



**12th International Conference on Thalassemia
and the Hemoglobinopathies
Antalya-Turkey**

**The MOLECULAR BASIS
of β -THALASSEMIA
in TURKEY**

Boğaziçi University Experience

A. Nazlı Başak

*Boğaziçi University
Department of Molecular Biology and Genetics
NeuroDegeneration Research Laboratory*



Inherited Disorders of Hemoglobin

- Most common/oldest monogenic disorders worldwide
- Two broad groups:
 - - **Structural variants**: alter the globin polypeptide without affecting its rate of synthesis.
 - - **Thalasseмии**: result from an array of molecular defects that reduce or completely abolish the synthesis of hemoglobin chains.



Mutations in the globin genes provide...



- Selective protection /heterozygote advantage against *P. falciparum*
- Malaria-endemic regions => High frequency of Hb-pathies ↑
- Worldwide gene frequency: 5.1% (~300 mio carriers)
- 300 000 affected children born annually (all Hbpathies)

Thalassemias...

- Quantitative disorders of Hb synthesis
- The most common AR diseases worldwide
- Lack of α - or β -globin chains in erythrocytes



α/β -chain imbalance !



α -Thalassemia



β -Thalassemia

(80 mio carriers)

Beta-Thalassemia

β -thalassemia is the result of deficient/absent β -chain synthesis, resulting in reduced Hb in RBCs.

It includes 3 main forms:

- Thal Major: Cooley's Anemia, Medit Anemia
- Thal Intermedia
- Thal Minor: Beta Thal trait/carrier

β -Thalassemia: History

- 1925: Thomas Cooley & Pearl Lee
- Children of Italian origin
- Form of severe anemia
- Splenomegaly & “peculiar bone changes”
- Majority of early cases of Mediterranean origin

- *thalassa* + *haima* \Rightarrow *Thalassemia*



β -Thalassemia: High Prevalence

A world map with a dark blue background and light blue landmasses. The map highlights several regions with a yellowish-green glow, indicating high prevalence of beta-thalassemia. These regions include the Mediterranean Basin, North Africa, the Middle East, the Indian Subcontinent and Burma, and Central and Southeast Asia (including China, Malaysia, and Indonesia).

- **Mediterranean Basin**
- **North Africa**
- **Middle East**
- **Indian Subcontinent & Burma**
- **Central and Southeast Asia: China, Malaysia, Indonesia**

1.5 % of the world population...

- 80-90 mio people are **beta-thal carriers**
- **60.000** symptomatic individuals born /year
- Annual incidence of patients 1/100.000
- Beta thal major patients come to medical attention within the first 2 years of life and require regular RBC transfusions to survive.
- Acc to Thal Int Federation **200.000** patients with beta thal major are registered only.

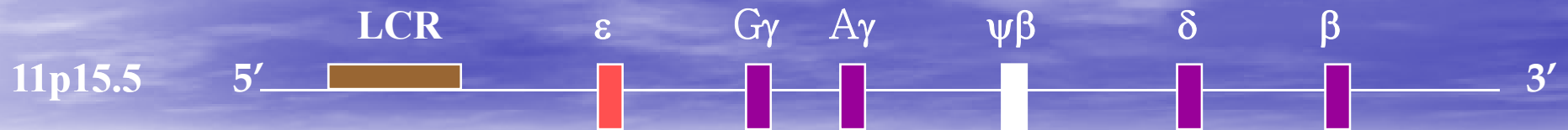


β -Thalassemia: Phenotype

extent of α /non- α chain imbalance

- the main pathophysiological determinant of the severity of β -thalassemia

Human β -Globin Gene Cluster



- **Location:** short arm of Chr. 11p15.5
- **Size:** 60 kb: 5 functional genes & a pseudo gene
- **Gene Arrangement:** the 5 functional genes are in chronological order of expression
- **Regulation:** 5' promoter with TATA, CAAT, CACCC
- **LCR:** 20 kb 5' of ϵ -globin gene, contains four erythroid-lineage-specific and one ubiquitous DNaseI hypersensitive sites and a strong enhancer.

β -Globin Gene



- **Size: 1.6 kb**
- **Composed of: 3 exons, 2 introns**

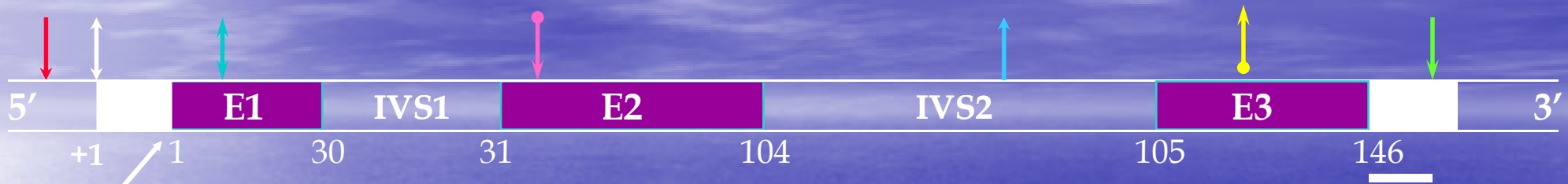
5' and 3' regulatory sequences (UTRs)

β -Thalasseмииas: very heterogeneous at molecular level!

- **> 200** disease-causing mutations
a variety of mechanisms !
- Mutations can be grouped acc. to the mechanism, by which they inactivate β -gene expression.



Mutations inactivate beta-globin gene expression by different mechanisms



- ↓ Transcription
- ↑ RNA Processing
- ↕ Cap Site +1 (A→C)
- ↓ RNA Cleavage
- ↗ Initiator codon ATG→ AGG, ACG, GTG
- ↕ Frameshift
- ↓ Nonsense Codon
- ↑ Unstable Globin
- Small Deletion

- Single nt subst, small in/del common: 180
- gross gene deletions rare: 13

Point mutations in beta-thal belong to three categories:

- Promoter and 5'UTR mutations lead to defective **beta-gene transcription**
- Splice-junction, cons. sequence, polyA and 3'UTR mts affect **mRNA processing**
- Nonsense, frameshift and initiation codon mts result in abnormal **mRNA translation**



The allelic heterogeneity at the β -globin locus results in....

- Two phenotypes:

- β^0 -thalassemia: mutations in exons, exon/intron junctions & large deletions

\Rightarrow no β -globin chain synthesis

- β^+ -thalassemia: mutations in introns and regulatory regions

\Rightarrow reduced β -globin chain synthesis.

Phenotypical variation may also derive from genetic factors OUTSIDE the β -globin gene/cluster

The manifestation of β -thal can be modulated by several factors:

- The nature of the mutation
- Co-inheritance of α -thal
 - ➔ reduction in α/β imbalance
- Co-inheritance of genetic factors
 - ➔ increasing γ -chain production
 - γ -chains will combine with excess α -chains
 - ➔ favourably influencing HbF.
- **Modifier genes:** iron absorption, bilirubin and bone metabolism, infection susceptibility

β -Thalassemia: Molecular Heterogeneity

great phenotypical variation



marked molecular heterogeneity

***Despite marked molecular heterogeneity:
molecular defects are limited in at-risk groups....***

- Heterogeneity is simplified
=> mutations are ethnic-group specific
- Thumb rule: **4-8 mts** in a given population
=> account for **90-95%** of β -globin genes

Preventive Programs

- Treatment of β -thal is still unsatisfactory
- Most important approach to its control is

Prenatal Diagnosis !



Implementation of Preventive Programs in Beta-thalassemia

Prenatal diagnosis programs



determination of common mutations
in a given population



Preventive Programs rely on:

- Mass education
- Heterozygote detection (carrier screening)
- Counselling
- Fetal diagnosis

➔ Very effective in reducing the birth rate of β -thal major

➔ Such programs are ongoing in several at-risk areas

Fetal Diagnosis

In the last 20 years the methodologies used in fetal diagnosis improved dramatically.

- Introduction of 1st trimester fetal sampling
- Development of procedures for mutation detection by DNA analysis
- Molecular definition of β -thal in at-risk populations



Prenatal diagnosis is routinely applicable



Fetal Diagnosis: DNA Analysis

The advent of PCR ,
combined with of ASO probes/ sequencing

has offered a variety of new approaches
for facilitating the speed and accuracy
of carrier detection and fetal diagnosis

High-Tech Methods

**Despite the apparent
“*high technology*”
of some of these approaches,
they have been established successfully
in many at-risk populations.**

β -Thalassemia in Turkey

- In the broad group of Mediterraneans:
~35 mutations described
- Allele frequencies vary among countries

What is the situation in Turkey?



β -Thalassemia in Turkey

- Major public health concern
 - Gene frequency: 2.1-10% (Thrace, Muğla, Antalya)
 - Many affected births:
 - *High birth rate (36:1000)
 - *Consanguineous marriages (21%)
- => Unlike other Mediterranean countries**
Turkey is very heterogenous at clinical level

Prenatal Diagnosis

**design and implementation
of
prenatal diagnosis strategies**



**molecular basis of β -thalassemia
in Turkey**



Turkey: Boğaziçi University Experience (1987-present)

>1500 patients with β -thal major
(>3100 chromosomes)

not pre-selected and not related
were investigated by DNA analysis

PCR-based ASO Hybridization

In mass-screening of β -thal populations,
in whom the mutant alleles are
at high frequencies, PCR-based technologies
great breakthrough!!

TR: Mutation Distribution / Frequencies

| Mutation | Frequency | PT | Mutation | Frequency | PT |
|------------------|-----------|-----------|------------------|-----------|-----------|
| IVS-I-110 (G-A) | 39.4 | β^+ | IVS-I-116 (T-G) | 0.2 | β^0 |
| IVS-I-6 (T-C) | 10.1 | β^+ | -101 (C-T) | 0.1 | β^+ |
| FSC8 (-AA) | 5.5 | β^0 | CD27 (G-T) | 0.1 | β^+ |
| IVS-I-1 (G-A) | 5.0 | β^0 | -28 (A-C) | 0.1 | β^+ |
| IVS-II-745 | 5.0 | β^+ | IVS-I-130 (G-A) | 0.1 | β^0 |
| IVS-II-1 (G-A) | 4.7 | β^0 | FSC36/37 (-T) | 0.1 | β^0 |
| CD39 (C-T) | 3.8 | β^0 | 290 bp deletion | 0.1 | β^0 |
| -30 (T-A) | 3.1 | β^+ | IVS-II-654 (C-T) | 0.1 | β^+ |
| FSC5 (-CT) | 2.1 | β^0 | Cd15(TGG-TAG) | 0.1 | β^0 |
| FSC8/9 (+G) | 1.3 | β^0 | Cd15(TGG-TGA) | 0.1 | β^0 |
| FSC44 (-C) | 1.3 | β^0 | FSC74/75 (-C) | 0.1 | β^0 |
| IVS-I-5 (G-C) | 1.1 | β^+ | FSC22-24 (-7 bp) | 0.1 | β^0 |
| -87 (C-G) | 0.8 | β^+ | 3'-UTR (-13 bp) | 0.1 | β^+ |
| Poly A (TAA-TGA) | 0.5 | β^+ | Cd41/42 (-4bp) | 0.1 | β^0 |
| FSC6 (-A) | 0.4 | β^0 | IVS-II-2 (T-A) | 0.1 | β |
| IVS-II-848 (C-A) | 0.4 | β^+ | HbS | 4.9 | β |



The Thumb Rule: Does Not Apply to Turkey

- In contrast to most other Mediterranean countries, Turkey is very heterogeneous at molecular level
- The 6 most common mutations add up to **69,7%**
- The overall frequency of the first 12 mutations, all having a frequency above 1%, is only **82,4%**.

Severe Mutations are Predominant

- The ratio of β^0 : β^+ mutations is 1:1
- Since the majority of beta-thal cases in TR bears the severe IVS-I-110, most of the mts give rise to β -thal major in homozygous or compound heterozygous combinations
- Several rare and 5 novel mutations
- Many unidentified cases (7%)



Are mutations region-specific?

**To simplify this complex picture ,
the mutation distribution in
six geographical regions of Turkey
was investigated.**



Common β -Globin Mutations in Turkey: Regional Distribution

IVS1-110 34.1%

IVS1-6 14.8%

IVS1-1 9.1%

FSC8 8.0%

IVS2-745 4.6%

Cd39 4.6%

IVS1-110 42.4%

IVS1-6 12.6%

IVS1-1 6.6%

IVS2-1 5.6%

FSC8 4.6%

IVS2-745 4.6%

IVS1-110 31.0%

IVS1-6 10.9%

IVS2-745 10.9%

-30 9.1%

-87 7.3%

IVS2-1 5.5%

IVS1-110 26.4%

IVS1-6 8.5%

-30 6.7%

IVS2-745 6.0%

FSC8 4.3%

FSC5 3.5%

IVS1-110 52.3%

IVS1-6 7.2%

IVS2-745 7.2%

FSC8 6.3%

IVS2-1 5.4%

IVS1-1 4.5%

IVS1-110 27.1%

IVS1-6 10.2%

-30 8.5%

FSC8 8.4%

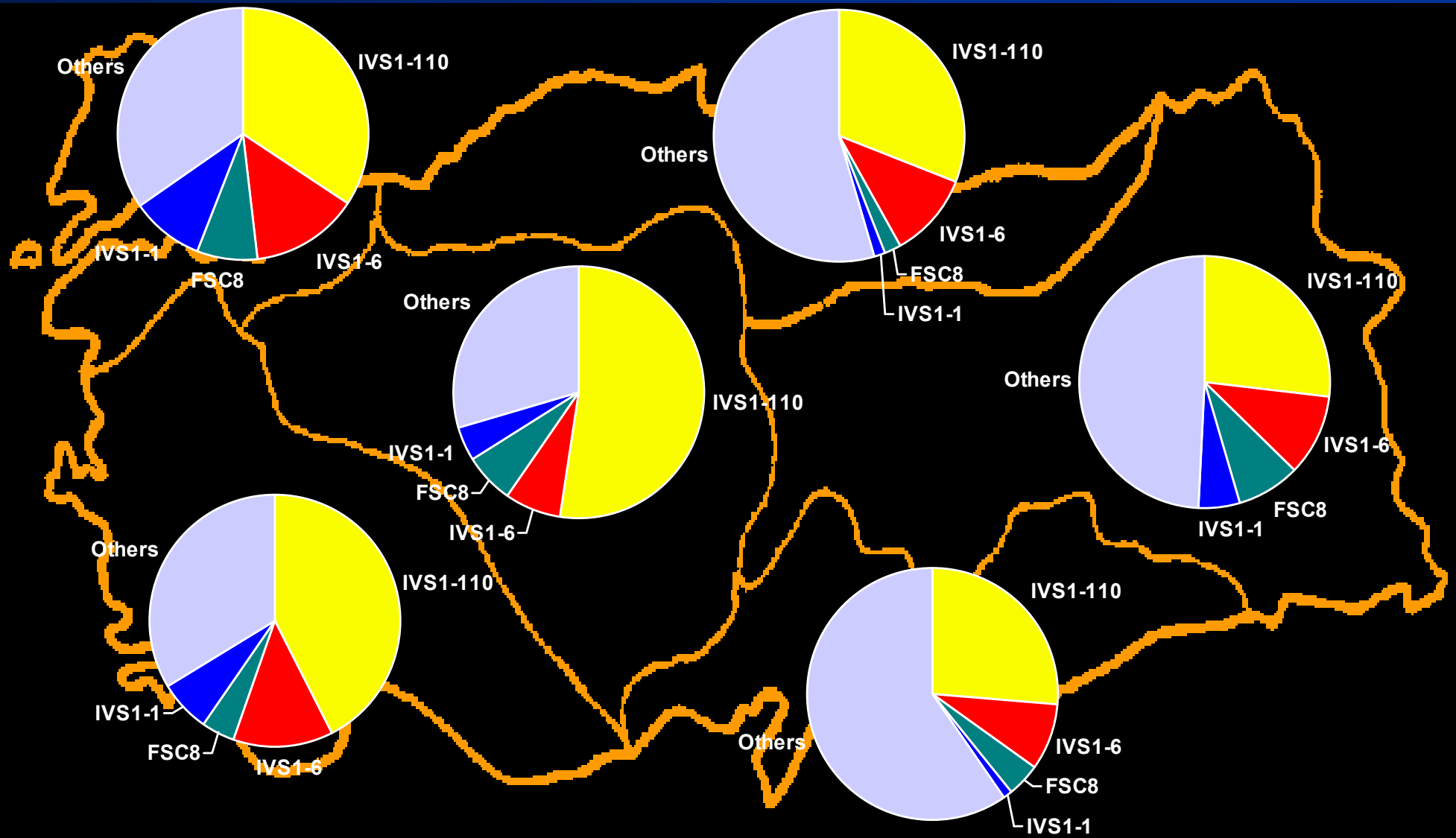
IVS1-1 5.1%

FSC8/9 5.1%

Distribution of Mutations in 6 regions




- Patients organized according to their origins
- Istanbul and the Western parts of Asia Minor are in accordance with the nation-wide distrib. pattern
- Central Anatolia and the Eastern parts of TR are different, IVS-I-110 decreases to 26% in East.
- Gradient of increasing heterogeneity from W to E.
- A map displaying the origins of rare and novel mutations, identified in TR, was established.

Common β -Globin Mutations in Turkey: Regional Distribution



Gradient: Increasing Heterogeneity from W to E



-  <6 Chromosomes/mutation
-  6-10 Chromosomes/mutation
-  >10 Chromosomes/mutation

β -Thalassemia: ***Regional Differences***

- **Migrations:** probable reason of large numbers of mutations in Turkey
- **Ethnic identities:** more preserved in the Northern and Eastern parts of Turkey
- **Unidentified mutations:** reflect the presence of rare and novel lesions
- **PCR-based techniques and DNA sequencing:** simplify the problem of molecular heterogeneity

Prenatal diagnosis is applicable!



Boğaziçi University Experience: 2001-present

- In a heterogeneous population, it is expensive and time-consuming to use Dot-Blot Hybridization in molecular and prenatal diagnosis.
- Reverse DOT Blot Hybridization, in which the ASO probes are immobilized on the membrane (instead of the DNA samples) obviates the need for multiple hybridizations.
- RDBH provides a rapid and simultaneous analysis of several muts. in a single hybridization reaction.

β -Globin Strip Assay (Vienna Lab)

- **One hybridization** simultaneously detects
- **22 Mediterranean-specific mutations**, fixed onto a membrane in form of probes
- **Reliable & fast: <1 day**
- **Diagnostic efficiency for TR >95%**

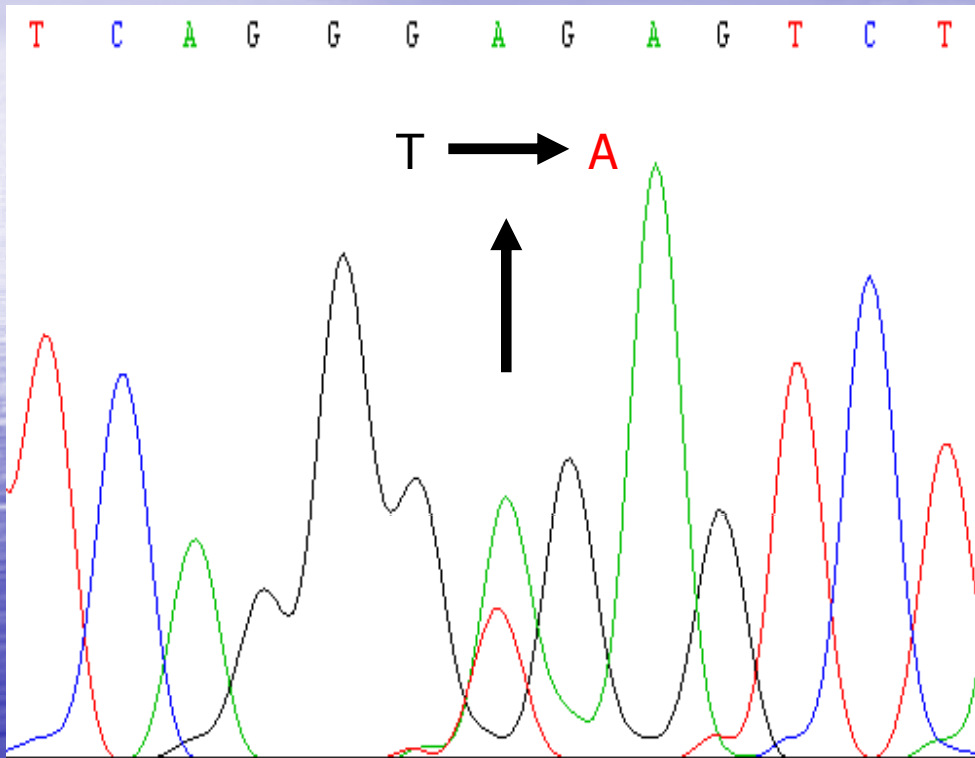


Mutations Covered by the Assay

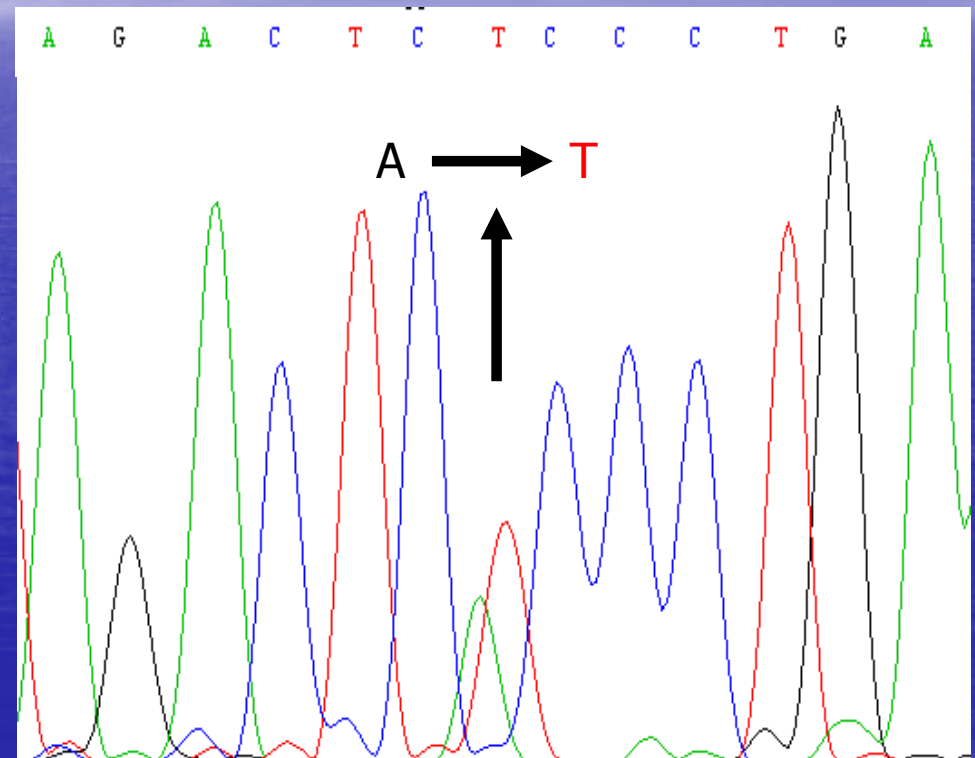
- ✓ -87 (C→G)
- ✓ -30 (T→A)
- ✓ Codon 5 (-CT)
- ✓ Hemoglobin C (G→A)
- ✓ Hemoglobin S (A→T)
- ✓ Codon 6 (-A)
- ✓ Codon 8(-AA)
- ✓ Codon 8/9 (+G)
- ✓ Codon 22 (del 7bp)
- ✓ Codon 30(G→C)
- ✓ IVS I.1 (G→A)
- ✓ IVS I-2 (T→A)
- ✓ IVS I.5 (G→C)
- ✓ IVS I.6 (T→C)
- ✓ IVS I.110 (G→A)
- ✓ IVS I.116 (T→G)
- ✓ IVS I-25 (del 25 bp)
- ✓ Codon 36/37 (-T)
- ✓ Codon 39 (C→T)
- ✓ Codon 44 (-C)
- ✓ IVS II.1 (G→A)
- ✓ IVS II.745 (C→G)



Strip Assay combined with genomic sequencing: very powerful!



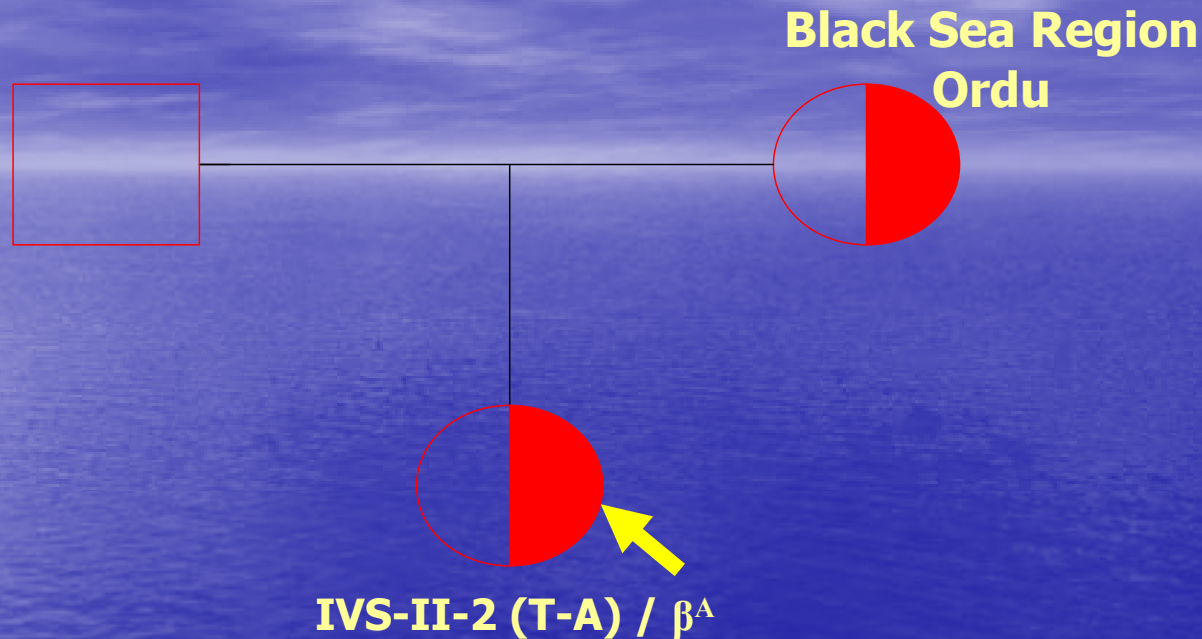
Forward Seq.



Reverse Seq.

Novel Mutation: IVS-II-2 (T-A)

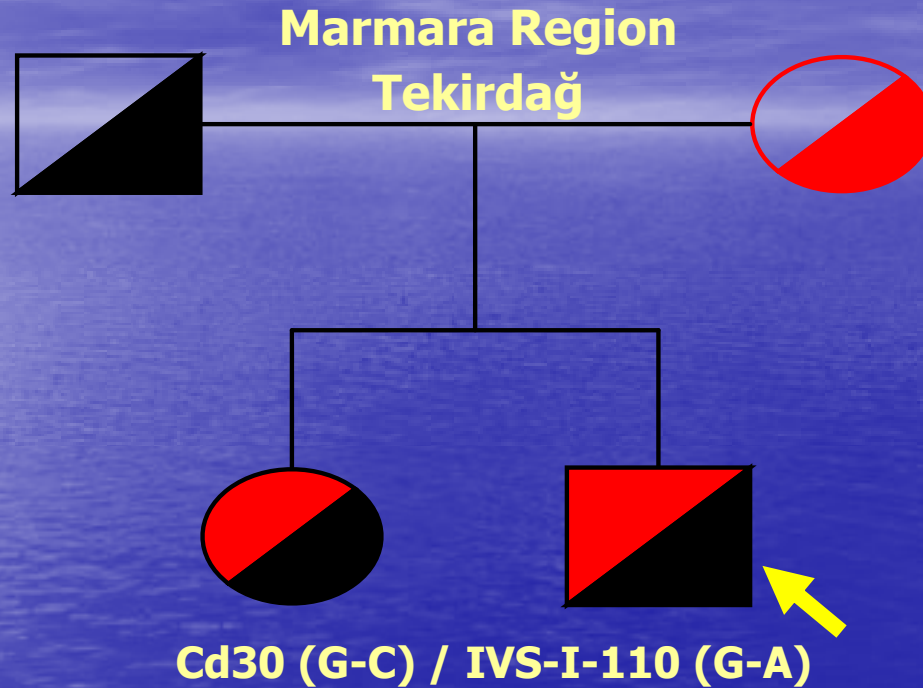
Family 1



- IVS-II-2 (T-A): Invariant “GT” → GA” substitution at IVS-II
- Phenotype : β -thalassemia carrier
- Elevated HbA₂ levels and decreased RBC indices

Rare Mutation: CD30 (AGG-AGC)

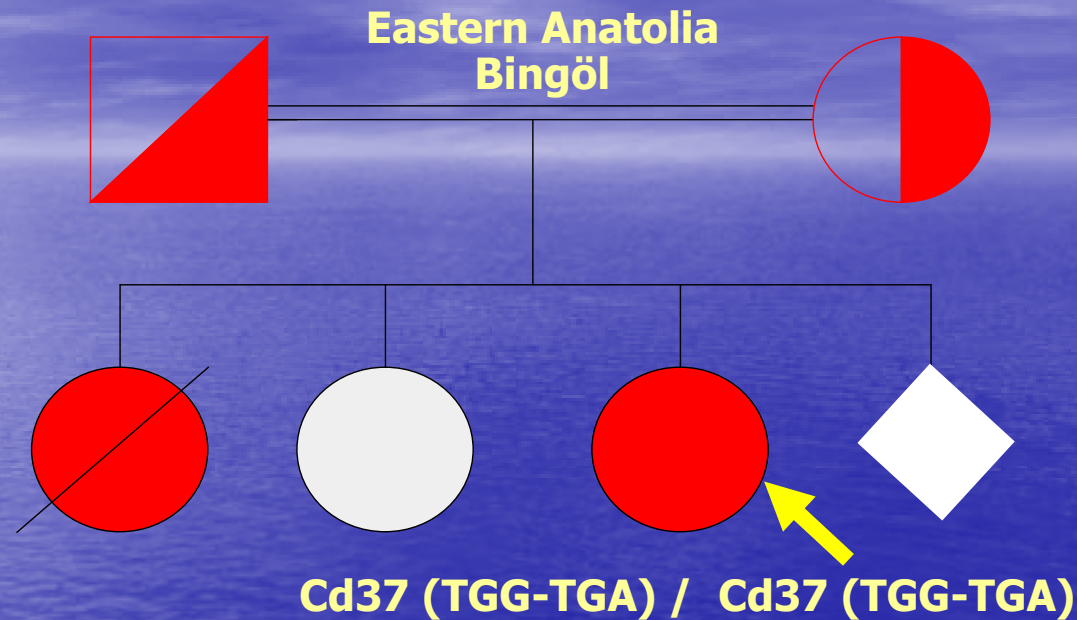
Family 2



- Codon 30 (AGG-AGC): Arg → Ser
- Phenotype: severe β^+ -thal

Rare Mutation: CD37 (TGG-TGA)

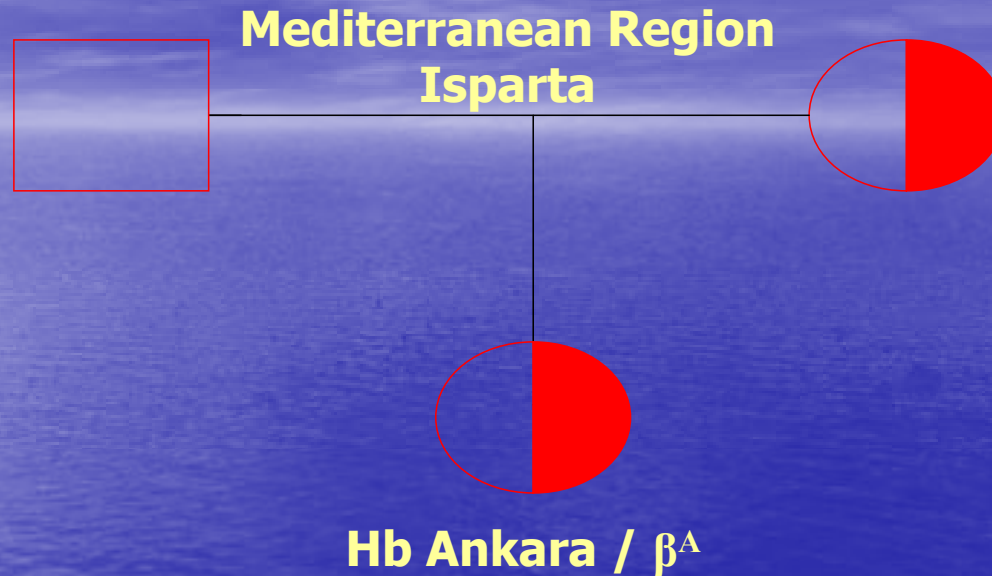
Family 3



- **Cd37 (TGG-TGA) : Trp → Stop Codon**
- **Phenotype: β^0 -thal**
- **First described in a Saudi Arabian family**

Hb Ankara (CD10 GCC-GAC)

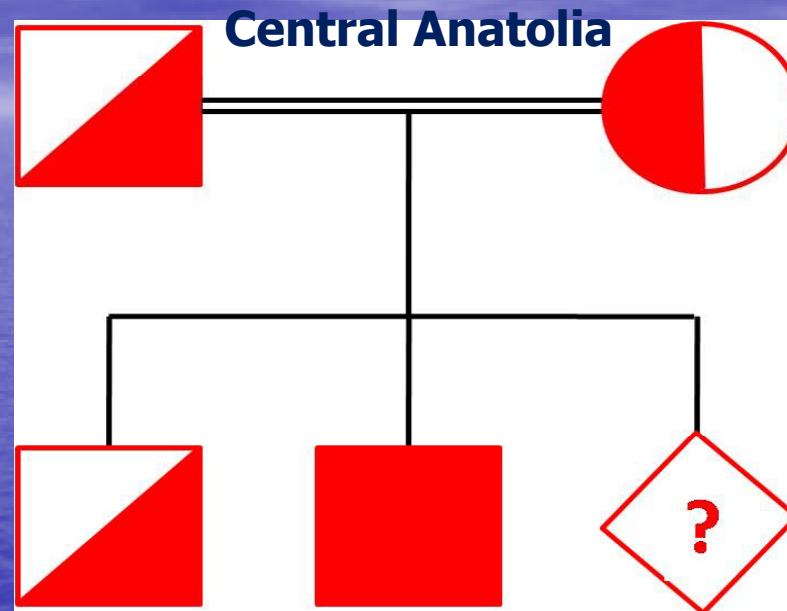
Family 4



- Hb Ankara (Codon 10 GCC-GAC): Ala → Asp
- Phenotype: normal in heterozygote

Novel mutation: CD 41/42 (-CTTT)

Family 5



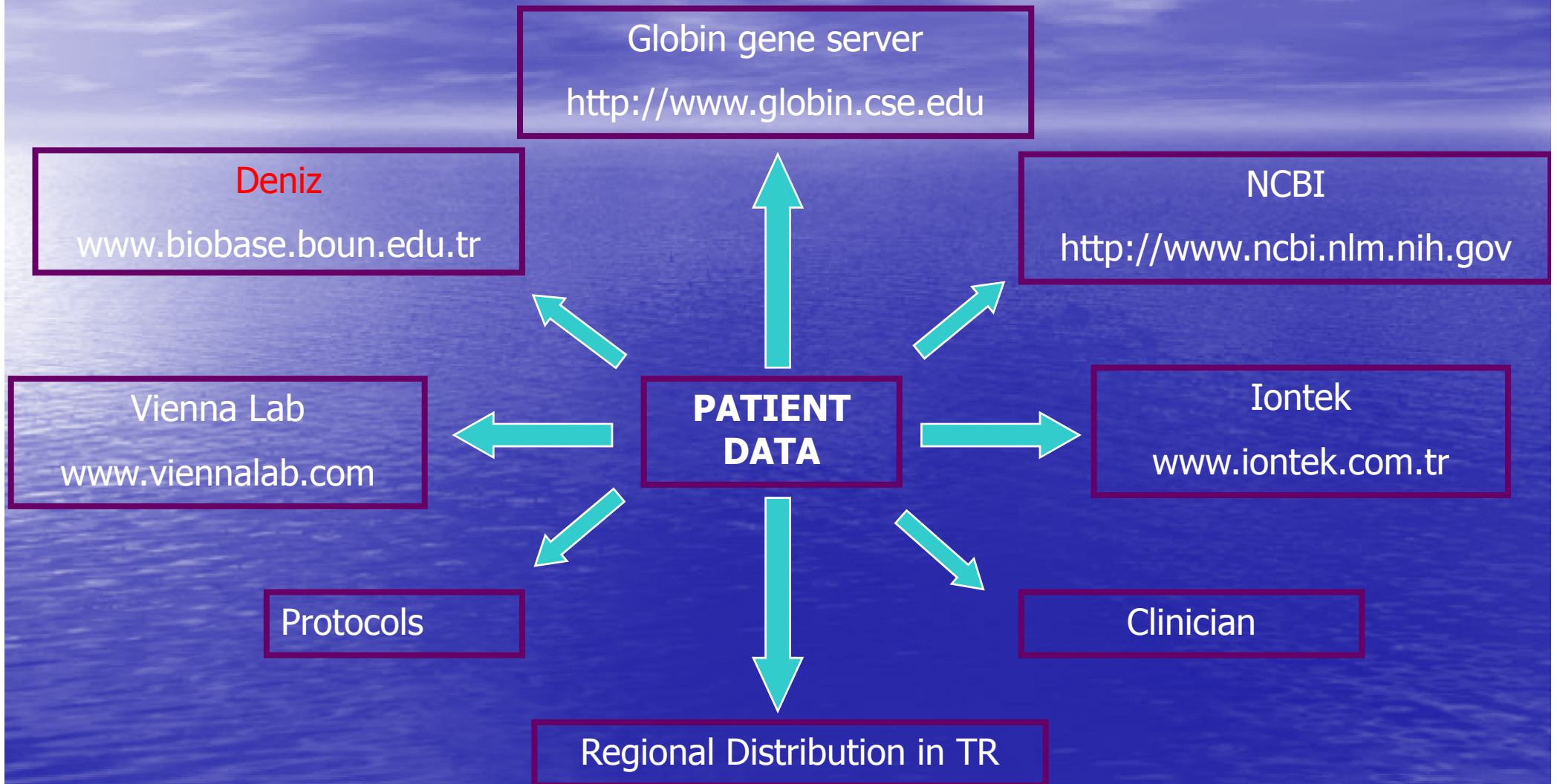
- Codon 41/42 (-CTTT, 4bp deletion)
- Phenotype: B-thal major in homozygote
- Prenatal Diagnosis on the fetus

Classification of Patient Data in Deniz Data-base:

Iranian, TR and Japanese populations carry the most heterogeneous beta-thal mutations

- To manage the large number of patient samples: DENIZ database construction
- Statistical analysis of 36.000 beta-thal chromosomes
 - displaying the frequency of mutations in a population/country/ continent- specific way....

Structure of the DENIZ Database



β -Thalassemia: Model System

- First disease studied at molecular level
- Ideal model for genotype/phenotype correlations
- Important part of clinical practice in tropical countries
- Survival to the 5th decade of life represents a dramatic improvement in morbidity/mortality in genetic disease
- 85 years after the initial description of “peculiar bone changes”, β -thal still emerges as a huge public health concern worldwide
- In terms of cure, β -thal- remains a therapeutic challenge in the 21. century.



β -Thalassemia: Model System

- The mapping and sequencing of the human genome has opened new avenues in understanding disease pathologies in recent years.
- Despite notable progress in this field and the new technologies available, we are still far from understanding the key processes orchestrating developmental regulation in the globin gene clusters
- Novel scientific strategies are needed to facilitate the translation of globin research from bench to bedside, like in all other diseases.

Understanding the molecular mechanisms involved in globin gene regulation, will be an important step towards finding a cure for β -thalassemia.





THANK YOU...