Sinlge gene disorders may be classified into four categories:

a. Defects in membrane receptors and transport systems

b. Enzyme defects and their consequences

c. Alterations in the structure, function, or quantity of nonenzyme proteins

d. Mutation resulting in unusual reactions to drugs.

Molecular Pathology

Alterations in the structure, function,
or quantity of nonenzyme proteins

THE HEMOGLOBINOPATHIES

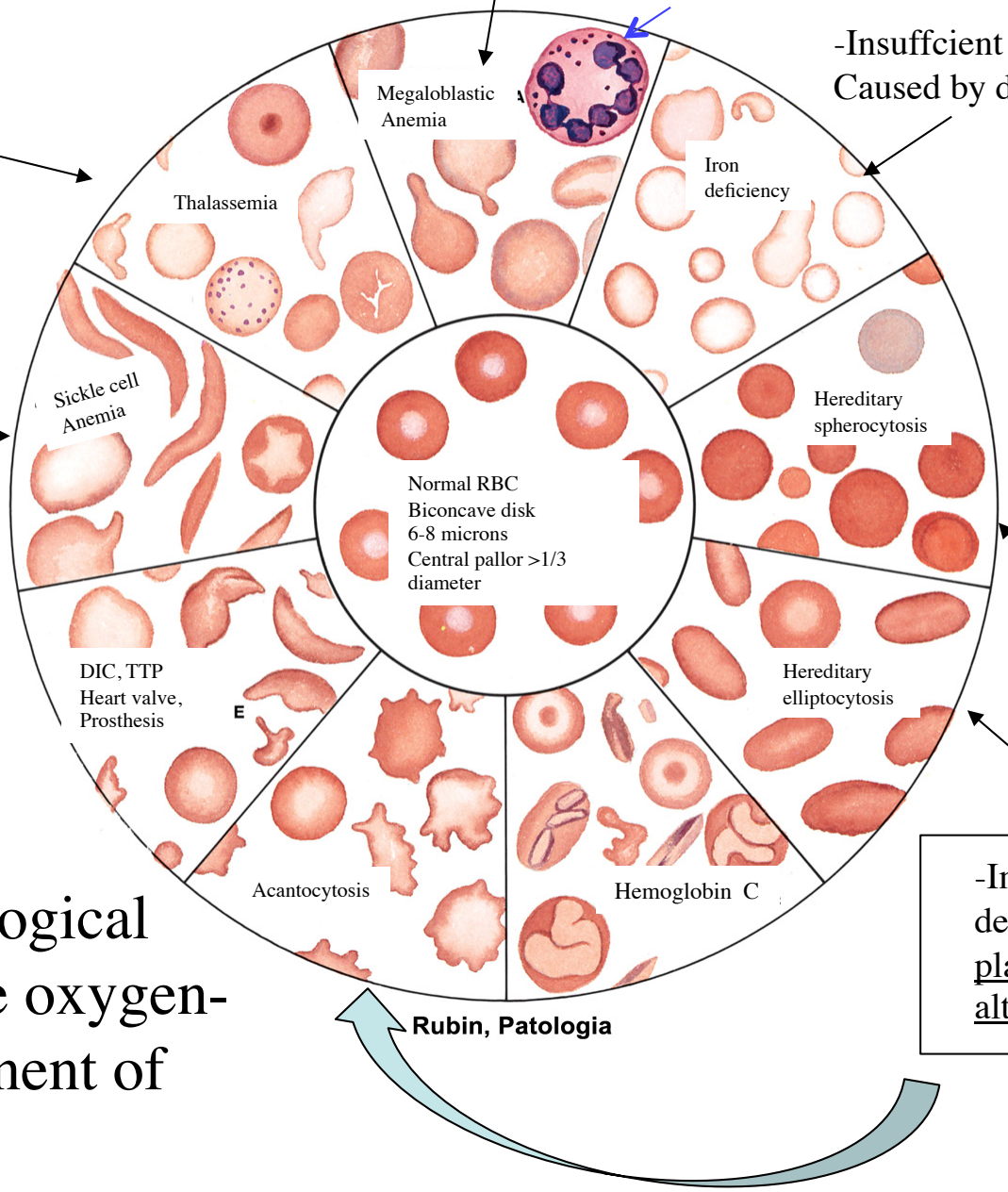
MODELS OF MOLECULAR DISEASE

-Insuffcient production of erythrocytes
Caused by B12 deficit

-Insuffcient production of erythrocytes
Caused by defective HEME synthesis

-Insuffcient production of erythrocytes
Caused by defective globin synthesis

-Increased Erythrocytes destruction caused by structurally abnormal globins



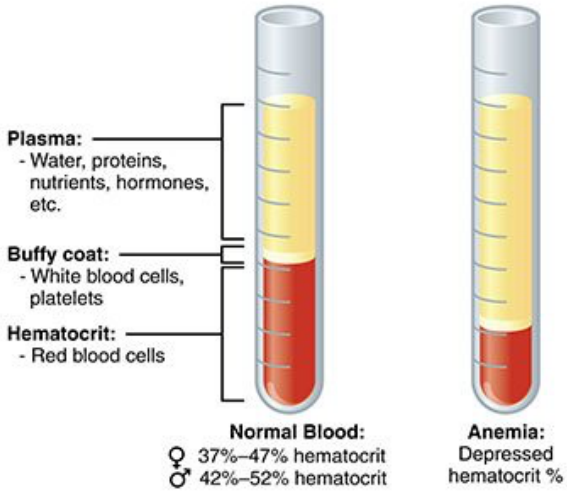
Poikilocytes: abnormally shaped red blood cells

-Increased Erythrocytes destruction caused by plasmamembrane alterations(cytoskeleton)

Anemia: Pathological deficiency in the oxygen-carrying component of the blood.

Adult reference ranges for Red Blood Cells

Unit	Men	Women
Hemoglobin(gr/dl)	13.6-17.2	12.0-15.0
Hematocrit(%)	39-49	33-43
Red Cell count(x10^6/ul)	4.3-5.9	3.5-5.0
Reticulocyte count(%)	0.5-1.5	



Hemoglobinopaties

The disorders of the human hemoglobins

The most common genetic disease
(5% of the world population are carriers)

Substantial morbidity

Pathogenetic mechanism knwon at the molecular level

Structural mutations of hemoglobins

Altered globin gene expression (chains α and β)

FUNCTION OF HEMOGLOBIN

Oxygen carrier in vertebrate red blood cells
(6 isoforms)

HEMOGLOBIN STRUCTURE

Tetrameric molecule with a globular structure

Consists of two each of two different subunits

SUBUNIT

1 polypeptide chain: GLOBIN

1 prosthetic group: HEME

THE HEMOGLOBINOPATHIES

1. STRUCTURAL VARIANTS:	alteration of the globin structure, without affecting its rate of synthesis
2. THALASSEMIAS:	decreased synthesis of one or more of the globin chains ---> inbalance of the relative amounts (α : β)
3. HPFH	Hereditary persistence of fetal hemoglobin

1. STRUCTURAL VARIANTS

Point Mutations
More Complex Mutations of one of the structural gene

A. Variants that cause HEMOLYTIC ANEMIA
alteration of the physical properties of Hb

- HbS
- HbC

Unstable Hemoglobins
- Hb Hammersmith

B. Mutants with ALTERED OXYGEN TRANSPORT

- Hb M
- Hb Kempsey
- Hb Kansas

C. Variants with a THALASSEMIA PHENOTYPE

- Hb E
- Hb Lepore

Hemolytic Anemias:

- Premature Red Blood Cell (RBC) destruction
- Accumulation of products of the Hemoglobin catabolism
- Elevated erythropoiesis as a compensative mechanism to reduce RBC loss

Extravascular Hemolysis

Anemia

Splenomegaly

Jaundice

Intravascular Hemolysis

HEMOGLOBIN WITH NOVEL PHYSICAL PROPERTIES

HEMOGLOBIN S (Hb S)

Sickle cell disease (80-95% HbS)
recessive transmission
impaired growth and development

Clinical features (Homozigosity)
- Hemolytic anemia (18-30%)(6-9gr/dl)
- Hepato-splenomegaly
- recurrent infections causative of aplastic crisis
- “Hand-foot” syndrome

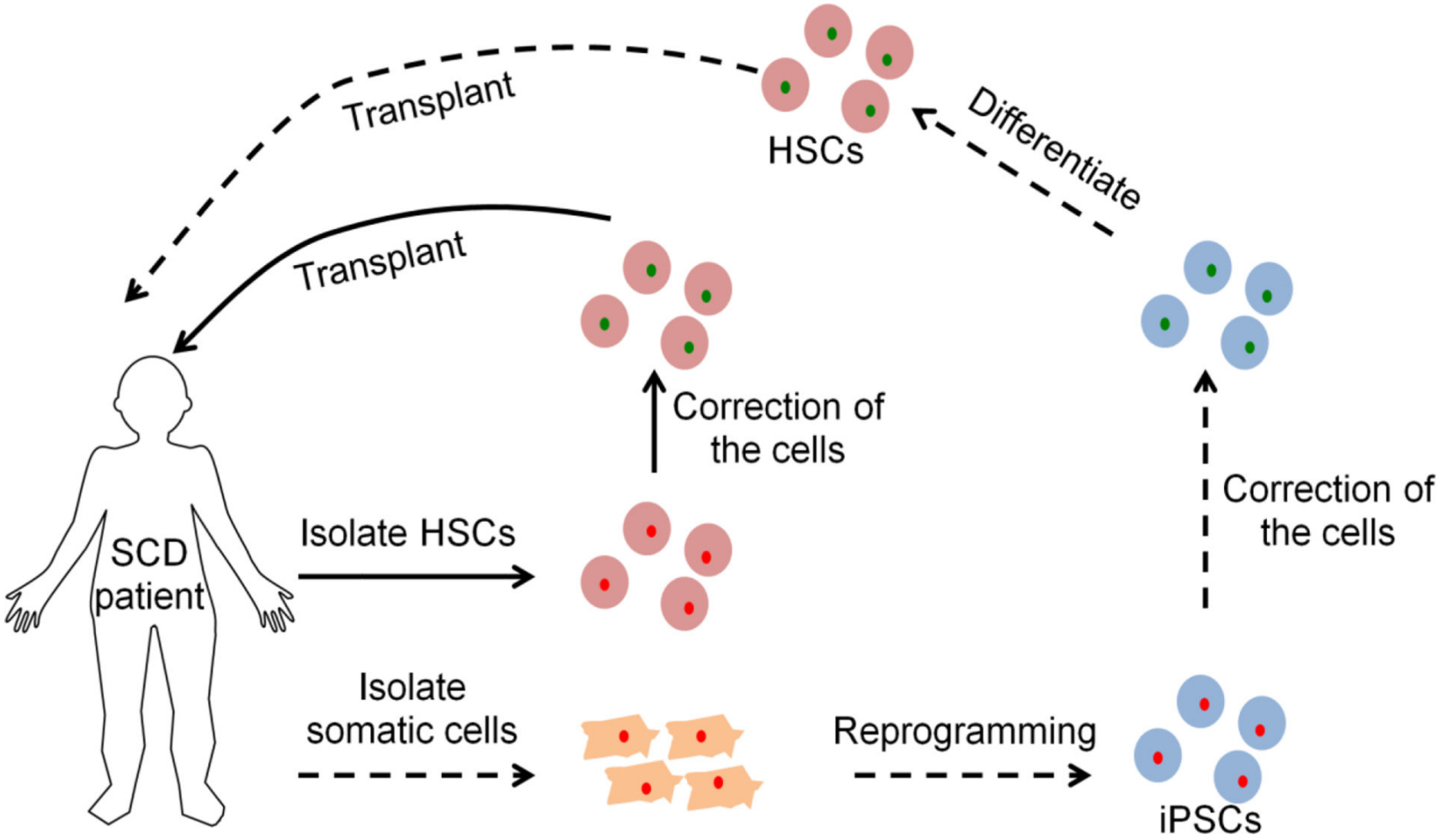
Heterozigosity: risk in particular conditions (HbS-HbA)
(sickle cell trait)

Geographical distribution
High frequency in equatorial Africa
(selective advantage by malaria)

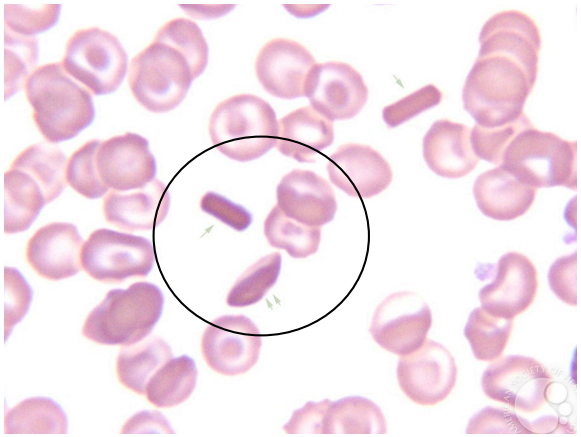
Nobel Prize for Chemistry 2020 for the development of the CRISPR/Cas9 genome-editing system

Emanuelle Charpentier and Jennifer A. Doudna

Genome-editing tools to threat sickle cell anemia



OTHER MUTATIONS OF THE MOLECULE SURFACE



HEMOGLOBIN C (Hb C)

Milder Hemolitic disorder

MOLECULAR BASIS :

Point mutation codon 6 Glu ---> Lys (non polar)

↓

solubiliity ----> crystallization

reduced deformability of eritrocytes

Hb SC: milder hemolitic anemia than Sickle cell anemia

vaso-occlusive complications

retinopathy

UNSTABLE HEMOGLOBIN

Denaturated Hb --→ Heinz bodies

HAMMERSMITH HEMOGLOBIN
Autosomal dominant

CLINICAL FEATURES:

severe hemolytic anemia

cyanosis:

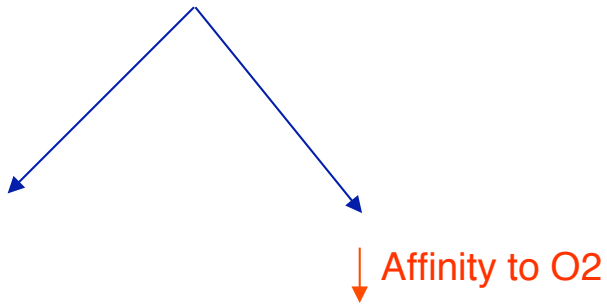
Molecular Basis:

point mutations codon 42 β globin

Subst. Phe ----> Ser

Phe42 residue CD1

Alteration of the HEME pocket



Instability of Hb

↓ Affinity to O2

VARIANTS WITH ALTERED OXYGEN TRANSPORT

METHEMOGLOBINS (Hb M)

Autosomal dominant

OXYHEMOGLOBIN ⇌ DESOXYHEMOGLOBIN

Fe +2 reduced status (ferrous)

METHEMOGLOBIN (do not bind O2)

Fe +3 oxydated status (ferric)

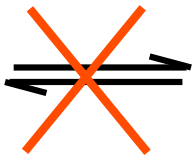
Spontaneously

Methemoglobin reductase

HEMOGLOBINS WITH ALTERED O2 AFFINITY

Interface α1 β2: **significant movemet between the chains**

+ O2
Relaxed state



- O2
Tense state

Mutations
interface α1 β2

Examples:

Hb Kempsey

Hb Kansas

Inverse molecular effects and clinical outcomes

Hb Kempsey

β 99 Asp ----> Asn ---> Relaxed

↑ affinity for O2 -----> ↓02 in tissues

Polycithemia

Hb Kansas

β 102 Asn ----> Thr ---> Tense

↓ affinity for O2

Asymptomatic, cyanosis

VARIANT HEMOGLOBIN WITH THALASSEMIA PHENOTYPE

Hemoglobin E (Hb E)

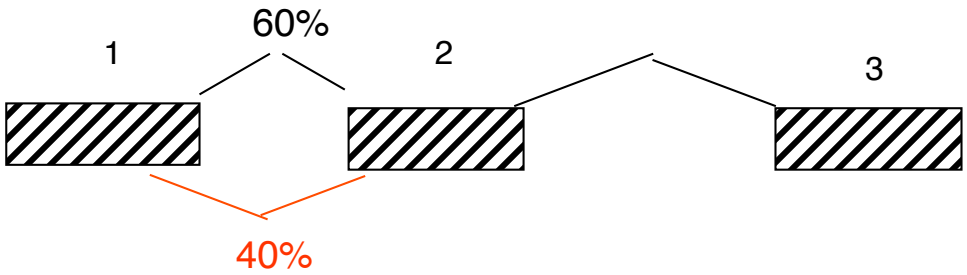
autosomal recessive

High frequency

compound heterozygosity with β thalassemia

mutazion β 26 Glu ---> Lys

alternative splicing



Homogosity: asinptomatic or mild anemia

THALASSEMIA

The most common monogenic disorder

Reduced synthesis of globins α o β

Altered ratio $\alpha : \beta$

Relative excess of globin
Precipitation
HEMOLYTIC ANEMIA

Reduced Hb
HYPOCHROMIC ANEMIA
MICROCYTIC

α-THALASSEMIA

β-THALASSEMIA

Geographic distribution:

MEDITERRANEAN
MIDDLE EAST AFRICA
INDIA
ASIA

Heterozygos selective advantage against MALARIA

Caused by red blod cells alterations,

That impedes the normal reproductive cycle

ALPHA - THALASSEMIA

Defects of fetal and adult Hb

Hb H = β4

HB Barth = γ4

Clinical conditions	α functional genes	Genotype	α Chain
normal	4	αα/αα	100%
Silent carrier	3	αα/α−	75%
α-thalassemic trait (mild anemia , microcytosis)	2	αα/−− α−/α−	50%
Hb H (moderately severe hemoliyticca anemia,)	1	α−/−−	25%
Homozygous α-talassemia (Hydrops fetalis Hb Bart)	0	−−/−−	0%

−−/αα frequent in south-east Asia ----> HOMOZIGOUS

Condition	Hemoglobin A, %	Hemoglobin H (β4), %	Hemoglobin Level, g/L (g/dL)	MCV, fL
Normal	97	0	150 (15)	90
Silent thalassemia: –α/αα	98–100	0	150 (15)	90
Thalassemia trait:–α/–α homozygous α-thal-2[a]	85–95	Rare red blood cell inclusions	120–130 (12–13)	70–80
or				
––/αα heterozygous α-thal-1[a]				
Hemoglobin H disease: ––/–α heterozygous α-thal-1/α-thal-2	70–95	5–30	60–100 (6–10)	60–70
Hydrops fetalis: ––/–– homozygous α-thal-1	0	5–10[b]	Fatal in utero or at birth	

FETAL HYDROPS

Accumulation of edema fluid in the fetus during intrauterine growth.

Immune

Rh blood group incompatibility between mother and fetus.
Antigen D prophylaxis.

Non Immune

Three major causes: cardiovascular defects, chromosomal anomalies, and fetal anemia.

THE BETA - THALASSEMIAS

Defects of adult Hemoglobin

Post-natal pathology (before the age of 2 years)

reduced β ------> Hypocromic anemia
microcytic anemia
altered ratio α : β

Relative excess α ------> peripheral hemolysis
ineffective erythropiesis

α2 δ2 Hb A2 increased (3.3-7%)

α2 γ2 Hb F increased (1-5%) → **Selective Survival of erythrocytes**

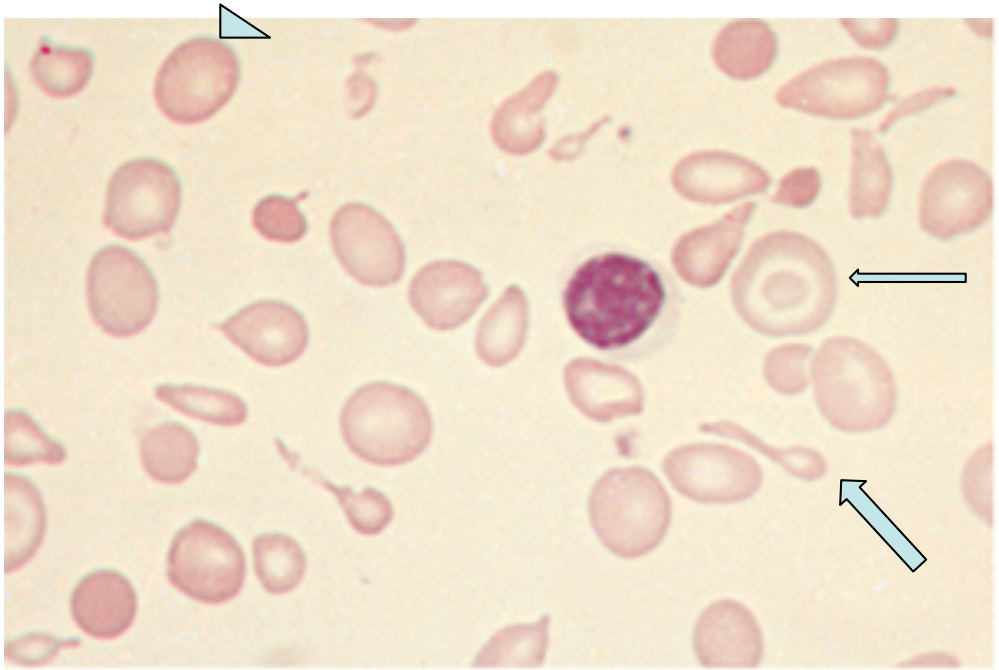
Great genetic variability of alleles

homozygotes

compound heterozygotes (+ frequent) } **TALASSEMIA MAJOR**

heterozygotes **TALASSEMIA MINOR**
Mild anemia
Microcytic anemia
Hypocromic red cells

β Thalassemia intermedia.



Molecular mechanisms:

- Promoter mutations
- α−Thalassemia coinheritance
- Coinheritance of genetic determinants increasing γ-chain synthesis

β –THALASSEMIA MOLECULAR BASIS

DELETIONS (not frequent)

SIMPLE THALASSEMIA

Hb Lepore

deletion 619 bp Asiatic Indians

COMPLEX THALASSEMIA

Great deletions of clustered genes

TALASSEMIA $\delta\beta^0$

TALASSEMIA $\gamma\delta\beta^0$

} severe

deletion $\delta\beta$ → $\delta\beta^0$ (17 % Hb F)

→ HPFH Fetal Persistence of Hemoglobin
Benign state($\alpha 2\ \gamma 2$)

Absence of postnatal switch
Hb F persist and compensate Hb A

HPFH no deletion
Promoter mutations of γ gene (CAAT Box)
Clinically normal

Splice Junction Mutations

1. INTRON/EXON JUNCTION (β^0)

Intron 2 Exon 3

CAG / CTC----------CGG / CTC

Acceptor

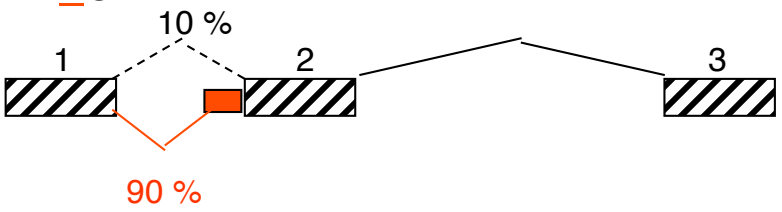
Use of cryptc splice sites (intron 2)

Longer Protein

2. NEW SPLICING SITE (β^+)

Intron I

GG ------> AG



Altered Protein +19 nucleotides --> stop

3. Hb E (β^+, β^E)

60% Hb A 40% Hb E

I. Structural hemoglobinopathies—hemoglobins with altered amino acid sequences that result in deranged function or altered physical or chemical properties
    A. Abnormal hemoglobin polymerization—HbS, hemoglobin sickling
    B. Altered O_2 affinity
        1. High affinity—polycythemia
        2. Low affinity—cyanosis, pseudoanemia
    C. Hemoglobins that oxidize readily
        1. Unstable hemoglobins—hemolytic anemia, jaundice
        2. M hemoglobins—methemoglobinemia, cyanosis

II. Thalassemias—defective biosynthesis of globin chains
    A. α Thalassemias
    B. β Thalassemias
    C. δβ, γδβ, αβ Thalassemias

III. Thalassemic hemoglobin variants—structurally abnormal Hb associated with coinherited thalassemic phenotype
    A. HbE
    B. Hb Constant Spring
    C. Hb Lepore

IV. Hereditary persistence of fetal hemoglobin—persistence of high levels of HbF into adult life

V. Acquired hemoglobinopathies
    A. Methemoglobin due to toxic exposures
    B. Sulfhemoglobin due to toxic exposures
    C. Carboxyhemoglobin
    D. HbH in erythroleukemia
    E. Elevated HbF in states of erythroid stress and bone marrow dysplasia