Nomination of Alpha Thalassemia: Hemoglobin H Disease

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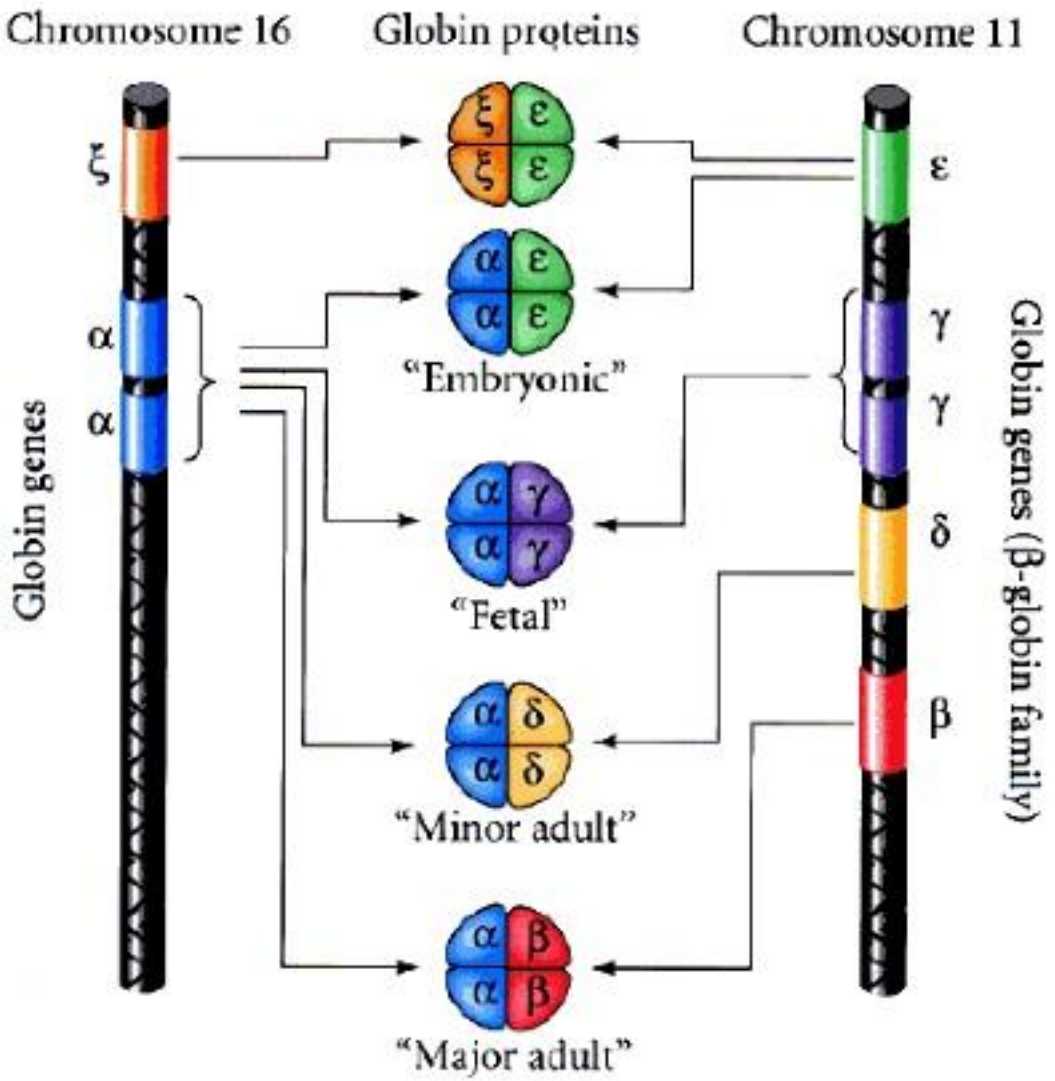
Nomination of Alpha Thalassemia: Hemoglobin H Disease

Name of Proponent:	Elliott Vichinsky, MD
Organization:	Children's Hospital Oakland
Date:	4/28/09
Condition:	Alpha-thalassemia / Hb H
Type of Disorder:	Hemoglobinopathy
Screening Method:	Newborn, Dried Blood Spot
Treatment strategy:	Early referral for comprehensive care before onset of illness

Human Globin Genes and Products



Gower 1: $\zeta_2 \varepsilon_2$

Gower 2: $\alpha_2 \varepsilon_2$

Portland: $\xi_2 \gamma_2$

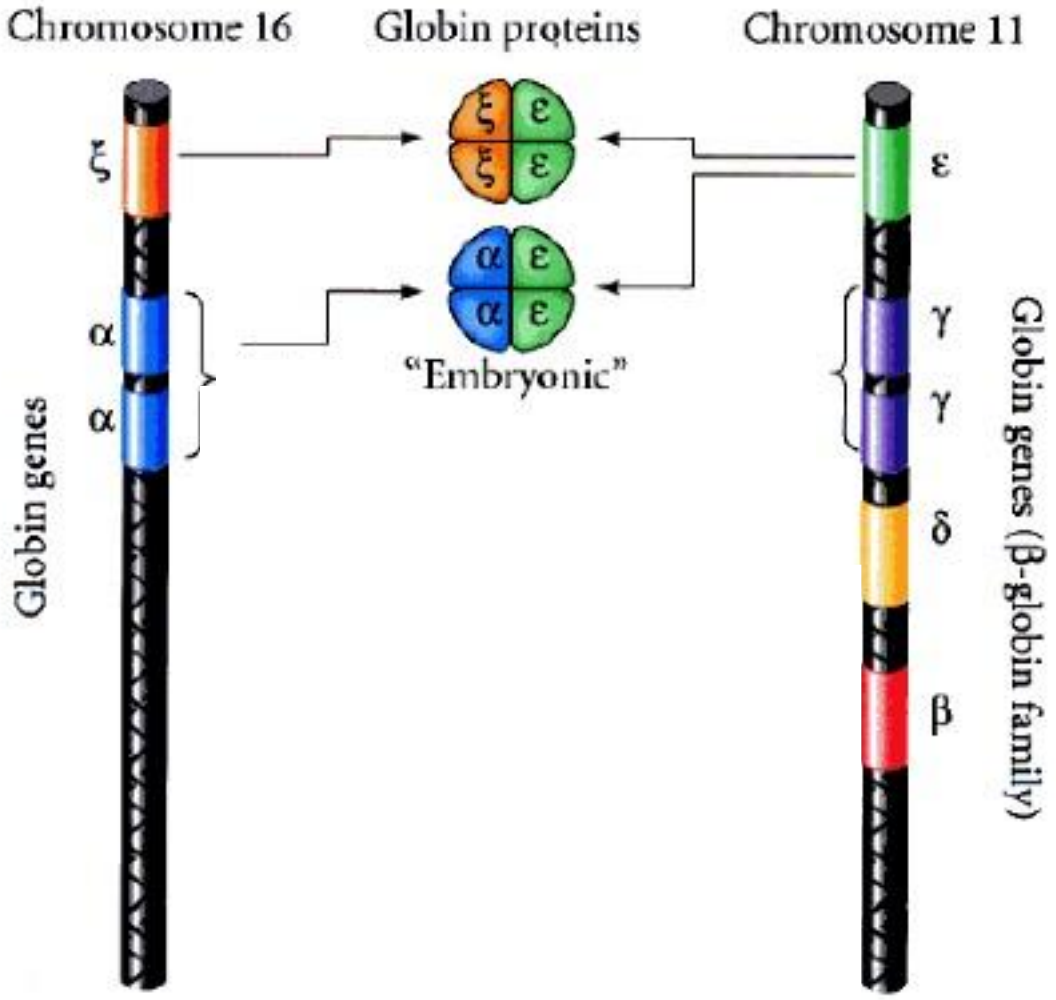
F: $\alpha_2 \gamma_2$

A2: $\alpha_2 \delta_2$

A: $\alpha_2 \beta_2$

Hemoglobins in Development

Human Hemoglobin Genes and Products



Gower 1: $\zeta_2 \varepsilon_2$

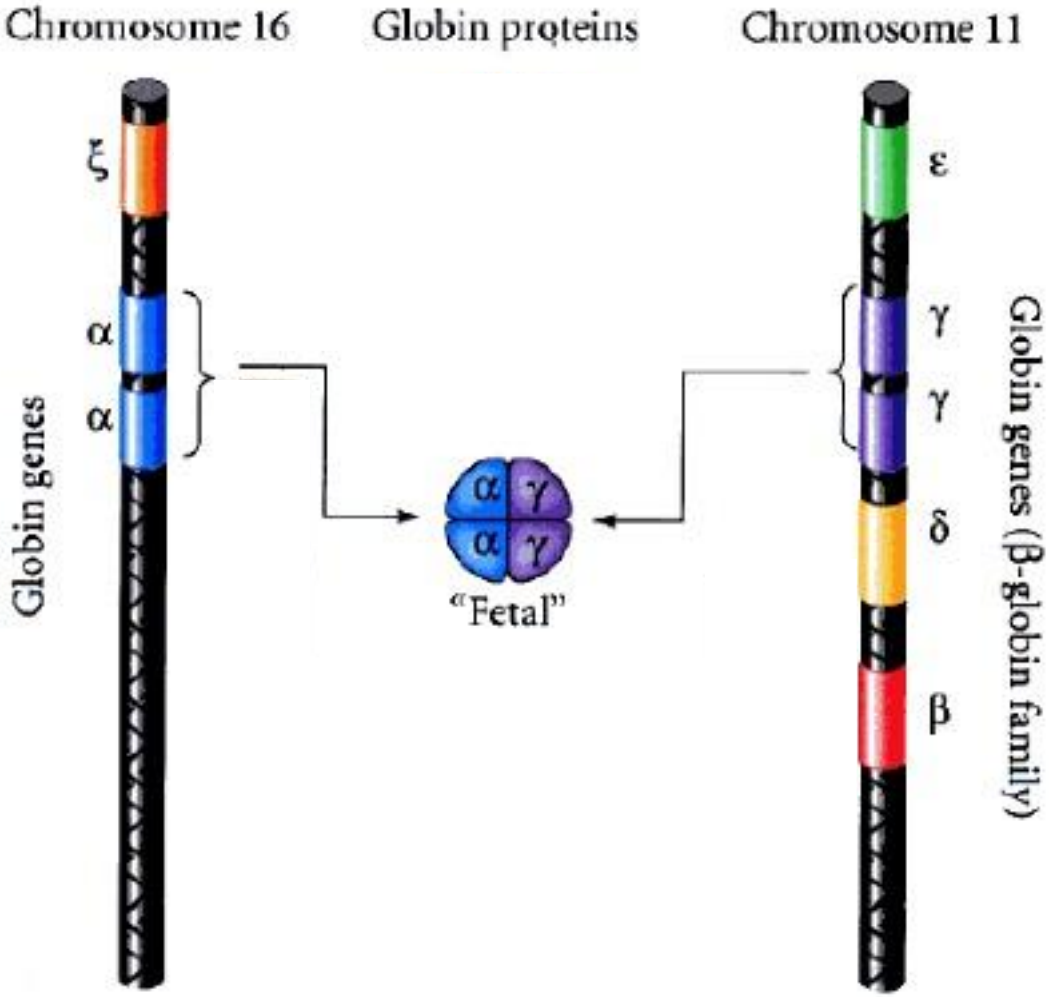
Gower 2: $\alpha_2 \varepsilon_2$

Portland: $\xi_2 \gamma_2$

Embryonic Hemoglobins (early pregnancy)

Hemoglobins in Development

Human Hemoglobin Genes and Products

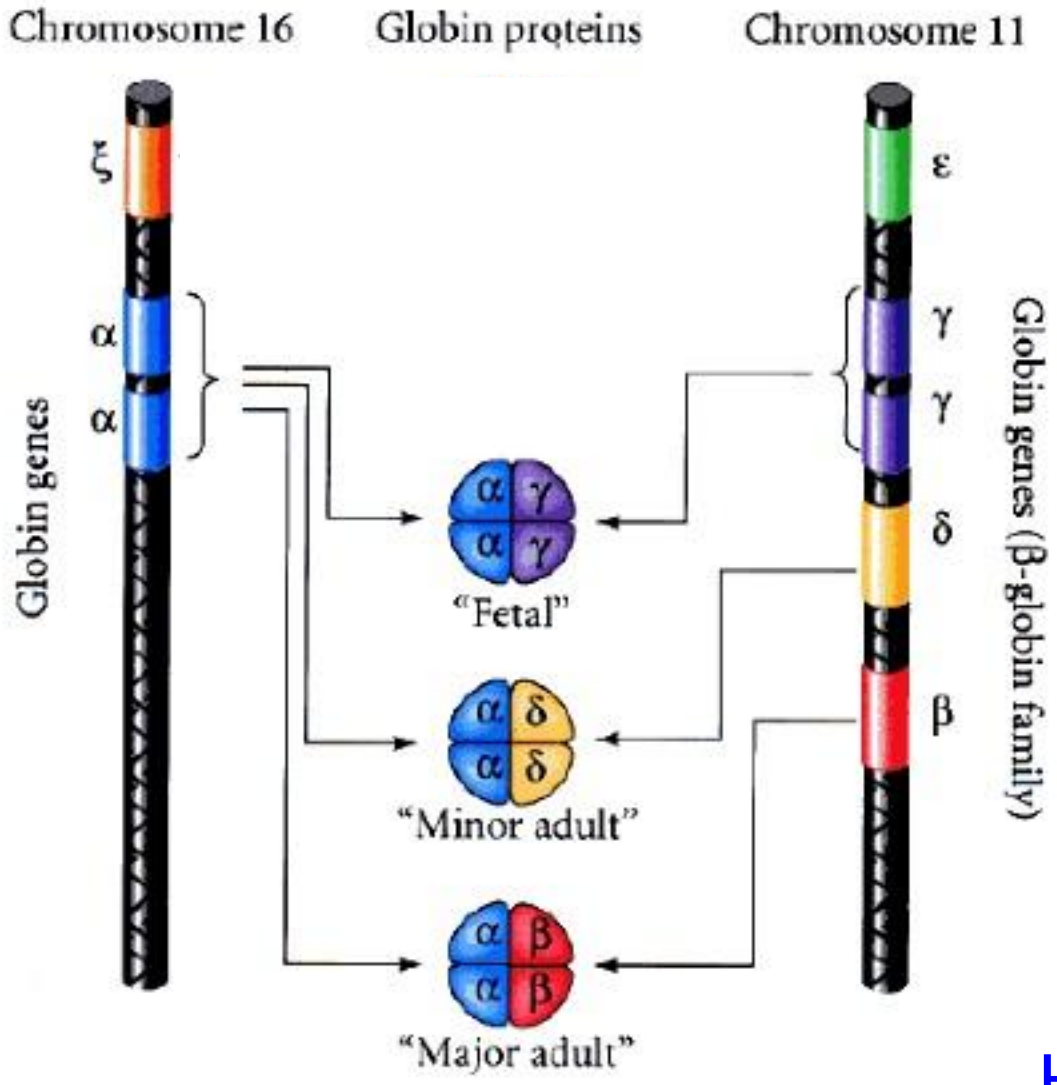


F: $\alpha_2 \gamma_2$

Fetal hemoglobin (Hb F

Hemoglobins in Development

Human Hemoglobin Genes and Products

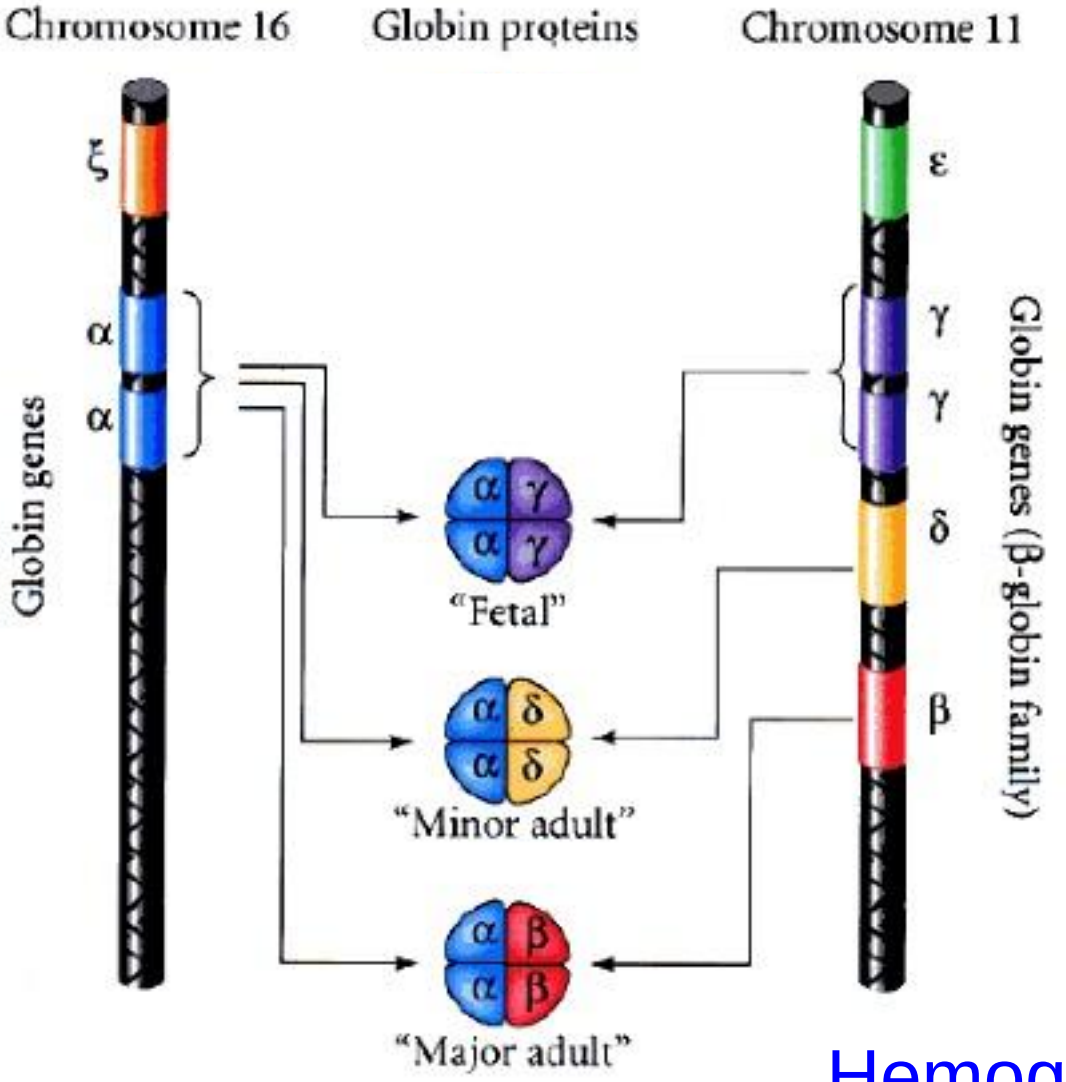


F:	$\alpha_2 \gamma_2$	60-90%
A2:	$\alpha_2 \delta_2$	< 1%
A:	$\alpha_2 \beta_2$	10-40%

Hemoglobins at Birth

Hemoglobins in Development

Human Hemoglobin Genes and Products



F:	$\alpha_2 \gamma_2$	< 2%
A2:	$\alpha_2 \delta_2$	~ 3%
A:	$\alpha_2 \beta_2$	96%

Hemoglobins by age ≥ 1

Alpha thalassemia results from a deficiency in α-globin production

Globin Imbalance

Normal Hb	Globin composition	α Thalassemia	Abnormal Hb
F	$\alpha_2 \gamma_2$	Excess γ	Bart's (γ_4)
A	$\alpha_2 \beta_2$	Excess β	H (β_4)

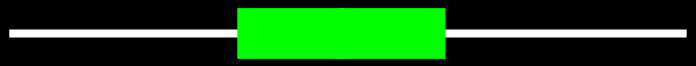
Alpha Thalassemia

Causes of α Thalassemia

Deletional (90%)

Normal: α α

α^{+}-thalassemia: – α



α^{o}-thalassemia: – –

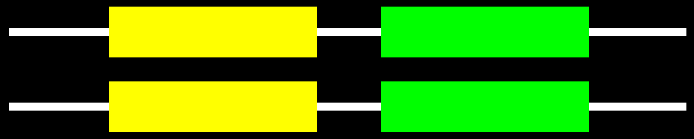
Non-deletional (10%)

Hb Constant Spring: α α^{cs}

Alpha Thalassemia

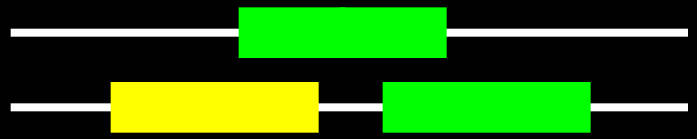
α-Gene Complement and Deletions (1)

Normal: α α/α α



Silent Carrier of α thalassemia — *1 α–gene deletion*

Heterozygous α^+-thal: α α/– α



α-Thalassemia trait — *2 α–gene deletion*

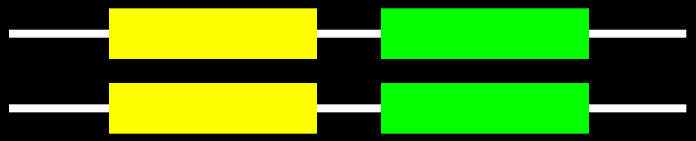
Homozygous α^+-thal: – α/– α

Heterozygous α^o-thal: – –/α α

Alpha Thalassemia

α-Gene Complement and Deletions (2)

Normal: α α/α α



Hb H Disease

3 α-gene deletion

α^{+}/α^{o}-thal: – –/– α

Hydrops fetalis

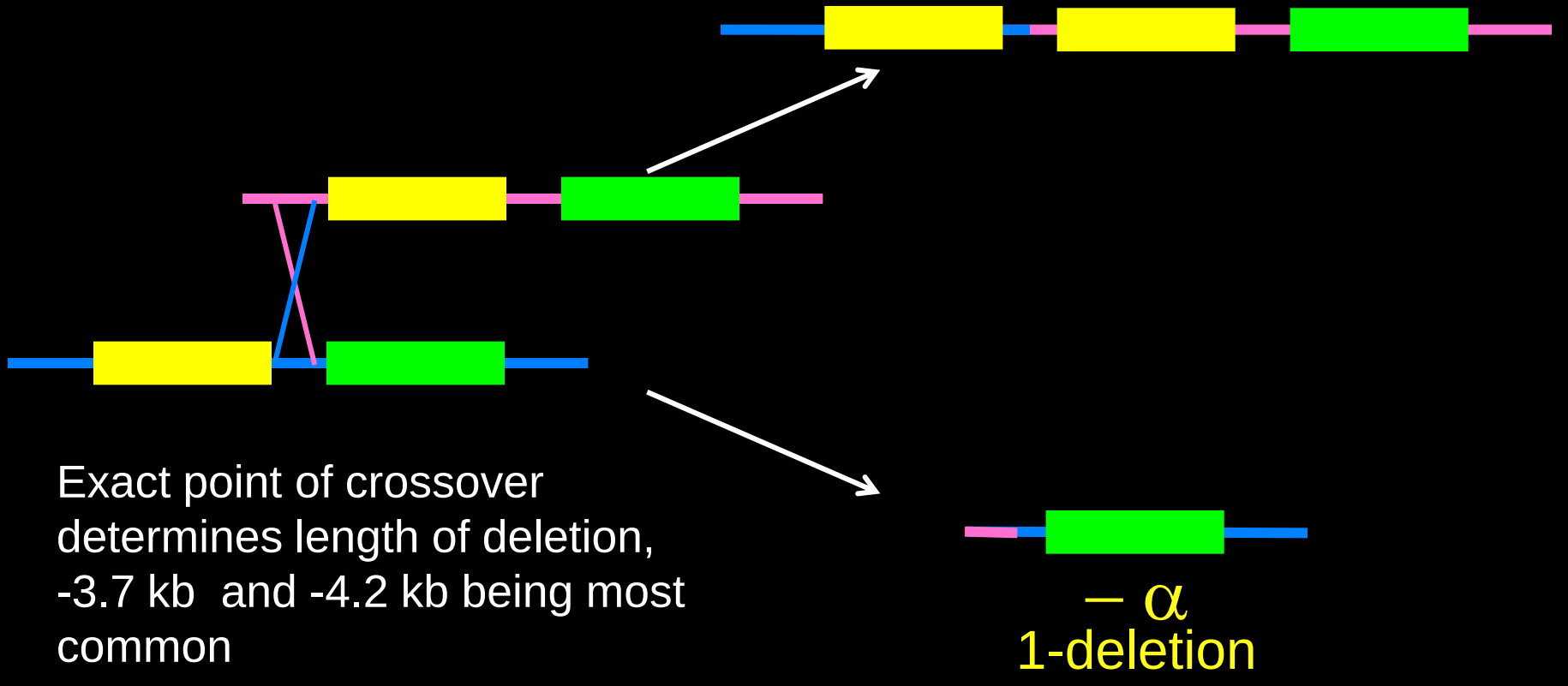
4 α-gene deletion

Homozygous α^{o}-thal: – –/– –

Alpha Thalassemia

Mechanism of Deletional α^+ Thalassemia

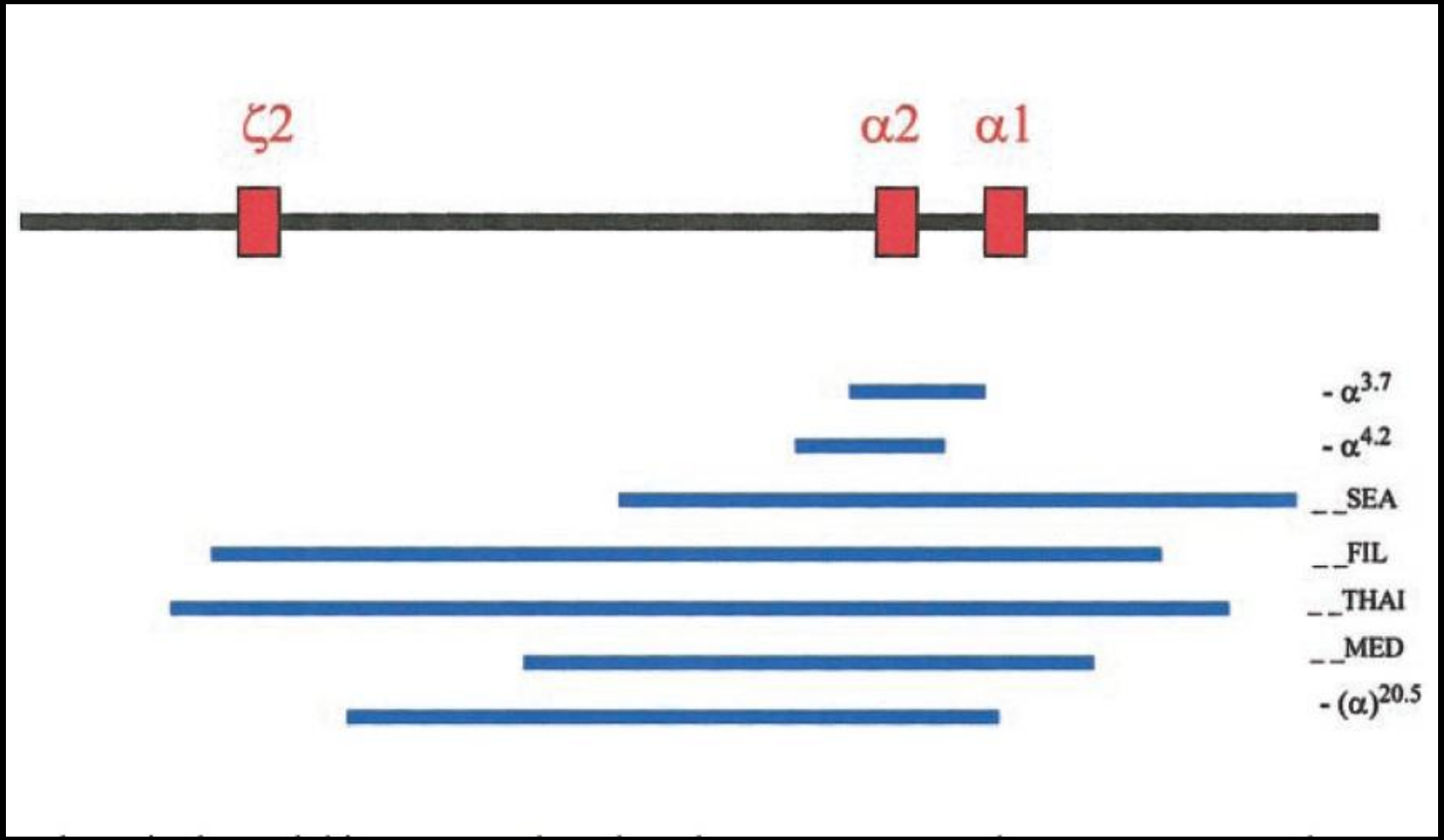
Misalignment of α gene alleles and unequal crossover during prophase of meiosis



Exact point of crossover determines length of deletion, -3.7 kb and -4.2 kb being most common

Alpha Thalassemia

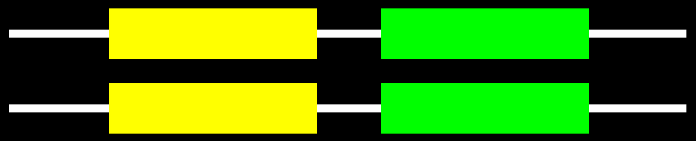
Examples of Deletional α^0 Thalassemia



Chui et al., Blood, 2003;101; 791

Alpha Thalassemia

Hb H Disease

Normal: $\alpha\ \alpha/\alpha\ \alpha$ 

Deletional

3 α-gene deletion

α^{+}/α^{o}-thal: $--/-\ \alpha$ 

"Non-deletional"

Hb Constant Spring: $--/\ \alpha\ \alpha^{cs}$ 

Geographic Distribution of α Thalassemia

Gene Frequency of α thalassemia				
Region	α^o thal	- -MED %	α^+ thal	- $\alpha^{3.7}$ %
Sub-Saharan Africa	0		0.1 – 0.33	90-100
Mediterranean Basin	0.001 – 0.01	50 – 100	0.011 – 0.1	20-96
Arabian Peninsula	0 – 0.005		0.27 – 0.67	67-95
India	0		0.09 – 0.92	33-100
Southeast Asia	0.009 – 0.07	98-100	0.01 – 0.16	28-100
China	0.002 – 0.15	21-100	0.003 – 0.04	23-100
Oceania	0		0.03 – 0.38	14-100
Distribution mirrors that of regions with history of endemic malaria				

Hb H Disease Genotypes

α-Globin genotypes in 319 Hb H patients from California, Hong Kong, and Ontario: Deletional Syndromes

Hb H disease	No. of patients	%
Deletional	266	83
$(--^{SEA}/-\alpha^{3.7})$	175	55
$(--^{SEA}/-\alpha^{4.2})$	37	12
$(--^{FIL}/-\alpha^{3.7})$	36	11
$(--^{MED}/-\alpha^{3.7})$	8	2
$(--^{THAI}/-\alpha^{3.7})$	2	< 1
$(--^{BRIT}/-\alpha^{3.7})$	1	< 1
$(--^{SA}/-\alpha^{3.7})$	1	< 1
$(--^{de\ novo}/-\alpha^{3.7})$	1	< 1
$(--^{SEA}/-\alpha^{4.2}$ Q-Thailand [Codon 74 GAC>CAC or Asp→His])	4	1
$(--^{FIL}/-\alpha^{4.2}$ Q-Thailand [Codon 74 GAC>CAC or Asp→His])	1	< 1

Chui et al., Blood, 2003;101; 791

Hb H Disease Genotypes

α-Globin genotypes in 319 Hb H patients from California, Hong Kong, and Ontario: Non-deletional Syndromes

Hb H disease	No. of patients	%
Nondeletional	53	17
(--^{SEA}/α Constant Spring [Codon 142 TAA>CAA or Ter→Gln]α)	31	10
(--^{SEA}/α Quong Sze [Codon 125 CTG>CCG or Leu→Pro]α)	7	2
(--^{SEA}/α Codon 30 deletion of GAGα)	3	< 1
(--^{TOT}/α Codon 30 deletion of GAGα)	1	< 1
(--^{SEA}/α Initiation codon ATG>AGα)	1	< 1
(--^{THAI}/α Initiation codon ATG>AGα)	1	< 1
(--^{SEA}/α Codon 31 AGG>AAG or Arg→Lysα)	2	< 1
(--^{FIL}/α Codon 35 TCC>CCC or Ser→Proα)	1	< 1
(--^{MED}/α IVS I deletion of TGAGGα)	1	< 1
(--^{SEA}/α Codon 59 GGC>GAC or Gly→Aspα)	1	< 1
(--^{SEA}/α Pakse [Codon 142 TAA>TAT or Ter→Tyr]α)	2	< 1
(--^{THAI}/α Constant Spring [Codon 142 TAA>CAA or Ter→Gln]α)	1	< 1
($\alpha^{Hb\ Sallanches}$ [Codon 104 TGC>TAC or Cys→Tyr] α/$\alpha^{Hb\ Sallanches}\alpha$)	1	< 1

Chui et al., Blood, 2003;101; 791

Hemoglobin H Disease

Pathophysiology

1. Deficiency of α globin mRNA and α-globin chains
   - α/β mRNA ratio < 0.5
   - α/β globin chain ratio, 0.2 to 0.7

2. Fetal development:
   Excess γ-globin chains for γ_4 tetramers (Hb Bart's)

3. Switch from Hb F to Hb A predominance:
   Excess β-globin chains for β_4 tetramers (Hb H)

4. Hb H
   - High O_2 affinity, insignificant tissue O_2 delivery
   - Unstable, oxidized to form precipitates (Hb H inclusion bodies)
   - Hb H precipitates cause
     - early erythroid cell death
     - ineffective erythropoiesis
     - membrane damage leading to hemolysis

Hemoglobin H Disease

Clinical Presentation

Wasi, 1974:

Thailand: 1,002 patients (200 adults, 502 children)

- Age at presentation: Birth to 74 years
- Infants: Near normal Hb at birth, no hepatosplenomegaly

Chen, 2000:

- Hong Kong: 114 patients
- Only 24% presented with symptoms; 76% discovered incidentally
- 13% growth failure in 13%, < 3rd %

Most patients discovered through routine hematologic tests or with symptoms of acute or chronic hemolytic anemia: pallor, fatigue, jaundice

Hb H Disease

Hemoglobin Levels

Hb H disease	Hemoglobin, g/L (n)	
	Male	Female
Deletional	111 ± 11 (28)	94 ± 12 (59)
	105 ± 10 (40)	88 ± 11 (47)
	106 ± 13 (10)	91 ± 9 (41)
	100 ± 12 (67)	
Nondeletional	105 ± 10 (6)	85 ± 7 (5)
	86 ± 19 (11)	83 ± 20 (14)
	96 ± 11 (64)	

Chui et al., Blood, 2003;101; 791

Hb H Disease

MCV and MCH Levels

MCV, fL (n)		MCH, pg (n)	
Male	Female	Male	Female
65 ± 7 (121)		19 ± 2 (120)	
63 ± 5 (40)	64 ± 6 (47)	19 ± 1 (40)	19 ± 2 (47)
62 ± 4 (10)	62 ± 5 (41)	19 ± 2 (10)	19 ± 2 (41)
67 ± 7 (56)		19 ± 2 (56)	
68 ± 6 (14)		19 ± 2 (14)	
73 ± 7 (11)	72 ± 7 (14)	21 ± 2 (11)	20 ± 2 (14)
77 ± 5 (47)		21 ± 2 (47)	

Chui et al., Blood, 2003;101; 791

Hemoglobin H Disease

Clinical Course

Wide clinical spectrum

- Acute anemia with febrile illness, oxidising agents
  - usually hemolytic, but also erythroid aplasia
- Splenomegaly common, hepatomegaly uncommon
- Iron overload, common in SEA uncommon in Mediterranean genotypes, not related to transfusion but increased absorption
- Cholelithiasis
- Anemia may worsen in pregnancy; pre-eclampsia, and CHF reported

Hemoglobin H Disease

Treatment

Primarily preventive and supportive

- Folic acid supplementation
- Education about signs of acute anemia, palpation of spleen
- Avoidance of oxidative medications
- No iron therapy, unless iron deficiency is documented
- Monitoring for iron overload; iron chelation therapy if needed
- Episodic red cell transfusion as needed for acute illness
- Chronic transfusion therapy
- Close monitoring of pregnant women
- Splenectomy for those with hypersplenism

Hemoglobin H Disease

Indications for Chronic Transfusion

1. Confirmation of diagnosis of severe thalassemia
2. Anemia: Hb levels persist at < 7 g/dL
3. Poor growth and weight gain
4. Bony changes
5. Extramedullary hematopoiesis

Hemoglobin H Disease

Screening Test in Neonates

Identification and Quantitation of Hb Bart's

HPLC of dried blood samples

- California experience
  - Hb Bart's > 25% for DNA-based analyses

Isoelectric focusing

- Quantitation of Bart's by scanning of gel, or,
- Quantitation of Bart's by HPLC

Other less common methods

Alpha Thalassemia

Levels of Hb Bart's and Hb H

Definition	Genotype	Hb Phenotype
1 gene deletion - silent carrier	– α/α α	Newborn: Bart's 1%–2%
2 gene deletion - a thalassemia trait	– α/– α or – –/α α	Newborn: Bart's 3%–10%
3 gene deletion - Hb H disease	– –/– α	Newborn: Bart's 20–30% Older child: Hb H 10%–15% Hb F 10%
Hb H + Constant Spring - 2 gene deletion and Constant Spring mutation	– –/α α^{cs}	Older child: Hb CS 2%– 3% Hb H 20%–40%
4 gene deletion - Hydrops Fetalis	– –/– –	Newborn: Bart's 80%–100%
Nondeletional mutation	α α/α $\alpha^{variant}$	~2% variant Hb

Hemoglobin H Disease

Confirmatory Tests

DNA-based Molecular Analyses

Several techniques required to identify specific lesions

- Southern blot with specific probes for ζ and α-globin genes
- PCR with deletion-specific primers for the 2-gene cis deletions
  - California: multiplex gap PCR genotyping for common deletion and for non-deletion CS mutation
- Non-deletion syndromes by gene amplification and direct sequencing of allele-specific dot-blot hybridization

Newborn Screening for Hemoglobinopathies in California

Hemoglobin Genotypes Confirmed Jan 1998 – Jun 2006 (1)		
Diagnosis	No.	Incidence per 100,000 screened
Sickle cell disease	688	15.2
Alpha thalassemia (Hb H) syndromes	502	11.1
Beta thal syndromes	79	1.8
Other mutations	862	19.1

Michlitsch et al, Pediatr Blood Cancer 2009;52:486–490

Hemoglobin H Disease

Nomination for Newborn Screening

1. Condition medically serious?	Yes; variable course
2. Pilot data available?	Yes; California
3. Clinical spectrum known	Yes; not easily predicted
4. Screening test specificity	False negative rate unclear
5. Severe cases easily identifiable?	Over time; anemia
6. Treatment	Symptomatic; most patients will not require medical intervention in infancy

Hemoglobin H Disease

Nomination for Newborn Screening

1. Current newborn screening labs are capable of measuring Hb Bart's level with little or no additional equipment.

2. State programs can include specific training for quantitation and reporting of Hb Bart's and referral of babies with elevated Bart's for DNA-based studies

3. Reference laboratory service for DNA-based tests

4. Education and counseling regarding α thalassemia