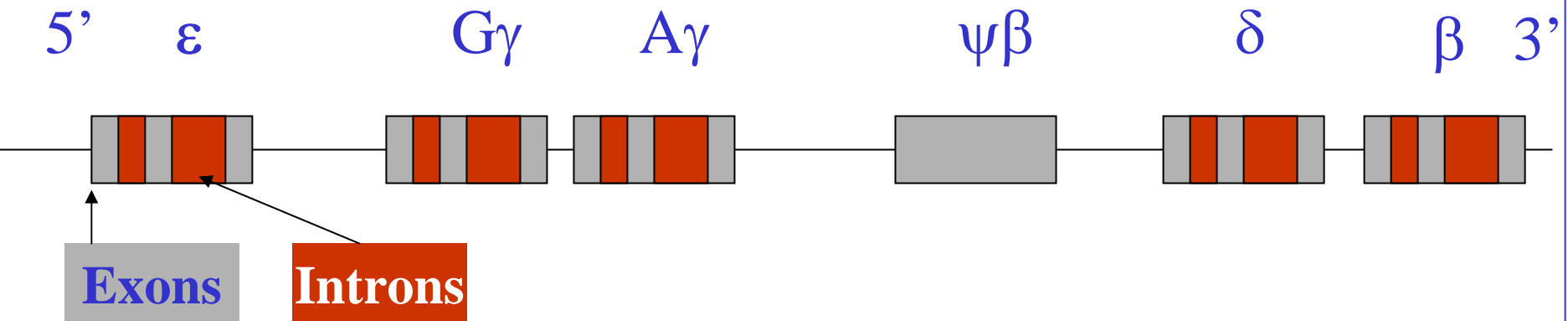


Sequence Diversity in beta-globin gene fragments in Saudi Population

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Introduction

- The β -globin like gene cluster occupies about 60 Kb and is located on the short arm of Chromosome 11.
- It is composed of five functional genes (ϵ -G γ -A γ - δ - β) and a pseudogene ($\psi\beta$).



The β -like globin gene cluster

Introduction (Contd.)

- A number of restriction fragment length polymorphisms (RFLPs) and mutations are distributed along the entire length of of the β -globin gene cluster.
- Hundreds of the mutations produce structurally abnormal haemoglobins (e.g. HbS, HbC, HbE, HbD, HbO-Arab), while a number of mutations affect the rate of globin chain synthesis, hence producing 'thalassaemias' (β -, $\delta\beta$ -, $\gamma\delta\beta$ -).
- In additions, several of the mutations do not have any clinical consequences.
- These RFLPs and mutations are a major cause of genetic diversity both in normal human features and in disease clinical presentation.

Introduction (Contd.)

- References of the ethno-geographic variations in the base sequence of DNA are crucial in the understanding of the pathological diversions from the normal conditions in different populations.
- In addition, intra-ethnic variations have been reported within the same population.

*Our
Sequences*



Objectives

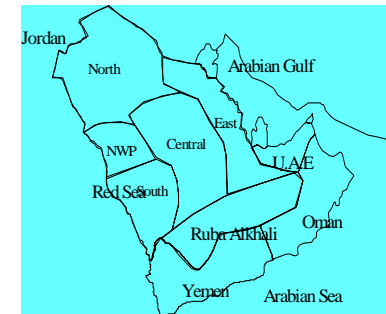


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graph TD; A[Objectives] --> B([To investigate intra-ethnic genome diversity in normal Saudis and in patients suffering from β- thalassaemia.]);
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To investigate intra-ethnic genome diversity in normal Saudis and in patients suffering from β - thalassaemia.

Material and methods

- **No. Investigated: Normal:** 30,179 males and females Saudis
 β Thal patients: 68
- **Haematological parameters**
 - Red cell indices
 - Discriminant factor
 - Hb electrophoresis:
 - Alkaline pH
 - Acid pH
 - Hb A₂
 - Hb F
 - α/β ratio
 - DNA Extraction
- **Detection of β mutations in the β globin-gene and β globin-gene cluster**
 - ARMS
 - RFLP
 - Dot blot analysis
 - DNA sequencing



Results

Mutations identified in β -globin gene cluster in Saudis

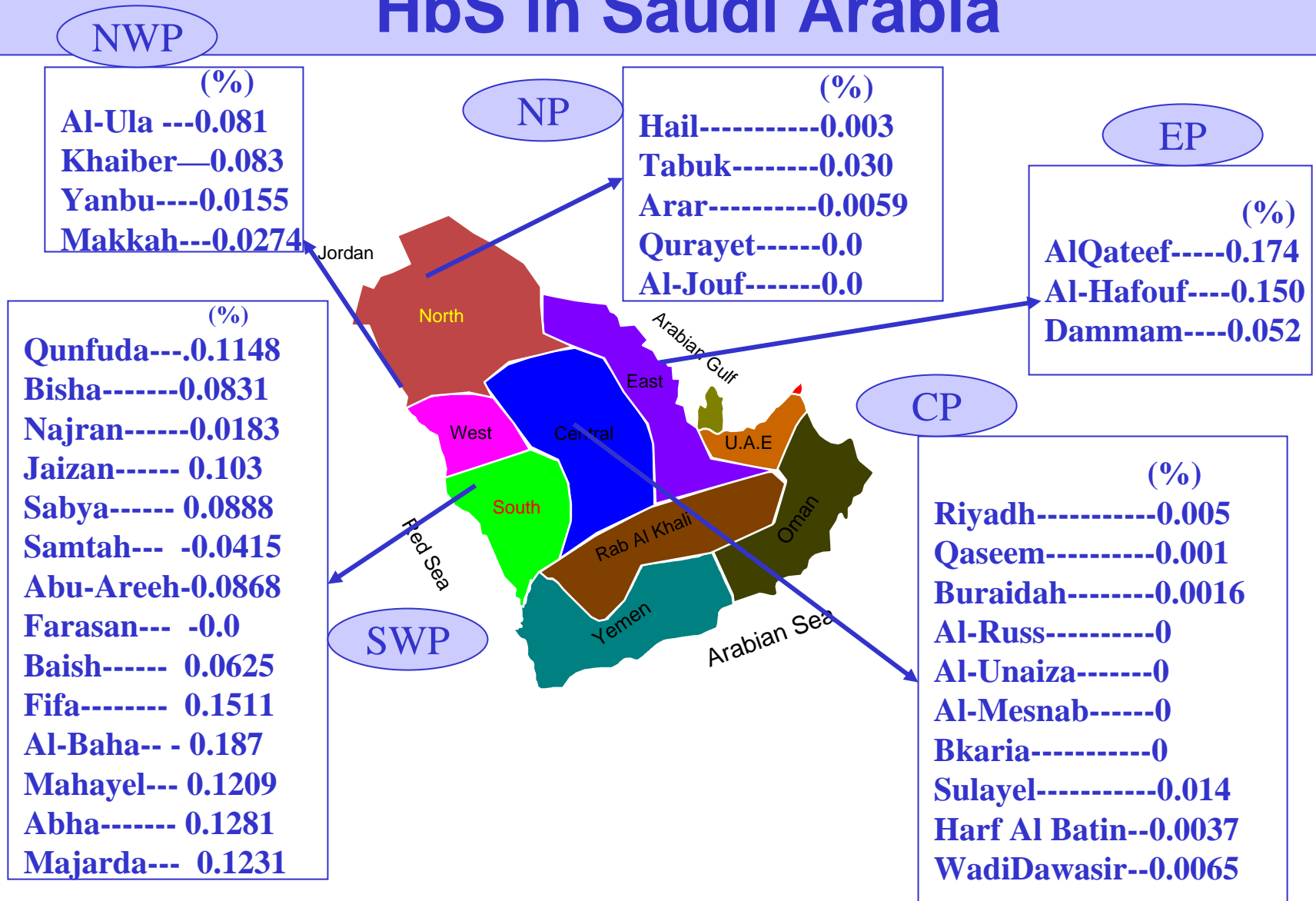
Structural variants of Hb

- HbS (β^6 GAG-GTG)
- HbC (β^6 GAG-AAG)
- HbE (β^{26} GAG-AAG)
- HbD Punjab (β^{121} GAA-CAA)
- HbO-Arab (β^{121} GAA-AAA)
- Hb-Riyadh (β^{120} AAA-AAC or AAT)
- HbF-Dammam (γ^{79} GAG-GTG)
- Hb-Handsworth (α^{18} GGC-CGC)
- Hb-Setif (α^{97} GAC-TAC)

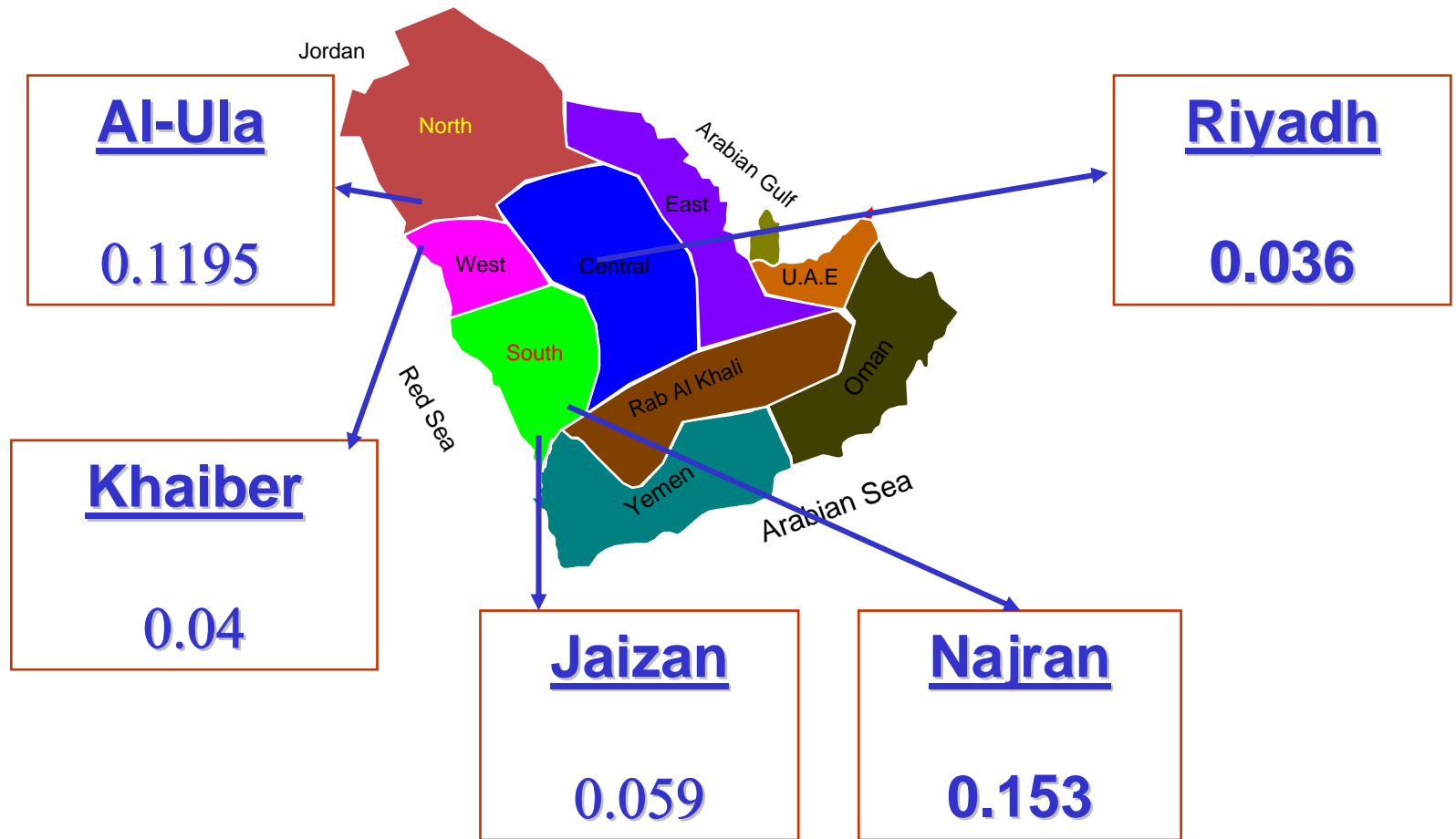
Biosynthetic variants of Hb

IVS-I-110 (G → A)	Internal IVS change	β^+
IVS-I-5 (G → C)	Consensus change	β^+
CD-39 (C → T)	Nonsense mutant	β^0
IVS-1-3'end (- 25)	Splice junction	β^0
IVS-II-1 (G → A)	“ “	β^0
CD 6 (-A)	Frame shift	β^0
CD 8/9 (+G)	Frame shift	β^0
IVS-I-1 (G → A)	Splice junction	β^0
IVS-I-6 (T → C)	Consensus change	β^+
CD 44 (-C)	Frameshift	β^0
CD 5 CCT → (C)	Frameshift	β^0
CD 26 (+T)	Frameshift	β^0

Intra-ethnic variation in the frequency(%) of HbS in Saudi Arabia



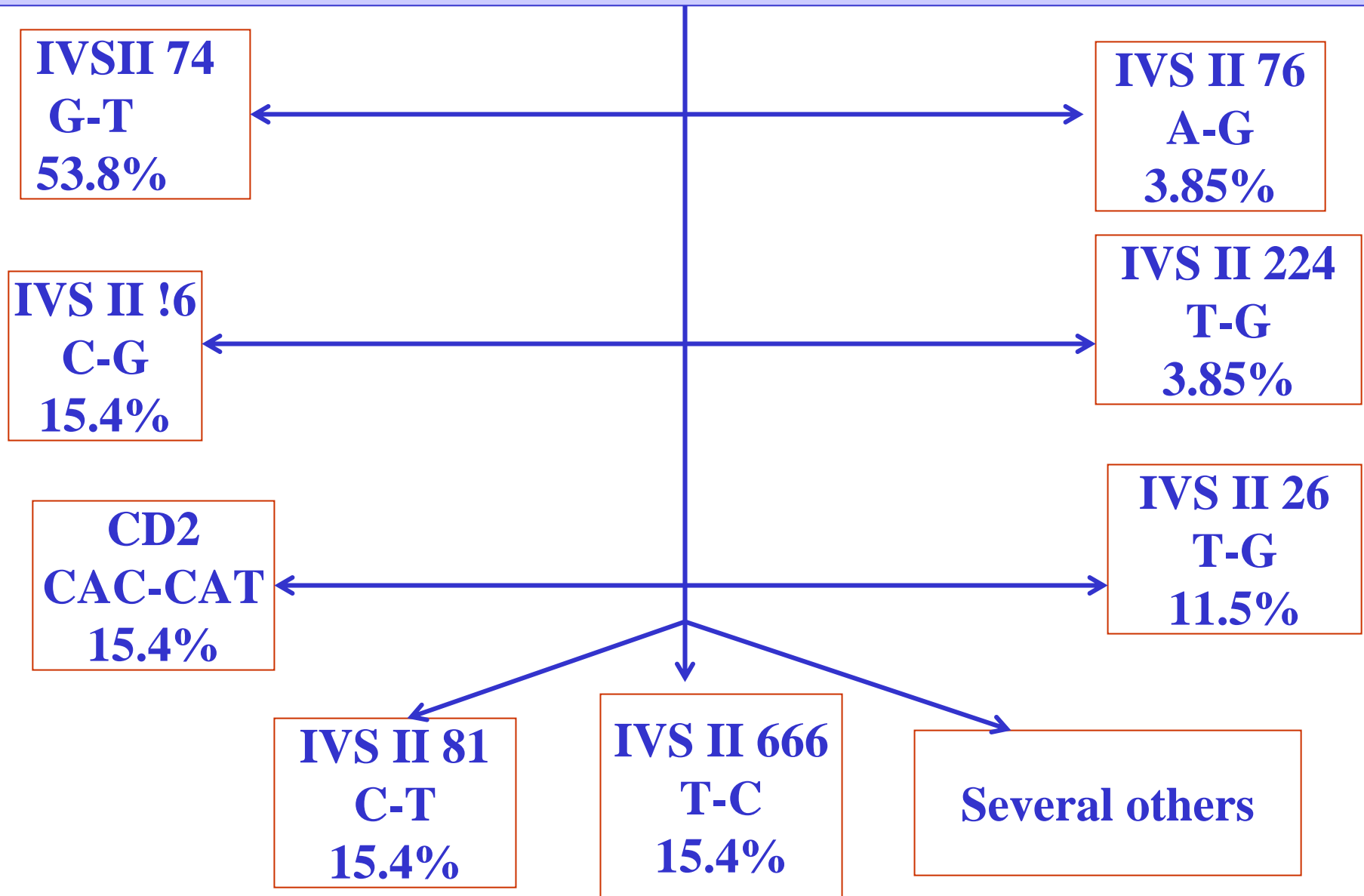
Intra-ethnic variation in the frequency(%) of β -Thalassaemia in Saudi Arabia



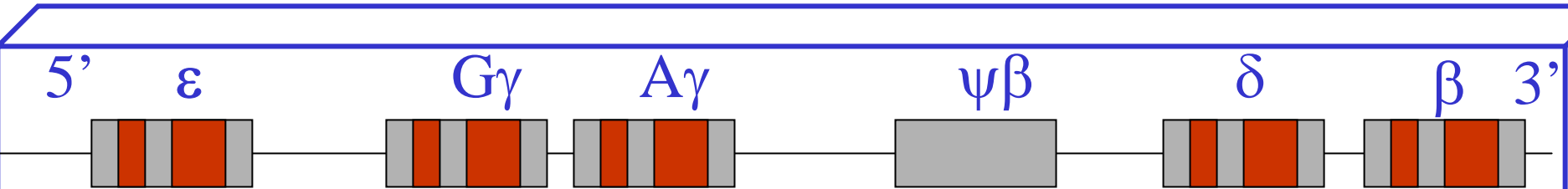
Frequency of the mutations identified in Saudis

Mutations	Number	Percent
IVS-I-110	26	27.4
IVS-I-5	26	27.4
CD-39	27	14.5
IVS-I-3'end	0	12.9
IVS-II-1	30	16.1
CD6	8	4.3
CD 8/9	2	1.07
IVS-I-1	1	0.5
IVS-I-6	1	0.5
CD 44	2	1.07
CD 5	4	2.14
CD 26	1	0.5
Total:		95 %

Single Nucleotide Polymorphisms in the β -Globin gene in Saudis



Extensive Polymorphism in β -globin gene in Saudis



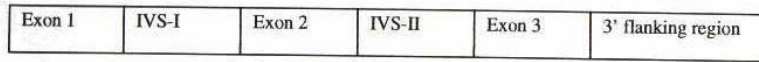
- Saudis have extensive Polymorphisms in the β -globin gene and the β -globin gene cluster.
- In a group of β -thalassaemia patients, 23.1% had five polymorphic sites each.
- These were: IVSII 16, IVSII 74, CD2, IVSII 81 and IVSII 666.

Extensive Polymorphism in β -globin gene in Saudis

Thalassemia

Case 4 (#609):

IVS I-1 (G \rightarrow A) heterozygous
 CD 39 (C \rightarrow T) heterozygous



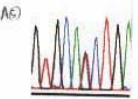
CD 2 (CAC \rightarrow CAT) IVS-I-1 (G \rightarrow A) CD 39 (CAG \rightarrow TAG)
 IVS-II-16 (C \rightarrow G) IVS-II-74 (G \rightarrow T)* IVS-II-81 (C \rightarrow T) IVS-II-666 (T \rightarrow C)

*denotes homozygous

Sequencing Results:

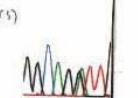
CD 2 (CAC \rightarrow CAT)

3 TGC A N C T G A
 160



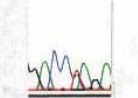
IVS-I-1 (G \rightarrow A)

3 G C A G N T T G
 160



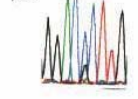
CD 39 (CAG \rightarrow TAG)

G A C C N A G A G
 400



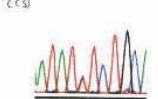
IVS-II-16 (C \rightarrow G)

3 G G A C C C T T G
 100



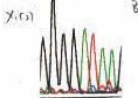
IVS-II-666 (T \rightarrow C)

3 A T A T N T C T G C
 190

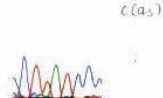


IVS-II-74 (G \rightarrow T)*

3 G G G A T A A G
 160

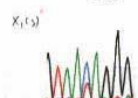


Antisense
 5' G A A C A A A A A A 3'
 3' C C C C T T T T T T 5'
 3 C T T A T C C C
 380



IVS-II-81 (C \rightarrow T)

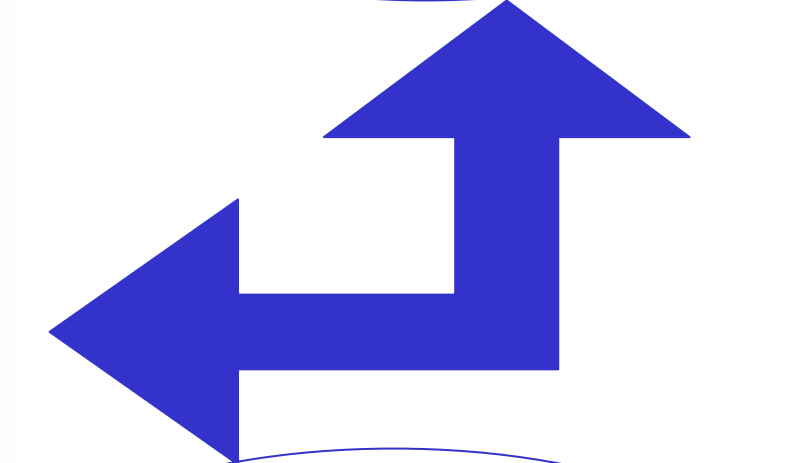
3 T T A A C A G G G
 170



Antisense
 5' T A A C A G G 3'
 3' A T T A T C C 5'
 3 C C C T G T T A
 370

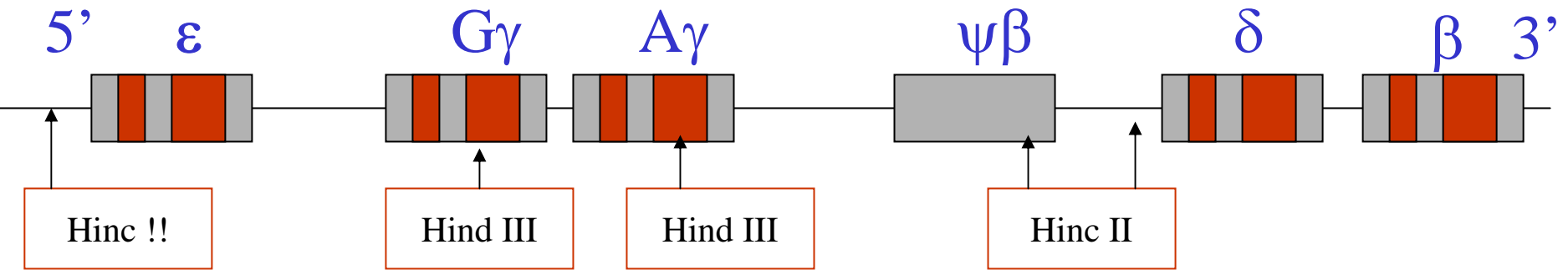


A β -thal patient
 Double heterozygous to
 IVS I 6 & IVSII 848

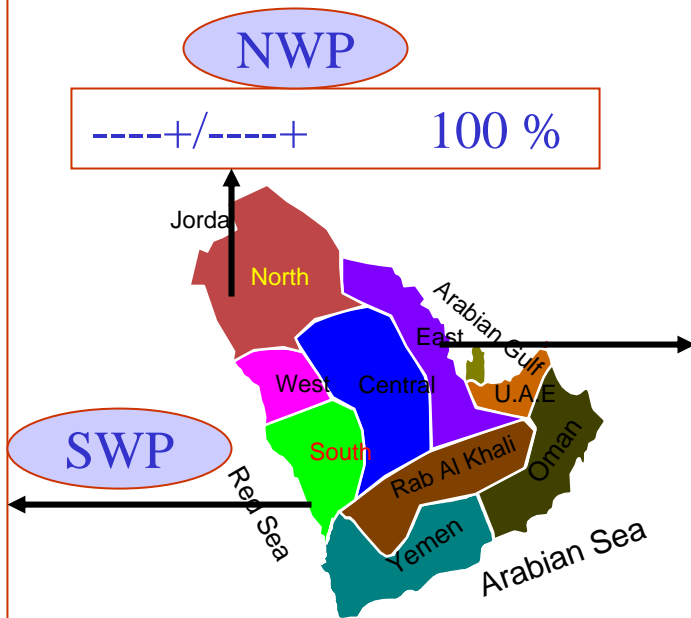


Nine Polymorphic Sites in
 the Globin gene

Intra-ethnic variations in β -globin haplotypes in Saudi Arabia



----+ / ----+	44.8%
----+ / ----	11.9%
----+ / +----	13.4%
----+ / +----+	4.5%
----+ / -+++	3.0%
----+ / --+-	3.0%
----+ / -++-	1.4%
----+ / -+--	3.0%
----+ / +++--	1.5%
----+ / --++	1.5%
-+---- / -+--	3.0%
+---- / +----	1.5%
+---- / +----	1.5%
Others	6.0%



NWP	
----+ / ----+	100%

EP	
++-++ / ++-++	50.0%
++-++ / ----+	36.4%
++-++ / +----+	4.5%
++-++ / ----	4.5%
----++ / ----+	4.5%

Conclusions

- **Sequence diversity appears to be very frequent in the Saudi Population.**
- **With-in the β -globin gene cluster, extensive polymorphisms occur and create intra-ethnic variations in globin gene expression.**
- **With-in the β -globin gene:**
 - * **mutations producing structural and biosynthetic variants of haemoglobin are wide spread.**
 - * **extensive polymorphic sites are observed resulting in sequence diversity in the β -globin gene.**