# Haplotypes of the β-globin gene as prognostic factors in sickle-cell disease

*M.A.F. El-Hazmi,[1] A.S. Warsy,[2] N. Bashir,[3] A. Beshlawi,[4] I.R. Hussain,[5] S. Temtamy [5] and F. Qubaili [6]*

SUMMARY We collaborated with researchers from Egypt, Syrian Arab Republic and Jordan in a study of patients with sickle-cell disease from those countries, and from various parts of Saudi Arabia, in order to investigate the influence of genetics on the clinical presentation of the disease, and to attempt to determine the origin of the sickle-cell gene in Arabs. Our results suggest that β-globin gene haplotypes influence the clinical presentation of sickle-cell disease, and that there are at least two major foci for the origin of the sickle-cell gene, one in the eastern part of Saudi Arabia, and the other in the populations of North Africa and the north-western part of the Arabian peninsula.

## Introduction

The sickle-cell gene has been identified in Arab countries at frequencies which vary widely. The clinical presentation of sickle-cell disease (SCD) has two major forms; severe SCD and mild SCD.

The β-globin gene cluster is located on the short arm of chromosome 11 in a 50-kb region. The arrangement of the genes comprising the β-globin gene cluster is shown in Figure 1 [*1–3*].

Variations in the β-globin gene cluster of individuals are produced by sequence differences that result from alterations in the recognition sites of particular restriction endonucleases [*4–8*]. Sickle-cell haemoglobin (Hb S) results from a point mutation, GAG→GTG, in the 6th codon of the β-globin chain of Hb A, where a glutamate residue is replaced by valine.

Despite the same mutation in SCD patients, a widely variable clinical presentation of the disease is found [*9–12*]. Several factors are implicated as possible ameliorating agents. One of these is the β-globin gene haplotype, which is believed to influence the severity of SCD in patients from different populations [*9,13,14*]. The Hb S mutation is shown to occur on chromo-

[1]*Medical Biochemistry Department and World Health Organization Collaborating Centre for Haemoglobinopathies, Thalassaemias and Enzymopathies, College of Medicine and King Khalid University Hospital, Riyadh, Saudi Arabia.*
[2] *Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia.*
[3]*Department of Medical Technology and Biochemistry, Faculty of Medicine, University of Science and Technology, Irbid, Jordan.*
[4]*Faculty of Medicine, University of Cairo, Cairo, Egypt.*
[5]*Human Genetics, National Research Centre, Giza, Egypt.*
[6]*University of Damascus, Damascus, Syrian Arab Republic.*
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somes carrying different β-globin gene haplotypes. The haplotypes are constructed using different restriction endonucleases. If the restriction endonuclease cuts the DNA, it is given the (+) sign, and if absent, the (–) sign is recorded. Combination of two or more such sites produces a specific pattern referred to as a haplotype.

This study was conducted with two objectives: to determine the β-globin gene haplotypes associated with Hb S in Arabic-speaking populations from different countries (Egypt, Syrian Arab Republic and Jordan), and to compare the results with those of Saudi SCD patients from different regions of Saudi Arabia, where both mild and severe forms of the disease exist.

## Patients and methods

The study group of 126 SCD patients comprised 14 Egyptians, 9 Syrians, 10 Jordanians and 93 Saudis. Some of the Egyptians, Syrians and Jordanians were living in Riyadh, while others were living in their own countries, from where buffy coats were received. The Saudis were from three areas of Saudi Arabia where the sickle-cell gene has been reported at a high frequency, these being eastern (22 patients), south-western (67) and north-western (4) regions.

Blood samples were collected by venepuncture in EDTA tubes, and haematological parameters and red cell indices estimated using a Coulter Counter ZF6 (Beckman Coulter, California, United States of America). The haemoglobin pattern of each individual was reconfirmed by electrophoresis at alkaline [*15*] and acid pH [*16*]. The red cells were separated from the buffy coat and plasma by centrifugation, and washed twice with cold physiological saline. Fresh haemolysate was prepared by the addition of cold distilled water, and the levels of Hb $A_2$ [*15*] and Hb F [*17*] estimated.

Buffy coat was used to extract DNA [*18*]. Portions of DNA were first subjected to polymerase chain reaction (PCR) amplification, and the PCR product was restricted using *Ava* II, *Hin*dIII, *Hin*cII, *Hpa* I and *Xmn* I, following the procedure published earlier [*19,20*]. The presence or absence of each site (shown in Figure 1) was identified, based on the size of the fragments produced (Table 1). All DNA extraction and analysis was carried out in Riyadh, while routine haematological analysis was conducted in the respective local areas.

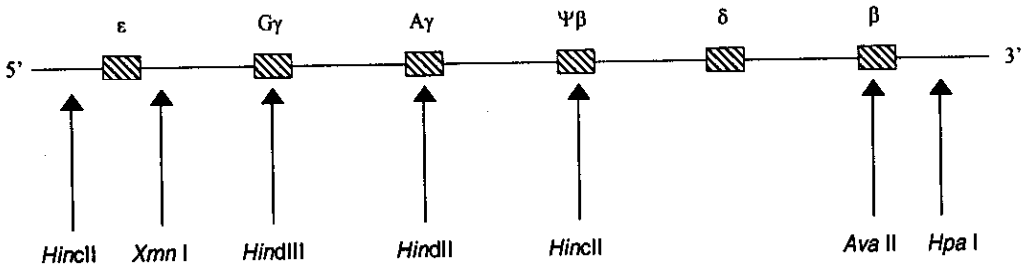


Figure 1 **β-globin gene haplotypes**

Table 1 **Fragments generated from amplified DNA to presence (+) and absence (–) of the restriction site of various restriction endonucleases**

| Restriction endonuclease | Location | Fragment size (bp) (+) | (–) |
|---|---|---|---|
| *Ava* II | 3' to β | 214 101 | 315 |
| *Hin*dIII | in Gγ | 235 980 | 323 |
| *Hin*dIII | in Aγ | 400 360 | 760 |
| *Hin*cII | 5' to ψβ | 687 107 | 794 |
| *Hin*cII | 3' to ψβ | 480 434 | 914 |
| *Hpa* I | 3' to β | 460 160 | 620 |
| *Xmn* I | 5' to Gγ | 450 200 | 650 |

## Results

All patients were classified as SCD patients based on the results of electrophoretic profiles. On the basis of disease presentation, the patients were grouped as having benign or severe SCD (Table 2).

Using a combination of the presence (+) or absence (–) of the seven restriction sites in the β-globin gene, haplotypes were constructed for each of the groups from the different countries. The frequencies of the major haplotypes in the different patients are presented in Table 3. The two main haplotypes were Benin (– – – – + – –) and Saudi–Indian (++–++++).

The Benin haplotype was found in patients with severe disease, either as homozygous or in combination with another haplotype. The majority of Syrians and Jordanians had the Benin haplotype, and severe disease. However, one in three Syrians and one in five Jordanians had a milder disease, and the Saudi–Indian haplotype was identified. All Saudi patients from south-western and north-western areas, where the disease is generally severe, had the Benin haplotype in the homozygous or heterozygous state. Of the Saudi patients from the eastern area, where a mild form of SCD exists, only 9% had the Benin haplotype. The remainder had the Saudi–Indian haplotype, either in its homozygous or heterozygous state.

Table 2 **Clinical and haematological parameters in patients with severe or benign sickle-cell disease**

| Parameter | Severe disease | Benign disease |
|---|---|---|
| *Clinical findings* | % | % |
| Pain in bones and joints | 82.4 | 85.7 |
| Abdominal pain | 80.7 | 42.9 |
| Vaso-occlusive crisis | 82.4 | 0.0 |
| Pallor | 82.2 | 42.9 |
| Jaundice | 33.3 | 14.3 |
| Hepatomegaly | 50.9 | 14.3 |
| Hand–foot syndrome | 26.3 | 0.0 |
| *Haematological findings* | **Mean ± *s*** | **Mean ± *s*** |
| Red blood cells ($\times 10^{12}$/L) | 3.0 ± 0.8 | 3.9 ± 0.9 |
| Haemoglobin (g/dL) | 6.4 ± 1.2 | 10.6 ± 2.2 |
| Packed cell volume (L/L) | 0.23 ± 0.05 | 0.3 ± 0.05 |
| Mean corpuscular volume (fL) | 8.3 ± 12.8 | 78.5 ± 10.5 |
| Hb F (%) | 10.3 ± 7.0 | 11.3 ± 6.2 |

*s = standard deviation*

## Discussion

Restriction endonuclease restriction sites have provided a useful insight into the nor-

Table 3 **Frequency (%) of the major haplotypes (homozygous or heterozygous) in patients with sickle-cell disease from different countries**

| Country of origin | Form | Benin haplotype | Saudi–Indian haplotype |
|---|---|---|---|
| Saudi Arabia | | | |
| South-western | Severe | 98.5 | 1.5 |
| Eastern | Mild | 9.0 | 91.0 |
| North-western | Severe | 100.0 | 0.0 |
| Egypt | Severe | 100.0 | 0.0 |
| Syrian Arab Republic | Severe | 66.7 | 33.3 |
| Jordan | Severe | 80.0 | 20.0 |

mal polymorphic variations in the DNA surrounding various gene loci, where a combination of two or more polymorphic sites has led to the identification of specific haplotype patterns [*13,14*]. This has been of significance in the study of the regions surrounding the β-globin gene (i.e. the β-globin gene cluster), where several polymorphic sites have been identified, and population differences have been found on analysis of the haplotype pattern [*9*]. An interesting observation is that the sickle cell mutation has occurred on chromosomes carrying different polymorphic sites and different β-globin gene haplotypes, and this seems to play a role in the clinical expression of SCD [*9*].

We compared the haplotype pattern of SCD patients from different Arabic-speaking countries. Benin haplotype was the major haplotype in all countries with a severe presentation of SCD and it was present in both the homozygous and heterozygous state. This was true for those SCD patients from south-western and north-western areas of Saudi Arabia, and for those from Egypt, Jordan and Syrian Arab Republic. On the other hand, patients from the eastern part of Saudi Arabia, who present with a significantly milder clinical picture, carried the Saudi–Indian β-globin gene haplotype either in its homozygous or heterozygous state.

The precise mechanism by which the haplotype influences clinical presentation is not clear — it may be through modulation of the globin gene expression, particularly the γ-globin gene. However, further detailed studies at the molecular level are required to unveil the mechanisms that influence the clinical presentation of SCD in Arabs.

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