Appraisal of sickle-cell and thalassaemia genes in Saudi Arabia

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SUMMARY A comprehensive national survey of the distribution of the sickle-cell (Hb S) gene and thalassaemia genes was initiated in 1982, with more than 30 055 blood samples collected. The Hb S, α- and β-thalassaemia gene frequency range was 0.005–0.145, 0.01–0.40 and 0.01–0.15 respectively in various areas of Saudi Arabia. We present here an appraisal of sickle-cell and thalassaemia gene occurrence in the Saudi population, based on our studies conducted over 10 years in different regions of Saudi Arabia.

Introduction

Since the first report of the sickle-cell (Hb S) gene in Eastern Province, Saudi Arabia by Lehman et al., its presence in several other regions of Saudi Arabia has also been documented [*1*]. It has been shown that, in addition to sickle-cell genes, α- and β-thalassaemia genes also occur frequently, with a strong correlation existing between the frequency of these genes and malaria endemicity [*2–10*]. Also highlighted by those earlier studies conducted during the 1960s and 1970s, was the mild presentation of sickle-cell disease (SCD) in Eastern Province [*11–13*].

These studies led to an extensive national programme to determine the frequency of sickle-cell and thalassaemia genes in the different regions of Saudi Arabia, in order to study the molecular defects and natural history, and to identify the possible factors ameliorating SCD in Saudis.

This paper summarizes the results of our studies conducted over 10 years in different regions of Saudi Arabia.

Materials and methods

We screened 30 055 Saudi males and females (age range 2–60 years), living in different provinces of Saudi Arabia. Blood samples were collected by venepuncture in EDTA tubes. Fresh blood was used in a Coulter Counter ZF6 (Beckman Coulter, California, United States of America) to determine the haematological parameters and red cell indices. The buffy coat, red cells and plasma were separated by centrifugation and stored until required for analysis. The red cells were washed with cold physiological saline and haemolysed using cold distilled water. The fresh haemolysate was used to estimate haemoglobin types by electrophoresis at alkaline [*14*] and acid pH

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[*15*], and to determine the levels of Hb F [*16*] and Hb A_2 [*14*]. The buffy coat was used to extract DNA using the method employed by Kunkel et al. [*17*]. Analysis of the β-thalassaemia mutations was carried out using an amplification refractory mutation system (ARMS) [*18*] and dot-blot analysis [*19*]. The α-thalassaemia gene deletion was determined by restriction fragment length polymorphism (RFLP) studies using *Bam*HI and *Bgl* II according to the previously published method [*20,21*].

Results

The results of haemoglobin electrophoresis were used to classify the individuals as Hb AA, Hb AS and Hb SS. The prevalence of each Hb phenotype was then calculated in the total population and in different provincial populations. Figure 1 presents the prevalence of Hb AS and Hb SS and the Hb S gene frequency in different provinces and in the total Saudi population. A statistically significant difference was observed for the Hb S gene frequency in the different provinces. The highest frequency was in the eastern provinces, and the lowest in the central provinces.

Interestingly, when the number of observed Hb SS cases was compared with the number of expected Hb SS cases calculated using Hardy–Weinburg equilibrium (Table 1) in each of the provinces (except the northern provinces), the number of observed Hb SS cases was significantly higher than the number expected ($P < 0.05$).

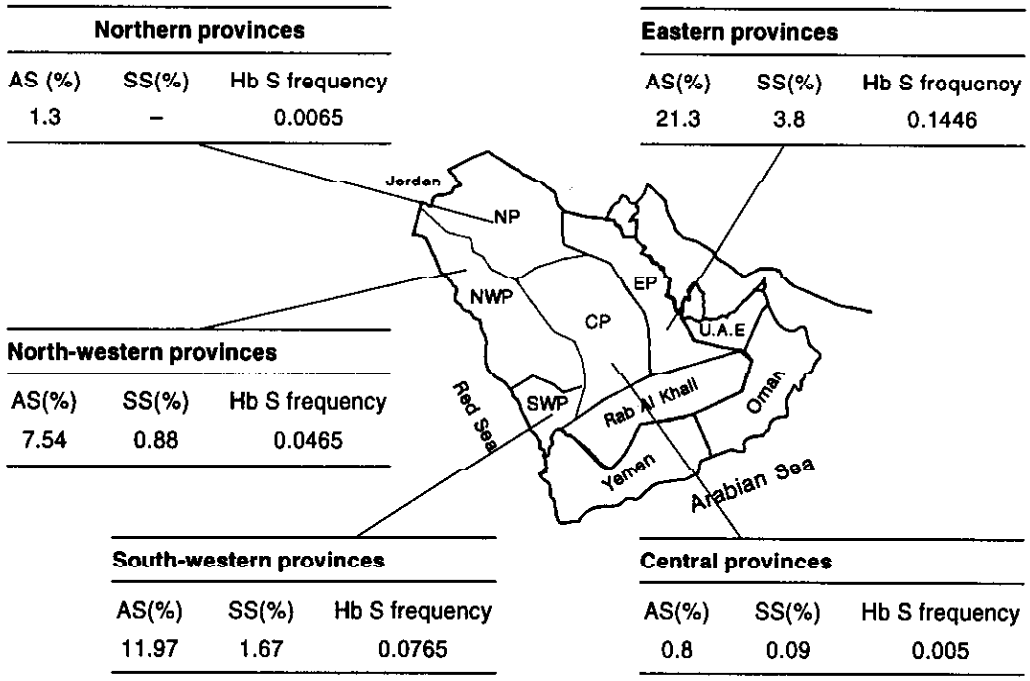

Figure 1 **Frequency of the Hb S gene in different provinces of Saudi Arabia**

Table 1 **Comparison between number of observed and expected Hb SS cases in different regions of Saudi Arabia**

Region	Observed	Expected[a]
Northern	0	0
North-western	30*	7.66
South-western	160*	47.14*
Central	9*	0.293*
Eastern	109*	43.09*

**Statistically significant*
[a]Calculated using Hardy–Weinburg equilibrium

Table 2 **Frequency (%) of α- and β-thalassaemia in different regions of Saudi Arabia**

Region	α-thalassaemia	β-thalassaemia
Northern	0.010	0.010
North-western	0.190	0.076
South-western	0.550	0.101
Central	0.010	0.036
Eastern	0.450	0.130

Table 3 **β-thalassaemia mutations identified in Saudi Arabia**

Mutation type	β-thalassaemia type	%
IVSI-110 (G→A)	+	26.9
IVSI-3' end (–25bp)	0	12.9
IVSI-5 (G→C)	+	12.9
CD39 (C→T)	0	12.9
IVSII-1 (G→A)	0	12.9
CD6 (–A)	0	4.50
CD8/9 (+1)	0	1.07
Cap + 1 (A→C)	+	1.07
IVSI-1 (G→T)	0	0.00
CD41/42 (–CTTT)	0	0.00
CD15 (TGG–TAG)	0	0.00
IVSI-6 (T→C)	+	0.00
CD16 (–1)	0	0.00
IVSII-745 (C→G)	+	0.00

Table 4 **Frequency (%) of α-thalassaemia gene deletion pattern in different regions of Saudi Arabia**

Region	α-thalassaemia gene deletion pattern		
	αα/αα	–α/αα	–α/–α
Al-Ula (NWP)	0.854	0.096	0.050
Khaiber (NWP)	0.860	0.100	0.060
Jaizan (SWP)	0.562	0.326	0.112
Al-Hafouf (EP)	0.482	0.388	0.127

NWP = north-western province
SWP = south-western province
EP = eastern province

Molecular pathogenesis of Hb S was investigated using restriction endonuclease *Mst* II. The G→T mutation in the 6th codon on the β-globin gene is recognized by *Mst* II, which produces a 1.15 kb fragment containing the β-gene in Hb AA individuals, 1.35 kb in Hb SS and both 1.15 kb and 1.35 kb fragments in Hb AS individuals. These results were obtained in all Hb SS, Hb AS and Hb AA individuals diagnosed by electrophoresis.

The clinical presentation of sickle-cell anaemia was investigated and two major clinical forms were identified. The severe form with several complications (significantly reduced haematological parameters and elevation in relevant biochemical parameters) was seen in the majority of the patients from the south-western provinces,

while in those from the eastern provinces, the disease was significantly milder.

The frequency of α- and β-thalassaemia was calculated using the results of red cell indices, Hb A_2 and, where possible, DNA analysis. The frequency of these genes for the different provinces of Saudi Arabia are presented in Table 2. A significant variation was seen in the frequency in the different provinces.

The molecular pathogenesis of β-thalassaemia mutation was investigated. The mutations identified are presented in Table 3. Both Mediterranean and Asian mutations were found to overlap in Saudis. The most frequently identified mutations were IVS1-110, IVSII-1, CD39, IVS1-5, IVI1-3' end and CD6. Together they accounted for almost 80% of the β-thalassaemia mutations in the survey population. Studies to clarify the molecular pathogenesis of α-thalassaemia in Saudis using *Bam*HI and *Bgl* II restriction endonucleases showed that the majority of α-thalassaemia was due to α-gene deletion (Table 4). A significant difference in the frequency of the α-thalassaemia deletion pattern was seen in the different regions of Saudi Arabia.

Discussion

The extensive investigations conducted over several years in different provinces of Saudi Arabia have revealed the wide distribution of the Hb S, α- and β-thalassaemia genes in the different provinces [*1–10*]. The studies have shown the high rate of occurrence of these genes in the eastern and western provinces, particularly in the south-western provinces. These areas have a history of malaria endemicity, and despite the fact that malaria has more or less been eliminated [*22*], the frequency of the gene's occurrence has remained high.

Two well-defined forms of SCD can be identified in Saudis [*23,24*]. In the eastern provinces the disease is generally milder (as reported earlier [*11–13*]), whereas in the western provinces the disease is severe [*23–27*] and similar to that reported in African populations. Several factors have been investigated to identify the possible causes of a mild clinical presentation. It appears that the polymorphic sites for different restriction endonucleases associated with the β^S globin gene and Gγ/Aγ ratio have a profound effect on the SCD phenotype, and hence on the clinical presentation of SCD [*28–30*]. Several other factors studied are listed in Figure 2. SCD in the western provinces, where the β^S is linked to the Benin haplotype, and the Gγ/Aγ ratio is low, is very closely related to the disease reported in the African population.

It is possible that the Hb S gene has arisen in the Saudi population as two separate events, with the Hb S mutation occurring on different types of chromosome 11, and this then influencing the SCD presentation. In other words, if the Hb S mutation occurs on chromosome 11 with the Benin haplotype, the disease presentation is severe, whereas if it occurs on the Saudi–Indian haplotype containing chromosomes, the disease presentation is milder.

Of special significance was the finding that the Hardy–Weinburg equilibrium was disturbed in the Saudi population. The occurrence of Hb SS observed during our investigations was significantly greater than that calculated using the Hardy–Weinburg equilibrium. Several possible factors may contribute to this. First, in Saudis there is a high rate of consanguineous marriage. This disturbs the Hardy–Weinburg equilibrium, in that the random nature of the screened population no longer applies [*31*]. Secondly, SCD is mild in the Saudi population, and the longer survival of Hb SS contributes sig-

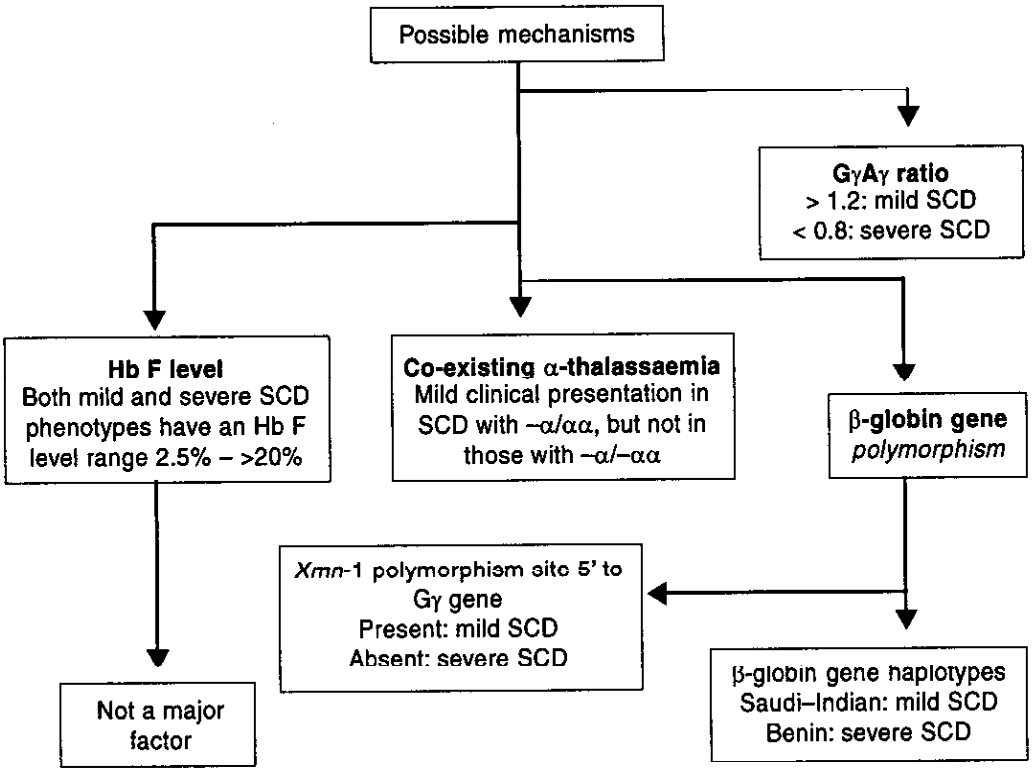

Figure 2 **Possible mechanisms leading to altered phenotypic expression of sickle-cell anaemia (SCD) in the Saudi population**

nificantly to the gene pool. Finally, it is also possible that this is due to some bias during sample collection. However, the main point to be stressed is that the Hardy–Weinburg equilibrium is disturbed in Saudis.

Studies on α- and β-thalassaemia in Saudis have also revealed considerable heterogeneity of the molecular pathogenesis, with most of the α-thalassaemia resulting from an α-gene deletion, while the majority of the β-thalassaemia is due to point mutations [*32–34*]. The –α/genotype, both in heterozygous (–α/αα) and homozygous (–α/α) forms, is commonly encountered, while cases of – –/genotype are very few [*32*]. Here then is a reason for the often-questioned absence of hydrops fatalis in Saudis. No cases have so far been reported, despite a high frequency of the α-thalassaemia gene in several of the areas. Some cases of Hb H disease (–α/– –) have been identified and are believed to result from an interaction between deletion/non-deletion type of α-thalassaemia mutation (–α/–α^T). The exact frequency of these non-deletional α-thalassaemia mutations is not known.

Beta-thalassaemia occurs frequently among Saudis, with the majority of cases having IVS1-110 mutation [*34*]. This is a β^+

mutation, and cases with homozygous mutation have mild to moderate anaemia. As severe anaemia often accompanies IVS1-100 homozygosity in other reports, this variation in Saudis may be due to associated α-thalassaemia or other polymorphisms known to influence the clinical presentation of haemoglobin disorders. In addition, double heterozygotes with this mutation and β° mutation present with moderate to severe anaemia.

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