Hemoglobinopathies

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NORMAL HEMOGLOBINS

- Consist of 2 alpha chains and 2 non alpha chains
- Hb A = $\alpha_2\beta_2$
- Hb F= $\alpha_2\gamma_2$
- Hb A2 = $\alpha_2\delta_2$

Hemoglobin Variants

Altered the conformational dynamic of globin: globin interaction

Altered oxygen affinity

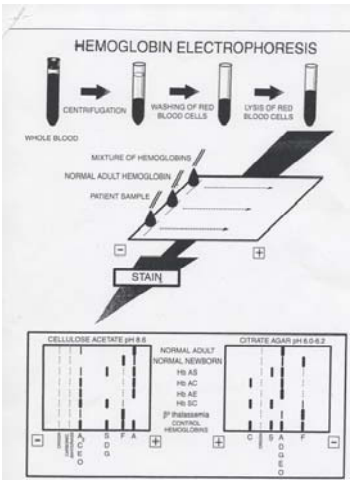
Altered the rheology and physiology of the red cell

Priniciple of Hemoglobin Electrophersis

- Hemolysate is prepared
- Globin is run on a gel

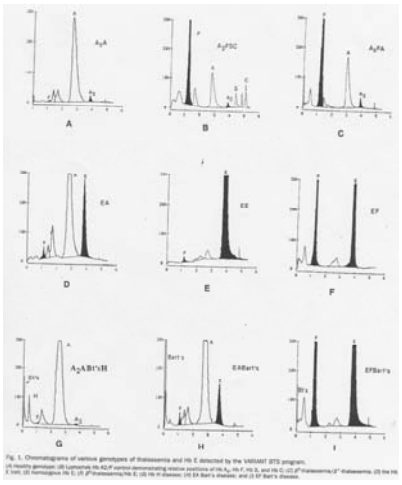
at acid and alkaline ph

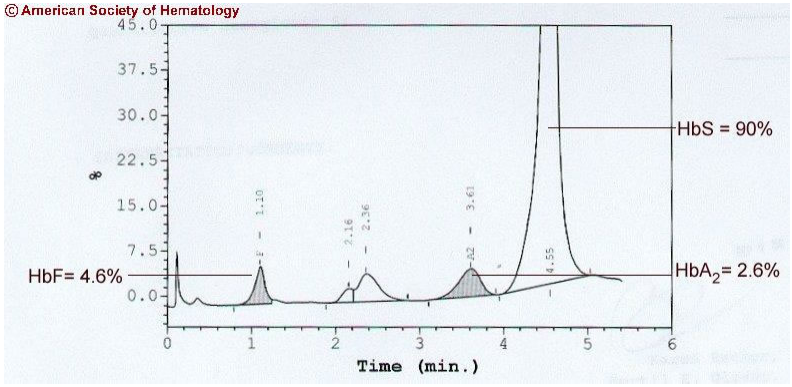
- Mobility base on charge



Abnormal Hb variant

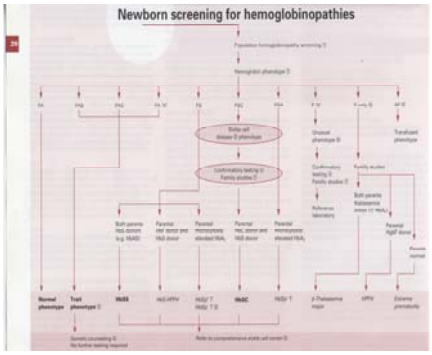
- Hemolysate run on HPLC column (cation)
- Molecule is split into heme and globin
- Globins are dissociated but not denatured
- Globins eluted based on mobility (charge difference)
- As Hb elutes it pass thru a photometer generate a chromatogram





New Born Screening

- New York State one of 43 states that test for hemoglobin variant as part of newborn screening
- NYS Newborn screening for sickle hemoglobin began in 1976



Molecular Genetics

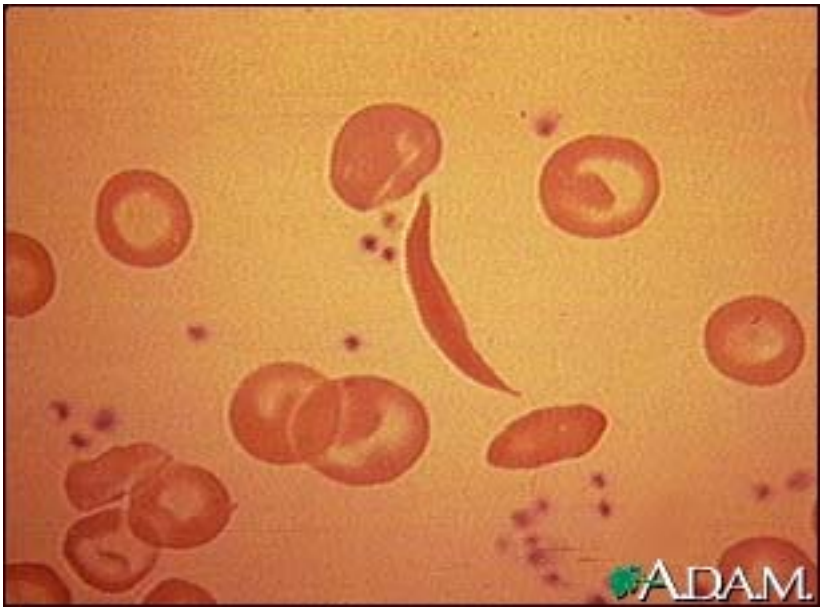
- SSD is due to the substitution of thymine for adenine in the glutamic acid DNA codon (GAG ↑GTG) resulting in valine substitutiion at the β6 position
- HbS = $\alpha_2\beta_2$ $^{(6glu\uparrow val)}$

Genetics of Sickle Cell Disease

- Autosomal recessive inheritance
- 8-10% African American (AA) carry the Hb S gene
- Gene arose as evolutionary selection for resistance to falciparum malaria
- Approximately 1in 600 AA are affected with SCD
- The offspring of two carriers has 1:4 chance being affected

Hb S

- Hb S tends to aggregates (valine)
- Deoxygenated Hb S in solution forms gel causes elongation of the cells and resulting in a distorted red cell
- Sickling is also influenced by amount and type of Hb in the cell
- 2,3 DPG



Clinical Laboratory SCD

- Chronic hemolytic anemia
- Steady state Hb 5-11gm/dl
- Normochromic anemia
- Reticulocytosis
- Normal MCHC
- Decrease amount of erythropoeitin relative to degree of anemia

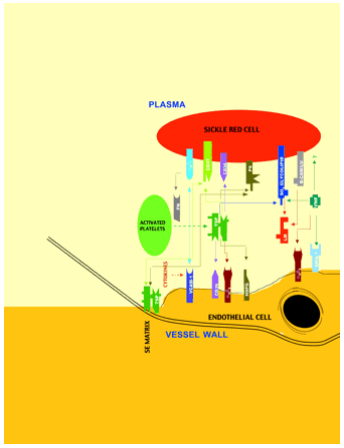
Table 1. Sickle Hemoglobinopathies: Neonatal Screening and Diagnostic Test Results.

Disorder	Neonatal screening results	Hematologic studies by 2 years			
		MCV[2]	Hb A_2[3] (%)	Hb F (%)	Hb F distribution
SS	FS	N or I	<3.6	<25	Heterocellular
SC	FSC	N or D	NA	<15	NA
S **beta**-thal	FSA or FS [7]	N or D	>3.6	<25	NA
S **beta**^{0}-thal	FS	D	>3.6	<25	Heterocellular
S delta/**beta**--thal	FS	D	<2.5	<25	Heterocellular
S-HPFH	FS	N or D	<2.5	<25	Pancellular

Hb = hemoglobin, thal = thalassemia, N = normal, I = increased, D= decreased

New Model of Vaso-occlusion

- Unlike normal red cells sickle cells adhere to the endothelium cells in vitro
- Membrane damage induces oxygen radical generation
- Increased expression of adhesion molecules



Role of Leukocytes

- Suggestion by clinical observation

-Severity of disease

-Increased leukocytes associated with increased mortality

-Increased silent infarcts

- Neutrophils binds sickle cell in vitro

Natural History of SCD

- FIRST DECADE
- Infection/Sepsis
- Cerebral infarct
- ACS
- Arthropathy
- Cholelithiasis
- Priapism
- Poor growth /Development

- SECOND DECADE
- Chronic organ damage
- Renal disease
- Leg ulcers
- Neurologic disease
- Sudden death

Functional asplenia

- The red pulp of the spleen is hypoxic and and acidotic and favors sickling .
- Sickle cells lack the deformability necessary for passage through the splenic sinus gaps.
- Sluggish flow, increased viscosity and low O2 and pH lead to chronic congestion of the red pulp and results in multiple micro-infarcts and hemorrhages.

Splenic Sequestration

- Occurs when there is rapid enlargement of the spleen accompanied by an acute fall in hemoglobin.
- The mechanism for the triggering of these episodes are not clear but be accompanied by viral illnesses
- 10-20% of children with sickle cell disease between the ages of 6 mons to 3 years will have at least one episode.

Stroke in Sickle Cell Disease:

- Affects as many as 15-25% of patients with SS disease (rare in Hgb SC)
- Hemorrhage or thrombosis
- Peak age for cerebral thrombosis and infarction is 7-10 years
- Subarachnoid hemorrhage more common in the second decade.

Patho-physiology of Stroke in Sickle Cell Disease:

- Intimal and medial hyperplasia and proliferation secondary to damaged endothelial from sickle cells.
- Narrowing or complete occlusion of the cerebral vessels; anterior and and/or middle cerebral arteries.
- Multi-vessel involvement is common even in patients will unilateral findings.

Transcranial Doppler (TCD) in SCD

- A measure of cerebral blood flood velocity
- Abnormal TCD predicts increased risk of stroke in SCD.
- Velocity in excess of 200cm/sec is associated with increased risk of stroke.

Prevention of Stroke in SCD

- STOP I (Stroke Prevention) Study :A randomized clinical trial of patients with abnormal TCD, compared chronic transfusion to reduce Hb S level $\leq$ 30% to a non transfusion arm.
- There were 92% fewer strokes in the transfusion arm compared to the non transfused arm.

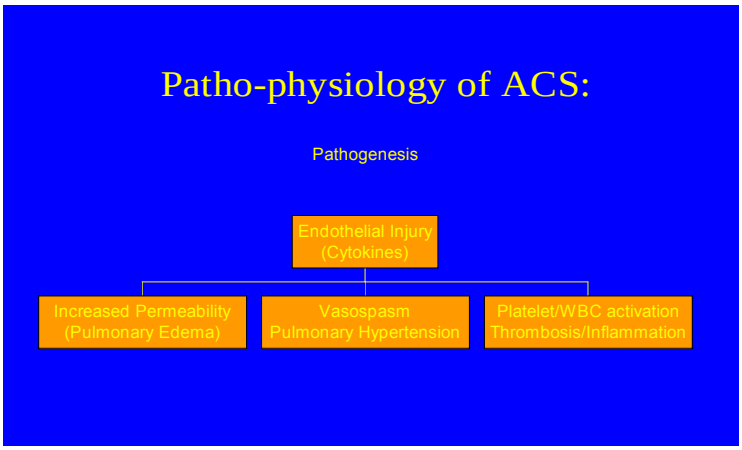
Prevention of Stroke in SCD

STOP II

- Patients with abnormal TCD who received transfusion therapy for 36 months randomized to continue or discontinue Tx after 36 months.
- Study was halted early because of increase stroke in discontinued Tx arm.

Acute Chest Syndrome

- Pulmonary disease (ACS) is the second most common reason for admission to the hospital in patients with SCD.
- It accounts for 25-40% of premature deaths in SCD.



Transfusion for SCD

- **Goals**

-Improve anemia and oxygen carrying capacity

-Reduce or prevent the occurrence of the complications

-Reduce Hb S levels

- **Methods**

Episodic

Chronic

Exchange

Indications for Episodic Transfusion

- ACS
- Surgery
- Erythroid aplasia
- Splenic/ Hepatic sequestration
- Intractable Pain
- Priaprism

Indications: Chronic Transfusion

- Primary Stroke Prevention
- Prevention of recurrent stroke
- Intractable pain
- Symptomatic anemia
- Pulmonary Hypertension/Sickle Cell Chronic Lung
- Recurrent Splenic Sequestration

Exchange Transfusion

- **Advantages**

Fast : allows emergent intervention

Eliminates risks of increase in viscosity and volume

Decreased iron loading compared to simple transfusion

- **Major role**

Treatement/prevention of life threatening events

Complications of Transfusion

- Transfusion reactions
- Iron Overload
- Alloimmunization
- Hypersplenism
- Hyperviscosity
- Transfusion transmitted infections

Pharmacological Agents that Stimulate Fetal Hemoglobin Production

- 5-Azacytidine
- Hydroxyurea
- Butyrate
- Erythropoietin
- Combinations

Why is Fetal Hemoglobin Beneficial in Sickle Cell Disease

- **Hb F interferes with polymerization of Hb S in vitro.**
- **Infants with SCD do not have complications during the first 6 months of life.**
- **SCD patients from Saudi Arabia and India with high Hb F levels have milder clinical disease.**
- **CSSCD showed an inverse correlation between Hb F levels and clinical severity**

Hydroxyurea

- Hydroxyurea (HU) is widely used in the treatment of sickle cell disease (SCD) in the US and abroad.
- The Multi-center Hydroxyurea Study (MSH), a randomized clinical trials demonstrated a reduction in frequency and severity of ACS and vaso-occlusive crisis by 30% and 50% respectively.
- Clinical benefit in the HU treated patients correlated with cyto-reductive effects

MSH follow-up Study

299 of the 500 patients enrolled in the original MSH study

9 year follow- up period

- -40% decrease in mortality in patients treated with HU for at least 3 yrs
- -28% of all deaths were pulmonary

PHT : Risk Factor of death in SCD

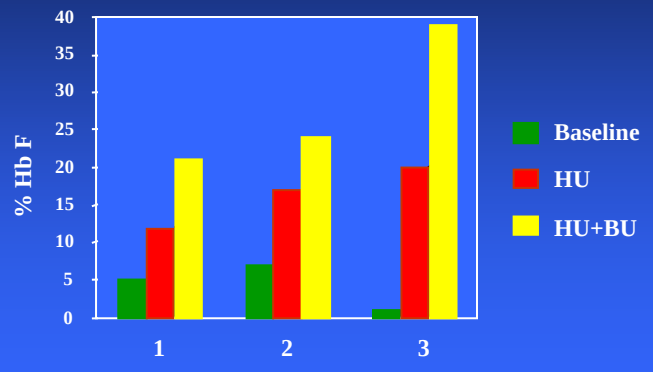
Gladwin et al NEJM 350:9 2004

-Prevalent in 32 % adult pts with SCD

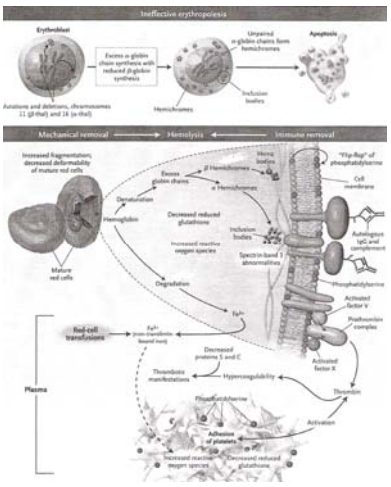
-Development of PHT in SCD associated with anemia, chronic hemolysis and either existing cardiac or renal disease

- Hb F levels or HU therapy did not appear to have protective effect.

Summary of HU + BU Combination Therapy



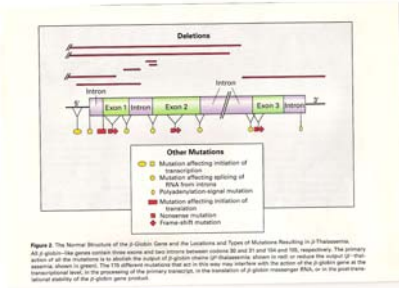
Patho-physiology Thalassemia



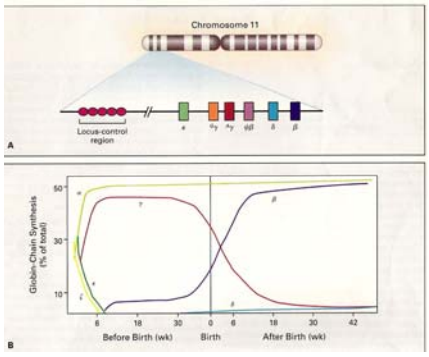
- Disease results from and imbalance in the synthesis of alpha and beta globin chains

Molecular Genetics of Thalassemia

- There are over 200+ different thalassemia mutations.
- Beta thalassemia from a single base substitutior



Beta Globin Gene Expression



- Genes arranged in the order they are expressed during development
- Developmental switching of human globin

Clinical Manifestation

- When Hb F levels fall which are normal offset by increased Hb A levels

Findings:

Extra-medullary hematopoeisis

Hemolysis

Hyperbilirubinemia

Congestive heart failure.

Types of Beta Thalassemias

Type	Hb levels	Clinical Features
$\beta^0\beta^0$ Thal Major	Hb F 100%	Tx dependent
$\beta^+ \beta^0$ Thal intermedia	Hb F 40-80%	+/- Tx
$\beta^+ \beta^+$ Thal intermedia	Hb F 30-95%	+/- Tx

Complications of Disease

- Bone expansion
- Short stature
- Osteoprosis/Arthopathy
- Delayed puberty
- Endocrinopathy/Hypopituitary

-IDDM, Hypothyrodism, Hypo

-Testicular and ovarian failure

- Cardiomyopathy
- Pulmonary hypertension

Therapy

Transfusion:

Mainstay of the therapy to correct the anemia.

Suppress the ineffective erythropoiesis

Prevent excessive marrow expansion

When to transfuse ?

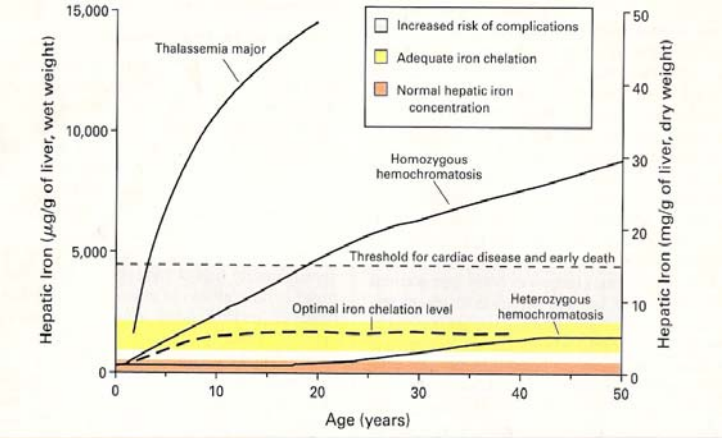
- Thal intermedia Hb levels 6-9 gm/dl
- Thal Major Hb levels 3-4 gm/dl

Hb Level that are insufficient and that lead to cardiovascular compromise, impaired linear growth and excessive marrow expansion.

Transfusion therapy

- 10-20 cc/kg every 4-6 weeks to maiantin Hb in 9.5-11 gm/dl range.
- Suppression of the erythropoeitic drive
- Decrease GI iron absorption, less bone demineralization and
- Splenectomy can reduce transfusion requirements.

Iron Balance in Thalassemia



Other Therapies for Thalassemia

- Erythropoetin
- Fetal Hb Augmentation
- Antioxidant

Curative: BMT/ HPSC

Support Therapies

- Chelation Therapy

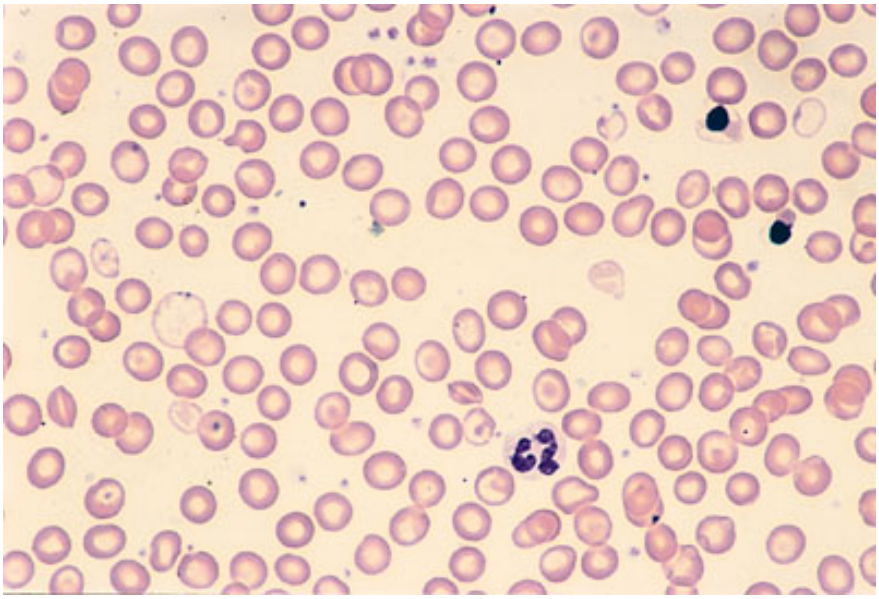
Subcutaneous Desferoxamine

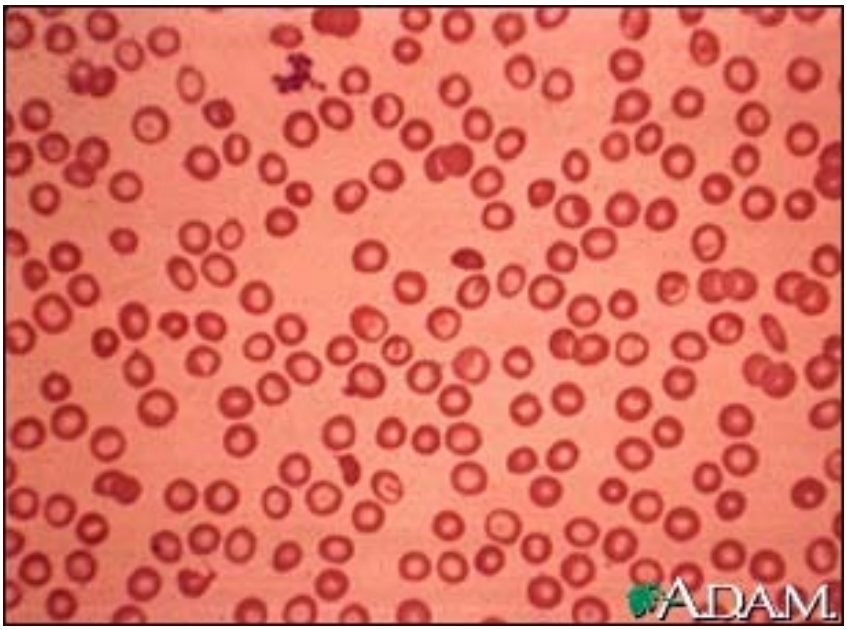
Deferipone, L1

Defarasirox (oral chelator)

- Osteoclast repelacment therapy bisphosphonates and Vitamin D

- Hormonal repalcement





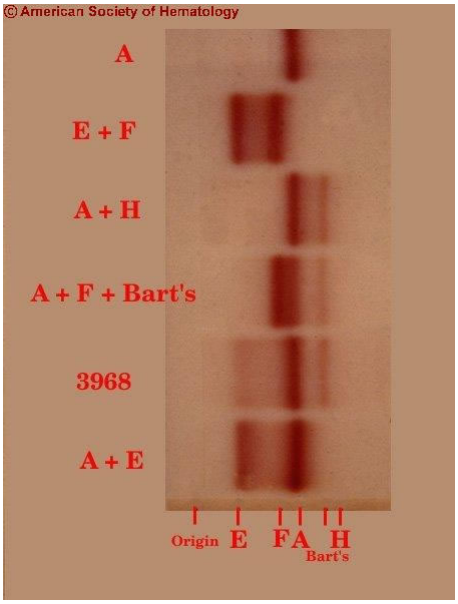
α-Thalassemia Syndromes

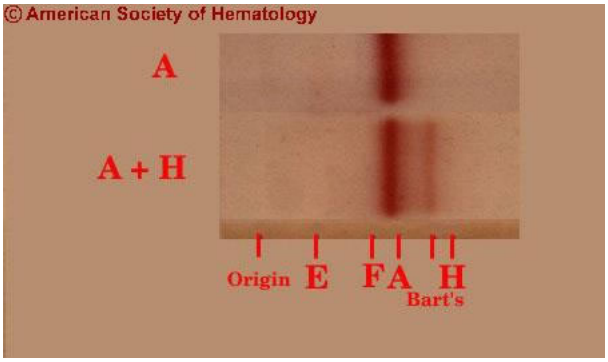
α Gene Map	α Genotype	α Clinical Syndrome
	Normal	Normal
	Heterozygous α - Thal - 2 (α^+ also called)	Silent Carrier of α Thalassemia
	Heterozygous α - Thal - 1 (α^o also called)	α - Thalassemia Trait
	Homozygous α - Thal - 2 (α^+ also called homozygous)	α - Thalassemia Trait
	Compound Heterozygous α - Thal - 1 & 2 (α^+/α^o also called)	Hb - H Disease
	Homozygous α - Thal - 1	Hydrops Fetalis

The interaction of Hb E and α thalassemia results in a variety of genotypes (see table below) presence of α thalassemia reduces the amount of Hb E usually found in Hb E heterozygotes.

Some interactions of hemoglobin E with α thalassemia [2]

Genotype	Clinical findings	Hemoglobin
$\alpha\alpha/\alpha\alpha\ \beta^A\beta^E$	Normal Red cells slightly hypochromic	A + E Hb E 25-30%
$-\alpha/\alpha\alpha\ \beta^A\beta^E$	Normal Hypochromic red cells	A + E Hb E 20-25%
$--/\alpha\alpha\ \beta^A\beta^E$	Normal Hypochromic red cells	A + E Hb E 17-20%
$--/-\alpha\alpha\ \beta^A\beta^E$	Hb E/Hb H disease *(see below)*	A + E + Bart's Hb E about 14%
$-\alpha/\alpha\alpha\ \beta^E\beta^E$	As for homozygous Hb E (mild anemia)	E + trace Bart's
$--/-\alpha\ \beta^E\beta^E$	Severe thalassemia intermedia	E + F + Bart's Hb E 80%, HbF 13%

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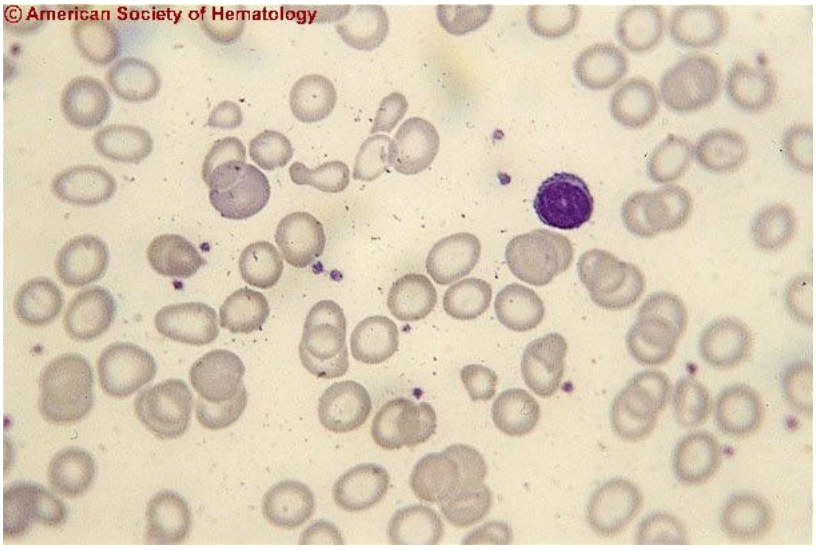
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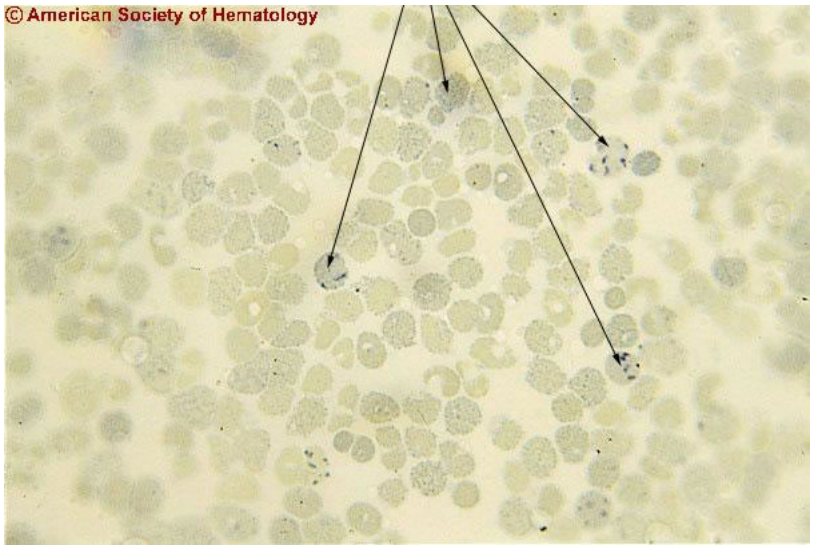
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