

## Protective effects of the sickle cell gene against malaria morbidity and mortality

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**The high frequency of the sickle-cell haemoglobin (*HbS*) gene in malaria endemic regions is believed to be due to a heterozygote (*HbAS*) advantage against fatal malaria. Data to prospectively confirm the protection associated with *HbAS* against mortality are lacking. We show that *HbAS* provides significant protection against all-cause mortality, severe malarial anaemia, and high-density parasitaemia. This significant reduction in mortality was detected between the ages of 2 and 16 months, the highest risk period for severe malarial anaemia in this area. These data are important in understanding the role of malaria in the selection and maintenance of the sickle cell gene.**

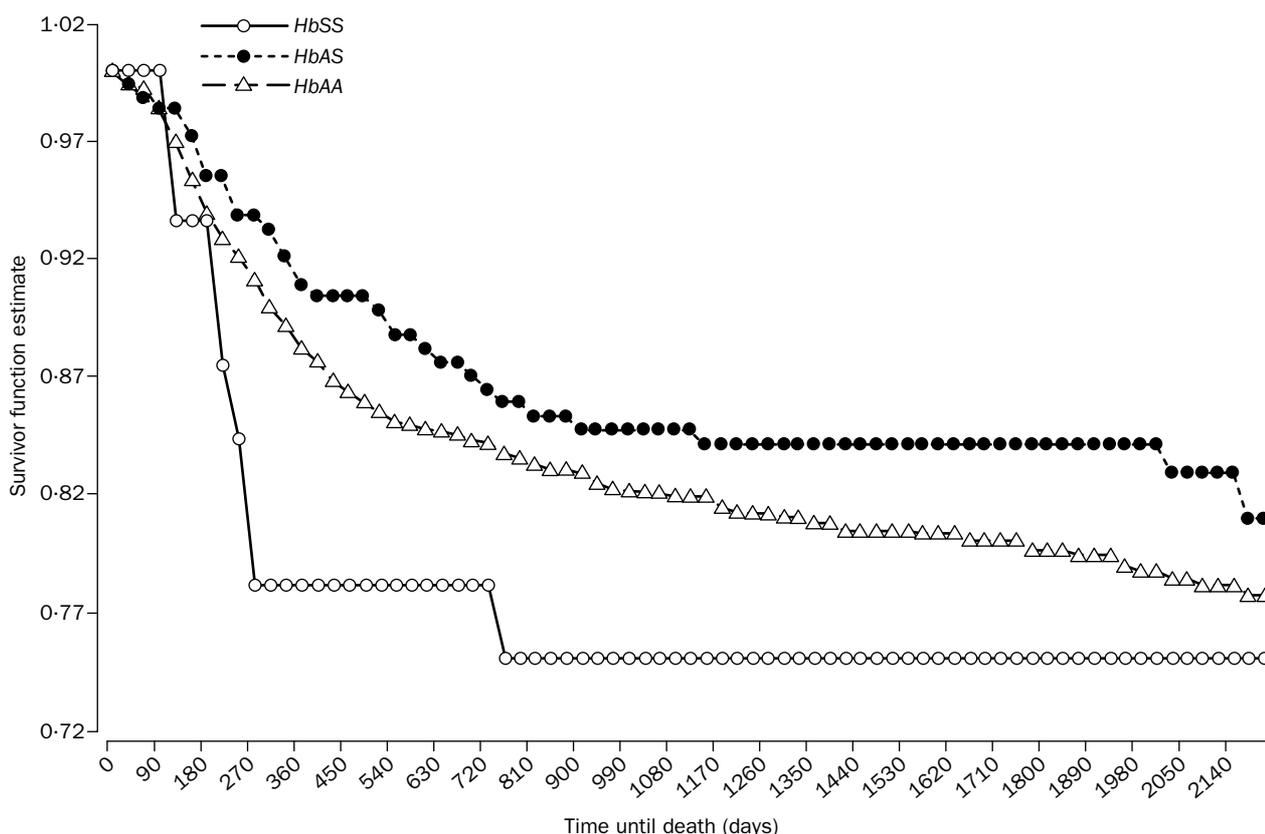
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The high frequency of the gene for sickle cell haemoglobin (*HbS*) in malaria endemic regions, despite the high mortality rate among homozygotes, is thought to be due to a selective advantage conferred by *HbAS* against malaria mortality.<sup>1</sup> The evidence in support of this hypothesis, however, is incomplete. First, although the protection against mortality conferred by *HbAS* has been indirectly estimated using *HbS* frequencies, the degree to which

*HbAS* protects against mortality has not been determined using cohort studies. Second, although it has been suggested that *HbAS* would provide a protective advantage early in life before the acquisition of clinical immunity to malaria,<sup>1</sup> definitive data to support this assumption, especially in high malaria transmission areas, are lacking.

We identified 1022 children from a birth cohort, the Asembo Bay Cohort Project, in Kisumu, Kenya, that had data on malaria morbidity and records of all-cause mortality from birth up to 3–5 years of life.<sup>2,3</sup> Details of the Asembo Bay Cohort Project have been previously described.<sup>2</sup> We did a survival analysis using sickle cell trait as a risk factor for all-cause mortality in a Cox regression model with time-dependent covariates. All-cause mortality was used because disease-specific mortality data were not available. Compared to *HbAA*, *HbAS* was significantly associated with a reduction in all-cause mortality only during the period from 2 to 16 months of age (risk ratio 0.45 [95% CI 0.24–0.84];  $p=0.0001$ , figure). However, when compared with *HbAA*, there was no *HbAS*-associated reduction in mortality during the first 2 months or >16 months of age (1.2 [0.7–2.1];  $p=0.5$ ). At ages 2–16 months, *HbSS* was not associated with any survival advantage when compared with *HbAA* (figure).

A multivariate Poisson regression model controlling for birthweight was used to determine the relationship between haemoglobin genotype and morbidity. Children with genes for *HbAS* and *HbSS* had significantly fewer episodes of severe malarial anaemia (haemoglobin <6 g/dL plus 10 000 parasites/ $\mu$ L) than children with *HbAA* in the first 5 years of life (table). *HbAS* but not *HbSS* was associated



### Survivor function estimates for haemoglobin genotypes

Genotyping was by PCR using sequence specific primers for *HbA* and *HbS*. The frequencies of *HbAA*, *HbAS*, and *HbSS* were 79.2%, 17.4%, and 3.3% respectively. Among the 1022 children genotyped, confirmed survival status to include in the analysis was available for only 1002 children. Covariates considered in the multivariate model were gestational age, birthweight, sex of child, mother's education (<5 or  $\geq 5$  years) and mother's survival status. Only mothers' survival status and gestational age were controlled for in the final model.

	Crude incidence/1000 person-months			Adjusted relative risk (95% CI)			
	HbAA	HbAS	HbSS	HbAS vs HbAA	p	HbSS vs HbAA	p
Severe malaria anaemia episodes	4.0	2.0	1.5	0.40 (0.30-0.60)	0.0001	0.29 (0.1-0.9)	0.04
All severe anaemia episodes (Hb <6 g/dL plus any parasitaemia)	8.8	6.8	7.5	0.61 (0.46-0.80)	0.0006	0.63 (0.35-1.2)	0.15
High density parasitaemia episodes	20	17.3	15.8	0.73 (0.65-0.84)	0.0001	0.52 (0.36-0.74)	0.0002

Hb=haemoglobin. HbSS was associated with lower parasite incidence than HbAA haemoglobin levels and parasitaemia were determined using routine monthly finger-prick blood samples and samples collected any time the children were reported ill. All data points collected monthly for the entire time children participated in the study were used in data analyses unless indicated otherwise. Only birthweight among the various covariates considered (same as for survival analysis) was controlled for in the final model.

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with a reduced risk of severe anaemia episodes that occurred in the presence of any level of parasitaemia (table). Both *HbAS* and *HbSS* were significantly associated with reduced risks of high-density (>10 000 parasites/ $\mu$ L) parasitaemia when compared with *HbAA* (table). Furthermore, *HbSS* (risk ratio 0.83 [95% CI 0.70–0.97];  $p=0.02$ ), and not *HbAS* (0.96 [0.90–1.0]), was significantly associated with lower parasite rates than *HbAA*.

We show that *HbAS* is associated with protection against all-cause mortality among children during the period when they are most at risk of severe falciparum malaria (2–16 months).<sup>4</sup> Although we did not have cause-specific mortality data, the finding that *HbAS* is associated with protection against severe malarial anaemia, a major cause of mortality, and high-density parasitaemia, supports the hypothesis that the lower mortality risk associated with *HbAS* is probably due to its protection against malaria-related mortality. Indeed, the association of *HbAS* with protection against severe anaemia in the presence of any level of parasitaemia was most evident from ages 2–16 months (risk ratio 0.52 [95% CI 0.31–0.86];  $p<0.05$ ), the period during which we noted a protective association against mortality. This result is consistent with the hypothesis that *HbAS*-mediated protection will be evident mainly before a significant level of clinical immunity is achieved.<sup>1</sup> Unlike *HbAS*, the apparent lack of an association of *HbSS* with protection against mortality despite its association with protection against severe malarial anaemia most probably reflects the high mortality rate due to sickle cell disease.

The lack of an apparent protection against mortality among children with the *HbAS* gene in the first 2 months of life could be due to maternally transferred protective immunity or the presence, in the first few months, of high levels of fetal haemoglobin, which poorly supports the growth of *P falciparum*. The lack of an association of *HbAS* with mortality after 16 months of age probably reflects a reduction in malaria-associated severe morbidity and mortality as a consequence of the development of clinical immunity rather than the absence of a protective effect. Alternatively, the apparent absence of a survival advantage after 16 months may be due to a reduction in the number of the susceptible *HbAA* population as a result of earlier malaria-related deaths.

We believe that the difference in the level of protection conferred by *HbAS* against severe malarial anaemia in our study and in The Gambia<sup>5</sup> (60% vs 90%) can be explained by differences in the study design (longitudinal vs case control) and transmission intensity (high and perennial vs low and seasonal). Furthermore, in the Gambian study, the 90% protection from severe malaria included protection from cerebral malaria, which our study does not address. In this study, all fevers in the presence of a positive blood film were treated with the antimalarial drug sulfadoxine-pyrimethamine (25 mg pyrimethamine: 500 mg sulfadoxine per tablet). Therefore, the observed incidence of anaemia is likely to be an underestimate of

what would be observed in a similar cohort without access to effective treatment.

This study confirms predictions from epidemiological observations that interventions to reduce malaria mortality and morbidity will probably be most effective when administered in the first years of life, especially in high perennial transmission areas such as western Kenya.

### Contributors

M Aidoo, F O ter Kuile, S Kariuki, B L Nahlen, A Lal, and V Udhayakumar designed the study. M Aidoo did the genotyping. D J Terlouw, M Kolczak, and P D McElroy did the statistical analysis. M Aidoo and V Udhayakumar wrote the paper.

### Conflict of interest statement

None declared.

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