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## Sickle Cell Disease in Saudi Arabia: The Phenotype in Adults with the Arab-Indian Haplotype is not Benign

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### Summary

Sickle cell disease (SCD) in Saudi patients from the Eastern Province is associated with the Arab-Indian (AI) *HBB* ( $\beta$ -globin gene) haplotype. The phenotype of AI SCD in children was described as benign and was attributed to their high fetal haemoglobin (HbF). We conducted a hospital-based study to assess the pattern of SCD complications in adults. A total of 104 patients with average age of 27 years were enrolled. Ninety-six percent of these patients reported history of painful crisis; 47% had at least one episode of acute chest syndrome, however, only 15% had two or more episodes; symptomatic osteonecrosis was reported in 18%; priapism in 17%; overt stroke in 6%; none had leg ulcers. The majority of patients had persistent splenomegaly and 66% had gallstones. Half of the patients co-inherited  $\alpha$ -thalassaemia and about one third had glucose-6-phosphate dehydrogenase deficiency. Higher HbF correlated with higher rate of splenic sequestration but not with other phenotypes. The phenotype of adult patients with AI SCD is not benign despite their relatively high HbF level. This is probably due to the continued decline in HbF level in adults and the heterocellular and variable distribution of HbF amongst F-cells.

### Keywords

Sickle cell disease; Saudi Arabia; Phenotype; Arab-Indian Haplotype; Fetal Haemoglobin

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## Introduction

Fifty years have passed since the first description of sickle cell disease (SCD) in Saudi Arabia (Lehmann, *et al* 1963). When the HbS mutation was associated with the Arab-Indian (AI) *HBB* ( $\beta$ -globin gene) haplotype, HbS homozygotes or compound heterozygotes for HbS and  $\beta^0$  thalassaemia were reported to have a milder phenotype than patients with a HbS mutation of African origin (Padmos, *et al* 1991, Pembrey, *et al* 1980, Perrine, *et al* 1972, Perrine, *et al* 1978). This was attributed to their high fetal haemoglobin (HbF) level. In some reports, SCD in the Eastern Province of Saudi Arabia, where the AI haplotype might have originated, was labelled as “benign” (Perrine, *et al* 1972, Perrine, *et al* 1978). Most patients in these reports were children, raising the question of whether the “benign” course of AI haplotype SCD extended to adults. Some studies suggested worsening of the disease as patients passed the first decade of life (Adekile, *et al* 2007, Adekile 2011, Marouf, *et al* 2003a, Marouf, *et al* 2003b). We studied the prevalence of SCD complications in Saudi patients with the AI haplotype whose average age was about 27 years, and examined the association between common complications and selected clinical and laboratory variables. We compared our results to African American patients and Saudi SCD patients from the Southwestern Province where the HbS mutation was associated with African *HBB* haplotypes. SCD in Saudi adult patients is not “benign”, and is probably due to the heterocellular and variable distribution of HbF amongst F-cells.

## Methods

### Inclusion criteria

Patients (HbSS and HbS- $\beta^0$  thalassaemia) with the AI haplotype, older than 12 years of age who presented to participating institutions in Al-Hofuf, Eastern Province, Saudi Arabia (Figure 1) in either the clinic or inpatient setting from July 2010 were enrolled after informed consent was obtained. The study was approved by the institutional review boards of all participating institutions.

### Laboratory evaluation

Steady state blood counts and erythrocyte glucose-6-phosphate dehydrogenase (G6PD) level were obtained as part of the clinical evaluation. HbF was measured by high performance liquid chromatography (HPLC) at the time of study enrollment. The Gentra Puregene blood kit was used for DNA extraction. AI haplotype was determined as previously described (Ngo, *et al* 2013). We screened for common  $\alpha$ -thalassaemia deletions ( $\alpha^{3.7}$  and  $\alpha^{4.2}$ ) and non-deletional poly A signal mutation ( $\alpha^T$  Saudi) as described (Tan, *et al* 2001, Viprakasit, *et al* 2002).

### Data collection

A comprehensive medical history and physical examination was undertaken and medical records reviewed. Age, gender, medications (including hydroxycarbamide), history of asthma and blood pressure measurement at time of enrollment were recorded. Duration and dose of hydroxycarbamide were not recorded. SCD complications that were recorded and analysed included acute vaso-occlusive painful events that occurred in the previous year and

required hospital admission, visit to emergency department or administration of pain medication at home (including crises that were self-reported or documented in the patient's chart); osteonecrosis proven by x-ray or magnetic resonance imaging (MRI) in symptomatic patients; acute chest syndrome, defined as an acute respiratory illness with a new pulmonary infiltrate on chest x-ray; overt stroke confirmed by MRI/magnetic resonance angiogram (MRA) in symptomatic patients; gallstones confirmed by ultrasound during routine screening or in symptomatic patients; splenic sequestration, defined as acute enlargement of spleen that was associated with a fall in haemoglobin level; persistent splenomegaly, defined as presence of palpable spleen at the time of assessment ; systemic infections, such as meningitis, bacteraemia, osteomyelitis, or septic arthritis, confirmed by positive culture or by MRI in osteomyelitis; priapism, defined as a sustained unwanted painful erection; leg ulcers.

### Statistical analysis

Clinical and laboratory characteristics were evaluated for association with SCD-related complications. As these analyses are descriptive and exploratory in nature,  $p=0.05$  was used as a threshold for nominal significance in all cases. Crude associations between outcomes and continuous variables were assessed using t-tests. For categorical variables, chi-square tests were used. SCD complications considered include pain, osteonecrosis, gallstones, acute chest syndrome, splenic sequestration, priapism, thrombosis, stroke, sepsis, and persistent splenomegaly. Each of these outcomes was assessed for association with age, gender, hydroxycarbamide use, history of asthma or hypertension, G6PD deficiency, white blood count (WBC), haemoglobin (Hb), mean corpuscular volume (MCV), HbF, total bilirubin, and co-inheritance of  $\alpha$  thalassaemia. Logistic regression was also performed in order to assess the relationship between each of the outcomes and HbF, adjusting for age and gender. All statistical analyses were conducted using R statistical software (R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>).

### Results

Clinical characteristics are shown in Table I and the laboratory findings are reported in Table II. Nearly 27% of patients were prescribed hydroxycarbamide. HbF level stratified by age of participants, in those who were not receiving hydroxycarbamide, is shown in Figure 2. The mean total Hb and HbF were 92 g/l and 15.6%, respectively. Acute painful episodes occurring within one year prior to joining the study were reported in 96% of patients. HbF level measured at enrollment was not associated with number of pain crisis (Figure 3). Forty-seven percent of cases had at least one episode of acute chest syndrome but only 15% had two or more episodes. The majority of patients had a palpable spleen and 66% had gallstones. All cases of osteonecrosis were in the hip, except two cases in the shoulder. Osteomyelitis or septic arthritis was observed in 8 patients and 13 patients had bacteraemia caused by organisms such as *Klebsiella pneumoniae*, *Salmonella*, *Staphylococcus aureus* or *epidermidis*. About one-third of patients tested had G6PD deficiency and 51 % had  $\alpha$ -thalassaemia.

Variables that were associated with different SCD subphenotypes are shown in Table III. HbF level was higher among patients with a history of splenic sequestration, which was probably related to the preservation of splenic function, though this association did not persist after adjustment for age and gender ( $p=0.051$ ) (Supplemental Table I). As expected, older patients had experienced more osteonecrosis and stroke events. Patients with co-existing systemic hypertension, defined as blood pressure  $>140/90$ , had higher rate of osteonecrosis and overt stroke. All 6 patients who had stroke were female. G6PD deficiency was observed more among cases with history of osteonecrosis, and this remained significant after adjusting for other variables.

## Discussion

Adults with the AI haplotype of SCD have stable HbF levels that are 3-times higher than those in African American patients and 1.5-times higher than Saudi Arabs with the Benin *HBB* haplotype. Nevertheless, the phenotype of their disease is not “benign” (Alsultan, *et al* 2012a, Alsultan, *et al* 2011). Table IV provides a summary of previous reports of clinical events in AI haplotype patients and a comparison with other patient groups. Except for the absence of leg ulcers, reduced prevalence of overt stroke and higher rate of persistent splenomegaly, the differences between adult AI haplotype and African haplotype patients were minor. Absence of leg ulcers was also observed in Saudi patients with African origin haplotypes, raising the possibility of the lack of an environmental trigger in Saudi Arabia compared with tropical Jamaica where patients have a higher rate of leg ulcers (Alsultan, *et al* 2012b, Nolan, *et al* 2006, Powars, *et al* 2005, Serjeant 1974). A formal study of mortality in the AI haplotype has not been done so it is not possible to compare survival in AI haplotype patients with other patient groups. In one analysis, the leading cause of death in Saudi patients was acute chest syndrome, followed by sepsis (Al-Suleiman, *et al* 2005, Al-Suliman, *et al* 2006). Adult patients with AI haplotype SCD bear some resemblance to patients with HbSC, given their higher hemoglobin levels, less haemolysis and a lower incidence of stroke and leg ulcers. Most children with AI haplotype SCD maintain near normal splenic function compared with African haplotype patients who commonly have early auto-splenectomy; this is probably related to the sustained high HbF in this population (Adekile 2011, Al-Awamy, *et al* 1984, Al-Jam'a, *et al* 2000, Alsultan, *et al* 2012b, Babiker, *et al* 1985, Mallouh, *et al* 1984). Persistent splenomegaly and hypersplenism sometimes require splenectomy (Al-Salem 2006, Al-Salem, *et al* 1996, Mallouh and Salamah 1985). In the first four years of life, the risk of infection was similar in AI SCD patients and normal Saudi controls (el Mouzan, *et al* 1989). Acute chest syndrome was observed in nearly half of our patients and was recurrent in 15%, slightly higher than previous reports (Padmos, *et al* 1991a, Pembrey, *et al* 1980, Perrine, *et al* 1978). Overt stroke was rare in our patients; however, silent brain infarct has been reported to be higher in adults with AI haplotype (Adekile, *et al* 2002, Marouf, *et al* 2003a).

Painful episodes during the year prior to commencement on study were not associated with HbF (Figure 3). Previous studies of pain in SCD with African origins of the HbS mutation reported that HbF was associated with a reduction of painful episodes (Steinberg and Sebastiani 2012), and HbF levels were not associated with painful events, acute chest syndrome, or survival in an earlier study of young African Americans with SCD (Powars, *et*

al 1980). Our failure to find the expected relationship between HbF and pain might be due to the short period (1 year) chosen in which to enumerate painful episodes, a relative small patient sample compared with previous studies in African Americans, ascertainment bias and the narrow and Gaussian distribution of HbF in our patients. It might also be that the definitions of painful episodes have changed over time, as has accessibility to medical care. HbF in patients with the AI haplotype is near 30% throughout the first 5-10 years of life and afterwards stabilizes at a mean level of 15-20% (Adekile, *et al* 2007, Pembrey, *et al* 1978) (Figure 2). This probably explains the benign course of the disease during early childhood and the change in phenotype in adults (Adekile 2011). With a mean haemolysate HbF of 15-20%, why is the protective effect of HbF not maintained in all adults? DeoxyHbS polymerization is prevented at a concentration of about 9-12 pg HbF/F-cell (Poillon, *et al* 1993). The number of F-cells containing this polymer-sparing concentration of HbF is the major determinant of the protective potential of HbF (Maier-Redelsperger, *et al* 1994, Poillon, *et al* 1993). Individuals with HbS-hereditary persistence of fetal Hb (HPFH) are nearly normal haematologically and clinically. Their HbF is uniformly distributed amongst all their erythrocytes and each of their cells should contain about 10 pg of HbF. This amount of HbF has been calculated to inhibit HbF polymerization at physiological venous and capillary O<sub>2</sub> saturations of 40-70% (Maier-Redelsperger, *et al* 1994). SCD with the AI haplotype, in contrast to HbS-HPFH, has a heterocellular distribution of HbF with a widely variable F-cell proportion, which ranges between 30 and 90% (Ngo, *et al* 2012, Pembrey, *et al* 1978). Depending on the distribution of HbF/F-cell, some cells will be protected from HbS polymerization and its consequences, while others will not.

This was not a prospective study and patients were restricted to those who attended clinic or were hospitalized. This might overestimate the prevalence of SCD complications. The patients enrolled represented about 9% of the clinic population in Alhassa where the number of SCD patients is estimated to be about 6000 (Al-Qurashi, *et al* 2008). Testing for certain complications, such as osteonecrosis, was restricted to symptomatic patients and so the very early stages of this complication might be missed. We also analysed the influence of HbF, measured at time of study admission, on different SCD complications; this analysis might be biased because HbF level could have varied during the period of the complication of interest. Adult patients with SCD patients in the Eastern Province of Saudi Arabia who have high HbF levels appear to have a frequency of complications comparable to other SCD populations, albeit with a higher rate of splenomegaly, rare overt strokes and absence of leg ulcers. In these particular patients, HbF appeared to have limited influence on their phenotype. This is probably a result of inconsistent distribution of protective levels of HbF amongst F-cells. Future studies are needed to study the influence of HbF on various organ functions in this population and on the overall risk of mortality.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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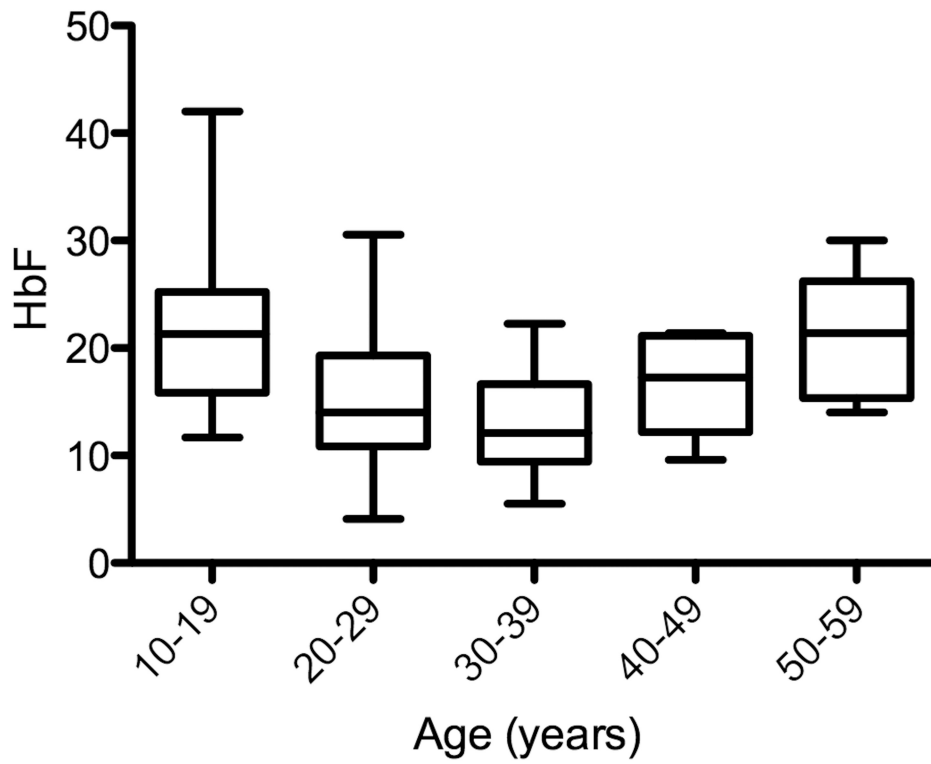
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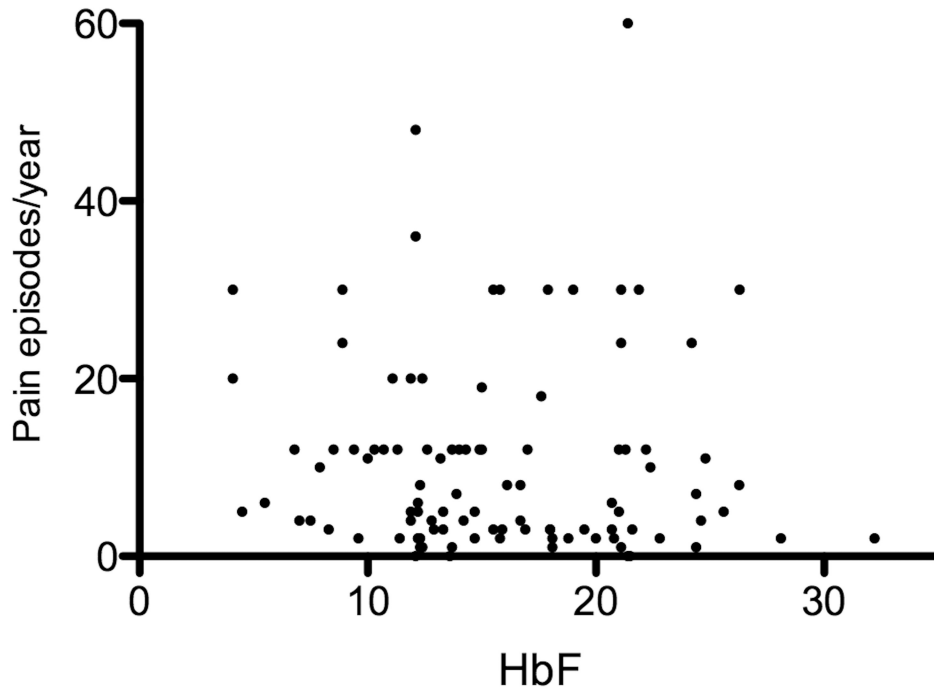




**Figure 1.** Map of Saudi Arabia showing the city of Al Hofuf (Al-Ahsa) in the Eastern Province where sickle cell disease (SCD) patients of the Arab-Indian haplotype were recruited for the current study. Saudi SCD patients from Southwestern Province (SWP) have the African origin *HBB* haplotypes, most commonly Benin.



**Figure 2.** HbF trend in relation to age in sickle cell disease patients with Arab-Indian haplotype who are not receiving hydroxycarbamide. Median HbF levels vary significantly between different age groups ( $P=0.0005$ ).



**Figure 3.** Number of pain episodes per year relative to HbF level. The number of painful episode was not associated with HbF ( $R^2=0.003$ ).

**Table I**

Clinical characteristics of Saudi patients with sickle cell disease of Arab-Indian Haplotype (n=104).

Characteristics	Number of patients (%)
Gender ratio: Male/Female	47/57 (45/55)
Mean age, years $\pm$ SD (range)	26.9 $\pm$ 10.6 (12-54)
Folic acid supplement	100 (96)
Penicillin prophylaxis	3 (3)
Hydroxycarbamide use	28 (27)
History of painful crisis	100 (96)
Median pain episodes/year (range)	6 (0-60)
Osteonecrosis	19 (18)
Acute chest syndrome	
At least one episode	49 (47)
Recurrent ( $\geq$ 2)	16 (15)
Gall stones/Cholecystectomy	69 (66)/39 (38)
Splenic sequestration	16 (15)
Splenectomy	20 (19)
Priapism	8 (17)
Leg ulcers	none
Serious infections	18 (17)
Deep venous thrombosis	4 (4)
Overt stroke	6 (6)
Persistent splenomegaly n=59	42 (71)

**Table II**

Laboratory characteristics of Saudi patients with sickle cell disease of Arab-Indian Haplotype (n=104).

Characteristics	n (%) or Mean ± SD (range)
HbSS/HbS-β <sup>0</sup>	74/30 (71/29)
WBC (× 10 <sup>9</sup> /l)	12.7±7.6 (2.6-52.5)
Hb (g/l)	92±17 (37-147)
MCV (fl)	76±11 (49-97)
Reticulocytes %	9.1±5.1 (2.6-17.0)
HbF	15.6±5.7 (4.1-32.2)
Alpha thalassaemia n=80	41 (51)
αα/−α <sup>3.7</sup>	7
−α <sup>3.7</sup> /−α <sup>3.7</sup>	16
αα/−α <sup>4.2</sup>	1
−α <sup>3.7</sup> /α <sup>TSaudi</sup> <sub>α</sub>	1
αα/α <sup>TSaudi</sup> <sub>α</sub>	15
α <sup>TSaudi</sup> <sub>α</sub> /α <sup>TSaudi</sup> <sub>α</sub>	1
G6PD deficiency n=46	13 (28)
Total bilirubin μmol/l	47±72 (5.4-534)
Serum creatinine μmol/l	41±17 (4-102)
LDH (iu/l) n=86	432±235 (146-1762)

Abbreviations: SD=standard deviation; WBC=white blood cells count; Hb=haemoglobin concentration; MCV=mean corpuscular volume; HbF=fetal haemoglobin; G6PD= glucose-6-phosphate dehydrogenase; LDH=lactate dehydrogenase.

**Table III**

Variables associated significantly ( $P < 0.05$ ) with sickle cell disease phenotype in Saudi patients with Arab-Indian haplotype.

Clinical History	Variable	Phenotype + n (%) or mean±SD	Phenotype – n (%) or mean±SD	P value
Osteonecrosis	Age	32.9±13.4 years	25.1±9.6 years	0.026
	G6PD deficiency	7 (64)	6 (18)	0.008
	Hypertension	6 (33)	4 (5)	0.003
Splenic sequestration	HbF	18.2±5.3	15.0±5.7	0.041
Priapism	Hydroxycarbamide use	5 (63) 85±27 g/l	6 (18) 101±17 g/l	0.011 0.045
	Hb			
Gallstones	Total bilirubin	55±86 µmol/l	30±15 µmol/l	0.023
Thrombosis	Hydroxycarbamide use	4 (100)	24 (26)	0.006
Overt Stroke	Age	38.0±13.3 years	26.3±10.5	0.012
	Female gender	6 (100)	41 (49)	0.029
	Hypertension	3 (50)	7 (8)	0.017

Abbreviations: SD=standard deviation; G6PD= glucose-6-phosphate dehydrogenase; Hb=haemoglobin concentration;

Table IV

Comparison of sickle cell disease patients with the Arab-Indian haplotype and those with African origin haplotype.

Variable	SCD with AI haplotype					Saudi SCD (African origin haplotype) (Alsultan, <i>et al</i> 2012b)	African American SCD studies
	Current study	Perrine, <i>et al</i> (1978)	Pembrey, <i>et al</i> (1980)	Padmos, <i>et al</i> (1991)	Kuwaiti studies		
Number	104	270	15	33		159	
Average age	26.9 years	80% <15 years	12 years	22.4 years		17.8 years	
Male/Female (%)	45/55	48/52	44/56	51/49		48/52	
Pain	96%	Rare < 3 years	13% hospitalized for pain	36% > three admissions for pain	Rare < 4 years Commonest cause of admission (Akar and Adekile 2008)	98%	61% (Platt, <i>et al</i> 1991)
Osteonecrosis	18%	4.4% between 10-19 years	n/a	31% (only hip AVN was evaluated)	25% (children) 50% (adults) (Adekile, <i>et al</i> 2001, Marouf, <i>et al</i> 2003b)	14%	10-21% (Milner, <i>et al</i> 1991, Powars, <i>et al</i> 2005)
Acute chest syndrome	47% but only 15% 2 episodes	30%	26%	15%		22%	38-50% (Castro, <i>et al</i> 1994, Gill, <i>et al</i> 1995, Powars, <i>et al</i> 2005)
Gallstones	66%	8% using oral cholecystography	n/a	n/a		34%	28% (Powars, <i>et al</i> 2005, Rennels, <i>et al</i> 1984)
Splenic sequestration	15%	n/a	n/a	3%		23%	10-20% (Gill, <i>et al</i> 1995, Powars, <i>et al</i> 2005)
Priapism	17%	0.8%	n/a	3%		6%	13-35% (Fowler, <i>et al</i> 1991, Powars, <i>et al</i> 2005)
Leg ulcers	none	none	n/a	none		none	10% (Nolan, <i>et al</i> 2006, Powars, <i>et al</i> 2005)
Serious infections	17%	4.1%	n/a	9%		11.5%	12% (Powars, <i>et al</i> 2005)
Stroke	6% overt stroke	0.9% overt stroke	n/a	n/a	Silent infarct 3.3% (children) Silent infarct 20% (adults) (Adekile, <i>et al</i> 2002, Marouf, <i>et al</i> 2003a)	7.5% overt stroke	11% overt stroke (Powars, <i>et al</i> 2005) About 30% silent infarct (DeBaun, <i>et al</i> 2012)
Persistent splenomegaly	71%	n/a	n/a	53%		11%	Autosplenectomy in majority (Frempong & Steinberg 2001)
HbSS/HbS-β <sup>0</sup>	both	HbSS only	HbS-β <sup>0</sup> only	both	both	both	both
Hb (g/d)	92	95	93	102	105 (Adekile, <i>et al</i> 2007)	86	86 (Lette, <i>et al</i> 2008)

Variable	SCD with AI haplotype						Saudi SCD (African origin haplotype) (Alsultan, <i>et al</i> 2012b)	African American SCD studies
	Current study	Perrine, <i>et al</i> (1978)	Pembrey, <i>et al</i> (1980)	Padmos, <i>et al</i> (1991)	Kuwaiti studies			
MCV (fl)	76	n/a	n/a	82.4	78.9 (Adekile, <i>et al</i> 2007)	81	89.4 (Lettre, <i>et al</i> 2008)	
Reticulocytes%	9.1	6.2	5.8	5.9		7.2		
HbF %	15.6	22	17.3	13.3	28.9 <5 years 21.5 >15 years (Adekile, <i>et al</i> 2007)	11.4	6.4 (Lettre, <i>et al</i> 2008)	
$\alpha$ -thalassaemia	51%	n/a	n/a	62%	42% (Adekile, <i>et al</i> 2007)	38%	30% (Steinberg and Embury 1986)	
G6PD deficiency	28%	n/a	n/a	21%		2.5%	10% (Steinberg, <i>et al</i> 1988)	

SCD=sickle cell disease; G6PD= glucose-6-phosphate dehydrogenase; MCV.=mean corpuscular volume; n/a= not reported or not applicable.