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Original Article

The burden and consequences of inherited blood disorders among young children in western Kenya

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Abstract

Although inherited blood disorders are common among children in many parts of Africa, limited data are available about their prevalence or contribution to childhood anaemia. We conducted a cross-sectional survey of 858 children aged 6–35 months who were randomly selected from 60 villages in western Kenya. Haemoglobin (Hb), ferritin, malaria, C-reactive protein (CRP) and retinol binding protein (RBP) were measured from capillary blood. Using polymerase chain reaction (PCR), Hb type, –3.7 kb alpha-globin chain deletion, glucose-6-phosphate dehydrogenase (G6PD) genotype and haptoglobin (Hp) genotype were determined. More than 2 out of 3 children had at least one measured blood disorder. Sickle cell trait (HbAS) and disease (HbSS) were found in 17.1% and 1.6% of children, respectively; 38.5% were heterozygotes and 9.6% were homozygotes for α^+-thalassaemia. The Hp 2-2 genotype was found in 20.4% of children, whereas 8.2% of males and 6.8% of children overall had G6PD deficiency. There were no significant differences in the distribution of malaria by the measured blood disorders, except among males with G6PD deficiency who had a lower prevalence of clinical malaria than males of normal G6PD genotype ($P = 0.005$). After excluding children with malaria parasitaemia, inflammation (CRP > 5 mg L^{-1}), iron deficiency (ferritin < 12 μg L^{-1}) or vitamin A deficiency (RBP < 0.7 μg L^{-1}), the prevalence of anaemia among those without α^+-thalassaemia (43.0%) remained significantly lower than that among children who were either heterozygotes (53.5%) or homozygotes (67.7%, $P = 0.03$). Inherited blood disorders are common among pre-school children in western Kenya and are important contributors to anaemia.

Keywords: sickle cell disorders, haemoglobinopathies, thalassaemia, G6PD deficiency, haptoglobins, anaemia.

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Introduction

Anaemia is a major cause of death and disease among young children in Africa (WHO/UNICEF/UNU 2001). Previous studies in Kenya have found that approximately 66% of children aged 6–35 months were anaemic (Kenya Ministry of Health 2002; CDC 2007). Although iron deficiency is the most common cause of anaemia, many other factors, including malaria and other infections, vitamin A deficiency, inflammation, and inherited blood disorders, play variable roles in different settings (WHO/UNICEF/UNU 2001).

Inherited blood disorders are common in sub-Saharan Africa, such as α^+-thalassaemia and the sickle mutation of the β-globin gene (β^s) that leads to the production of HbS (Weatherall 2008). Every year, an estimated 230 000 children are born in this region

with sickle cell disease (SCD, HbSS), which is approximately 85% of the global total (Modell & Darlison 2008). In addition, haemoglobinopathies contribute approximately 6.4% to the death rate of children younger than 5 years in Africa (Modell & Darlison 2008). Although detailed estimates of the allele frequencies for both β^s and α^+-thalassemia are available from the coast of Kenya (Williams *et al*. 2005b; Veenemans *et al*. 2008), few data are available about the prevalence of these and other genetic disorders in other parts of Kenya.

The current gene frequencies of haemoglobin (Hb) and other blood disorders can be explained by the survival advantage they provide against death from *Plasmodium falciparum* malaria; natural selection has resulted in population frequencies that are broadly proportional to the historic exposure to the disease (Weatherall 2008). However, susceptibility to malaria is likely affected by multiple host factors, and the mechanisms for this protection are not completely understood. For example, although SCD may be partially protective against infection with malaria, affected children who do become infected are at substantial risk of subsequent death (Williams & Obaro 2011). In addition, α^+-thalassaemia has also been shown to ameliorate malaria risk (Wambua *et al*. 2006b), possibly by limiting the decline in Hb concentration that normally results from malaria infection (Mockenhaupt *et al*. 2004; Fowkes *et al*. 2008; Veenemans *et al*. 2008). Glucose-6-phosphate dehydrogenase (G6PD) deficiency, also common in Africa, may protect against malaria, although it can also result in serious clinical outcomes caused by red blood cell haemolysis (Guindo *et al*. 2007). Finally, haptoglobin (Hp) binds free Hb following malaria-induced haemolysis and, thus, serves an important role in iron recycling, immune function and protection against oxidant stress (Gutteridge 1987). Polymorphisms in the Hp gene (e.g. Hp 2-2) have also been associated with malaria protection (Atkinson *et al*. 2007).

Limited data are available about the prevalence of these multiple inherited blood disorders and their relationships with anaemia and malaria among children in Africa. In our study, we aimed to determine the prevalence of the inherited blood disorders HbS, α^+-thalassaemia, G6PD deficiency and Hp 2-2 genotype in a cross-sectional sample of pre-school children in western Kenya to learn more about their associations with anaemia and malaria.

Materials and methods

Participants

The study was conducted in Nyando Division, a largely rural region within Nyanza Province in western Kenya that includes approximately 80 000 people and 15 000 households. Most residents are subsistence farmers and part of the Luo ethnolinguistic group. Intense malaria transmission occurs in this region throughout the year, peaking in the rainy seasons. The study was part of a larger longitudinal study, the Nyando Integrated Child Health and Education Project (NICHE), which evaluated the effectiveness of the promotion and sale of evidence-based health products, including micronutrient powders in 60 study villages during 2007–2010. Details of NICHE are described elsewhere (CDC 2007; Suchdev *et al*. 2010; Suchdev *et al*. 2012).

Key messages

- More than 2 out of 3 pre-school children in western Kenya have one or more disorders: HbAS, HbSS, α^+-thalassaemia, G6PD deficiency or the Hp 2-2 genotype.
- α^+-thalassaemia was strongly associated with anaemia with adjusted haemoglobin (Hb) concentrations 4–6 g L^{-1} lower in α^+-thalassaemics compared with normal children.
- G6PD deficiency in males was associated with a lower prevalence of clinical malaria, and sickle cell trait was protective against malaria-associated Hb decline.
- Programmes to combat anaemia and malaria should consider inherited blood disorders as part of an integrated and multifactorial approach.

Data and sample collection and processing

A cross-sectional survey was conducted in the 60 study villages during August 2010. Using an updated household census, 19 compounds were randomly selected per village. Lists of selected compounds were provided to the field team, and all children aged 6–35 months living in these compounds were approached for enrolment. Trained fieldworkers administered questionnaires to the mothers of study participants to collect data on demographic and socio-economic factors, hygiene, sanitation, child feeding practices and child morbidity during the preceding 24 h. Anthropometric measurements were also made, and capillary blood samples were collected for Hb measurements and the preparation of malaria smears. Aliquots were stored for the later measurement of C-reactive protein (CRP), iron and vitamin A status, and genotyping for blood disorders.

Details of the laboratory analyses are described in detail elsewhere (Grant *et al*. 2012). Briefly, Hb levels were measured in the field by using HemoCue® photometers (Angelholm, Sweden), and children were classified as aanemic if their Hb was <110 g L^{-1} (McLean *et al*. 2009). Malaria blood slides were read at the CDC laboratory in Kisian, Kenya. Parasite densities were estimated from the ratio of parasites to leukocytes, assuming a leukocyte count of 8000 μL^{-1}. Children with severe anaemia (i.e. Hb < 70 g L^{-1}) or with clinical malaria (fever with a positive malaria smear) were referred for treatment to the nearest hospital or clinic.

Frozen plasma samples were transported to a laboratory in Germany (VitA-Iron Lab), where levels of ferritin, retinol binding protein (RBP) and CRP were measured by sandwich enzyme-linked immunosorbent assay, as described in detail previously (Erhardt *et al*. 2004). The following thresholds were used to define abnormal values for these biochemical indicators: ferritin <12 μg L^{-1}; RBP <0.7 μmol L^{-1}; and CRP > 5 mg L^{-1} (Erhardt *et al*. 2004). Because ferritin and RBP levels are influenced by the presence of infection or inflammation, subjects with a CRP level of >5 mg L^{-1} were excluded from estimates of iron or vitamin A deficiency in the population (Thurnham *et al*. 2003; WHO/CDC 2004). For the purposes of this analysis, and in line with current WHO/CDC recommendations, we used low ferritin as the indicator of choice for iron deficiency (WHO/CDC 2004).

Packed red blood cells were transported at –20°C to the KEMRI Centre for Geographic Medicine Research in Kilifi for genetic diagnosis of the inherited blood disorders. Genotyping for HbS, the common African 3.7-kb α-globin α^{+}-thalassaemia deletion, and Hp were conducted by polymerase chain reaction (PCR) as described in detail previously (Chong *et al*. 2000; Waterfall & Cobb 2001; Williams *et al*. 2005c; Atkinson *et al*. 2006). A child with one α-globin deletion (-α/$\alpha\alpha$) was defined as heterozygous α-thalassaemia, and a child with two α-globin deletions (-α/-α) was defined as homozygous α-thalassaemia. Typing for the common G6PD variants that are prevalent in the Kilifi population ($G6PD^{B}$, $G6PD^{A}$ and $G6PD^{A-}$) was performed by using a novel ARMS PCR method as follows. By using SMS Primer2 software (Stothard 2000), we designed two sets of allele-specific oligonucleotide primers for the wild- and mutant-type SNPs at positions 202 (rs1050828) (*WT202:* TGCCCGAAAAC ACCTTCATCG and *MT202:* TGCCCGAAAAC ACCTTCATCA) and 376 (rs1050829) (*WT376:* ACCCCAGGTGGAGGGCATT and *MT 376:* ACC CCAGGTGGAGGGCATC). In all reactions, we included two additional primers (*Lis F* and *Lis R*) that target the *Lis 1* gene (Chong *et al*. 2000) as a positive control for the presence of genomic DNA. We used the following PCR conditions: denaturation at 95°C for 5 min; 30 cycles of 94°C (0:45 s), 65°C (1:30 s) 72°C (2:00 s), and a final elongation step of 72°C for 7 min. We used three separate PCR reactions to identify the three allelic forms of G6PD (B, A and A–), the primer combination for each reaction being as follows: $G6PD^{B}$ (WT202 and WT376), $G6PD^{A}$ (MT376 and WT202) and $G6PD^{A-}$ (MT202 and MT376). In all three reactions, the amplification of a 766 bp fragment in the presence of *lis 1* gene amplicon (2.1 kb) was considered as positive for the targeted allele and negative when only the *lis 1 gene* amplicon was present. On the basis of recent phenotypic studies (S. Shah, unpublished observations), we classified males as G6PD deficient if they were hemizygous for the $G6PD^{A-}$ allele, and females as G6PD deficient if

they were homozygous for the G6PD^{A-} allele (A–/A–), or compound heterozygous for the G6PD^{A-} and G6PD^{A} alleles (Chong *et al*. 2000; Stothard 2000). Analyses of the effects of G6PD deficiency and malaria were stratified by sex, as malaria protection has only been demonstrated in hemizygous males, not females (Guindo *et al*. 2007). In addition, a sample of capillary blood was spotted onto filter paper strips and transported at ambient temperatures to CDC Atlanta for Hb analysis with isoelectric focusing (IEF) by using the Resolve® Hemoglobin Kit (Perkin Elmer, Turku, Finland), according to the manufacturer's protocol. Phenotypes of the samples were based on the migration of the Hb and comparison with controls for HbA, HbS, HbC and HbD (Analytical Control Systems, Inc., Fishers, IN, USA). HbS status by IEF was used in the analysis for subjects with insufficient blood volumes for PCR.

Data handling

Statistical analyses were conducted by using SPSS (version 14, SPSS Inc., Chicago, IL, USA), accounting for cluster sampling. We used the WHO Child Growth Standards (WHO Anthro, Geneva, Switzerland) to calculate *z*-scores, and we categorised underweight as a weight-for-age *z*-score of <–2, stunting as a height/length-for-age *z*-score of <–2 and wasting as a weight-for-height/length *z*-score of <–2. The means of normally distributed data were compared by using the Student's *t*-test or analysis of variance. Categorical variables were compared by using Pearson's chi-square or Fisher's exact tests, as indicated. We assessed the effect of malaria infection on Hb concentrations by blood disorder genotype in a multivariate linear regression model, accounting for cluster sampling that adjusted for age, height-for-age *z*-score, ferritin and RBP. In this model, we evaluated whether the effect of malaria depended on blood disorder genotype by examining interaction terms (i.e. genotype times malaria status). We did not adjust for the presence of inflammation because the effect of infection on Hb concentration may be mediated, at least in part through inflammation. *P*-values < 0.05 were considered significant.

Ethical considerations

The study was approved by the Kenya Medical Research Institute National Ethical Review Committee and by the institutional review board of the US Centers for Disease Control and Prevention. Written informed consent was obtained from the parents or caregivers of all subjects. This trial is registered at clinicaltrials.gov, identifier NCT01088958.

Results

A total of 1348 children were assessed for eligibility, and 882 were enrolled. Of the 466 children not enrolled, 57.7% were outside the age range, 26.6% were not encountered on three attempted household visits, parental consent was not obtained in 7.1%, and 8.6% were not enrolled for other reasons. An additional 24 children were excluded because of missing Hb data, which led to 858 children included in the final analysis. Characteristics of the study population are shown in Table 1. The mean age of children was 21.5 months; 50.3% were male and approximately 75% were anaemic. Iron deficiency, vitamin A deficiency and malaria parasitaemia were detected in 27.2%, 16.8% and 32.5% of children, respectively.

Prevalence of inherited blood disorders

Sufficient sample was available for genotyping for 99.5% (854/858) of the children included in our final analysis. The prevalence of inherited blood disorders among these children is summarised in Table 2. At the *HBB* locus, 81.3% were of normal genotype (HbAA), 17.1% had sickle cell trait (HbAS) and 1.6% had SCD (HbSS). A total of 51.9% of subjects were of normal α^{+}-thalassaemia genotype ($\alpha\alpha/\alpha\alpha$), 38.5% were heterozygotes ($-\alpha/\alpha\alpha$) and 9.6% were homozygotes ($-\alpha/-\alpha$). Hp 2-2 genotype and G6PD deficiency were found in 20.4% and 6.8% of children, respectively. There were no significant differences in the prevalence of the inherited blood disorders by sex, including G6PD deficiency (8.2% in males and 5.4% in females, $P = 0.12$). The mean age of children with HbSS was 15.8 months, compared with 22.4 and 21.4 months for those with HbAS and HbAA, respectively

Table 1. Characteristics of enrolled children aged 6–35 months in western Kenya, 2010

$N = 858$	N	% or mean (95% CI)
Male sex, %	432	50.3 (46.9–53.8)
Age (months), mean	858	21.5 (20.9–22.1)
Ever breastfed, %	761	91.1 (88.1–94.2)
Currently breastfeeding, %	418	54.1 (50.2–57.9)
ITN use, %	765	92.3 (90.6–94.8)
Fever in last 24 h, %	232	27.1 (23.3–30.9)
Hb (g per L), mean	858	96.4 (95.1–98.2)
Anaemia (Hb<110 g L^{-1}), %	614	71.6 (68.1–75.1)
Iron deficiency (ferritin < 12 μg L^{-1}) %	162	19.1 (16.0–22.7)
Iron deficiency* (ferritin < 12 μg L^{-1}), %	152	27.2 (23.0–31.5)
Vitamin A deficiency (RBP < 0.7 μg L^{-1}), %	262	30.9 (27.2–35.0)
Vitamin A deficiency* (RBP < 0.7 μg L^{-1}), %	94	16.8 (13.4–20.3)
Malaria positive, %	276	32.5 (28.5–36.7)
Malaria positive and self-reported fever, %	122	14.4 (12.0–17.2)
Elevated CRP (>5 mg L^{-1}), %	289	34.1 (29.8–38.7)
Stunted (HAZ < –2), %	252	29.5 (26.4–32.7)
Wasted (WHZ < –2), %	30	3.5 (1.9–5.1)
Underweight (WAZ < –2), %	103	12.0 (9.7–14.4)

ITN, insecticide-treated nets; Hb, haemoglobin; CRP, C-reactive protein; WAZ, weight-for-age *z*-score; HAZ, height-for-age *z*-score; WHZ, weight-for-height *z* score. Data are presented as percentage or mean (95% confidence interval). *Restricted to $n = 558$ children with CRP ≤ 5 mg L^{-1}.

Table 2. Prevalence of sickle cell haemoglobin and α^+-thalassaemia, haptoglobin and G6PD genotypes among children aged 6–35 months in western Kenya, 2010

	Overall	Males	Females
Haemoglobin type ($n = 854$)			
HbAA	694 (81.3)	351 (81.6)	343 (80.9)
HbAS	146 (17.1)	69 (16.0)	77 (18.2)
HbSS	14 (1.6)	10 (2.3)	4 (0.5)
α^+-thalassaemia genotype ($n = 823$)			
Normal ($\alpha\alpha/\alpha\alpha$)	427 (51.9)	210 (50.7)	217 (50.8)
Heterozygote ($-\alpha/\alpha\alpha$)	317 (38.5)	164 (39.6)	153 (37.4)
Homozygote ($-\alpha/-\alpha$)	79 (9.6)	40 (9.7)	39 (9.5)
Haptoglobin genotype ($n = 802$)			
Hp 1-1	251 (31.3)	127 (31.6)	124 (31.0)
Hp 1–2	387 (48.3)	189 (47.0)	198 (49.5)
Hp 2-2	164 (20.4)	86 (21.4)	78 (19.5)
G6PD genotype (n = 826)			
Normal	770 (93.2)	381 (91.8)	389 (94.6)
Deficient	56 (6.8)	34 (8.2)	22 (5.4)

HbAA, normal genotype; HbAS, sickle cell trait; HbSS, sickle cell disease; Hp, haptoglobin; G6PD, glucose-6-phosphate dehydrogenase. Data are shown as number of cases (%) by sex.

($P = 0.015$). Overall, 70.1% of the 809 children for whom data were available for all four conditions – HbS, α^+-thalassaemia, G6PD deficiency and Hp 2-2 – were positive for one or more of these conditions.

Associations with malaria and anaemia

There were no significant differences in the distribution of malaria by the measured blood disorders, except for G6PD among males for whom clinical malaria was significantly less common among those with G6PD deficiency than those of normal genotype ($P = 0.005$) (Table 3). There was no effect of G6PD deficiency on malaria in females (data not shown). There were no differences in mean parasite densities by the measured blood disorders.

The α^+-thalassaemia was associated with a significantly increased prevalence of anaemia (82.3% in homozygotes, 75.4% in heterozygotes and 66.7% in normal; $P = 0.005$). Children with HbSS, however, had a similar prevalence of anaemia as those with HbAA (71.4% vs. 72.2%), even among those <24 months of age (70.0% vs. 75.2%). No association was found between Hp 2-2 or G6PD deficiency and the prevalence of anaemia (data not shown). Children with any one of the four measured blood disorders were 1.45 times more likely to be anaemic compared with those without a blood disorder (95% CI = 1.01–2.09).

To assess the residual anaemia associated with the blood disorders, we excluded 563 children with either malaria, inflammation (CRP > 5 mg L^{-1}), iron deficiency or vitamin A deficiency. There remained a significant difference in anaemia among those with normal α^+-thalassaemia genotype (43.0%), heterozygotes (53.5%) and homozygotes (67.7%, chi-square trend $P = 0.012$) (Table 4). We found no significant differences in anaemia by genotype for SCD, G6PD deficiency or Hp 2-2 (data not shown).

In a multivariate analysis, the drop in Hb concentration adjusting for confounders was compared according to inherited blood disorder (Table 5). Children heterozygous or homozygous for α-thalassaemia had a lower Hb than those with normal genotype,

Table 3. Distribution of malaria among children aged 6–35 months in western Kenya according to HbS, α^+-thalassaemia, haptoglobin type and G6PD genotype, 2010

	Positive malaria	Clinical malaria*	Parasite density‡
Haemoglobin type			
HbAA	222 (32.2)	97 (14.0)	3.5
HbAS	50 (34.2)	21 (14.4)	3.6
HbSS	4 (30.8)	4 (28.6)	3.0
P-value	0.89	0.31	0.38
α^+-thalassaemia genotype			
Normal ($\alpha\alpha/\alpha\alpha$)	137 (32.3)	68 (16.1)	3.5
Heterozygote ($-\alpha/\alpha\alpha$)	110 (34.9)	42 (13.4)	3.6
Homozygote ($-\alpha/-\alpha$)	19 (24.4)	7 (9.0)	3.5
P-value	0.20	0.21	0.68
Haptoglobin genotype			
Hp 1-1 or Hp 1–2	203 (32.0)	92 (14.6)	3.5
Hp 2-2	53 (32.9)	20 (12.4)	3.6
P-value	0.85	0.49	0.45
G6PD genotype†			
Normal	125 (33.0)	64 (16.9)	3.5
Deficient	8 (23.5)	0 (0.0)	3.4
P-value	0.25	0.005	0.60

Data are shown as number of cases (%) by malaria status. Differences in proportions using chi-square test. *Positive malaria smear and reported fever in last 24 h. †G6PD cases restricted to males. ‡Mean of $\log_{10}$-transformed parasitaemia per μL of whole blood.

Table 4. Residual anaemia among children aged 6–35 months in western Kenya as explained by α^+-thalassaemia, 2010

	Normal ($\alpha\alpha/\alpha\alpha$)	Heterozygote ($-\alpha/\alpha\alpha$)	Homozygote ($-\alpha/-\alpha$)	*P*
% Anemic	43.0	53.5	67.7	0.012
Mean Hb (g per L)	109.3	103.1	105.3	0.018

CRP, C-reactive protein; RBP, retinol binding protein. Data represent $n = 260$ children where no malaria, no inflammation (CRP ≤ 5 mg L^{-1}), normal iron status (ferritin ≥ 12 μg L^{-1}) and no vitamin A deficiency (RBP ≥ 0.7 μmol L^{-1}). Proportion data compared by using chi-square for trend, and means compared by using ANOVA.

although this observation did not reach statistical significance ($P = 0.058$). Hp 2-2 was associated with a lower Hb compared with those with Hp 1–2 or 1–2 genotype ($P = 0.018$). There were no significant differences in the drop in Hb that was associated with malaria parasitaemia, except in the case of HbS, where parasitaemia was associated with a drop of 2.5 g L^{-1} among children with sickle cell trait compared with 12 g L^{-1} among those with HbAA (*P* for interaction = 0.014) (Table 6).

Discussion

In common with reports from other parts of Kenya, we found a high prevalence of inherited blood disorders among pre-school children in Nyando Division, western Kenya, where more than 2 out of 3 children have one or more of the disorders HbAS, HbSS, α^+-thalassaemia, G6PD deficiency or the Hp 2-2 genotype. Most notably, α^+-thalassaemia was strongly associated with anaemia, and after eliminating other known causes of anaemia, such as iron and vitamin A deficiencies, malaria and inflammation, Hb concentrations were 4–6 g L^{-1} lower in α^+-thalassaemics than in normal children.

To our knowledge, this is the first published study in which the prevalence of multiple blood disorders has been described in a single population within western Kenya. In keeping with previous reports from the Asembo Bay area of western Kenya (Aidoo *et al.* 2002; Desai *et al.* 2005), we found that the prevalence of HbAS (17.1%) appeared to be slightly higher than that described in coastal populations (15%) (Scott *et al.* 2011). However, the prevalence of α^+-thalassaemia was lower than that among coastal

Table 5. Mean adjusted haemoglobin concentration among children aged 6–35 months in western Kenya according to α^+-thalassaemia, HbS, haptoglobin and G6PD genotype, 2010

	N	Adjusted mean Hb (95% CI)	*P**
Normal	427	96.1 (94.7–98.1)	0.058
α^+-thalassaemia hetero/homo	396	92.9 (90.8–95.1)	
HbAA	694	94.4 (93.0–95.8)	0.409
HbAS	146	95.8 (92.5–99.0)	
Hp1-1/Hp1–2	638	95.2 (93.7–96.7)	0.018
Hp 2-2	164	92.2 (90.2–94.3)	
Normal	381	92.5 (90.5–94.4)	0.212
G6PD deficient†	34	95.3 (90.7–99.9)	

Data are presented as adjusted mean Hb in g per L (95% CI). *Effect of blood disorder on haemoglobin by using multiple linear regression adjusting for age, height-for-age *z*-score, malaria, ferritin and retinol binding protein, accounting for cluster sampling. †G6PD cases restricted to males.

Table 6. Haemoglobin concentration among children aged 6–35 months in western Kenya according to α^+-thalassaemia, HbS, haptoglobin and G6PD genotype by malaria positivity, 2010

	n	*(-) Malaria*	*N*	*(+) Malaria*	Change in Hb (g per L)	*P**
		Mean Hb		Mean Hb		
Normal	287	101.5 (98.9–104.1)	137	90.2 (86.8–93.5)	–11.3	0.241
α^+-thalassaemia hetero/homo	264	97.5 (94.6–100.5)	129	88.9 (86.0–91.8)	–8.6	
HbAA	467	100.1 (98.1–102.0)	222	88.1 (85.6–90.5)	–12.0	0.014
HbAS	96	98.2 (93.8–102.7)	50	95.7 (90.4–101.0)	–2.5	
Hp1-1/Hp1–2	632	100.4 (98.1–102.7)	203	90.1 (87.5–92.8)	–10.3	0.580
Hp 2-2	108	97.9 (95.1–100.8)	53	86.2 (82.1–90.2)	–11.7	
Normal	254	97.4 (94.3–100.4)	125	87.6 (84.1–91.1)	–9.8	0.212
G6PD deficient†	26	100.6 (95.3–105.9)	8	89.2 (78.5–100.0)	–11.4	

Data are presented as adjusted mean Hb in g per L (95% CI). *Interaction *P*-value for models by using multiple linear regression, adjusting for age, height-for-age *z*-score, malaria, ferritin and retinol binding protein, accounting for cluster sampling. †G6PD cases restricted to males.

populations where the prevalence of heterozygous and homozygous α^+-thalassaemia has been estimated at 39–53% and 15–17%, respectively (Wambua *et al*. 2006b; Veenemans *et al*. 2008). This observation is interesting because, like HbAS, α^+-thalassaemia might also be expected to be higher in Nyanza province than on the coast, given greater selection pressure from malaria. Although we can speculate that this might be explained by negative co-evolution of these two conditions, as suggested by several recent studies (Williams *et al*. 2005b; May *et al*. 2007; Penman *et al*. 2009), further studies will be required in multiple populations to examine this question in more detail.

Given the high mortality that is traditionally associated with undiagnosed SCD in sub-Saharan Africa (Grosse *et al*. 2011), we were surprised by the high prevalence of HbSS (1.6%) found among our study population, a prevalence close to that reported at birth in previous studies among a nearby population (Aidoo *et al*. 2002). Because there is no active programme of diagnosis or treatment for SCD, we had anticipated that survivorship would have been lower. The observed number of survivors might potentially be explained by the young age of our sampled population or by the improved nutrition and hygiene measures introduced through the NICHE study (Suchdev *et al*. 2012).

In agreement with previous studies (Williams *et al*. 2005a; Wambua *et al*. 2006b), we found no evidence for a protective effect of either HbAS or

α^+-thalassaemia against the prevalence of either symptomless malaria parasitaemia or clinical malaria infection (Table 3). Although previous studies have shown that α^+-thalassaemia protects against the decline in Hb concentration associated with malaria infection (Wambua *et al*. 2006b; May *et al*. 2007), we did not observe this effect (Table 6). However, our data did show a protective effect of sickle cell trait (HbAS) against malaria-associated Hb decline, which may be a mechanism to explain the lower risk of malaria-specific mortality in subjects with HbAS compared with HbAA (Williams & Obaro 2011).

Compared with Hp 1-1 or Hp 1–2, we found that the Hp 2-2 genotype was associated with a significantly higher drop in Hb concentration among this malaria-endemic setting, an observation that accords with that made by Atkinson and colleagues in a cohort study conducted in The Gambia (Atkinson *et al*. 2006). Because Hp 2-2 binds less efficiently to Hb, it may be less efficient at scavenging free Hb iron following malaria-induced haemolysis (Okazaki & Nagai 1997).

Our study has a number of limitations. First, it is possible that other inherited blood disorders might be present among the population for which we did not measure. For example, we did not test for β-thalassaemia because previous studies from the region have not found evidence of this condition (Wambua *et al*. 2006a); nevertheless, there are no reports that exclude the possibility that this condition might be prevalent in this part of Kenya. Second, this study was cross-sectional, so it is not possible to evaluate the effects of the inherited blood disorders on malaria or anaemia risk. Finally, data are representative of a single division in Kenya and are not representative of Kenya as a whole. As in other countries in Africa, it is likely that ethnic diversity will have a major effect on the prevalence of inherited blood disorders in Kenya, which need to be further evaluated in large population-based surveys.

In conclusion, inherited blood disorders, including SCD, α^+-thalassaemia, G6PD deficiency and the Hp 2-2 genotype, are common among pre-school children in western Kenya and have mixed associations with anaemia and malaria. Further studies are needed to measure and classify these blood disorders in resource-poor countries and to understand further the mechanisms through which they associate with malaria and anaemia. Despite the general lack of information on the global burden of inherited blood disorders, progress is being made to establish population screening and education programmes (Odame 2010). Programmes to combat anaemia and malaria should consider inherited blood disorders as part of an integrated and multifactorial approach.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

PSS and LJR designed and conducted the research; ME, AM and TNW performed the blood disorder analyses; PSS analysed the data, wrote the manuscript

and had primary responsibility for the final content. All authors read and approved the final manuscript. This paper is published with the permission of the Director of KEMRI. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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