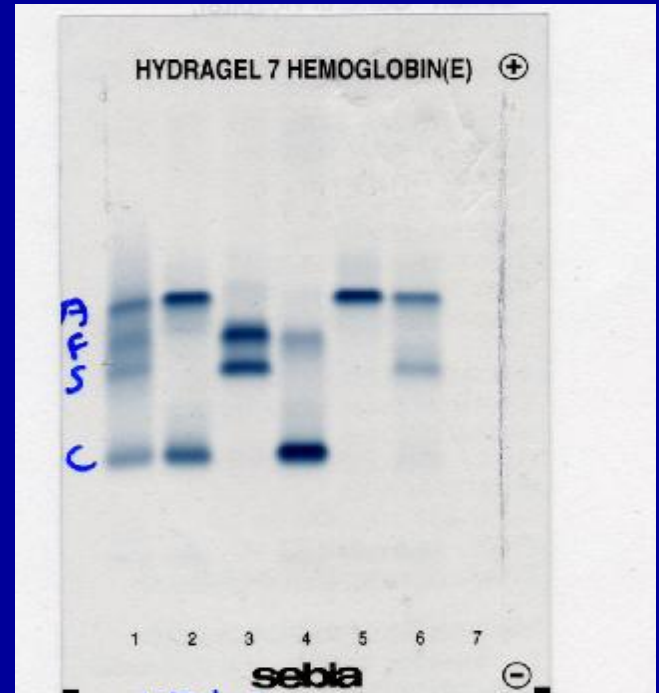
Haemoglobinopathy screen:

- HPLC

- Major peak with RT of 3.64mins (co-eluting with Hb A2)
- Minor peak with RT of 1.16mins – Hb F
- No Hb A peak!



- Haemoglobin Electrophoresis:
 - Major band detected with mobility of Hb C/E/A2
 - Minor band with mobility of Hb F
 - Hb A absent!

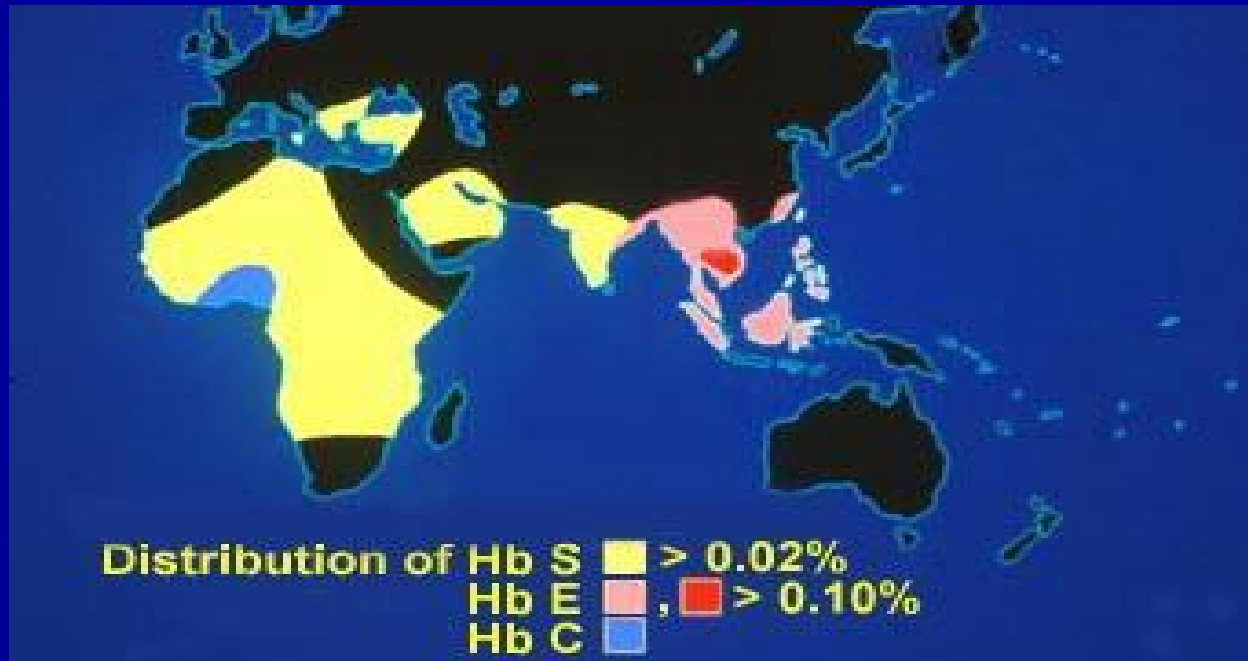


Differential diagnosis:

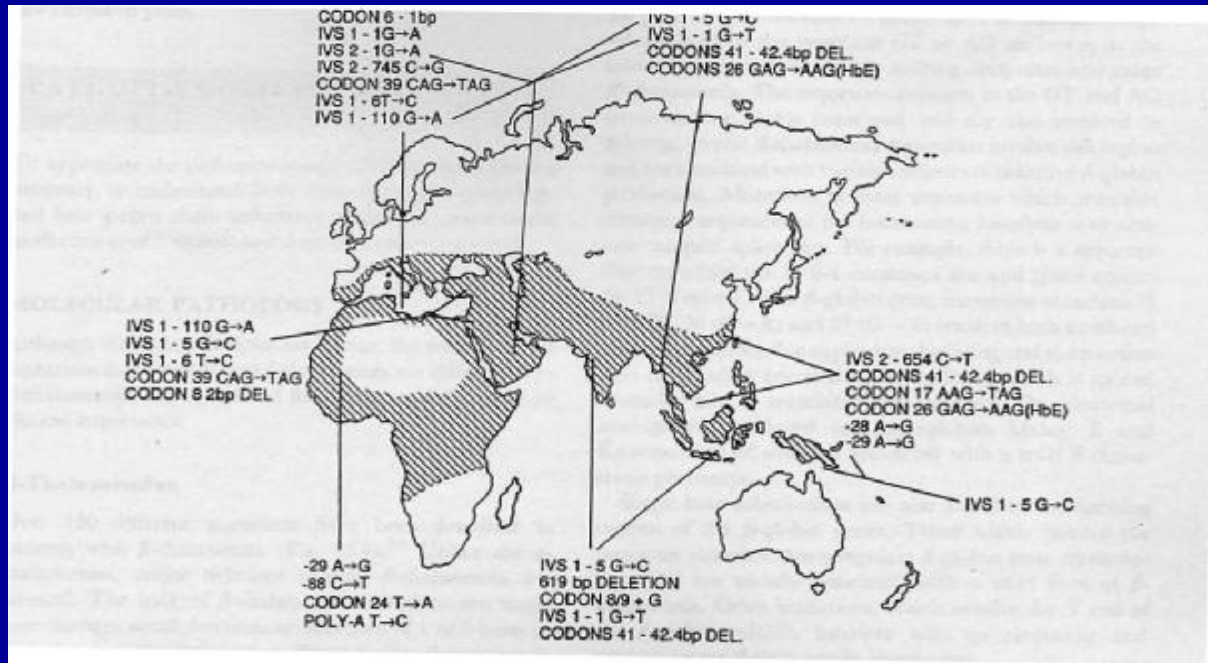
1. Homozygosity for Hb E
2. Compound heterozygosity for Hb E and Beta⁰ Thalassaemia

Parental samples requested for analysis.

Geographical distribution of Hb E



Geographical distribution of Beta-Thalassaemia alleles

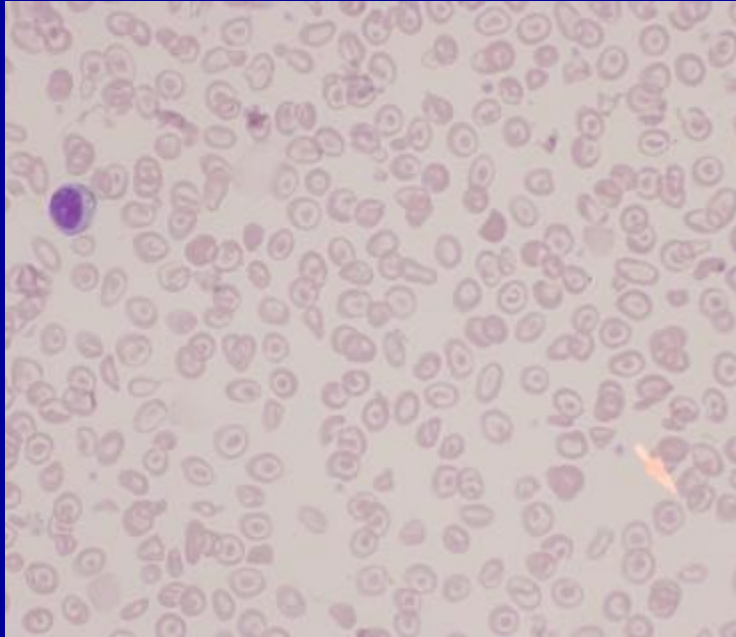


Parental screening results

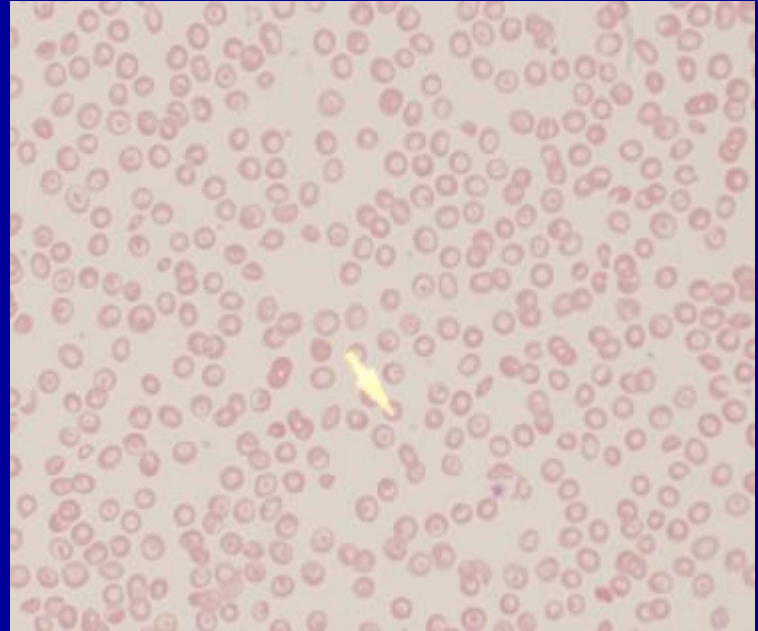
| | Paternal | Maternal |
|----------------------------|----------|----------|
| Hb g/L: | 12.4 | 11.2 L |
| RBC x 10 ¹² /L: | 8.32 H | 5.55 H |
| MCV fL: | 46.6 L | 58.7 L |
| MCH pg: | 15.9 L | 20.1 L |
| MCHC g/L: | 342 | 343 |

RBC morphology

Paternal

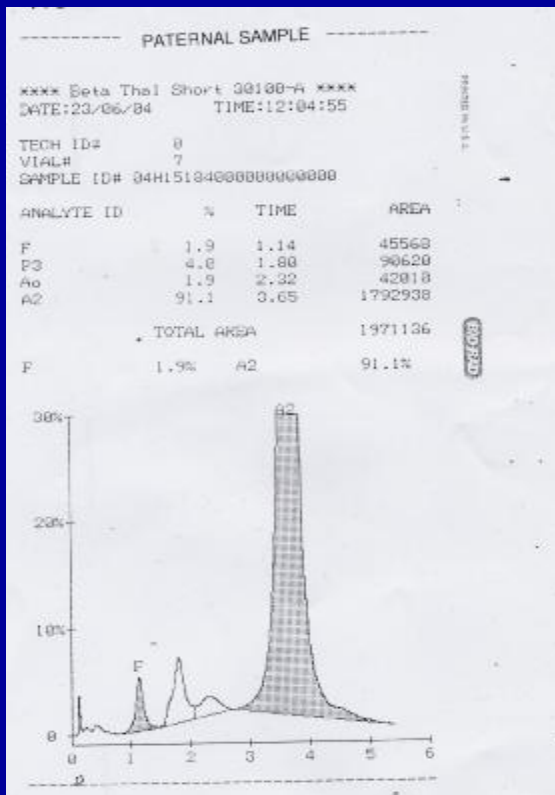


Maternal

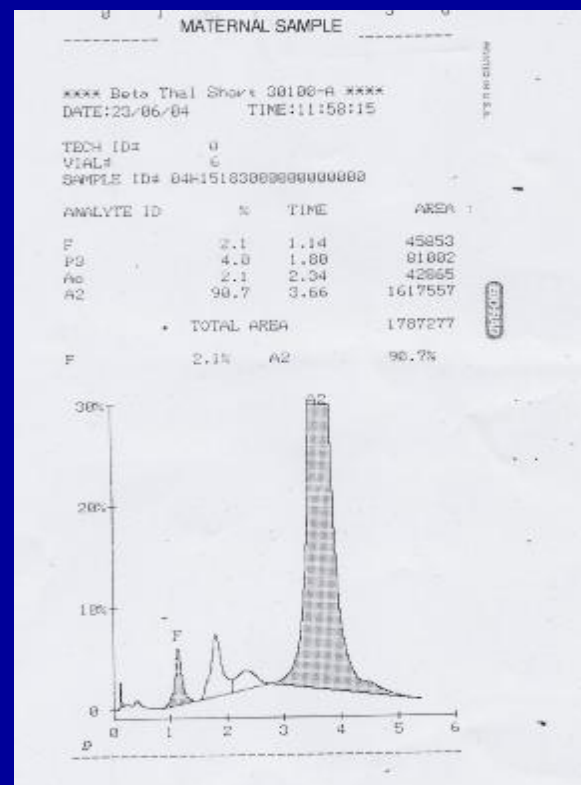


HPLC:

Paternal



Maternal



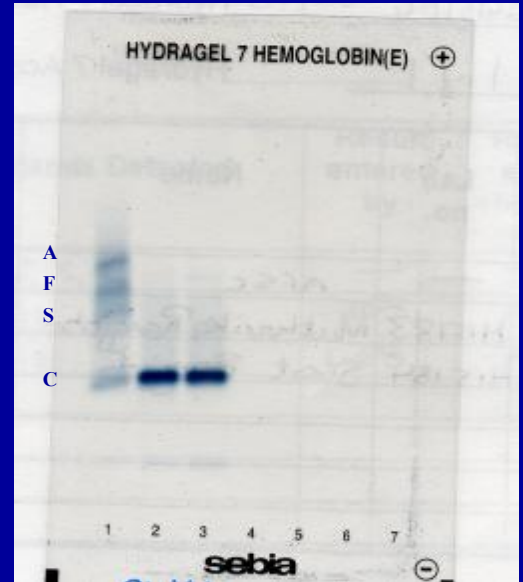
Haemoglobin Electrophoresis:

Paternal sample

Lane 2: one band
with mobility of Hb C/E/A2

Maternal sample

Lane 3: One band
with mobility of Hb C/E/A2



Diagnosis ??



- Both parents apparently homozygous for Hb E – Clinical phenotype mild
- Compound heterozygosity for Hb E and Beta⁰ Thalassaemia not consistent with clinical phenotype.
- RBC indices and distinctive morphology in paternal and patient samples indicative of possible co-existent Alpha Thalassaemia

Molecular Diagnostics

- Detection of β^E mutation by ARMS-PCR

– Patient/ Paternal/ maternal samples:

Positive result for mutant β^E primer

Negative result for normal β^E primer

All homozygous for Hb E (genotype $\beta^E\beta^E$)

Molecular Diagnostics cont'd

- Detection of Alpha Thalassaemia deletions by Gap-PCR
 - Maternal: Results show the genotype: $\alpha\alpha/\alpha\alpha$
 - Paternal: Results show the genotype: $--^{SEA}/-\alpha^{3.7}$
 - Patient: Results show the genotype: $--^{SEA}/\alpha\alpha$

Conclusions:

- Maternal samples show homozygosity for Hb E
- Gap PCR shows a normal complement of α -globin genes
- Mild clinical phenotype

Conclusions cont'd:

- Paternal samples show homozygosity for Hb E and co-existing HbH disease – usually gives rise to a phenotype of severe Thalassaemia Intermedia
- Father is clinically well with no medical Hx
- Some unidentified factor modifying the clinical Phenotype!

Conclusions cont'd:

- Patient samples show homozygosity for Hb E
- Patient has also inherited the South-East Asian α^0 -thalassaemia deletion mutation from his father
- Genotype: $-\text{SEA}/\alpha\alpha, \beta^E\beta^E$
- Mild clinical phenotype
- Carries significant Alpha Thal allele

References

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- Weatherall D. Single gene disorders or complex traits: lessons from the thalassaemias and other monogenic diseases. (2000) *BMJ*:321;1117-1120.
- Bank A. Understanding globin regulation in β -Thalassaemia: it's as simple as α , β , δ , γ . (2005). *J.Clin.Invest.* 115:1470-73.