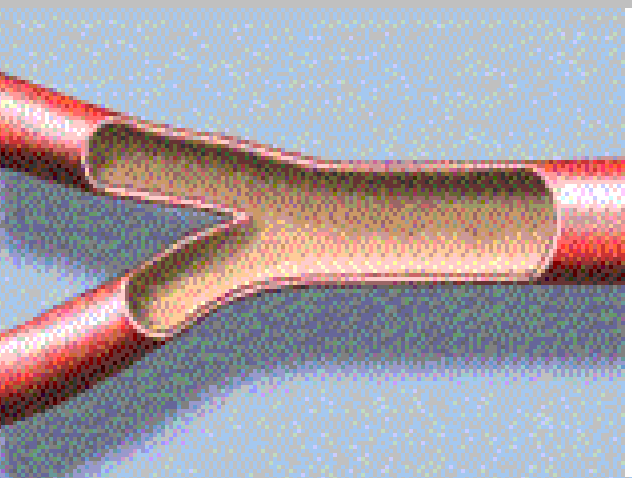
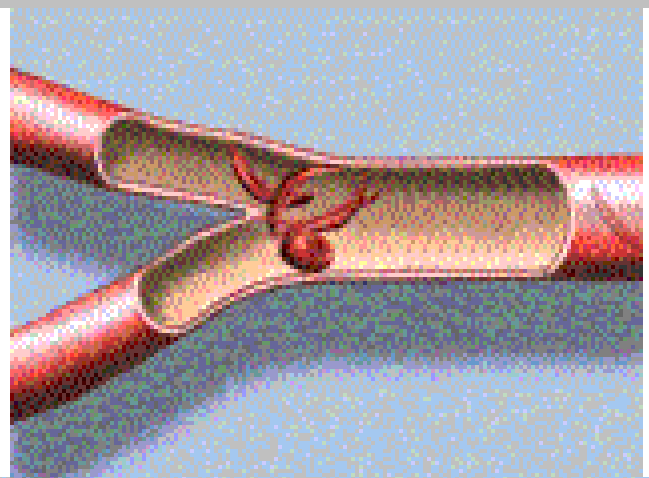
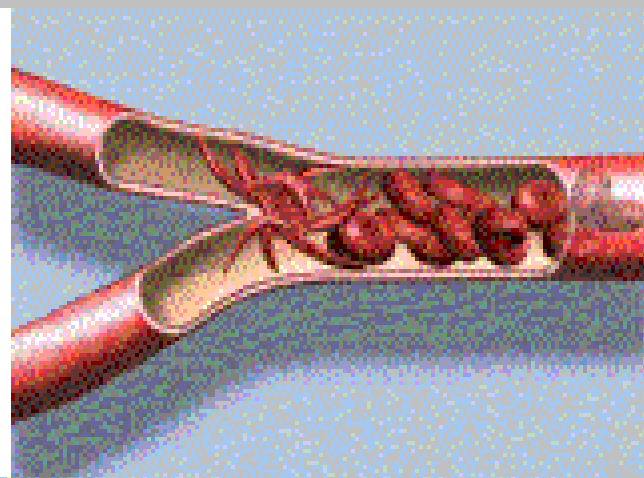
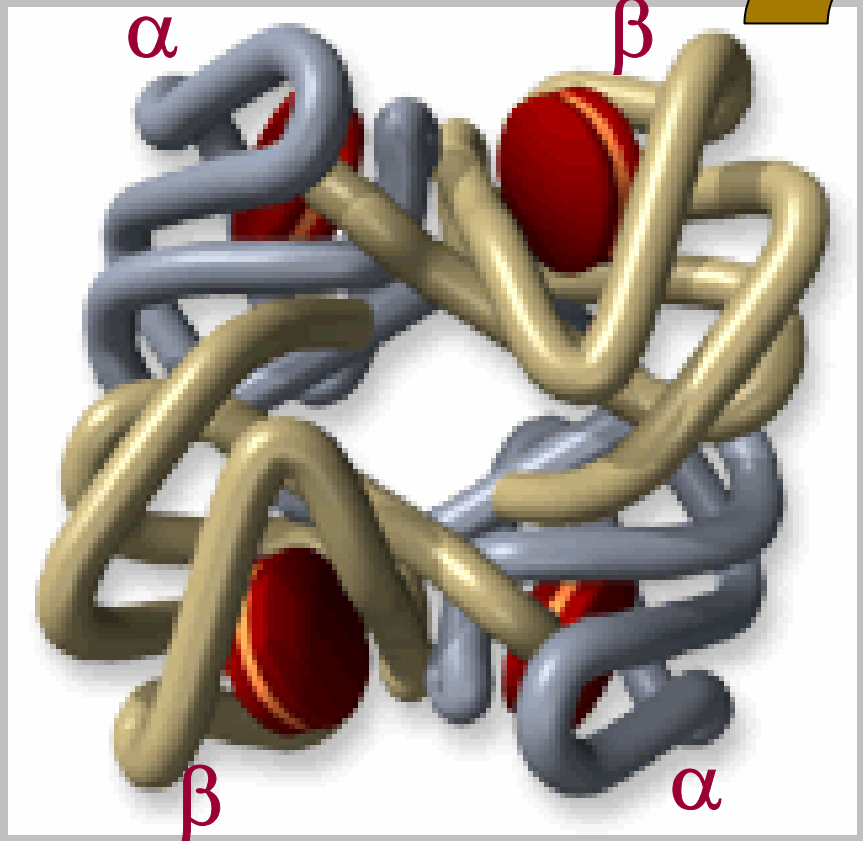
SICKLE CELL ANAEMIA IN SAUDI ARABIA - TWO FORMS AND CLINICAL PRESENTATION



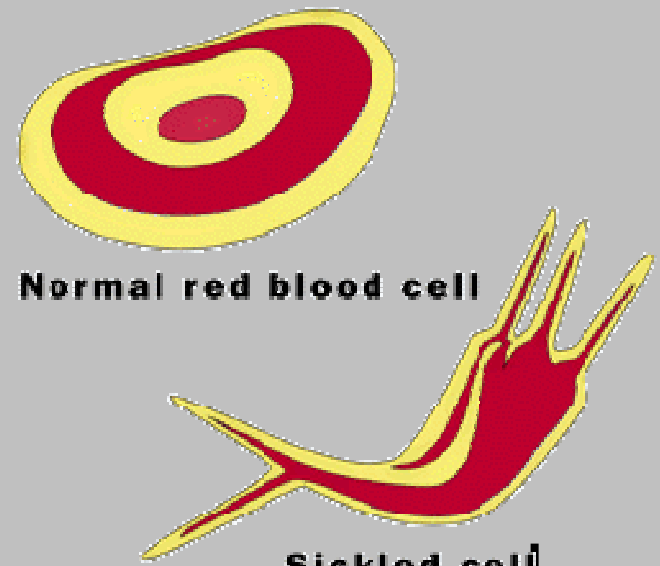




Sickle Cell Haemoglobin results from a mutation in the 6th codon of the β-globin gene,this replaces a glutamic acid by valine in the β-globin chain, producing HbS, which in homozygous state, causes sickling of the red cells

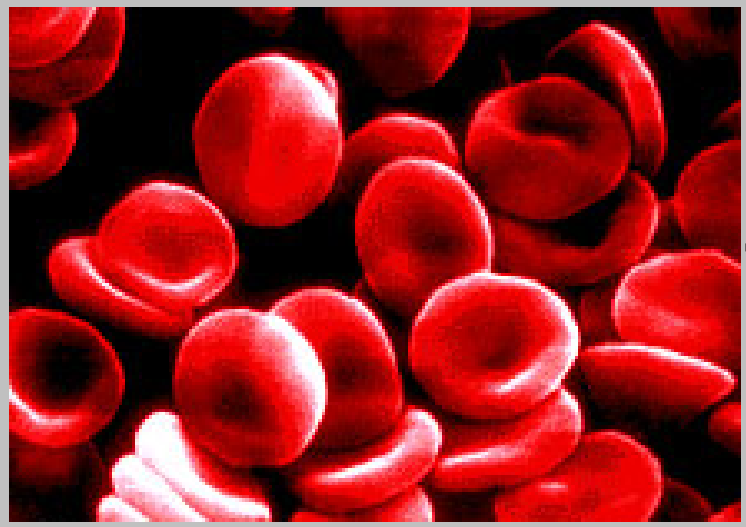


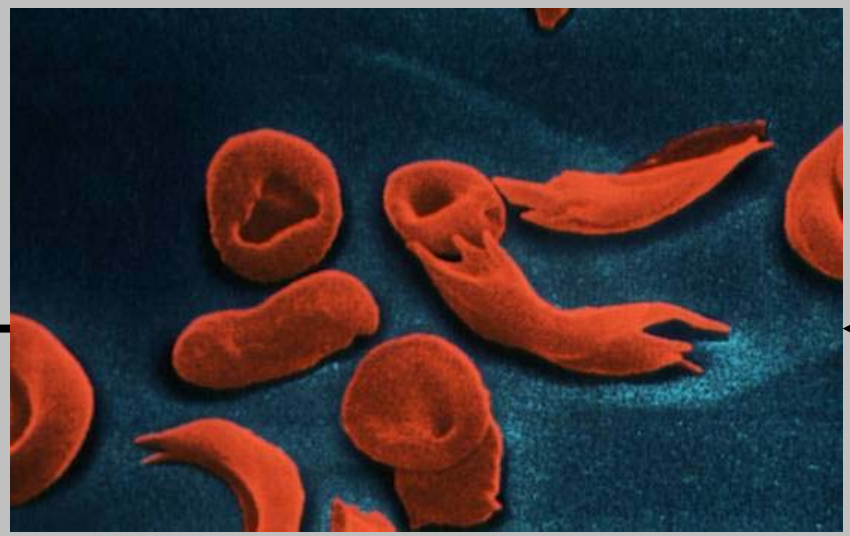
β^{6} GAG → GTG



Haemolysis

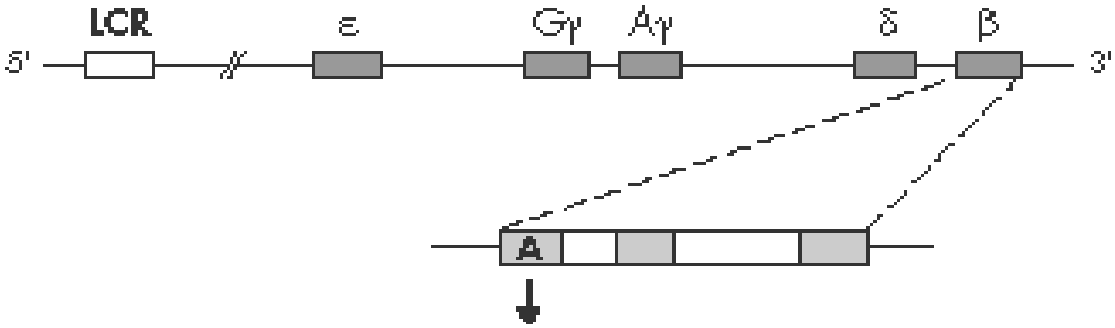
Red cell sickling





Lungs
↑pO_2

Tissues
↓pO_2



Chromosome 11

SCD

Gene β^S

GTG Codon 6

Haemoglobin S (Hb S)

Oxy

Deoxy

β^S Glu → Val

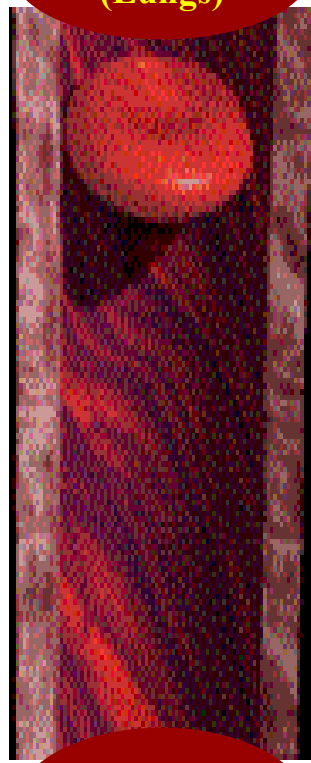
Chains of β^S

Polymerisation

-↓Deformation
-Rigidity
-Fragmentation

Haemolytic Anaemia

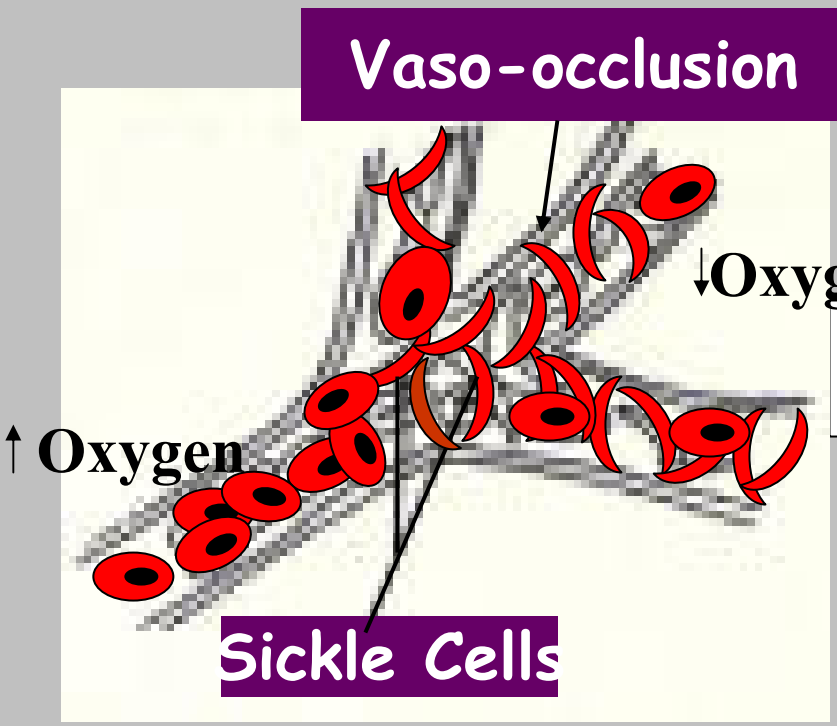
Oxygenated (Lungs)



DeOxygenated (Tissues)

Vaso-occlusion

Vaso-occlusion in Sickle Cell Disease

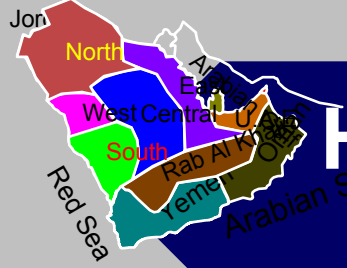


Sickle cell crises

Pain

Redness

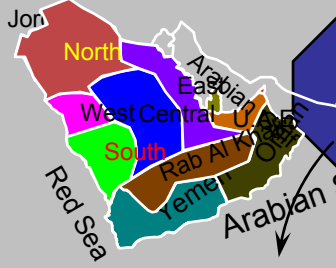
Swelling



Haemoglobin S in Saudi Arabia- Historical

- HbS first reported in Eastern Province by Lehmann et al (1963).
- Gelpi reported a mild SCD in 1965.
- Weatherall et al in 1969 related this to the presence of associated α-thalassaemia in Eastern Province.
- During 1970's HbS was identified in several areas in Saudi Arabia and three major foci of HbS gene were identified by El-Hazmi and coworkers.
- During 1980- 2003 several screening studies have clarified the picture about the haemoglobin variants in Saudi Arabia and have shed light on the natural history of these genes in the Saudis. (ElHazmi et al., Padmos et al., Al Awamy et al.,Sejeny et al.)

Sickle Cell Gene in Saudi Arabia



Occurs almost in all regions at variable frequency

Frequency of HbS correlates with past or present endemicity to malaria

Coexists with other abnormal genes

α–Thal.

β⁺ - & βº- Thal.

HbS/ β⁺- Thal

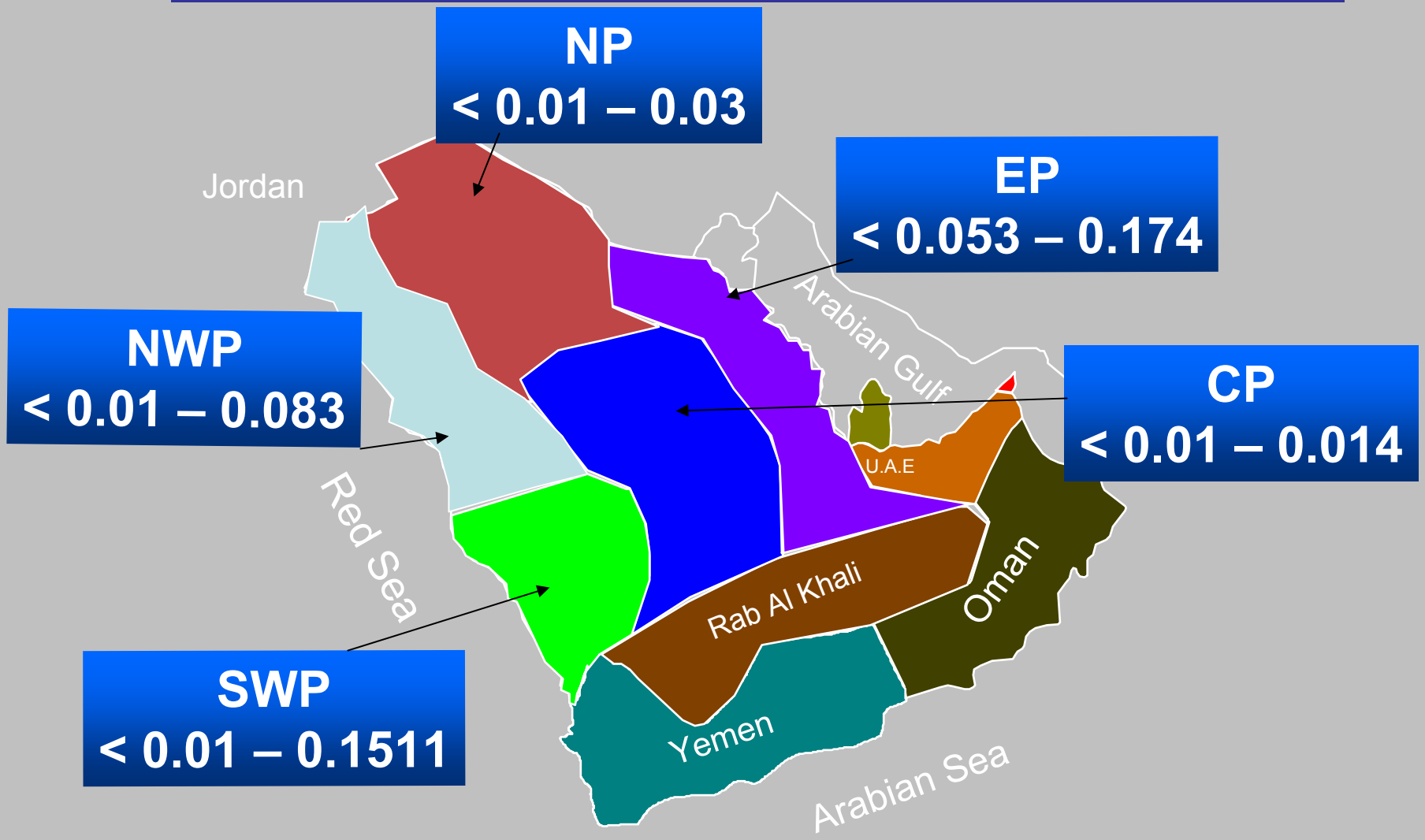
HbS/ βº- Thal

Shows variable clinical presentation

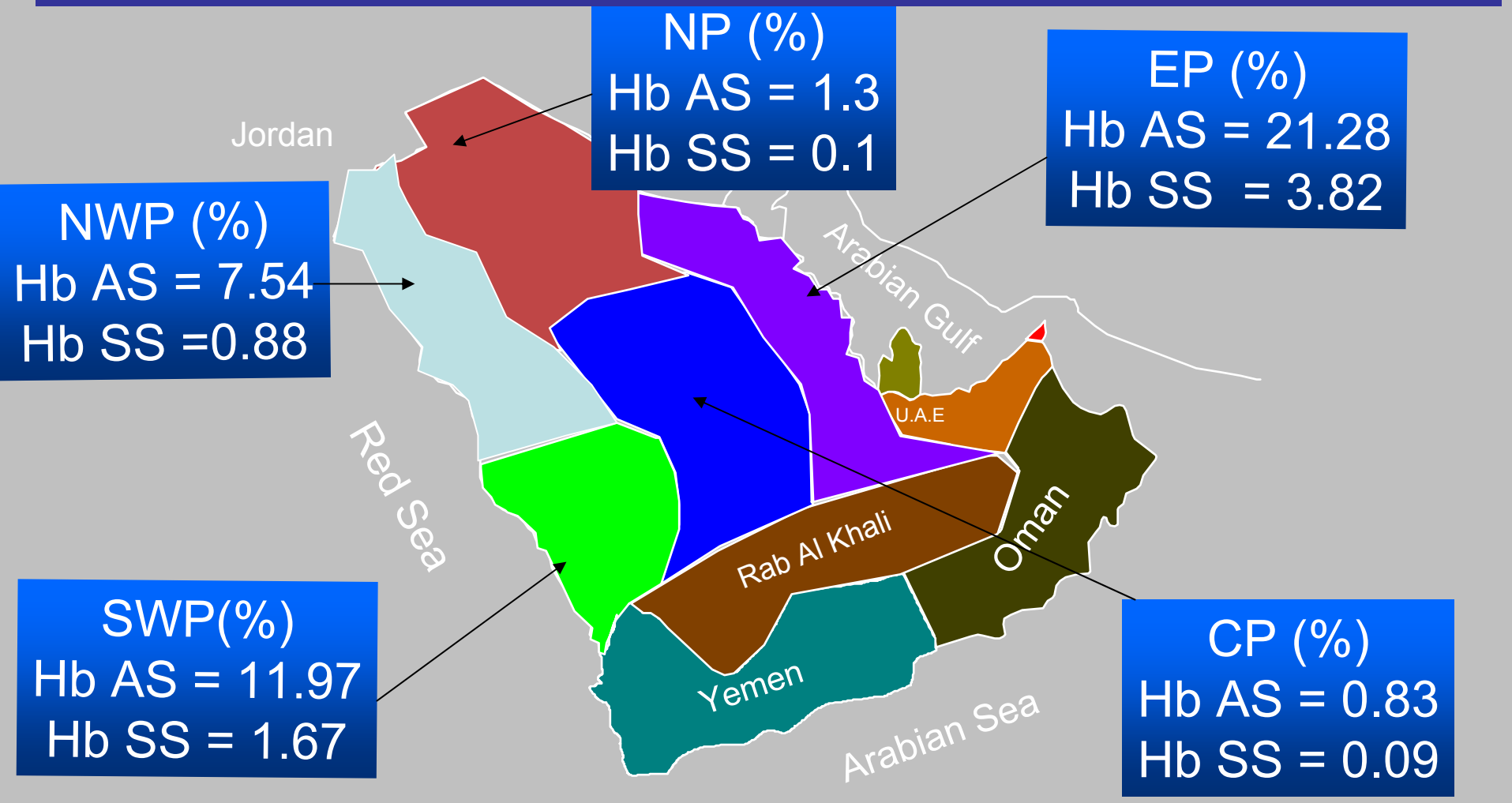
E.P. Benign disease

W.P. Severe disease

Hb S Gene Frequency in Saudi Arabia



Prevalence of Hb AS and Hb SS in different Regions of Saudi Arabia



Micro-distribution of HbS in Saudi Arabia

NWP

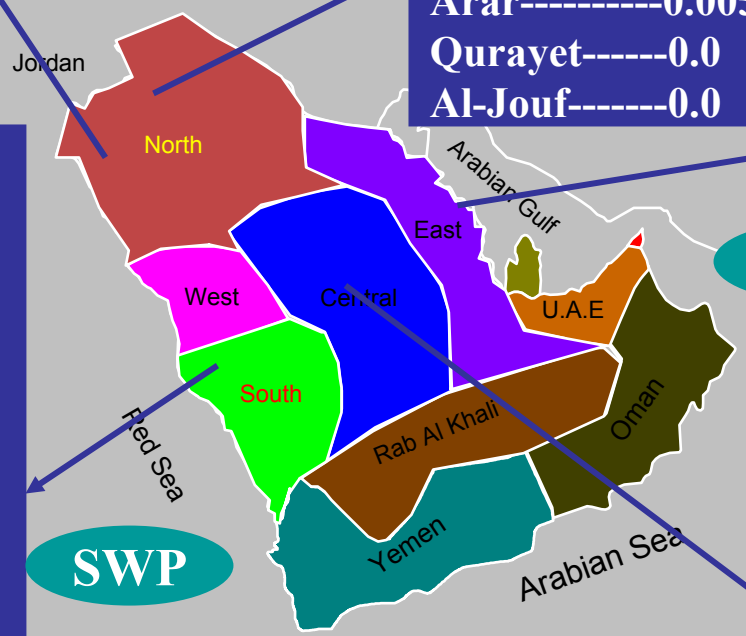
(%)
Al-Ula ---0.081
Khaiber—0.083
Yanbu----0.0155
Makkah---0.0274

NP

(%)
Hail----------0.003
Tabuk--------0.030
Arar----------0.0059
Qurayet------0.0
Al-Jouf-------0.0

EP

(%)
AlQateef-----0.174
Al-Hafouf----0.150
Dammam----0.052



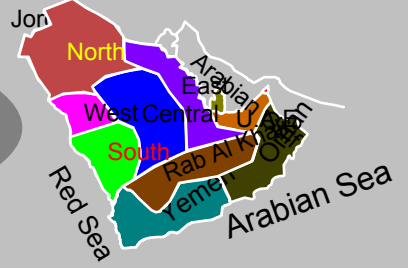
CP

(%)
Riyadh----------0.005
Qaseem----------0.001
Buraidah--------0.0016
Al-Russ----------0
Al-Unaiza-------0
Al-Mesnab------0
Bkaria-----------0
Sulayel----------0.014
Harf Al Batin--0.0037
WadiDawasir--0.0065

SWP

(%)
Qunfuda---.0.1148
Bisha-------0.0831
Najran------0.0183
Jaizan------ 0.103
Sabya------ 0.0888
Samtah--- -0.0415
Abu-Areeh-0.0868
Farasan--- -0.0
Baish------ 0.0625
Fifa-------- 0.1511
Al-Baha-- - 0.187
Mahayel--- 0.1209
Abha------- 0.1281
Majarda--- 0.1231

Estimates of HbS in Saudi Arabia

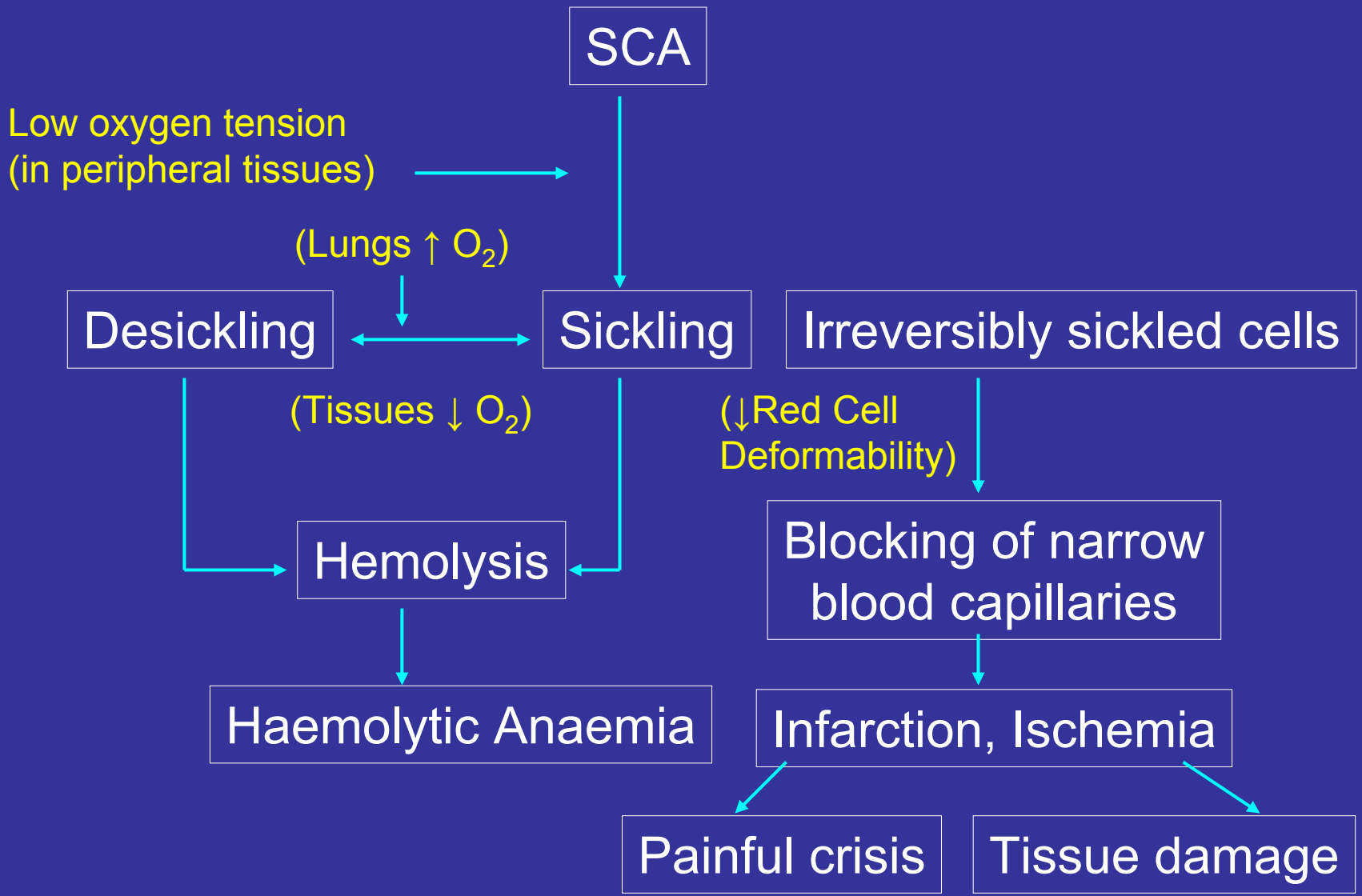


Carrier (% of population) *	1 - 25
Hb S Carrier born/year (/thousands):**	59
Total Carriers (/Thousands):**	1410
Hb SS for total Saudi population:	
- Births/1000	5
- Births/year	2105
Hb S/β-Thal.for total Saudi population:	
- Birth/year	398

* El-Hazmi et al, 1997.

** Based on guidelines for control of Hb disorders, Geneva W.H.O. document WHO/HDP/HB/GL/GL-1 (1994)

CLINICAL CONSEQUENCES OF SICKLE CELL ANAEMIA



Clinical Presentation of SCD in Saudi Arabia

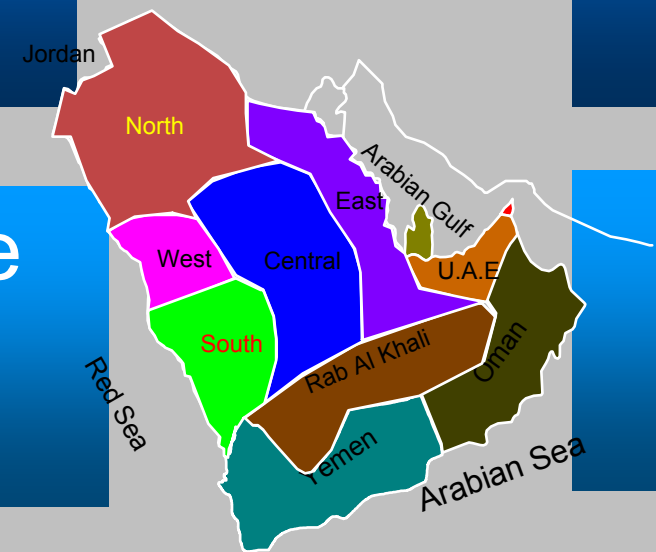
Variable-Two major forms

Severe Presentation

Mainly in the Western province

Mild (benign) Presentation

Mainly in the Eastern province



MAJOR FEATURES OF MILD AND SEVERE SCA IN SAUDI ARABIA

Clinical Presentation	Mild	Severe
• Anaemia	Mild	Severe
• Splenomegaly	Frequent	Frequent + Autosplenectomy
• Hepatomegaly	Rare	Frequent
• Crises	Low frequency short duration	Frequent
• Hand-foot syndrome	Rare	
• Leg ulcers	Rare	
• Hospitalization	Rare	
• Blood transfusion requirement	Non-existent	

Two forms of SCD in Saudi Arabia - Haematological Parameters

Severe SCD

Western Province

Parameter	mean±SD
RBC($x10^{12}$/l)	2.6 ±0.6*
Hb (g/dl)	8.3 ±1.1*
PCV (l/l)	0.22 ±0.04*
MCV (fl)	87.6 ±7.9*
MCH (pg)	31..7 ±4.8
MCHC (g/dl)	36.9 ±2.81
HbA_2 (%)	3.0 ±0.75*
HbF (%)	9.9 ±6.4

Mild SCD

Eastern Province

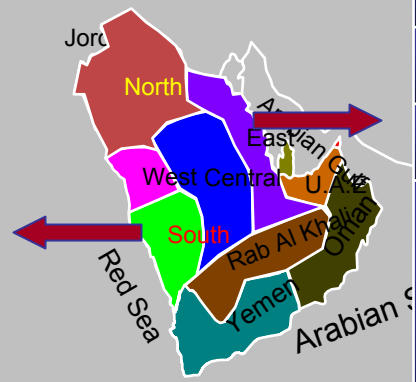
Parameter	mean±SD
RBC($x10^{12}$/l)	4.0 ±0.74*
Hb (g/dl)	10.5 ±2.0*
PCV (l/l)	0.3 ±0.05*
MCV (fl)	74.7 ±9.3*
MCH (pg)	26.2 ±4.4
MCHC (g/dl)	34.7 ±3.1
HbA_2 (%)	2.9 ±0.48*
HbF (%)	11.1 ±5.7

(* $p < 0.05$)

Two forms of SCD in Saudi Arabia – Clinical Presentation

Western Province

Sign/Symptoms	(%)
Anaemia	100
Jaundice	13.2
Splenomegaly	77.4
Hepatomegaly	66.0
Hand/foot Synd.	33.9
Crises	
-Vasoocclusive	75.5
-Infective	9.4
-Haemolytic	18.9
Hospitalization	96.2
Blood Transfusion	84.9
-2-5 times/year	43.4

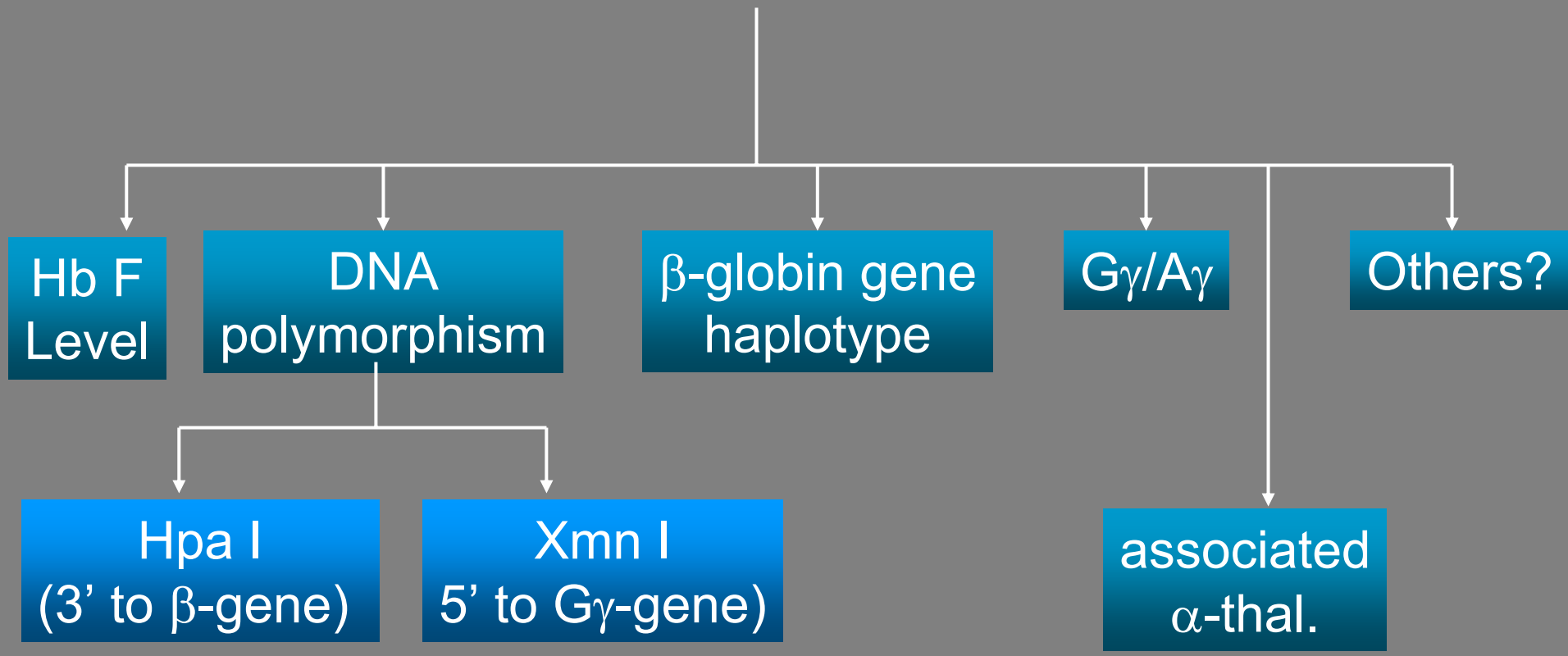


Eastern Province

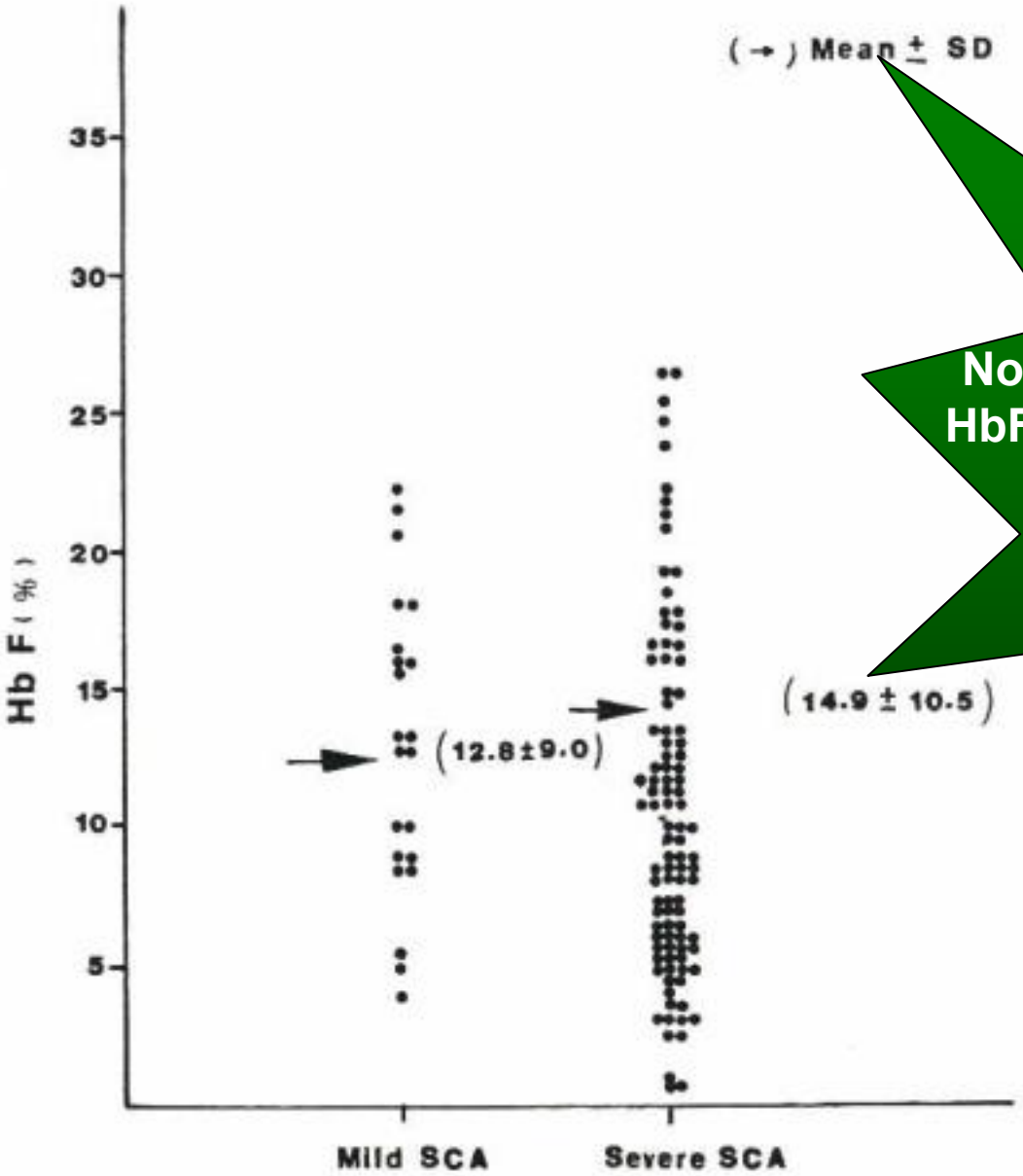
Sign/Symptoms	(%)
Anaemia	56.1
Jaundice	17.1
Splenomegaly	31.7
Hepatomegaly	36.6
Hand/foot Synd.	Nil
Crises	
-Vasoocclusive	Rare
-Infective	Rare
-Haemolytic	Rare
Hospitalization	41.5
Blood Transfusion	48.8
-2-5 times/year	---

POSSIBLE MODULATING FACTORS OF SCA?

Possible factors influencing the clinical presentation of SCA in Saudis

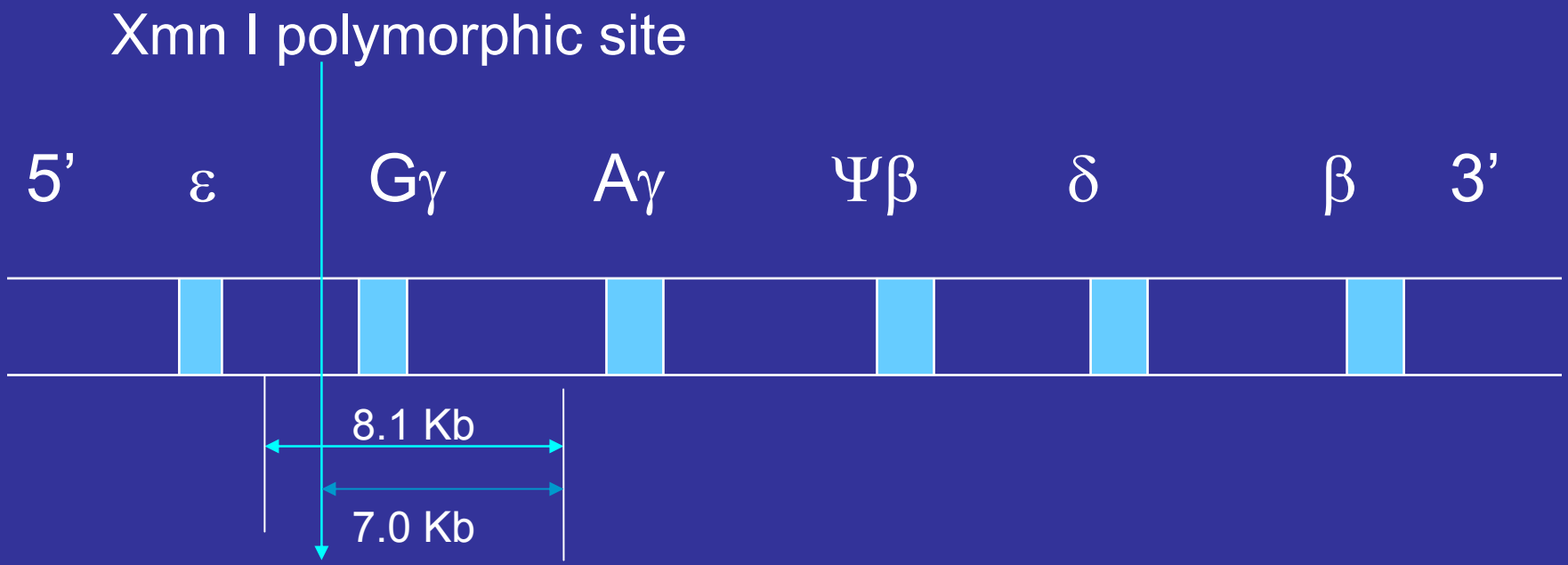


Hb F level in Saudi SCA patients



No major difference seen in the HbF level in patients with mild or severe Disease in Saudi population

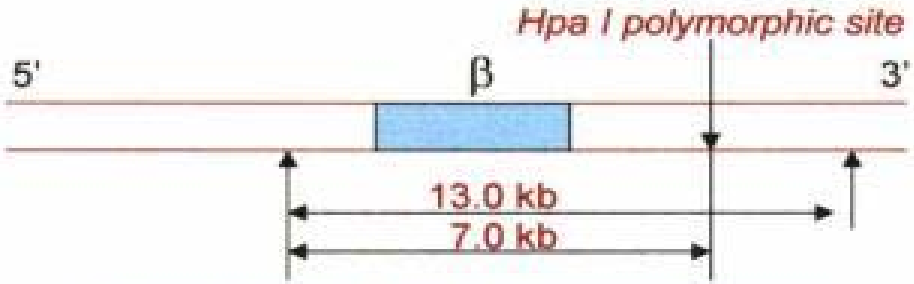
Xmn I polymorphic site in Saudi SCA patients with mild and severe SCA



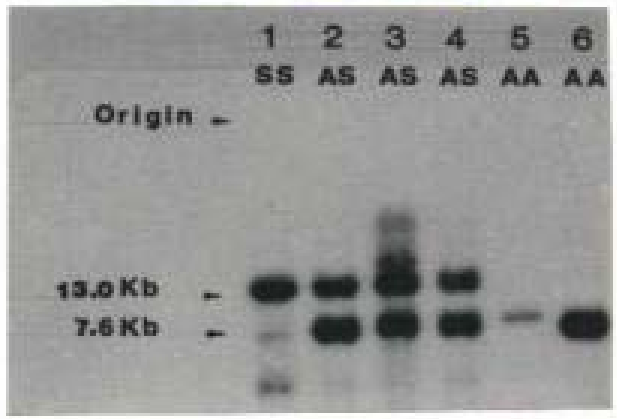
Xmn I Polymorphic	Fragment size (Kb)	Frequency (%)		P
		Mild SCA	Severe SCA	
Present (+)	7.0	86.54	2.86	<0.0001
Absent (-)	8.1	13.46	97.32	<0.0001

Hpa I polymorphic site in Saudi SCA patients with mild and severe disease

- Hpa I polymorphic site 3' to the β-globin gene.



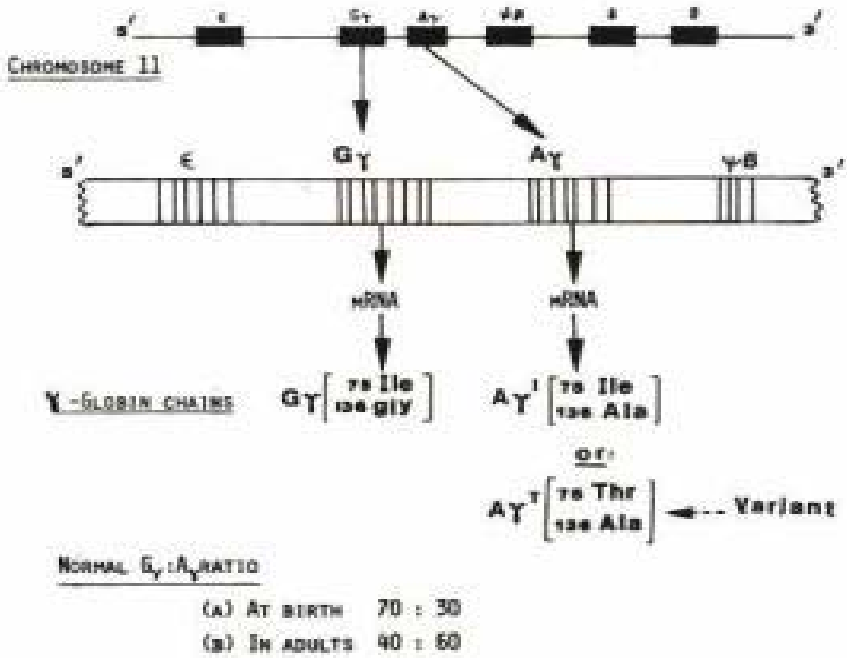
Autoradiograph of Hpa I digestion fragment containing the β-globin gene



Frequency of Hpa I polymorphic site in Saudi SCA patients

Polymorphic site	Fragment size (kb)	Frequency (%)	
		Mild SCA	Severe SCA
Present (+)	7.6	87.5	10.0
Absent (-)	13.0	12.5	90.0

The γ-globin genes: Gγ and Aγ

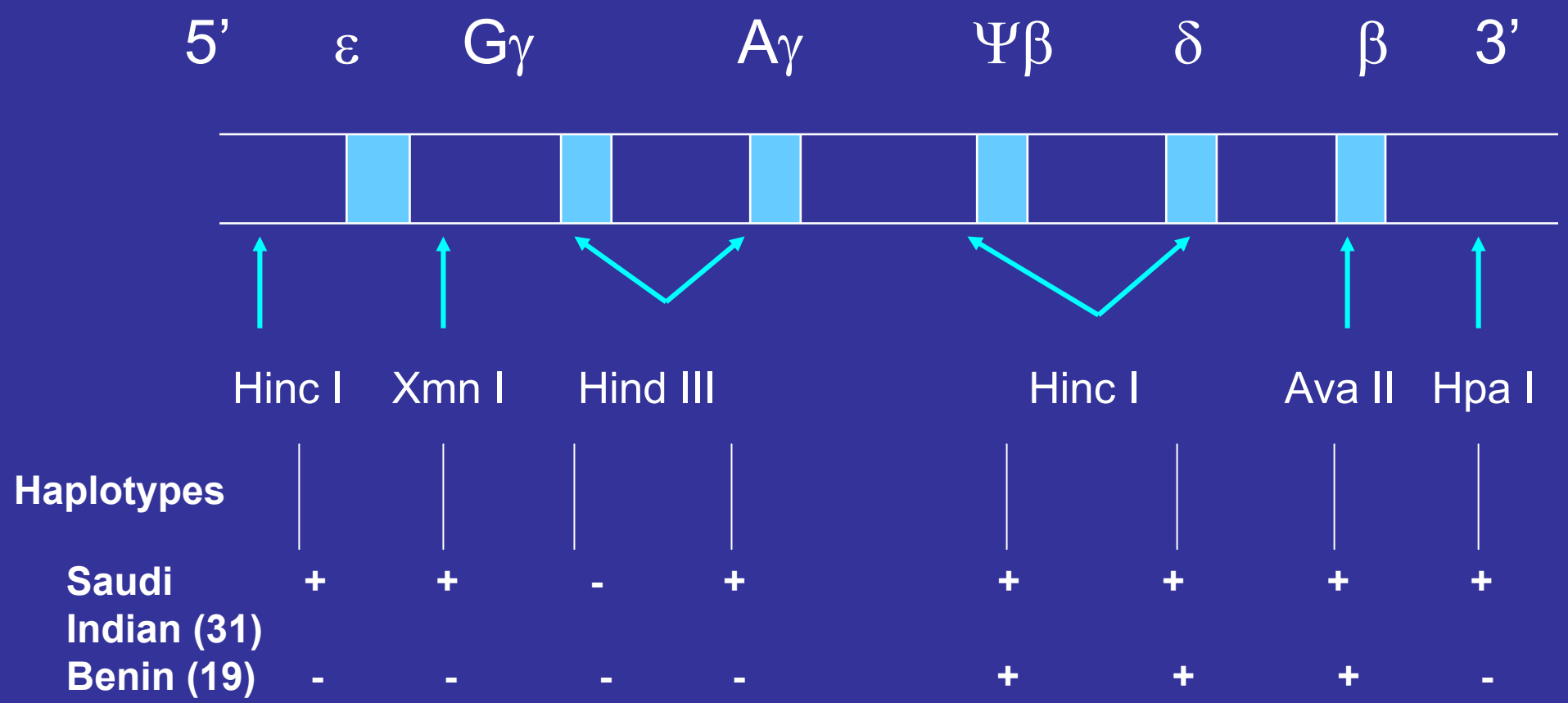


The Gγ/Aγ ratio, Aγ^{I} and Aγ^{T} in Saudi SCA patients

	Mean ± SD		
	Mild	Severe	p
▪ Gγ/Aγ	1.244±0.47	0.839±0.38	0.001
▪ Aγ^{I}(%)	19.8 ± 6.9	52.5 ± 12.6	0.0001
▪ Aγ^{T}(%)	29.9 ± 13.8	9.3 ± 5.4	0.0001

INFLUENCE OF β-GLOBIN GENE HAPLOTYPES ON CLINICAL PRESENTATION OF SCA

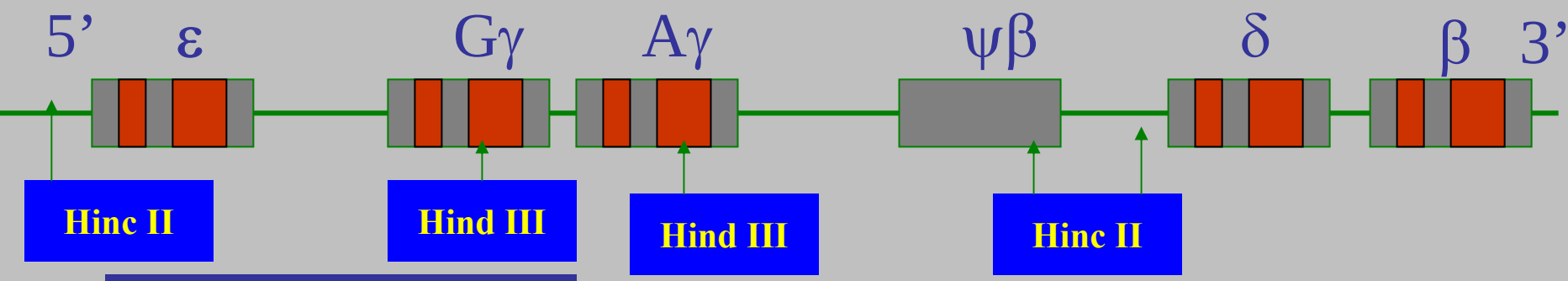
- Two major haplotypes identified in Saudi SCA patients are the Saudi/Indian haplotype and the Benin haplotype.



FREQUENCY OF THE DIFFERENT HAPLOTYPES IN SAUDI SCA PATIENTS

Frequency			p
Haplotype	Mild SCA	Severe SCA	
Benin	22.7	66.45	< 0.001
Saudi-Indian	72.6	0.75	< 0.001
Others	4.5	32.8	< 0.001

Intra-regional variations in β-globin haplotypes associated with HbS 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%

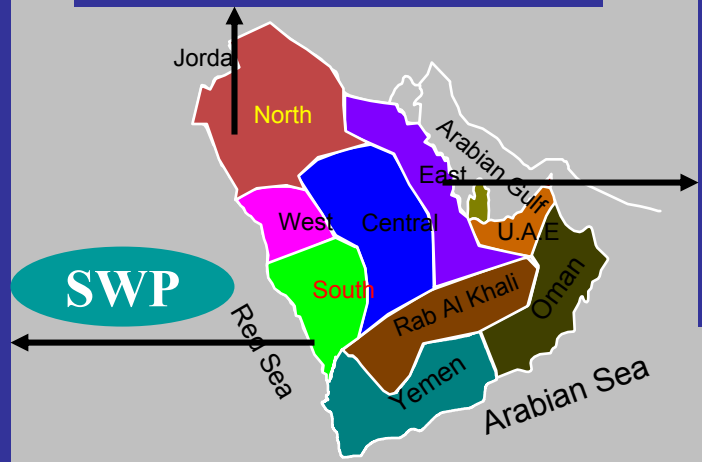
NWP

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

EP

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

SWP



AFFECTS OF ASSOCIATED α-THAL. IN SAUDI SCA PATIENTS (1)

(A) Clinical Presentation

Parameters	SCA (%) (Without α-Thal.)	SCA (%) (With α-Thal.)
Severe anaemia	56	30
Pain in bone and joints	90	70
Abdominal pain	64	29
Hepatomegaly	36	-
General weakness	90	86
Jaundice	18	14
Oedema feet	9	14
Blood Transfusion	73	30
Osteomyelitis	45	30

AFFECTS OF ASSOCIATED α-THAL. IN SAUDI SCA PATIENTS (2)

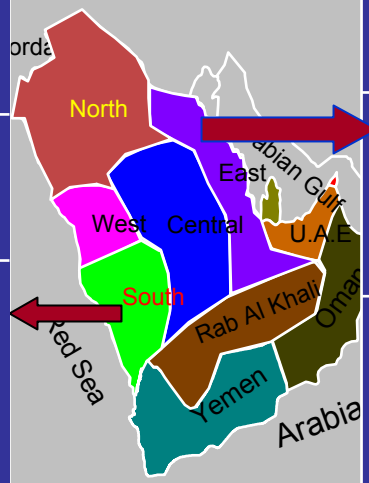
(B) Haematological Presentation

Parameter	SCA (Without α-Thal.)	SCA (-α/αα)	SCA (-α/-α)
RBC (x10^{12}/l)	3.3 1.15*	3.84 0.61*	4.35 1.01*
Hb (g/dl)	10.0 3.51	10.84 1.6	10.0 1.9
PCV (l/l)	0.27 0.08*	0.30 0.03*	0.31 0.04*
MCV (fl)	82.8 2.0*	79.8 6.90	72.5 5.4
MCH (pg)	30.3 1.5*	28.3 2.7*	25.0 3.6*
Hb A_2 (%)	2.8 0.13*	2.5 0.26*	3.25 0.6*
Hb F (%)	15.25 2.56	14.14 6.24	7.6 4.2*

Associated α-thal. ameliorates SCA and decreases major complications (*p <0.05)

Genetic differences in the two major forms of Sickle cell Disease in Eastern and Western Saudi Arabia

Differences at gene level	Severe SCD
β-globin gene haplotypes	Mainly Benin
Xmn 1 polymorphic site 5' to γ- globin gene	Absent in > 90 %
Hpa 1 polymorphic site 5' to β-globin gene	Absent in > 90 %
G γ /A γ Ratio	< 0.8
HbF level	Low to high



Differences at gene level	Mild SCD
β-globin gene haplotypes	Mainly Saudi-Indian
Xmn 1 polymorphic site 5' to γ- globin gene	Present in > 90 %
Hpa 1 polymorphic site 5' to β-globin gene	Present in > 90 %
G γ /A γ Ratio	> 1.2
HbF level	Low to high

USEFULNESS OF THE MODULATION FACTORS AND THEIR APPLICATION IN COUNSELLING AND CARE PROGRAMS

- Diagnosis of SCA
  - Prenatal diagnosis
  - Neonatal screening
  - Premarital screening
  - Population screening
- Diagnosis of carriers

Analysis of the modulating factors

- Xmn I – polymorphic site
- Hpa I – polymorphic site
- β-globin gene haplotype
- Gγ/Aγ ratio
- $A\gamma^1$ and $/A\gamma^T$
- α-thal. (1 or 2 α-gene deletions
- Hb F level ?

- Xmn I poly site (+)
- Hpa I poly site +)
- Saudi-Indian haplotype
- ↑Gγ/A, ↑$A\gamma^T$, ↓$A\gamma^1$
- 1 or 2 α-gene deletion

- Xmn I – poly site (-)
- Hpa I – poly site (-)
- Benin haplotype
- ↓Gγ/A, ↓$A\gamma^T$, ↑$A\gamma^1$
- No α-gene deletion

Disease will be mild

Disease will be severe

Treat accordingly

Counsel accordingly

CONCLUSIONS

- We conclude that SCA in Saudi Arabia is significantly influenced by genetic variation and polymorphism around the β-globin gene in addition to possible physical influence of associated α-thalassaemia.
- In the SCA patients with mild disease from the Eastern Province, majority of the patients have:
  - Xmn I polymorphic site
  - Hpa I polymorphic site
  - High Gγ/Aγ ratio.
  - Higher level of AγT
  - Lower level of $A\gamma^1$
  - α-thal. is present

(contd..)

CONCLUSION

- **While in the patients from Western province, with severe disease in majority of the patients:**
  - **Xmn I is absent**
  - **Hpa II is absent**
  - **$G\gamma/A\gamma$ ratio of lower**
  - **Level of $A\gamma^T$ is lower**
  - **Level of $A\gamma^1$ is higher**
  - **α-thal. is generally absent**

  "This may play an important role in counselling and care programmes"

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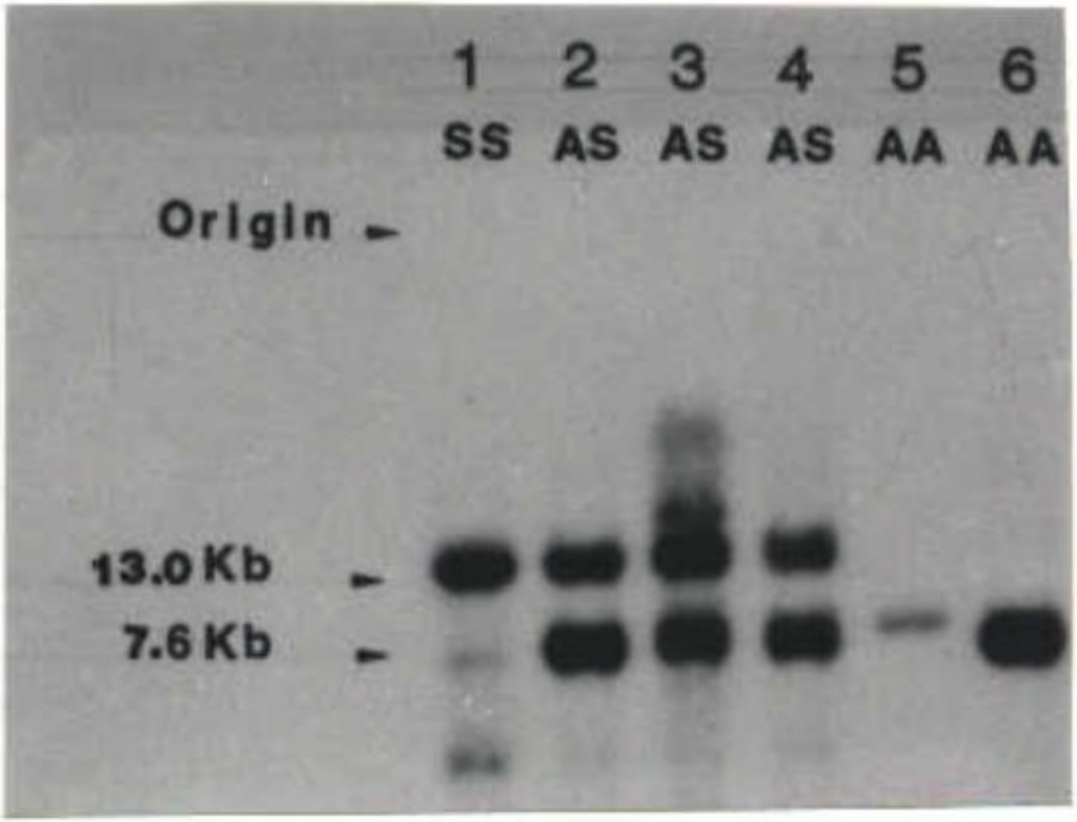
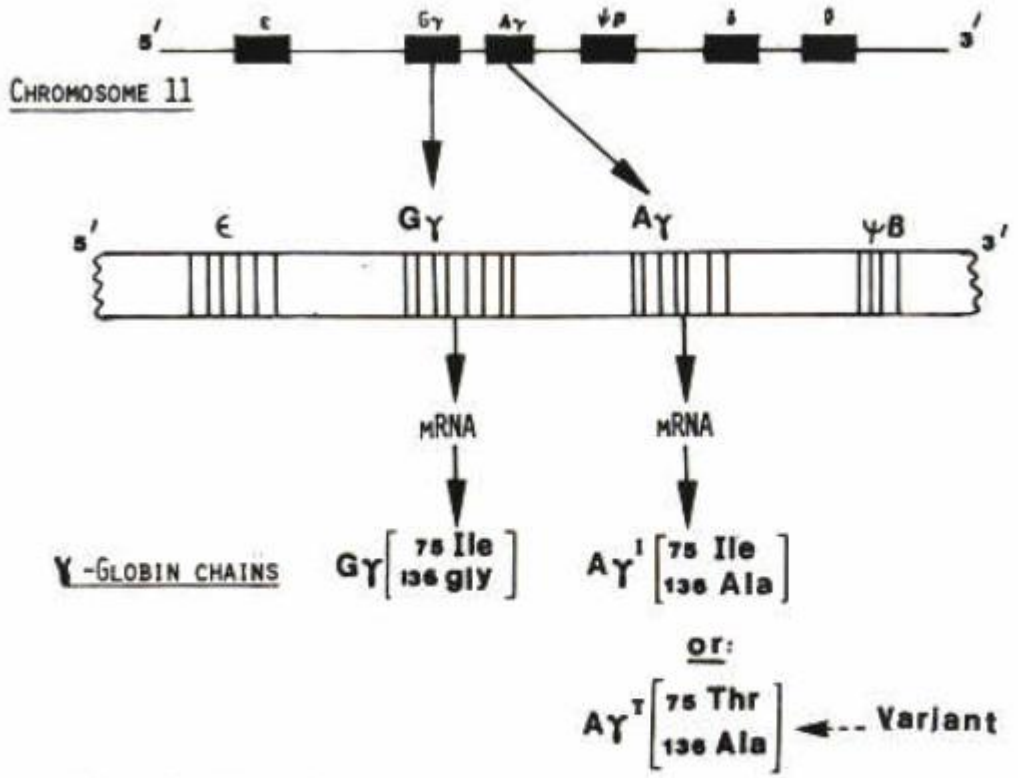
Autoradiograph of Hpa I digestion fragment containing the β-globin gene





NORMAL $G_\gamma : A_\gamma$ RATIO

(A) AT BIRTH 70 : 30

(B) IN ADULTS 40 : 60

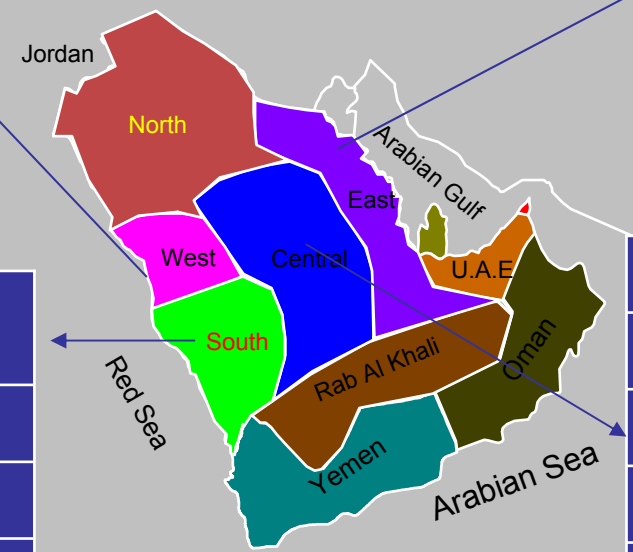
Cases identified with Rare Abnormal Haemoglobins

NWP

Hb Type	No.
AE	11

EP

Hb Type	No.
AC	4
HbF-Dammam	?
Hb-Setif	?
Hb-Handsworth	?



SWP

Hb Type	No.
AE	25
AC	8
SE	2
AD	1

CP

Hb Type	No.
AC	4
AE	3
A O-Arab	2
AD	4
SO	1
Hb Riyadh	1 Family

HAEMATOLOGICAL AND BIOCHEMICAL FEATURES IN MILD AND SEVERE SCA (1)

Parameter	Mild SCA	Severe	P
T. Hb (g/dL)	10.7 ± 2.2	9.6 ± 1.4	0.0001
RBC x $10^{12/l}$	3.8 ± 0.8	2.7 ± 0.67	0.0001
WBC x $10^{9/l}$	8.5 ± 4.4	11.8 ± 4.5	0.001
PCV (1/l)	0.29 ± 0.05	0.23 ± 0.04	NS
MCV (fl)	80.0 ± 8.8	83.3 ± 9.1	NS
MCH (pg)	29.0 ± 4.8	32.1 ± 4.6	NS
MCHC (g/dl)	36.0 ± 3.0	37.6 ± 4.0	NS
Hb A_2 (%)	2.7 ± 5.3	3.0 ± 0.5	NS
Hb F (%)	12.4 ± 5.3	13.8 ± 10.0	NS

NS = Non-significant

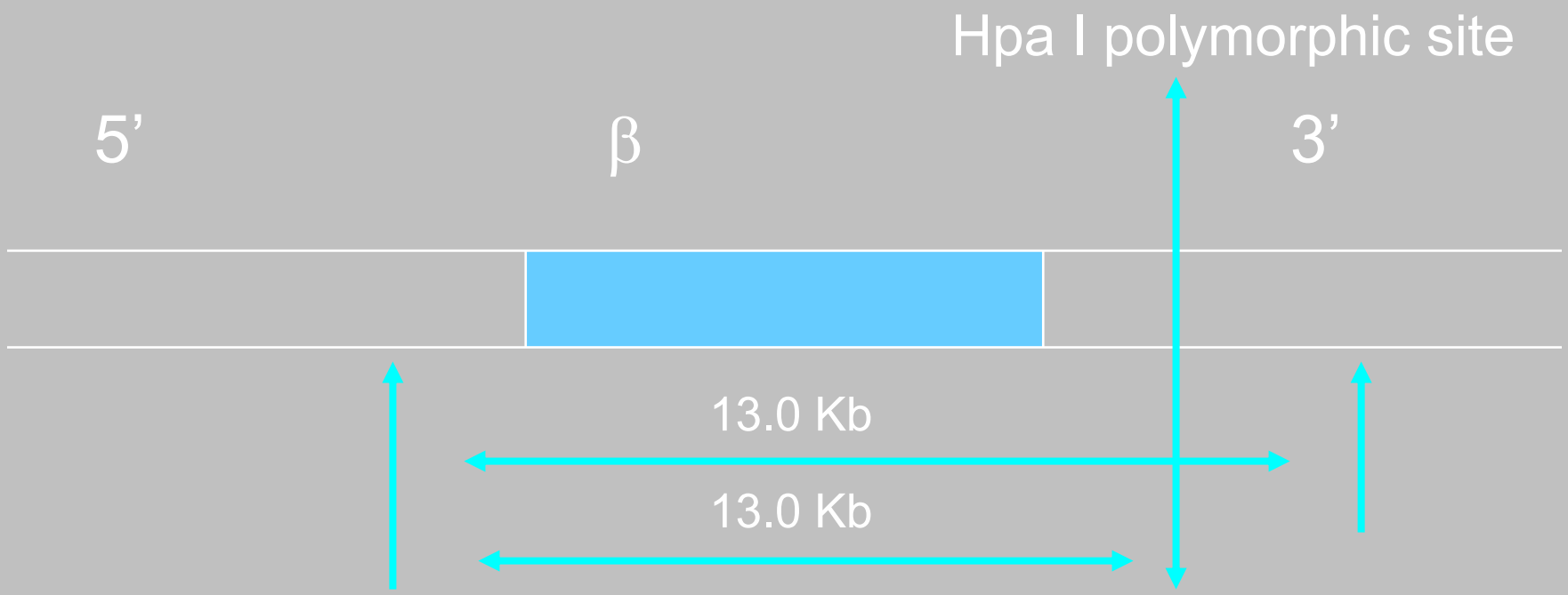
HAEMATOLOGICAL AND BIOCHEMICAL FEATURES IN MILD AND SEVERE SCA (2)

Parameter	Mild SCA	Severe	P
T. Bil (mmol/l)	9.9 ± 7.7	32.5 ± 19.3	0.01
D. Bil (mmol/l)	2.0 ± 1.3	9.95 ± 7.53	0.01
T. Protein (g/l)	80.7 ± 7.8	66.6 ± 15.8	0.01
Albumin (g/l)	47.0 ± 4.6	38.0 ± 9.0	0.01
SGOT (U/l)	40.8 ± 17.3	98.4 ± 56.6	0.01
SGPT (U/i)	23.1 ± 19.5	11.3 ± 12.1	NS
Severity Index	3.9 ± 1.2	11.09 ± 3.78	0.0001

NS = Non-significant

Hpa I polymorphic site in Saudi SCA patients with mild and severe disease

- Hpa I polymorphic site 3' to the β-globin gene



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FREQUENCY OF Hpa I POLYMORPHIC SITE IN SAUDI SCA PATIENTS

Polymorphic site	Fragment Size (kb)	Frequency (%)	
		Mild SCA	Severe SCA
Present (+)	7.6	87.5	10.0
Absent (-)	13.0	12.5	90.0