

# Homozygous Sickle Cell Disease in Uganda and Jamaica

## A Comparison of Bantu and Benin Haplotypes

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### ABSTRACT

**Objective:** To compare the haematological and clinical features of homozygous sickle cell (SS) disease in Bantu and Benin haplotypes in a cross-sectional study of 115 Ugandan patients attending the Sickle Cell Clinic at Mulago Hospital, Kampala, Uganda, with 311 patients in the Jamaican Cohort Study.

**Methods:** This involved comparison of clinical features and haematology with special reference to genetic determinants of severity including fetal haemoglobin levels, beta-globin haplotype and alpha thalassaemia status.

**Results:** The Bantu haplotype accounted for 94% of HbS chromosomes in Ugandan patients and the Benin haplotype for 76% of HbS chromosomes in Jamaica. Ugandan patients were marginally more likely to have alpha thalassaemia, had similar total haemoglobin and fetal haemoglobin levels but had higher reticulocyte counts and total bilirubin levels consistent with greater haemolysis. Ugandan patients had less leg ulceration and priapism, but the mode of clinical presentation, prevalence of dactylitis, features of bone pain and degree of delay in sexual development, assessed by menarche, were similar in the groups. In Ugandan patients, a history of anaemic episodes was common but these were poorly documented.

**Conclusion:** The haematological and clinical features of the Bantu haplotype in Uganda were broadly similar to the Benin haplotype in Jamaica except for less leg ulceration and priapism and possibly greater haemolysis among Ugandan subjects. Anaemic episodes in Uganda were treated empirically by transfusion often without a clear diagnosis; better documentation including reticulocyte counts and observations on spleen size is necessary to evolve appropriate models of care.

**Keywords:** Alpha thalassaemia, Bantu haplotype, SS disease, Uganda

# La Anemia de Células Falciformes Homocigóticas en Uganda y Jamaica

## Comparación de Haplotipos Bantú y Benin

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### RESUMEN

**Objetivo:** Comparar los rasgos clínicos de la anemia de células falciformes homocigóticas (SS) en los haplotipos Bantú y Benin en un estudio transversal de 115 pacientes ugandeses que asisten a la Clínica de la anemia de células falciformes en el Hospital de Mulago, Kampala, Uganda, con 311 pacientes en un estudio de cohorte jamaicano.

**Métodos:** El estudio conllevó la comparación de los rasgos clínicos y hematológicos con referencia especial a los determinantes genéticos de la severidad, incluyendo los niveles de la hemoglobina fetal, haplotipos del gen de la globina beta, y el estado de la alfa talasemia.

**Resultados:** El haplotipo Bantú dio cuenta del 94% de los cromosomas HbS en los pacientes ugandeses, en tanto que los haplotipos Benin dieron cuenta del 76% de los cromosomas de HbS en Jamaica. Los pacientes de Uganda presentaron una probabilidad marginalmente mayor de alfa talasemia, tenían niveles similares de hemoglobina total y hemoglobina fetal, pero en cambio presentaban conteos más

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*altos de reticulocitos así como niveles mayores de bilirrubina total, en correspondencia con una mayor hemólisis. Los pacientes ugandeses presentaban menor ulceración de las piernas y priapismo, pero el modo de presentación clínica, la prevalencia de dactilitis, los rasgos de dolor óseo, y el grado de retraso en el desarrollo sexual, evaluado por la menarquia, fueron similares en todos los grupos. Los pacientes de Uganda se caracterizaron comúnmente por una historia de episodios de anemia, pobremente documentados.*

**Conclusión:** *Los rasgos clínicos y hematológicos del haplotipo Bantú en Uganda fueron considerablemente similares al haplotipo Benin en Jamaica, salvo por una menor presencia de ulceración de las piernas y priapismo, así como posiblemente mayor hemólisis entre los sujetos de Uganda. Los episodios de anemia en Uganda fueron tratados empíricamente mediante transfusión, a menudo sin un diagnóstico claro. Se necesita una mejor documentación – incluyendo conteos de reticulocitos – así como observaciones del tamaño del bazo, a fin de desarrollar modelos de cuidado apropiados.*

**Palabras claves:** Alfa talasemia, haplotipo Bantú, enfermedad de la hemoglobina SS, Uganda

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## INTRODUCTION

Analysis of the DNA structure flanking the  $\beta^S$  mutation site has revealed polymorphic sites characteristic of populations with HbS which are termed beta-globin haplotypes (1). These are named after the areas where they were first described and the common African patterns are known as Benin, Senegal and Bantu (2–6). A fourth, presumed independent occurrence of the HbS mutation, characterizes populations in the eastern Province of Saudi Arabia and central India and is known as the Asian haplotype (7, 8). It was hoped that these haplotypes might provide genetic markers for clinical severity and so provide a mechanism for understanding some of the marked diversity in clinical and haematological features of homozygous sickle cell (SS) disease. A generally recognized ameliorating factor in SS disease is the persistence of high levels of fetal haemoglobin [HbF] (9). An Xmn I site 5' to the  $G\gamma$  gene, which is strongly associated with high expression of the  $G\gamma$  gene, results in higher HbF levels in the Senegal and Asian haplotypes (5, 10, 11). Fetal haemoglobin levels in the three African haplotypes showed lowest levels in the Benin haplotype and values intermediate between Benin and Senegal haplotypes in the Bantu haplotype (12). Comparison of the three common African haplotypes has been limited by the dominance of the Benin haplotype and the relatively low frequencies of the Bantu and Senegal haplotypes among patients of African origin in North America and the Caribbean although approximately equal numbers with the Benin and Bantu haplotypes occur in Brazil (13). Furthermore, comparison of the haplotypes has been largely confined to the haematology (14, 15) and although some haematological differences emerge between Benin and Bantu haplotypes, there have been no comprehensive comparisons of clinical features except for the possible suggestion that the Bantu haplotype may run a more severe clinical course (15). Since the Bantu haplotype characterizes SS disease in Uganda (4), the current study has addressed this issue by

comparing haematological and clinical features of patients attending the Sickle Cell Clinic at Mulago Teaching Hospital with a population of predominantly Benin haplotype in the Jamaican Cohort study.

## SUBJECTS AND METHODS

The Ugandan patients attended the Sickle Cell Clinic of Makerere University Medical School at Mulago Hospital, Kampala, and were ascertained through clinic attendance or through a register maintained by the Sickle Cell Association of Uganda. This was necessarily a cross-sectional study which recorded data in 78 patients over 18 days in July 2003 and in 36 patients over 22 days in July 2004 for a total study group of 114 subjects. The protocol initially required 10 subjects of each gender in the first five decades but because of the lack of older patients, the protocol was modified to use all data collected (Table 1). In the final analysis, two subjects lacked clinical data leaving 112 subjects, and 17 lacked steady state haematology, eight because of non-steady state features (two recently transfused, one aplastic crisis, five with hypersplenism) and nine subjects under age five years were excluded because of the rapid age-related changes at that age, leaving a group of 97 subjects. A comparison group was provided by the Jamaican Cohort Study (16) with 311 cases of SS disease recruited during the screening of 100 000 consecutive non-operative deliveries at the main Government maternity hospital in Kingston, Jamaica. All cases of SS disease detected in this programme have been followed prospectively as a cohort study but losses by death or emigration has left 264 subjects which have been used for haematological and clinical comparisons. The study was explained to the subjects (parents for those under 16 years of age) and signed consent obtained in accordance with the Medical Research Council (MRC) Guidelines (MRC Ethics Series, Human Tissue and Biological Samples for Use in Research, 2001).

### Laboratory methods

Blood samples were taken by venepuncture into EDTA and plain tubes. The diagnosis of SS disease was based on a single major haemoglobin band in the position of HbS on alkali haemoglobin electrophoresis on cellulose acetate (Helena System) and a positive sickle test, and characteristic haematology, supported by the demonstration of the sickle cell trait in both parents when possible. Haematological investigations included red cell indices in a calibrated haematology analyser (Coulter AcT, Coulter Beckman, Nyon, Switzerland) and reticulocytes counted manually after incubation with brilliant cresyl blue. Packed red cell volumes (PCV) were determined differently in the two populations, conduction haematocrit being used in the Uganda subjects and centrifuged microhaematocrit in Jamaica and the mean cell haemoglobin concentration (MCHC) calculated from the different forms of haematocrit in the two countries so differences in haematocrit and MCHC should be treated with caution. Thick blood films were examined for malarial parasites and fetal haemoglobin (HbF) determined by alkali denaturation (17). Serum bilirubin (direct and total) was measured by the Diazo Sulphanilic Acid method on an Alcyon 300/300i Analyzer (Abbott Laboratories, Diagnostics Division, Abbott Park, Illinois 60064), and serum ferritin by the Imx assay based on Microparticle Enzyme Immunoassay technology (Abbott Laboratories). Previous parvovirus B19 infection was based on the detection of parvovirus B19 specific IgG in a commercial immunoassay with baculovirus-based, recombinant VP2 protein (Biotrin, Dublin, Ireland) which used calibrators at 3.1, 6.25, 25, 50 and 100 IU allowing expression of results in IU. Levels below 5 IU were assumed to be non-immune and seroconversion was associated with levels of 20 IU or greater although values exceeded 100 IU in most subjects.

Red cells were carried frozen to the United Kingdom where DNA was extracted using the standard organic solvent method (18). Beta-globin gene haplotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of seven polymorphisms: *Hind-II* ( $\epsilon$ -gene), *Hind-III* ( $G\gamma$ -gene), *Hind-III* ( $A\gamma$ -gene), *Hind-II* ( $5'\psi\beta$ -gene), *Hind-II* ( $3'\psi\beta$ -gene), *Ava-II* ( $\beta$ -gene) and *Hinf-I* [ $\beta$ -gene] (19, 20). Alpha-globin genotyping was performed by Southern blot analysis, following digestion of genomic DNA with *Bam HI* or *Bgl II* and hybridization with  $\alpha$  and  $\psi\zeta$ -gene probes to detect deletion forms of alpha thalassaemia and alpha-globin gene numbers (19).

### Clinical definitions

The aplastic crisis was a sudden fall in haemoglobin associated with absence of reticulocytes, or if present, a daily increase consistent with the recovery phase, confirmed by evidence of human parvovirus infection. Acute splenic sequestration (ASS) implied a sudden increase in splenic size (usually  $\geq 3$  cm below the costal margin), fall in haemoglobin by  $> 2$  g/dL and usually below 4.5 g/dL, and increase in

reticulocyte count (usually 2–3 times steady state values), with decrease in splenomegaly following transfusion or spontaneous resolution of the attack. Hypersplenism was applied to splenomegaly (usually  $\geq 4$  and often  $> 6$  cm below the costal margin), low haemoglobin (usually  $< 6.0$  g/dL) and high reticulocytes (usually  $> 20\%$ ) sustained for periods greater than three months. Acute chest syndrome (ACS) was based on a new pulmonary infiltrate or signs of consolidation, and was usually associated with a history of cough and dyspnoea. Dactylitis was based on painful swelling of the fingers or toes, hands or feet in any combination. The painful crisis referred to episodes of bone pain sufficient to interfere with function and require narcotic analgesia. Leg ulceration was defined as an active ulcer recorded at least twice over a minimum period of six months or a healed scar. Priapism was a prolonged painful penile erection unassociated with sexual desire for which a history was routinely enquired. Stroke was a neurological deficit persisting for more than 24 hours and usually permanently, most frequently manifest as a hemiplegia; transient ischaemic attacks were a similar neurological deficit which returned completely to normal within 24 hours. Septicaemias included a variety of invasive organisms (*Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Escherichia coli*, *Salmonella* spp, *Klebsiella*) isolated on blood culture and were combined with meningitis for analysis.

### Statistical methods

The alpha thalassaemia status was examined by a Chi-squared test. Average haematology was compared using a separate linear regression for each haematological index, adjusting for the potentially confounding effects of age, sex and alpha thalassaemia status.

## RESULTS

The age and gender distribution of the Ugandan patients (Table 1) shows fewer male subjects especially among the oldest age group, but this did not reach statistical significance (binomial test,  $p = 0.46$ ).

Table 1: Age and gender distribution of study subjects

Age Group (years)	Males	Females	Total
0–9	11	14	25
10–19	11	10	21
20–29	11	11	22
30–39	11	12	23
40+	9	15	24
<b>Total</b>	<b>53</b>	<b>62</b>	<b>115</b>

*Tribal grouping:* Baganda accounted for 92/114 (80.7%), other groups included Munyole 5, Soga 3, Teso 3, Lango 2, Samia 2, Ankole 2, Baamba 1, Japadola 1, and parents of disparate tribes 3. Since only 19% were not of Baganda origin, there were insufficient numbers of other tribal groups to allow comparison.

*Alpha-globin gene number:* Among 114 Ugandan SS subjects with known alpha thalassaemia status, 7 (6.1%) were homozygous for alpha thalassaemia, 49 (43.0%) heterozygous and 58 (50.9%) had a normal alpha-globin gene number. Comparable figures in 270 subjects from the Jamaican Cohort Study were 9 (3.3%), 90 (33.3%), and 171 (63.3%), respectively showing that Ugandans were marginally more likely to have heterozygous or homozygous alpha thalassaemia ( $p = 0.06$ ).

*Beta-globin haplotype:* Haplotypes could be assigned in 104 Ugandan subjects, of which 92 (89%) were homozygous for Bantu typical/Bantu atypical 6 haplotype, 11 (10%) were heterozygous for this pattern or Bantu typical/Bantu atypical and one heterozygous for a Bantu/Senegal pattern. None had the Benin or Asian haplotype. In the Jamaican Cohort Study, beta-globin haplotypes had been determined in all 223 available subjects (88 had died or emigrated before the technology became available) and showed that 339/446 (76%) chromosomes were of the Benin haplotype (125 homozygotes, 35 heterozygous with Bantu, 17 heterozygous with Senegal, 37 with other combinations or cross-overs), 37 were the Bantu haplotype (two homozygotes, 35 heterozygous with Benin), 23 were of the Senegal haplotype (one homozygote, 17 heterozygous with Benin, four other combinations) and 47 were a mixture of cross-overs or uncommon chromosome structures.

*Haematological indices:* Comparison with Jamaican data was confined to subjects aged  $\geq 5$  years when age-related changes are less rapid. Total haemoglobin levels were similar, PCV, mean corpuscular volume (MCV), platelets, proportional reticulocyte counts and total bilirubin levels were higher, and mean corpuscular haemoglobin (MCH) and MCHC lower compared with Jamaican Cohort subjects

(Table 2). Fetal haemoglobin levels did not differ between the populations.

*Malarial parasites:* Malarial parasites were seen in 7/108 (6%) graded semi-quantitatively as 1<sup>+</sup> parasites in six and 2<sup>+</sup> in one. These subjects were clinically well, three had splenomegaly, and haematologically these subjects did not differ from the whole group. Malaria does not occur in Jamaica so comparable data are not available.

*Serum parvovirus B19 data:* B19 antibodies were studied in 2003 when sera were available in 74/78 (95%) Ugandan subjects. Estimates of B19 IgG from Biotrin kits (courtesy of Dr Bernard Cohen, Health Protection Agency, Colindale, London) showed seroconversion in 18% of those aged 0–9 years, in 68% aged 10–19 years, 84% aged 20–29 years, 92% aged 30–39 years, and 100% of those aged  $\geq 40$  years. One patient aged 9.8 years when first seen had an Hb 5.2 g and reticulocytes of 1%, values falling to 3.2 g and 0% two days later, received immediate transfusion and when seen six months later had values of 8.2 g and 11% reticulocytes. These features were consistent with a classic aplastic crisis which was confirmed by 10<sup>11</sup> parvovirus DNA particles in the acute serum. Seroconversion rates to human parvovirus in the Jamaican Cohort were almost identical (21).

*Serum ferritin:* Assays in 60 females gave an average of 174 ng/ml (median 219, range 14 to > 1000) and in 49 males, an average of 258 ng/ml (median 142, range 19–1127). No patient had values below 10 ng/ml, consistent with iron deficiency. Values exceeded the upper limit of normal for females (80 ng/ml) in 45 (75%) and for males (250 ng/ml) in 17 (35%). Most had received transfusions but the recalled quantities were inaccurate except for one female aged 4.9 years who had received 28 transfusions for hypersplenism prior to splenectomy at age 3.9 years and whose ferritin level was > 1000 ng/ml.

Table 2: Comparison of haematology with Jamaican Cohort in patients five years and older

	Jamaican Cohort Steady state n = 264			Ugandan Clinic n = 97			Comparison*	
	mean	SD	range	mean	SD	range	difference	p
Hb (g/dL)	7.73	0.94	5.4–10.9	7.91	1.59	4.7–12.8	0.007	0.97
HbF (%)	5.93	4.58	0.27–25.35	6.22	4.46	0.4–22.6	-0.238	0.70
PCV	22.66	2.85	16.0–31.4	26.35	5.11	16.7–43.6	3.068	< 0.001
MCHC	34.28	1.34	29.5–38.0	30.11	1.50	26.0–34.0	-3.987	< 0.001
RBC ( $\times 10^{12}/l$ )	2.73	0.43	1.88–4.28	2.89	0.68	1.61–4.46	0.110	0.08
MCV (fl)	84.62	7.27	63.85–102.45	92.65	9.94	71–123	7.280	< 0.001
MCH (pg)	28.71	2.84	21.20–35.73	27.93	3.36	19.0–38.0	-0.911	0.01
NBC	13.99	3.46	5.65–36.00	13.14	4.07	5.5–28.6	0.303	0.54
Platelets	476.7	114.7	226–940	491.6	165.6	118–863	35.03	0.05
Reticulocytes (%)	11.4	3.1	4.5–27.0	16.29	6.59	3.0–33.0	6.238	< 0.001
Total bilirubin	47.7	22.0	14.0–143.3	68.05	58.9	2.9–308.2	16.07	0.001

\*Ugandan Clinic participants compared to Jamaican Cohort controlling for age, sex, and alpha thalassaemia status.

Hb: haemoglobin; HbF: fetal haemoglobin; PCV: packed cell volume; MCHC: mean cell haemoglobin concentration; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; NBC: normal B-cell

*Serum creatinine:* Assays were performed in the 46 subjects aged  $\geq 30$  years of whom two male subjects aged 39 and 43 years had values exceeding the normal upper range of 124  $\mu\text{mol/L}$  and so would be classified as chronic renal impairment. However, using the revised upper limits of normal for SS disease of 80  $\mu\text{mol/L}$  for males and 68  $\mu\text{mol/L}$  for females, derived from serum creatinine levels and glomerular filtration rates in the Jamaican Cohort (22), impaired renal function occurred in 10/20 (50%) males and in 2/26 (23%) females of the Ugandan subjects. Comparison of prevalence with the Jamaican Cohort was not possible because of their lower age distribution.

### Clinical Features

*Presentation:* Of 103 subjects recalling the mode of presentation, dactylitis (hand-foot syndrome) accounted for 55 (53%), bone pain and/or swelling for 20, fever for nine, anaemia for six, vague ill health for four, incidental diagnosis for three, jaundice for two, meningitis/stroke for two, and acute chest syndrome for two. These figures are very similar to the initial specific symptoms occurring in the Jamaican Cohort (23).

*Splenomegaly:* In Ugandan patients, the spleen could not be felt in 91 (81%), was 1–2 cm below the costal margin in 10, 3–5 cm in five, 6–15 cm in six, and one child aged 4.9 years had had a splenectomy at 3.9 years for hypersplenism. In the six subjects with a spleen 6–15 cm below the costal margin, three patients with splenomegaly of 6–8 cm and mean age of 5.9 years (3.8–11.8 years) were not convincingly hypersplenic whereas three subjects with spleens measuring 12–15 cm and a mean age of 7.8 years (5.3–11.8 years) had haematology and transfusion requirements consistent with hypersplenism. The crude prevalence of hypersplenism in patients under 10 years of age was similar in Uganda (12%) and the Jamaican Cohort (13%, unpublished data). The incidence of acute splenic sequestration which was a major complication in the Jamaican Cohort, affecting 30% by the age of five years (24), could not be assessed in Ugandan subjects because of the lack of diagnosis in acute anaemic episodes.

*Anaemia and transfusion:* In Ugandan patients, 37 (33%) denied transfusion, single transfusions were reported in 36 (32%), two transfusions in 15 (13%), 3–5 transfusions in 18 (16%) and more than five transfusions in seven (6%). Indications for transfusion were usually low haemoglobin levels without documentation of reticulocyte count or mean cell volume or a clear diagnosis. Overall transfusion rates were similar to those in the Jamaican Cohort (25).

*Dactylitis:* Fifty-seven (55%) of 103 Ugandan patients recalled dactylitis compared to a cumulative prevalence of 45% by the age of two years in the Jamaican Cohort (26). One 43-year old male had a shortened right 5<sup>th</sup> metacarpal consistent with premature fusion of the growing epiphysis characteristic of infection complicating dactylitis.

*Bone pain or swelling:* This were reported in 104 (92%) Ugandan patients and the nine patients denying bone pains

were either young (1.2–7.0 years) or hypersplenic with haemoglobin levels between 3.8 and 5.7 g/dL. In 84 patients with a history of bone pain, precipitating factors included cold (61), exertion/fatigue (27), fever/malaria (17), stress (11), menstruation especially the premenstrual period in 3/45 (7%) postpubertal women. In the 47 patients aged over 20 years with recorded history, pain declined with age in 40 (85%), got worse in five and was unchanged in two.

*Avascular necrosis of femoral head (ANFH):* Ugandan patients reported symptoms in 10 patients (two males) with current ages of 17 years (one patient), 20–29 years (two patients), 30–39 years (one patient), and over 40 years (six patients). Age at onset was reported as eight years (two patients), 10–19 years (three patients), 20–29 years (two patients), 30–39 years (one patient), and over 40 years (two patients). The right side was affected in four, the left in four, and bilaterally in two. Three had painful limitation of movement, two had received hip surgery and one was told that the surgery was dangerous in Uganda and should be performed elsewhere. Another patient aged 27 years treated with an Austin Moore prosthesis persisted with painful limitation of movement and X-ray evidence of *protrusio acetabuli*.

*Acute chest syndrome:* A history of pneumonia was recalled in 17 (15%) subjects, was multiple in three, and accounted for initial presentation of sickle cell disease in two (five months and 20 months). In the circumstances, it was not possible to confirm these events by review of hospital notes or X-rays.

*Leg ulcers:* Ugandan patients had active ulcers or ulcer scars in 10/112 (9%) patients, affecting 4/23 (17%) aged 30–39 years and 6/24 (25%) aged 40 years and above. Age of onset was reported as 10–19 years in three patients, 20–29 in two, 30–39 in two, over 40 years in one and was not recalled in two. The Jamaican Cohort Study showed a median age of onset of 18 years, and a greater overall prevalence of 30% (27).

*Priapism:* Priapism occurred in six males, four aged 16, 20, 28 and 37 years with short-lived, nocturnal, stuttering episodes and two aged 20 and 25 years had major attacks lasting two days or longer. There was a 15% prevalence among Ugandan males aged  $\geq 15$  years compared with 40% among postpubertal males in the Jamaican Clinic (28).

*Puberty and reproduction:* Of the 44 post-pubertal females, menarche commenced at a median age of 15.5 years (range 12–25 years) compared with 15.4 years in the Jamaican Cohort (29). The Ugandan group reported 33 pregnancies in 15 subjects (mean 2.2 pregnancies, range 1–4) compared with a mean of 1.8 pregnancies among those becoming pregnant in the Jamaican Cohort (30) but this figure is likely to rise.

*Other clinical features:* Evidence of marked bone marrow expansion occurred in five subjects (one aged 40 years with gnathopathy due to expansion of the maxilla, two aged 10 and 16 years with bossing due to expansion of the parietal bones, one aged 15 years with tower skull and one aged six

years with both tower skull and gnathopathy). The haematology of these five cases did not differ significantly from the rest of the group. Strokes had occurred in four subjects at ages 8, 8, 14 and 15 years, three with right hemiplegia and a 10-year old boy with spastic quadriplegia and hypersplenism. Osteomyelitis had been drained in four subjects.

*Surgery:* Twenty-two subjects had surgical procedures, six obstetric (one ectopic pregnancy, two lower segment Caesarean sections, three tubal ligation), eight orthopaedic (two hip surgery, four drainage of osteomyelitis, one carpal tunnel syndrome, one trauma), two skin grafting (split skin grafting in both), one splenectomy for hypersplenism, one cholecystectomy for gallstones, and four other procedures (umbilical hernia, circumcision, dorsal slits for major priapism, tonsillectomy).

## DISCUSSION

It is impossible to separate a genetic disease from its environment and sickle cell disease in Uganda occurs against a background of malaria, malnutrition, frequent infection, and generally poor socio-economic conditions. The most representative and accurate data for comparison were derived from the Jamaican Cohort Study, which has the advantage of meticulous and prospective recording of complications but a disadvantage in that the oldest Cohort study subjects were only 31 years at the time of this study. However, the Cohort does provide age-matched comparison data for 68 (60%) of Uganda subjects. More serious will be the much greater selection pressure for survival in Uganda. No formal figures are available for survival among Ugandan SS subjects although estimates suggest that the median survival may be less than five years, consistent with the difficulty of finding sufficient subjects in the older age groups. By comparison, median survival in Jamaican SS subjects has been estimated as 52 years for males and 58.5 years for females (31). It is inevitable therefore, that the Ugandan subjects represent a group which has survived malaria and other adverse environmental conditions. The different selection pressures in the two communities, Uganda and Jamaica, therefore demand caution in interpreting some of the differences. Further caution is required in this interpretation since values from the Jamaican Cohort were based on the mean of multiple steady state values whereas Ugandan values were single estimates during the presumed steady state. Against this background, some features of interest did emerge.

All Ugandan subjects possessed at least one Bantu chromosome and 89% were homozygous for the Bantu typical or Bantu atypical 6 according to the nomenclature of Nagel and Steinberg (32). Of the two genetic factors generally believed to influence expression of SS disease, there was no discernible difference in HbF levels but deletional alpha thalassaemia was marginally more common among Ugandan subjects. Total haemoglobin levels were similar but the MCV was higher in Ugandan subjects despite the greater prevalence of alpha thalassaemia which generally lowers

MCV. Reticulocyte counts and total bilirubin levels were higher in Ugandan subjects consistent with a greater haemolytic rate. A shortcoming of the Ugandan data was that, possibly because of limited resources, acute anaemic episodes were treated empirically by transfusion often without a clear underlying diagnosis, so the prevalence of acute splenic sequestration and of the aplastic crisis, which are important causes of acute anaemic episodes in Jamaica (24, 33) were undocumented in Uganda. Indeed, it was believed that aplastic crises did not occur but one acute parvovirus infection was documented during the present study and seroconversion rates to parvovirus in Ugandan subjects (34) was similar to that in Jamaica. In the patient with acute aplasia mentioned above, the reticulocyte count was not reported by the laboratory because no reticulocytes were seen leading to the assumption that the supravital staining method had failed to work whereas the real absence of reticulocytes was crucial to the diagnosis of aplasia. The natural history of aplastic crises elsewhere indicates that immunity to parvovirus is life-long and recurrence has never been observed thus simplifying the differential diagnosis of subsequent acute anaemic episodes in an individual patient.

Similar observations apply to cases with marked splenic enlargement and haematological evidence of hypersplenism. Elsewhere, it has been found that splenectomy is a more cost-effective and potentially less dangerous therapy than recurrent transfusion (35) and one subject with 32 admissions and 28 transfusions between ages 2.1–3.9 years has remained virtually asymptomatic without admissions or transfusions over the two years postsplenectomy. Episodes of acute splenic sequestration, once defined and the natural history better understood, may be more appropriately prevented by splenectomy, which should also be a treatment option for chronic hypersplenism. Similarly, iron or folate deficiency, once diagnosed, should respond to specific oral supplementation. To evolve appropriate models of clinical care for Uganda, there is an urgent need to document the diagnoses leading to acute anaemic episodes, which must include appraisal of clinical spleen size and also of routine reticulocyte counts.

Other clinical differences were the lower prevalence in Uganda of stuttering priapism and chronic leg ulceration. Stuttering priapism, referring to nocturnal painful erections, usually lasting two to three hours unassociated with sexual activity, are often underestimated because of patient embarrassment and the lack of awareness that this is a common feature of SS disease. Direct questioning has therefore become routine in Jamaica leading to a history of stuttering priapism in approximately 40% of post-pubertal males (36) but a similar approach in Uganda found this problem in 15% of males  $\geq$  15 years. The importance of this complication, apart from daytime somnolence from loss of sleep, is that it may be a prodrome for major attacks with erections exceeding 12 hours which are generally followed by damage to the vascular erectile system and permanent impotence.

Chronic leg ulceration was also less frequent in Ugandan patients than in the Jamaican Cohort where ulceration of  $\geq$  six months occurred in 30% with a median age of onset at 18 years (27). In contrast, active ulcers or healed ulcer scars occurred in 10 (9%) Ugandan patients and tended to occur at a later age. The prevalence of ulcers in the Cooperative Study in the United States was 5–10% (37) and the much higher prevalence in Jamaican SS disease remains unexplained although it includes poor socio-economic status (27). However, the lower prevalence among Ugandan subjects despite generally poor socio-economic conditions suggests other mechanisms may contribute to the high prevalence in Jamaica.

A striking clinical feature among some African patients is the clinical evidence of erythropoietic extension manifest in the maxillary expansion leading to gnathopathy (38), widening of the diploic space with bossing of the frontal and parietal bones (39) and of cephalohaematoma (40). Formal prevalence figures are not available for Jamaica but there is a clinical impression that these features are much less frequent than in reports from Africa. It is assumed that these changes reflect greater bone marrow activity which, in association with the relative reticulocytosis, are consistent with greater bone marrow activity among some African patients.

Apart from these differences, many aspects of the disease were similar in both environments. Delayed physical and sexual development, characteristic of SS disease, was similar judged by the onset of menarche in Ugandans and Jamaicans, the latter representing a 2.5-year delay compared to normal Jamaican controls (29). There was also a close similarity between the patterns of presentation and the prevalence of dactylitis (23). Furthermore, there were few haematological differences, bearing in mind the constraints of the techniques available.

Perhaps the major message of this study is the need for careful documentation of acute anaemic episodes which could help define the role of splenectomy in the management of acute splenic sequestration and of chronic hypersplenism and avoid the economic burden and potential dangers of unnecessary transfusion.

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