Bacteraemia in Kenyan children with sickle-cell anaemia: a retrospective cohort and case-control study

Thomas N Williams, Sophie Uyoga, Alex Macharia, Carolyne Ndila, Charlotte F McAuley, Daniel H Opi, Salim Mwarumba, Julie Makani, Albert Komba, Moses N Ndiritu, Shahnaaz K Sharif, Kevin Marsh, James A Berkley, J Anthony G Scott

Summary

Background In sub-Saharan Africa, more than 90% of children with sickle-cell anaemia die before the diagnosis can be made. The causes of death are poorly documented, but bacterial sepsis is probably important. We examined the risk of invasive bacterial diseases in children with sickle-cell anaemia.

Methods This study was undertaken in a rural area on the coast of Kenya, with a case–control approach. We undertook blood cultures on all children younger than 14 years who were admitted from within a defined study area to Kilifi District Hospital between Aug 1, 1998, and March 31, 2008; those with bacteraemia were defined as cases. We used two sets of controls: children recruited by random sampling in the same area into several studies undertaken between Sept 1, 1998, and Nov 30, 2005; and those born consecutively within the area between May 1, 2006, and April 30, 2008. Cases and controls were tested for sickle-cell anaemia retrospectively.

Findings We detected 2157 episodes of bacteraemia in 38 441 admissions (6%). 1749 of these children with bacteraemia (81%) were typed for sickle-cell anaemia, of whom 108 (6%) were positive as were 89 of 13 492 controls (1%). The organisms most commonly isolated from children with sickle-cell anaemia were Streptococcus pneumoniae (44/108 isolates; 41%), non-typhi Salmonella species (19/108; 18%), Haemophilus influenzae type b (13/108; 12%), Acinetobacter species (seven of 108; 7%), and Escherichia coli (seven of 108; 7%). The age-adjusted odds ratio for bacteraemia in children with sickle-cell anaemia was 26·3 (95% CI 14·5–47·6), with the strongest associations for S pneumoniae (33·0, 17·4–62·8), non-typhi Salmonella species (35·5, 16·4–76·8), and H influenzae type b (28·1, 12·0–65·9).

Interpretation The organisms causing bacteraemia in African children with sickle-cell anaemia are the same as those in developed countries. Introduction of conjugate vaccines against S pneumoniae and H influenzae into the childhood immunisation schedules of African countries could substantially affect survival of children with sickle-cell anaemia.

Introduction

Bacterial infections are a major cause of morbidity and mortality in children with sickle-cell anaemia. Several organisms, including Streptococcus pneumoniae, Haemophilus influenzae, and non-typhi Salmonella species, have been identified as important causative agents through studies undertaken in the USA. The introduction of penicillin prophylaxis and immunisation with conjugate vaccines directed against S pneumoniae and H influenzae type b have led to substantial improvements in the prognosis of patients born with sickle-cell anaemia in developed countries. In Africa, where more than 80% of all children with this disease are born, a scarcity of data has impeded development of evidence-based guidelines; however, available data suggest that the range of organisms causing invasive bacterial disease in African patients with sickle-cell anaemia might differ from that described in the USA, leading to calls for further studies to guide policy.

A potential bias in many of the studies undertaken in Africa has been their focus on people with an existing diagnosis of sickle-cell anaemia. Because diagnosis is often delayed in Africa, studies confined to known cases will under-report events in young children. Although cohort studies based on neonatal screening programmes could circumvent this problem, such programmes are expensive and time-consuming, and the medical follow-up received by affected children would probably change the natural history of the disease. Instead of looking for bacterial infections in a cohort of patients with sickle-cell anaemia, we have examined the sickle-cell anaemia status of a large group of consecutive patients admitted to hospital with bacteraemia from within a known denominator population. This approach allows an unbiased description of serious bacterial infections in children with sickle-cell anaemia, which we compared with that in children without sickle-cell anaemia. Further, we have quantified the risk of bacteraemia in children with sickle-cell anaemia, both in general and for specific bacterial pathogens, by undertaking case–control analyses with two large community-based control groups drawn from the same denominator population. Finally, we have estimated the incidence of bacteraemia in children with sickle-cell anaemia in our study population from the number of bacteraemia episodes in this group, the size of the denominator population, and the prevalence of this disease in controls.
Methods

Study setting

The study was undertaken at Kilifi District Hospital (KDH) on the Kenyan coast, which serves as a first-referral centre for 500 000 people. Common causes of admission to KDH have been reported previously.16 The burden of malaria, the most common diagnosis at the start of the study, decreased substantially as the study proceeded.17 7% of children admitted have bacteraemia (on the basis of findings from a study undertaken between Aug 1, 1998, and July 31, 2002), and five organisms (S pneumoniae, non-typhi Salmonella species, H influenzae, Escherichia coli, and Staphylococcus aureus) cause 70% of these infections.18 H influenzae type b conjugate vaccine was introduced into the immunisation schedule in November, 2001, but pneumococcal conjugate vaccine has not yet been deployed. Coverage for three doses of pentavalent vaccine (for diphtheria, tetanus, pertussis, H influenzae type b, and hepatitis B) was estimated at 88% in 2004.19 The prevalence of HIV infection in routine antenatal screening at KDH was 5–7% during 2004–07. In 2000, a system of epidemiological and demographic surveillance (Kilifi Epi-DSS) was established in a defined area of 891 km² surrounding KDH, with a population of about 100 000 children younger than 14 years living in five administrative divisions.20 About 80% of children who are admitted to KDH live in the Kilifi Epi-DSS area. Medical services for children with sickle-cell anaemia are scarce in Kilifi district; the only specialist clinic is at KDH.

Cases and controls

Since Aug 1, 1998, all children admitted to KDH have been investigated with blood cultures, apart from those admitted for elective procedures or for observation after minor accidents.18 Routine clinical and laboratory data have also been recorded systematically at admission. This study included all patients with bacteraemia aged younger than 14 years admitted between Aug 1, 1998, and March 31, 2008, who lived within the Kilifi Epi-DSS area. For the purpose of our case–control analyses, we defined these patients as cases. The diagnosis of sickle-cell anaemia was based on the finding of a major haemoglobin band in the position of HbS—for venepuncture samples on alkaline electrophoresis on cellulose acetate membrane (control group 1) or for capillary samples on HPLC (Variant Analyzer, BioRad, Hercules, CA, USA) with the β thalassaemia short program (control group 2). In both instances, HbS was confirmed by PCR.21 No patient had HbA (unless known to be acquired from transfusion), implying a diagnosis of homozygous HbS (SS) disease or sickle-cell β thalassaemia. Most patients will have had SS disease, but sickle-cell β thalassaemia has not been excluded; thus because of this uncertainty, we have chosen to use the term sickle-cell anaemia throughout this Article.

We used two separate sets of controls for our case–control analyses. Control group 1 consisted of children recruited by random sampling throughout the Kilifi Epi-DSS into several studies undertaken between Sept 1, 1998, and Nov 30, 2005, as described in detail previously;16,22–24 control group 2 included children born consecutively within the Kilifi Epi-DSS area between May 1, 2006, and April 30, 2008. Control group 2 remains under investigation for genetic susceptibility to infectious diseases. Control group 1 was representative of cases in terms of age, sex, and ethnic group but were less representative of the geographic area of residence than was control group 2, which was highly representative of cases in all variables apart from age. Age is an important confounder because of the high mortality associated with sickle-cell anaemia in early life and the pronounced variation in bacteraemia in all children with age.8 We therefore did our principal analyses with control group 1, with adjustment for age, sex, and ethnic origin, and used control group 2 to check for any residual confounding attributable to variation in geographical residence.

We obtained informed consent from the parents of all cases and controls. The study was approved by the the Kenya Medical Research Institute/National Ethical Review Committee.

<table>
<thead>
<tr>
<th>Bacterial isolates from patients with bacteraemia, stratified by sickle-cell anaemia status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolates from children without sickle-cell anaemia (%)</strong></td>
</tr>
<tr>
<td>Gram positive</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Group A streptococci</td>
</tr>
<tr>
<td>Group B streptococci</td>
</tr>
<tr>
<td>Other gram-positive organisms†</td>
</tr>
<tr>
<td><strong>Gram negative</strong></td>
</tr>
<tr>
<td>Non-typhi Salmonella species</td>
</tr>
<tr>
<td>Haemophiles influenza type b</td>
</tr>
<tr>
<td>Haemophiles influenza other</td>
</tr>
<tr>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Acinetobacter species</td>
</tr>
<tr>
<td>Pseudomonas species</td>
</tr>
<tr>
<td>Klebsiella species</td>
</tr>
<tr>
<td>Other gram-negative organisms†</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
</tr>
<tr>
<td>Any organism</td>
</tr>
</tbody>
</table>
We compared the means of normally distributed continuous data by use of Student’s t tests and medians of non-normally distributed data by the Kruskal-Wallis test. We calculated odds ratios (ORs) for sickle-cell anaemia in children with bacteraemia and each control group separately by both univariate and multivariable logistic regression analysis, with adjustment for sex, ethno-linguistic group, and, when possible, for division of residence or age in three strata. All analyses were done with STATA (version 10.1). We calculated the incidence of bacteraemia in children with sickle-cell anaemia from the midstudy population of the Kilifi Epi-DSS area and the age-specific prevalence of this disease in control group I.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During the study period, blood cultures were collected from 38,441 children younger than 14 years who were admitted to KDH from the study area. Important bacterial pathogens were cultured from 2157 (6%) cases in this analysis. Blood samples from 1999 (93%) children with bacteraemia were available for genotyping, which was successful in 1749 (87%). Of these children, 108 (6%) were homozygous for HbS, which was the only form of sickle-cell anaemia identified during this study. We noted no significant differences between patients with bacteraemia who did or did not have genotyping results in age, prevalence of severe anaemia (haemoglobin <50 g/L), or pattern of organisms isolated. However, because children who died at presentation were not investigated, typing was less complete in children who died in hospital (409/551, 74%) than in survivors (1340/1608, 83%; p<0.0001). The mass of blood cultured from children with bacteraemia who did or did not have genotyping results was greater than 300 ml in controls but not in patients with sickle-cell anaemia (p=0.05). The proportion of children with sickle-cell anaemia who died in hospital was not significantly different from that in children without sickle-cell anaemia (1340/1608, 83%; p=0.05). In 80 of 108 (74%) children with sickle-cell anaemia, the diagnosis had not previously been made at the time of admission.

Table 1 summarises the organisms isolated from patients with bacteraemia in the groups with and without sickle-cell anaemia. The proportion with sickle-cell anaemia varied significantly between patients infected with different pathogens (p<0.001), and was highest for those infected with Haemophilus influenzae type b, non-typhoid Salmonella species, and S pneumoniae (table 1). Five

Procedures

Blood was taken from children admitted to KDH and inoculated into culture bottles (BACTEC Peds Plus, Becton Dickinson, Franklin Lakes, NJ, USA) as described previously. A full blood count was done and an aliquot of blood stored at −80°C for later genetic tests. Culture bottles were weighed before and after blood inoculation and processed by an automated blood-culture system (BACTEC 9050, Becton Dickinson). Positive samples were subcultured on standard media by routine microbiological techniques. Quality assurance was provided by the UK National External Quality Assessment Service. Clinical indications for lumbar puncture were impaired consciousness or meningism in children younger than 5 years, prostration in children younger than 3 years, seizures (other than febrile seizures) in children younger than 2 years, and suspicion of sepsis in children younger than 60 days. In children with bacteraemia, bacterial meningitis was defined as a white-cell count in cerebrospinal fluid of greater than 50x10⁶/L or a ratio of cerebrospinal fluid to plasma glucose less than 0·1. Serotyping of S pneumoniae was done by use of the Quellung reaction (Statens Seruminstitut, Copenhagen, Denmark) and H influenzae by latex agglutination with polyclonal rabbit antiserum (Difco Laboratories, Detroit, MI, USA). DNA was extracted retrospectively from the frozen samples collected at admission by use of Qiagen DNA blood mini kits (Qiagen, Crawley, UK) and typed for sickle-cell anaemia by PCR.

Statistical analysis

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Vol 374   October 17, 2009

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organisms accounted for almost 90% of isolates from children with sickle-cell anaemia, with the highest ranked being *S pneumoniae*, non-typhi *Salmonella* species, *H influenzae* type b, *Acinetobacter* species, and *E coli* (table 1). A similar range of organisms was cultured from children with and without a previous diagnosis of sickle-cell anaemia. In the subset of 28 patients who were known to have the disease before admission, *S pneumoniae* was isolated from ten (36%), non-typhi *Salmonella* species from seven (25%), and *H influenzae* type b from three (11%).

Samples were available for 13 558 control children, of whom 13 492 (99·5%) were typed successfully. The proportion of children with sickle-cell anaemia in control group 1 was 0·3% (13/4741); it was 1·0% (eight of 782) in those aged 12–23 months, and 0·1% (four of 3677) in those aged 2–13 years. The proportion in control group 2 was 0·9% (76/8751).

The age-adjusted OR for sickle-cell anaemia in patients with bacteremia calculated with control group 1 was 26·3 (95% CI 14·5–47·6, p<0·0001; table 2). The strongest associations were for *S pneumoniae*, non-typhi *Salmonella* species, *H influenzae* (particularly type b), *Acinetobacter* species, and *E coli* (table 2). Adjustments for sex, division of residence, and ethnic origin (and for division of residence with control group 2) made no significant difference to the analysis (data not shown).

Table 3 shows the incidence estimates for admission to hospital with bacteremia in children with sickle-cell anaemia living within the Kilifi Epi-DSS area, both overall and for each of the three most common pathogens. By contrast with children without sickle-cell anaemia, the incidence of bacteremia in children with this disease was higher after infancy (table 3).

In patients with pneumococcal bacteremia, we noted no significant difference in distribution of serotypes between children with or without sickle-cell anaemia (table 4). 20 of 44 isolates (45%) from patients with sickle-cell anaemia and 146 of 425 isolates (34%) from patients without this disease were of serotypes represented in the seven-valent pneumococcal conjugate vaccine (p=0·17). The serotypes in the ten-valent pneumococcal conjugate vaccine covered 75% of pneumococcal isolates in patients with and without sickle-cell anaemia (table 4).

To examine the effect of *H influenzae* type b conjugate vaccine on the association between sickle-cell anaemia and *H influenzae* type b bacteremia, we arbitrarily divided the study into three time strata representing the periods before (before Nov 1, 2001), during (Nov 1, 2001, to Oct 31, 2002), and after (after Oct 31, 2002) the introduction of *H influenzae* type b vaccine into the immunisation schedule. The number of cases of *H influenzae* type b bacteremia detected during these periods was 88 before, 21 during, and 37 after the introduction; the proportion of sickle-cell anaemia in these cases was stable at 12%, 12%, and 10%, respectively (χ²=3·2; p=0·5).

Meningitis was diagnosed in 20 of 108 (19%) patients with bacteremia who had sickle-cell anaemia, and in 212 of 1641 (13%) without sickle-cell anaemia (p=0·1). Of these children with sickle-cell anaemia, *S pneumoniae* was isolated from 11, *H influenzae* type b from eight, and *E coli* from one. Isolates from blood and cerebrospinal fluid were concordant in all cases of meningitis in which an organism was isolated from cerebrospinal fluid. Osteomyelitis was diagnosed in four patients with bacteremia with sickle-cell anaemia, with non-typhi *Salmonella* species and *Acinetobacter* species each being cultured twice. In children with bacteremia who were anaemic, the proportion who had sickle-cell anaemia was 17% (30/175) compared with 5% (78/1574) in those without anaemia (p<0·0001). Similarly, anaemia was strongly associated with sickle-cell anaemia in patients with
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Infections, of which, *S pneumoniae* sickle-cell anaemia are highly susceptible to bacterial children with sickle-cell anaemia.

**Discussion**

Findings from our study confirm that, as in developed countries, African patients with sickle-cell anaemia are at high risk of invasive bacterial infections, especially those caused by *S pneumoniae*, *H influenzae*, and non-typhi *Salmonella* species. Furthermore, the incidence of pneumococcal bacteraemia in children with sickle-cell anaemia was similar to the range of 1.5–11.6 reported in patients with sickle-cell anaemia in sub-Saharan Africa despite the fact that more than 200 000 African children are born with this disease every year. *S pneumoniae* was the most common isolate in an early study undertaken in the Congo, but infections with *S aureus*, *E coli*, *Klebsiella* species, and non-typhi *Salmonella* species have predominated in recent studies from Uganda and Nigeria. In these studies, both *S pneumoniae* and *H influenzae* were detected infrequently, an observation that has challenged the potential benefits of antimicrobial prophylaxis in African patients with sickle-cell anaemia. However, two factors might account for this discrepancy. First, most studies have examined patients with an existing diagnosis of sickle-cell anaemia in a region in which infections with *H influenzae* type b and pneumococcus occur predominantly in young children but most patients die before a diagnosis of sickle-cell anaemia is made. Second, *S aureus* and *E coli* are easier to culture than are fastidious organisms such as pneumococcus and *H influenzae* type b, and in laboratories with few resources, this differential culture sensitivity might bias the distribution of the pathogens detected.

In this study, we took as our starting point children with bacteraemia who were detected through the surveillance of all children admitted to a general paediatric facility. This approach is unbiased by preconceptions about the disease status of admitted children, since our sampling frame included all children irrespective of their age or diagnosis. Moreover, the retrospective design means that in most cases the natural history of sickle-cell anaemia was unaffected by previous detection of *HbSS* and long-term medical management. Finally, we investigated bacteraemia in an established laboratory, meeting international quality assessment standards.

Our findings lend support to previous reports showing that African patients with sickle-cell anaemia are especially susceptible to bacteraemia caused by *H influenzae* type b. Vaccination against *H influenzae* type b has reduced the incidence of bacteraemia caused by this pathogen in Kilifi by 88%, and the consistent prevalence of sickle-cell anaemia in patients with *H influenzae* type b before, during, and after introduction of the vaccination programme suggests that the vaccine is equally effective in patients with and without this disease. This finding accords with previous studies showing a brisk immunological response to conjugate *H influenzae* type b vaccines in children with sickle-cell anaemia. As with pneumococcal conjugate vaccine, therefore, maintenance of the vaccine for *H influenzae* type b in Kenya will be especially useful to children with this disease.

Although children with sickle-cell anaemia are known to be at increased risk of infections caused by non-typhi *Salmonella* species, most reports are of osteomyelitis. In one study from Kingston, Jamaica, investigators noted no obvious bone involvement in half of all infections

<table>
<thead>
<tr>
<th>Isolates from children without sickle-cell anaemia</th>
<th>Isolates from children with sickle-cell anaemia</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>127 (30.6%)</td>
<td>10 (22.7%)</td>
</tr>
<tr>
<td>4†</td>
<td>16 (3.9%)</td>
<td>3 (6.8%)</td>
</tr>
<tr>
<td>5*</td>
<td>37 (8.9%)</td>
<td>3 (6.8%)</td>
</tr>
<tr>
<td>6A</td>
<td>30 (7.2%)</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>6B†</td>
<td>35 (8.4%)</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>7F†</td>
<td>1 (0.2%)</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>5 (1.2%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>14†</td>
<td>38 (9.2%)</td>
<td>6 (13.6%)</td>
</tr>
<tr>
<td>18C†</td>
<td>9 (2.2%)</td>
<td>2 (4.6%)</td>
</tr>
<tr>
<td>18F</td>
<td>2 (0.5%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>19A</td>
<td>14 (4.0%)</td>
<td>3 (6.8%)</td>
</tr>
<tr>
<td>19F†</td>
<td>20 (4.8%)</td>
<td>2 (4.6%)</td>
</tr>
<tr>
<td>23B</td>
<td>0</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>23F†</td>
<td>28 (6.8%)</td>
<td>2 (4.6%)</td>
</tr>
<tr>
<td>Others†</td>
<td>53 (13.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>415 (100%)</td>
<td>44 (100%)</td>
</tr>
</tbody>
</table>

Data are the number of isolates of each serotype (% of the total isolates for each category). We noted no significant difference in the distribution of serotypes between sickle-cell anaemia groups either overall (χ²=2.6, p=0.92) or for any serotype individually. Additional strains represented in the ten-valent pneumococcal vaccine. We noted no occurrences of serotype 9V in either the cases with or without sickle-cell anaemia.

**Table 4:** Serotypes of *Streptococcus pneumoniae* isolates by status of sickle-cell anaemia

bacteraemia (OR 23.6, 95% CI 14.4–37.3). Inpatient mortality did not differ significantly between patients with bacteraemia with (25/108, 23%) or without (384/1641, 23%) sickle-cell anaemia.
The dominance of just a few bacterial pathogens suggests infections make a large contribution to the morbidity and deaths will increase unless specific interventions are made. For example, the relative contribution of sickle-cell anaemia to childhood mortality in children younger than 5 years, the huge mortality associated with sickle-cell anaemia in invasive bacterial disease makes a very substantial contribution to take antibiotics without prescription, and the true culture of blood is an insensitive measure of the burden of invasive bacterial disease, especially in Africa where antibiotics are available without prescription, and the true incidence is likely to be much higher than our estimates. Finally, a quarter of the children with sickle-cell anaemia admitted with bacteraemia died in hospital, yet we know that only a third of children dying in the Kilifi Epi-DSS area present to hospital in their final illness. Taken collectively, these observations suggest that invasive bacterial disease makes a very substantial contribution to the huge mortality associated with sickle-cell anaemia in African children. Furthermore, as developing countries strive to meet the Millennium Development Goals and reduce mortality in children younger than 5 years, the relative contribution of sickle-cell anaemia to childhood deaths will increase unless specific interventions are developed to target the disease.

Our findings have shown that invasive bacterial infections make a large contribution to the morbidity and mortality of children with sickle-cell anaemia in Kenya. The dominance of just a few bacterial pathogens suggests that the health status and survival of children with this disease in Africa could be improved substantially by neonatal screening for affected children or by the accelerated introduction into immunisation programmes of *H influenzae* type b and pneumococcal vaccines. How best to identify children with sickle-cell anaemia and prevent bacterial infections in them should be a priority target for health research in Africa.

**Contributors**

TNW conceived the study, analysed the data, and drafted the report. SU, AM, CFM, and DHO contributed to sample analysis, data interpretation, and editing of the paper. CN helped with data analysis, data interpretation, and editing of the paper. SM contributed to the bacterial culture work, data interpretation, and editing of the paper. JM contributed to study conception and design, data interpretation, and editing of the paper. AK contributed to data interpretation and editing of the paper. NNM contributed to the pneumococcal serotype epidemiology, data interpretation, and editing of the paper. SSK contributed to data interpretation and policy implications and editing of the results, and editing of the paper. JAGS contributed to study design and data analysis, interpretation of the results, and editing of the paper.

**Conflicts of interest**

We declare that we have no conflicts of interest.

**Acknowledgments**

This study was supported by the Kenya Medical Research Institute (KEMRI) and the Wellcome Trust, UK. TNW is supported by the Wellcome Trust (grant number 070934), the EU Network 6 BioMalpar Consortium, and the MalariaGen Consortium funded by the Bill & Melinda Gates Foundation. JM (grant number 072068), KM (grant number 077092), JAB (grant number 083579), and JAGS (grant number 081835) are supported by the Wellcome Trust, UK. We thank the patients and staff of Kilifi District Hospital and the KEMRI/Wellcome Trust Centre for their support with this study; Emily Olori, Adan Mohammed, Metrine Tendwa, and Johnstone Makale for laboratory support; Hussein Kivogo, Emmanuel Mabibo, Gideon Nyutu, and other members of the Genetics Team for field work and data management; and David Weatherall, Bob Snow, Charles Newton, and Kathryn Maitland for helpful comments on earlier drafts. This paper is published with the permission of the Director of the Kenya Medical Research Institute (KEMRI).

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