Effect of Previous Exposure to Malaria on Blood Pressure in Kilifi, Kenya: A Mendelian Randomization Study

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Background—Malaria exposure in childhood may contribute to high blood pressure (BP) in adults. We used sickle cell trait (SCT) and αthalassemia, genetic variants conferring partial protection against malaria, as tools to test this hypothesis.

Methods and Results—Study sites were Kilifi, Kenya, which has malaria transmission, and Nairobi, Kenya, and Jackson, Mississippi, where there is no malaria transmission. The primary outcome was 24-hour systolic BP. Prevalent hypertension, diagnosed using European Society of Hypertension thresholds was a secondary outcome. We performed regression analyses adjusting for age, sex, and estimated glomerular filtration rate. We studied 1127 participants in Kilifi, 516 in Nairobi, and 651 in Jackson. SCT frequency was 21% in Kilifi, 16% in Nairobi, and 9% in Jackson. SCT was associated with −2.4 (95% CI, −4.7 to −0.2) mm Hg lower 24-hour systolic BP in Kilifi but had no effect in Nairobi/Jackson. The effect of SCT in Kilifi was limited to 30- to 59-year-old participants, among whom it was associated with −6.1 mm Hg (CI, −10.5 to −1.8) lower 24-hour systolic BP. In pooled analysis allowing interaction by site, the effect of SCT on 24-hour systolic BP in Kilifi was −3.5 mm Hg (CI, −6.9 to −0.1), increasing to −5.2 mm Hg (CI, −9.5 to −0.9) when replacing estimated glomerular filtration rate with urine albumin to creatinine ratio as a covariate. In Kilifi, the prevalence ratio for hypertension was 0.86 (CI, 0.76–0.98) for SCT and 0.89 (CI, 0.80–0.99) for αthalassemia.

Conclusions—Lifelong malaria protection is associated with lower BP in Kilifi. Confirmation of this finding at other sites and elucidating the mechanisms involved may yield new preventive and therapeutic targets. (J Am Heart Assoc. 2019;8:e011771. DOI: 10.1161/JAHA.118.011771.)

Key Words: ambulatory blood pressure monitoring • high blood pressure • hypertension • malaria • Mendelian randomization • sickle cell disease • sickle cell trait • thalassemia

High blood pressure (BP) is a major cause of morbidity and mortality worldwide, and its impact is particularly substantial in sub-Saharan Africa.1 Examining factors unique to, or more prevalent in, sub-Saharan Africa might reveal pathophysiological mechanisms underlying hypertension that could be exploited to reduce the burden of hypertension. More than half of Africa’s population lives in areas with moderate to high malaria transmission.2 Falciparum malaria is associated with low birth weight, childhood malnutrition, and chronic inflammation, each of which has been associated with the development of hypertension.3 In addition, children of women who experienced malaria in pregnancy have higher BP at 1 year of age than those whose mothers did not have malaria during pregnancy.4

Mendelian randomization studies, in which genetic polymorphisms are used to represent environmental exposures, are increasingly being applied in determining causality.5 These natural experiments, based on genes acquired at conception, overcome the limitations of observational studies while avoiding the expense and ethical concerns that prevent the conduct of randomized clinical trials.

Sickle cell trait (SCT) and αthalassemia are common genetic polymorphisms among African populations that...
What Is New?

- We tested the hypothesis that previous exposure to malaria is associated with increased blood pressure (BP).
- We compared BP in individuals with and without sickle cell trait, which protects against malaria, at 3 sites, where there is ongoing malaria transmission and 2 where there is no malaria transmission.
- We found that individuals with sickle cell trait had lower BP than those without sickle cell trait, but this was only in the area with malaria transmission, indicating that malaria is associated with high BP.

What Are the Clinical Implications?

- Confirmation of the existence of a link between previous exposure to malaria and high BP at other sites and elucidation of the mechanisms involved may help in finding new ways to prevent and treat high BP.

provide protection against malaria. Examining differences in BP among adults with and without these polymorphisms can allow inferences to be made as to whether malaria influences BP.\(^3\) In the present study, we tested whether SCT and \(\alpha\) thalassemia are associated with lower BP and a lower prevalence of hypertension in Kilifi, Kenya, where there is malaria transmission. For comparison, we investigated the same association in Nairobi, Kenya, and Jackson, Mississippi, 2 areas where there is no malaria transmission.

Methods

Data and Materials Availability

The authors confirm that, for approved reasons, some access restrictions apply to the data underlying the findings. The authors are unable to make the data more freely available because of the terms for data sharing included in the consent forms for this study. Data are available through the Data Governance Committee of the KEMRI Wellcome Trust Research programme where uses are compatible with the consent obtained from participants for data collection in this study. Requests can be sent to the coordinator of the Data Governance Committee on dgc@kemri-wellcome.org and the Jackson Heart Study on jhspub@umc.edu.

This Mendelian randomization study was performed among residents of 3 sites (Figure S1) with different levels of malaria transmission; Kilifi, Kenya, where there has been moderate malaria transmission;\(^6\)–\(^8\) and 2 sites (Nairobi, Kenya, and Jackson, Mississippi) where there is no malaria transmission.\(^9\)–\(^11\) We utilized SCT, which offers 50% to 90% protection against malaria episodes,\(^12\)–\(^13\) predominantly in childhood,\(^14\) to represent malaria exposure (Figure S2). In a secondary analysis among Kenyan participants, we used \(\alpha\) thalassemia, which provides a lower level of protection against malaria than SCT. To be eligible for the study in Kenya, individuals had to be 10 years or older at investigation and to be lifelong residents of Kilifi or Nairobi. In Kilifi, we recruited randomly selected residents of Chasimba and Junju locations within the Kilifi Health and Demographic Surveillance System.\(^15\) Kilifi participants were predominantly of the Chonyi subtribe of the Mijikenda ethnic group. In Nairobi, we recruited randomly selected residents of the Nairobi Urban Health and Demographic Surveillance System.\(^16\) Study participants in Nairobi were randomly selected from among those who had self-identified as belonging to ethnic groups known to have a high frequency of malaria-protective polymorphisms (Luhya, Luo, Teso, Mijikenda).\(^17\)–\(^18\) Data were collected in Kenya between December 2015 and June 2017. Participants in the United States were blacks aged 21 years and older recruited into the Jackson Heart Study between 2000 and 2004.\(^19\)

We used appropriately sized cuffs on the nondominant arm to undertake ambulatory BP monitoring (ABPM). We used the Arteriograph\(^24\) (TensioMed Ltd.) device in Kenya and the Spacelabs 90207 (Spacelabs) device in the United States. For the primary analyses, we defined completeness of ABPM recordings using European Society of Hypertension (ESH) criteria.\(^20\) These criteria require \(>20\) daytime (\(9\ AM–9\ PM\)) and \(\geq 7\) nighttime (\(1\ AM–6\ AM\)) readings.\(^20\) The respective time periods were used to determine mean daytime and nighttime BP. The mean 24-hour BP was calculated using all available readings. Prevalent hypertension was diagnosed among participants 16 years and older who met any of the ESH-defined thresholds for ABPM hypertension regardless of whether they were taking antihypertensive medication. The thresholds used were 24-hour systolic BP (SBP) \(\geq 130\) mm Hg or 24-hour diastolic BP \(\geq 80\) mm Hg, daytime SBP \(\geq 135\) mm Hg or daytime diastolic BP \(\geq 85\) mm Hg, and/or nighttime SBP \(\geq 120\) mm Hg or nighttime diastolic BP \(\geq 70\) mm Hg.\(^21\)–\(^22\) Detailed study procedures are described in the supplement.

Statistical Analysis

The primary outcome was the difference in 24-hour SBP by SCT status. We determined that a sample of 1115 participants in Kilifi and 1270 participants in Nairobi and Jackson combined would provide at least 80% statistical power to detect a 4-mm Hg difference in 24-hour SBP between participants with and without SCT at each of these sites, assuming an SCT frequency of 15% in Kilifi and 10% in Nairobi/Jackson and an SD for 24-hour SBP of 15 mm Hg. We defined secondary outcomes as the genotype-specific...
We used \( \chi^2 \) test and Student t test to compare categorical and continuous variables at each site by genotype. Hardy–Weinberg equilibrium was evaluated using a \( \chi^2 \) test. Nonnormally distributed variables were log-transformed before analysis.

Two types of analyses were conducted to test the hypothesis. First, we compared BP among participants with and without SCT at each of the 3 sites, while adjusting for confounders as described below. Second, we pooled data from the 3 sites and analyzed whether there was an interaction in the effect of SCT on BP by site.

In the first analyses, which were site-specific, we performed linear regression to determine whether SCT status was associated with 24-hour SBP, adjusting for age, sex, and estimated glomerular filtration rate (eGFR).\(^{23}\) (Figure S3), which were specified a priori as potential confounders. These covariates were also used in Poisson regression models with robust variance to assess whether SCT was associated with prevalent hypertension. As \( \alpha^+ \)thalassemia modifies the protective effect of SCT against malaria,\(^{24}\) we tested for statistical interaction in their effect under both dominant and additive conditions among Kilifi participants.

The second, pooled analyses were conducted as follows. Initially, we tested for heterogeneity in the effect of SCT on BP in the 2 sites with no malaria transmission, Nairobi and Jackson, by conducting a linear regression allowing interaction by site. As there was no evidence of heterogeneity in this analysis, we combined data from these 2 sites and then tested whether the effect of SCT on BP differed across sites with different levels of malaria transmission (Kilifi versus Nairobi/Jackson). This was conducted in a regression model that tested for the main effects of age, sex, eGFR, malaria site (Kilifi versus Nairobi/Jackson) and SCT, and an interaction term for SCT and malaria site.

Prespecified subgroup analyses included stratification by age category (10–29, 30–59, and \( \geq 60 \) years) and sex, and after exclusion of participants taking antihypertensive medication. These subgroup analyses were performed for Kilifi separately and for Nairobi and Jackson combined. We also examined the effect of replacing eGFR with log-transformed urine albumin to creatinine ratio as the covariate representing renal function in the pooled regression models.

In sensitivity analyses, we repeated the analyses using an expanded data set that included participants whose ABPM data met the less stringent IDACO (International Database of Ambulatory Blood Pressure in relation to Cardiovascular Outcome) study criteria for completeness. These criteria require a minimum of 10 daytime (10 AM–8 PM) readings and 5 nighttime (12 AM–6 AM hours) readings.

All analyses were conducted using Stata version 15 software (StataCorp).

The ethical review committees/institutional review boards of the Kenya Medical Research Institute, London School of Hygiene and Tropical Medicine, Jackson State University, Tougaloo College, and the University of Mississippi Medical Center approved the study. Analysis of Jackson Heart Study data was approved by the University of Alabama at Birmingham’s institutional review board. Written informed consent, and assent for participants younger than 18 years in Kenya, was obtained from study participants or parents.

Results

We identified 9543 lifelong residents in the Kilifi population register and, using a random number sequence, we invited 2790 to participate in the study (Figure 1). Characteristics of participants by consent to undergo ABPM and by completeness of ABPM recordings are summarized in Tables S1 and S2, respectively. Individuals with homozygous sickle cell disease (n=5) were excluded from all analyses. Complete data were available for 1127 participants. None of the participants were previously aware of their genetic status. Overall, 238 (21%) participants had SCT and 768 (67%) were either heterozygous (\(-\alpha/\alpha\alpha\)) or homozygous (\(-\alpha/-\alpha\)) for \( \alpha^+ \) thalassemia, equally distributed by SCT status (69% for those with SCT versus 67% for those without SCT, \( P=0.5 \)). There was no departure from Hardy–Weinberg equilibrium for either SCT (\( P=0.5 \)) or \( \alpha^+ \)-thalassemia (\( P=0.8 \)). Mean 24-hour SBP was 127±18 mm Hg.

Characteristics of participants by study site and SCT status are presented in Table 1. A higher proportion of participants without SCT versus participants with SCT in Jackson were taking antihypertensive medication. In Kilifi, eGFR was lower among participants with SCT versus participants without SCT. Urine albumin to creatinine ratio was higher among participants with SCT versus participants without SCT in Kilifi and Jackson.

Mean 24-hour SBP in Kilifi was 126±18 mm Hg in individuals with SCT and 127±18 mm Hg in individuals without SCT. In Nairobi/Jackson, mean 24-hour SBP was 123±13 mm Hg in individuals with SCT and 123±14 mm Hg in individuals without SCT. After adjusting for age, sex, and eGFR in regression analyses, SCT was associated with a –2.4 (95% CI, –4.7 to –0.2) mm Hg (\( P=0.037 \)) lower 24-hour SBP in Kilifi (Figure 2). In Nairobi and Jackson, there was no association between SCT and 24-hour SBP. As there was no heterogeneity between Nairobi and Jackson in the effect of SCT on any BP measure (test for interaction, \( P=0.409–0.944 \); Table S3) data from these sites...
were pooled in subsequent analyses. The proportion of variation in BP explained by the regression model as determined by the adjusted $r^2$ statistic was 25% in Kilifi, 16% in Nairobi, and 7% in Jackson.

The association between SCT and 24-hour SBP observed in Kilifi was unaffected by excluding participants taking antihypertensive medication (Table S4).

In Kilifi, SCT was associated with lower 24-hour SBP (−6.1 mm Hg; 95% CI, −10.5 to 1.8) among participants aged 30 to 59 years but not in participants in the other age categories (Table 2). In Nairobi and Jackson combined, there was no association between SCT and any BP measure in any age group except for 24-hour and daytime diastolic BP in those 60 years and older, where BP was higher among participants with SCT. In Kilifi, there was a numerically stronger association between SCT and lower 24-hour SBP in women compared with men ($P=0.218$, Table S5).

In the pooled analyses, SCT was associated with −3.5 mm Hg (CI, −6.9 to −0.1) lower 24-hour SBP ($P=0.041$) in Kilifi when compared with Nairobi and Jackson (Figure 3). This interaction model was associated with an adjusted $r^2$ statistic of 20%. The magnitude of the difference was larger (−5.2 mm Hg; CI, −9.5 to −0.9 [$P=0.019$]) when we adjusted for log-transformed urine albumin to creatinine ratio instead of eGFR (Table S6). Stratified by sex, the effect of SCT on 24-hour SBP in Kilifi compared with Nairobi/Jackson was not stronger in women compared with men ($P=0.419$, Table S7).

The prevalence of hypertension among study participants was 56% in Kilifi, 28% in Nairobi, and 62% in Jackson. In Kilifi, the adjusted prevalence ratio (PR) for hypertension among participants with SCT versus those without SCT was 0.86 (CI, 0.76–0.98, $P=0.027$). In Nairobi and Jackson, the corresponding adjusted PR was 0.96 (CI, 0.80–1.16; $P=0.706$). When using clinic BP to define hypertension (SBP ≥140 and/or diastolic BP ≥90 mm Hg), the adjusted PR for hypertension among participants with SCT versus those without SCT in Kilifi was 0.82 (CI, 0.64–1.05; $P=0.113$). In Nairobi and Jackson, the corresponding adjusted PR when using clinic BP was 1.45 (CI, 0.62–3.42; $P=0.396$).

$\alpha$-Thalassemia deletions were not associated with the primary outcome in either Kilifi and Nairobi (Table S8), but a significant association with prevalent hypertension was present in Kilifi. In Kilifi, the presence of $\geq 1$ thalassemia deletion was associated with an adjusted PR for hypertension of 0.89 (CI, 0.80–0.99; $P=0.036$). There was no effect of $\alpha$-thalassemia on hypertension in Nairobi (PR, 1.43; CI, 0.95–2.15 [$P=0.085$]).

There was no interaction between $\alpha$-thalassemia and SCT on 24-hour SBP in Kilifi (test for interaction, $P=0.865$).
Table 1. Characteristics of Study Participants With and Without SCT by Study Site

| Characteristic                           | Kilifi (N=1127) | Nairobi (N=516) | Jackson (N=651) |
|-----------------------------------------|----------------|----------------|----------------|
|                                         | SCT (n=238) | Non-SCT (n=889) | SCT (n=82) | Non-SCT (n=434) | SCT (n=58) | Non-SCT (n=593) |
|                                         | No. (%)     | No. (%)          | No. (%)      | No. (%)          | No. (%)      | No. (%)          |
| Women                                   | 126 (53)    | 528 (59)         | 40 (49)      | 235 (54)         | 38 (66)      | 392 (66)         |
| Smoker                                  | 17 (7)      | 78 (9)           | 3 (4)        | 7 (2)            | 5 (9)        | 74 (12)          |
| Previously diagnosed with hypertension*| 37 (16)     | 127 (14)         | 7 (9)        | 53 (12)          | 31 (53)      | 367 (62)         |
| Taking antihypertensive medication      | 9 (4)       | 26 (3)           | 0 (0)        | 8 (2)            | 26 (46)      | 338 (61)         |
| Mean (SD)                               | Mean (SD)   | Mean (SD)        | Mean (SD)    | Mean (SD)        | Mean (SD)    | Mean (SD)        |
| Age, y                                  | 41 (22)     | 39 (22)          | 20 (14)      | 23 (18)          | 61 (12)      | 60 (11)          |
| BMI, kg/m²                               | 20.6 (3.6)  | 20.6 (3.8)       | 20.2 (3.9)   | 20.5 (4.3)       | 31.1 (7.0)   | 30.9 (6.3)       |
| HbA1c, %                                | 5.2 (0.6)   | 5.1 (0.8)        | 5.2 (1.1)    | 5.2 (1.0)        | 6.0 (1.2)    | 6.1 (1.3)        |
| Hemoglobin, g/dL                        | 12.6 (2.0)  | 12.6 (1.6)       | 13.4 (1.7)   | 13.2 (1.7)       | 12.8 (1.4)   | 13.0 (1.4)       |
| WBC count ×10³/L                        | 5.7 (1.5)   | 5.7 (1.4)        | 5.4 (1.4)    | 5.4 (1.6)        | 4.9 (1.1)    | 5.3 (1.5)        |
| Platelet count ×10⁹/L                   | 267 (97)    | 262 (83)         | 283 (99)     | 287 (100)        | 230 (58)     | 243 (61)         |
| Plasma osmolality, mOsm/kg              | 290 (6.6)   | 290 (5.8)        | 291 (10)     | 291 (11)         | ... (…)      | ... (…)          |
| eGFR, mL/min per 1.73 m²                | 108 (35)    | 114 (42)         | 118 (27)     | 115 (24)         | 85 (26)      | 87 (26)          |
| Log UACR, mg/g                          | 1.3 (0.5)   | 1.2 (0.6)        | 1.3 (0.7)    | 1.5 (0.7)        | 1.06 (0.6)   | 0.90 (0.5)       |

Plasma osmolality measurements were not available for Jackson participants. BMI indicates body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; SCT, sickle cell trait; UACR, urine albumin to creatinine ratio; WBC, white blood cell.

*Answered “yes” to the question: Has a doctor or healthcare worker previously told you that you have high blood pressure?

Results Based on IDACO ABPM Criteria

The results of sensitivity analyses are presented in Tables S9 through S15. The number of participants who satisfied IDACO criteria for completeness of ABPM data was 2048 (86%). As expected, the associations between SCT and α-thalassemia and BP were weaker than in the primary analyses, but the associations with hypertension remained significant. In Kilifi, SCT was associated with an adjusted PR for hypertension of 0.92 (CI, 0.84–0.99; P=0.025). In Nairobi and Jackson, the corresponding adjusted PR was 0.96 (CI, 0.81–1.14; P=0.659). The presence of any α-thalassemia deletion among individuals in Kilifi was associated with an adjusted PR of hypertension of 0.92 (CI, 0.84–0.99; P=0.037). In Nairobi, the corresponding adjusted PR for α-thalassemia was 1.32 (CI, 0.95–1.86 [P=0.096]).

Discussion

In this study, SCT, a genetic polymorphism associated with partial protection against malaria, was associated with a 2.4-mm Hg lower mean 24-hour SBP and a 14% lower prevalence of hypertension in Kilifi, an area with malaria transmission, but not in Nairobi and Jackson, areas with no malaria transmission. α-Thalassemia, which provides a lower level of protection against malaria was associated with an 11% reduction in the prevalence of hypertension in Kilifi but not in Nairobi. This suggests that increased risk of malaria is associated with higher adult BP. In the absence of malaria, in Nairobi and Jackson, mean BP estimates were marginally higher among participants with SCT than those without SCT. Incorporating this baseline observation in a pooled analysis that compared malaria with nonmalaria sites, we estimate that malaria is responsible for a mean increase in 24-hour SBP of 3.5 mm Hg.

This difference in BP is roughly similar to those attributed to reduction of salt intake by ≤4 g/d/25 or a dose of 10 mg/d of ramipril in the HOPE (Heart Outcomes Prevention Evaluation) trial.26 At the population level, a reduction in SBP of 3 mm Hg may avert a substantial number of cardiovascular events including an ≈15% reduction in the incidence of stroke.27 However, several factors suggest that the actual effect of malaria on BP might be greater. First, SCT is only associated with a 50% reduction in incidence of nonsevere malaria13 and 90% reduction against severe malaria episodes,28 and this relative protection wanes with age.14 Second, the protection offered by SCT against malaria is reduced in individuals with concurrent α-thalassemia,24 who comprised 67% of participants in the current study who had SCT. The current study was not powered to analyze the effect of this interaction. In addition, because Kilifi has low to moderate malaria transmission,4 the effect of malaria on BP in other parts of Africa where malaria is endemic could be considerably higher.
In this epidemiological study, we were not able to study the physiological mechanisms by which malaria results in higher BP. However, there are a number of plausible hypotheses. For example, hypertension may be the consequence of chronic inflammation in childhood induced by malaria and inflammation, itself, has been associated with the development of hypertension. Malaria also causes stunting and malnutrition, which could influence BP, although anthropometric indices such as BMI were similar in the groups we studied. The numerically stronger association that we observed between SCT and BP in women could be explained by SCT-mediated protection against malaria in pregnancy. Malaria in pregnancy has been associated with gestational hypertensive disorders that place women at risk for chronic hypertension. CD4+ and CD8+ T-cells, which play a role in responses to malaria, as well as partially explain sex differences in hypertension, could possibly explain the sex differences that we observed. Theoretically, BP could be modulated by exposure to antimalarial treatment, but this seems an unlikely explanation because no commonly used antimalarial drug is

Table 2. Age-Specific Effects of SCT on BP by Study Site

| Age, y | Kilifi | Nairobi and Jackson pooled together* |
|--------|--------|-------------------------------------|
| 10 to 29 |        |                                    |
| 30 to 59 |        |                                    |
| ≥60     |        |                                    |

Results of linear regression models adjusted for age, sex, and estimated glomerular filtration rate. BP indicates blood pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure; SCT, sickle cell trait.

Figure 2. Effect of sickle cell trait (SCT) on blood pressure by study site. A, Systolic blood pressure and B, diastolic blood pressure. Linear regression models with adjustment for age, sex, and estimated glomerular filtration rate.

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SCT in Kilifi has been associated with BP traits; (2) studies in the United States show that SCT does not reduce the risk of hypertension-related outcomes such as stroke, heart failure, and chronic kidney disease, as would be expected if it lowered BP; and (3) most genetic polymorphisms influencing BP tend to have small effects.

Study Strengths

This study had several strengths. First was the use of ABPM, considered the reference standard for measuring BP. By performing multiple repeated measurements, ABPM gives more accurate estimates of BP. The largest differences were observed in the primary analyses that utilized more stringent quality criteria, which provided for better accuracy in measurement without introducing a selection bias. In addition, in both the primary and sensitivity analyses, the largest differences were observed when comparing nighttime measurements, which are less susceptible to interference by daytime activities. Nocturnal BP indices are also more predictive of cardiovascular outcomes than daytime or 24-hour values. Second, the Mendelian randomization approach that we used is a robust design for elucidating causal relationships. We used genetic variants that are strongly associated with malaria and showed associations with the outcomes that were consistent with the different levels of protection against malaria afforded by each variant. Third, study participants in Kilifi were of the same ethnicity, minimizing the possibility that population stratification could explain the differences observed. Fourth, we used prospectively collected health and demographic surveillance system records to ascertain residence in malaria/nonmalaria sites.

Study Limitations

Although Mendelian randomization is a well-established method for inferring causality, there are some residual limitations. As we did not have medical record data for the participants in Kilifi, we could not determine the timing, number, or severity of malaria episodes required to elevate BP in adult life. These questions could be investigated using sequential birth cohort studies that take into account the fact that there has been a marked reduction in malaria transmission in Kilifi from the year 2000. In addition, replication studies in other areas with malaria transmission are needed to confirm the observations we made in Kilifi.

Conclusions

SCT was associated with lower BP and reduced prevalence of hypertension in Kilifi but not in Nairobi, Kenya, or Jackson, Mississippi, an observation compatible with a causal association between malaria and higher BP. One implication is that...
malaria elimination would lead to health benefits well beyond those currently described. A second implication is that elucidating the mechanisms by which malaria leads to an elevation in BP could yield new preventive strategies for hypertension and consequent cardiovascular disease. 

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Disclosures

None.

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Supplemental Material
Study Procedures

a) Study Procedures in Kilifi and Nairobi, Kenya

Participants were recruited from December 2015 to June 2017. We recruited participants aged ≥10 years that were lifelong residents of the Nairobi Urban Health and Demographic Surveillance System\(^1\) and the Kilifi Health and Demographic Surveillance System\(^2\) respectively (locations shown in Figure S1). Lifelong residency was confirmed using prospectively collected residency data from enumeration rounds that are conducted every 3-4 months within the demographic surveillance systems. Continuous residency was required in order to minimize misclassification of exposure to malaria as Nairobi and Kilifi have markedly contrasting malaria transmission patterns. Study participants in Nairobi were randomly selected from those who had self-identified as belonging to ethnic groups known to have a high frequency of malaria protective polymorphisms (Luhya, Luo, Teso, Mijikenda) as a result of hailing from parts of Kenya that are known to be endemic for malaria.\(^3\),\(^4\) Study participants in Kilifi were predominantly from the Chonyi subtribe of the Mijikenda community. The prevalence of hypertension within the Kilifi Health and Demographic Surveillance System which covers an area of 900km\(^2\) is ~17%.\(^5\) However there are significant differences in the incidence to death due to stroke within the study area. Chasimba where >75% of study participants came from has an incidence of death due to stroke that is three times that of Kilifi township, suggesting that there are local geographical differences in the prevalence of hypertension which is the main risk factor for stroke.

In both Kilifi and Nairobi, trained study staff visited all individuals who had been selected to participate in the study at their homes and requested them to come to the
study clinic to undergo study procedures. Those who failed to come to the clinic within 3 months of being requested to do so were considered to have declined our invitation to participate in the study.

At the clinic participants first underwent an interview where they answered questions about their past medical history and their socioeconomic status based on the multi-dimensional poverty (MDP) index. Weight and height were measured using a validated SECA 874™ weighing machine and a portable stadiometer (Seca 213™), respectively. Body mass index was calculated as the weight in kilograms divided by height in meters squared (kg/m²). We did not classify BMI by age-category in the adolescents that we studied. Mid-upper arm circumference (MUAC) was measured in a standardized manner using TALC™ MUAC tapes. All participants were subsequently fitted with a validated Arteriograph24™ (TensioMed Ltd., Budapest, Hungary) device for 24-hour ABPM measurement. The devices were attached on the non-dominant arm and were programmed to take measurements every 20 minutes during daytime hours (0600-2200 hrs) and every 40 minutes at night (2200-0600 hrs). At the end of the 24-hour period, participants returned to the study clinic where the Arteriograph was removed and data downloaded onto computers that would later (within 12 hours) synchronize their data onto an MySQL database hosted on servers located at the KEMRI-Wellcome Trust Research Programme offices in Kilifi, Kenya.

We collected 10ml of blood from participants for full blood count, determination of sickle hemoglobin status and serum electrolytes. After performing automated full blood counts using an ACT 5™ machine, whole blood samples were frozen at -80ºC and then transported to the KEMRI-Wellcome Trust Research Programme laboratories in Kilifi, Kenya for determination of sickle hemoglobin status. DNA was
extracted retrospectively from the frozen samples by use of Qiagen™ DNA blood mini-kits (Qiagen, Crawley, United Kingdom) and typed for sickle hemoglobin and α-thalassemia using polymerase chain reaction. Glycosylated hemoglobin levels were determined using the Biorad™ D-10 machine (Bio-rad Laboratories Inc, Hercules, California).

Serum and urine samples collected from participants were frozen at -80°C within 4 hours of collection and later transported to the laboratories in Kilifi for analysis. We determined urea and creatinine levels in these samples using ion electrophoresis and the jaffe method, respectively. Creatinine measurements were performed using Isotope dilution mass spectrometry traceable methods. In addition, we determined albumin levels in the urine samples by immunoturbidimetry using a Quantex™ microalbumin kit. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in adults and the Schwartz equation in those aged ≤16 years.9, 10

b) Study Procedures in Jackson, Mississippi, USA

The Jackson Heart Study (JHS)11 is a population-based prospective cohort study, which was designed to evaluate cardiovascular disease risk among blacks. The JHS enrolled 5306 noninstitutionalized blacks, aged ≥21 years, between 2000 and 2004. The participants were recruited from the Atherosclerosis Risk in the Community site in Jackson, MS, and a representative sample of urban and rural Jackson, MS, metropolitan tricounty (Hinds, Madison, and Rankin counties) residents, volunteers, randomly selected individuals, and secondary family members.12 The current analysis was restricted to JHS participants who completed ABPM soon after the baseline study visit (visit 1).
During in-home interviews, trained African American interviewers administered standardized questionnaires to collect self-reported information on socio-demographics (e.g. age, sex, education, marital status and socioeconomic status), previously diagnosed co-morbid conditions and selected health-related behaviors (e.g. current smoking). Weight and height were measured during a clinic visit. At the clinic visit, blood samples were collected for full blood count, genetic studies and determination of serum sodium, potassium and creatinine concentrations. 24-hour urine samples were collected for determination of creatinine and albumin concentrations. Full blood counts were performed using the Coulter GenS machine (BeckmanCoulter, Hialeah, Florida, USA). DNA was extracted from whole blood samples using Puregene reagents (Gentra System, Minneapolis, USA) and genetic studies were performed as previously described. \[12\] Biochemical tests were performed using a Vitros 950 or 250 Ortho-Clinical Diagnostics analyzer (Raritan, New Jersey, USA). Urine albumin was measured on a Dade-Behring BN 11 nephelometer (dade-Behring, Newark, Delaware, USA). All tests were performed at the University of Minnesota laboratory with the exception of hematology tests, which were done at the University of Mississippi Medical Center. \[13\] Creatinine measurements were performed using Isotope dilution mass spectrometry traceable methods. Participants were fitted with an ABPM device (Spacelabs 90207, Spacelabs, Redmond, WA) on their non-dominant arm. Ambulatory BP was recorded every 20 minutes. After 24 hours, the device was removed, and data were downloaded onto a computer and processed with Medifacts International’s Medicom software (Rockville, MD).
Statistical Methods and Considerations

a) Reporting format
While there are no specific guidelines for reporting Mendelian randomization (MR) studies, the principles outlined in the Strengthening the Reporting of OBsErvational studies (STROBE)\textsuperscript{14} guidelines as well as the STROBE Extension for Genetic Association studies (STREGA)\textsuperscript{15} were used. Reporting was also guided by the review by Boef \textit{et al.} of the quality of reporting of MR studies.\textsuperscript{16}

b) Sample size estimation
The sample size calculation for Kilifi was based on a two-sample t-test comparing mean 24-hour systolic blood pressure in those with and without the sickle cell trait (SCT). The following assumptions were made:

- That the prevalence of SCT would be $\geq 15\%$\textsuperscript{17}
- That the standard deviation of 24-hour systolic BP would be $\leq 15$ mm Hg\textsuperscript{5, 18}

Based on these assumptions we calculated that, for Kilifi, we would need a minimum of 1115 participants with complete data in order to detect a statistically significant 4 mm Hg difference in 24-hour systolic BP with at least 80\% statistical power.

For participants in Nairobi/Jackson we assumed that the combined SCT prevalence for the two sites would be $\geq 10\%$.\textsuperscript{19} Other assumptions were similar to those for Kilifi. Based on these assumptions we calculated that for Nairobi/Jackson, we would need a minimum of 1270 participants with complete data in order to detect a statistically significant 4 mm Hg difference in 24-hour systolic BP with at least 80\% statistical power.

We assumed that with these numbers, we would achieve enough power for the primary outcome measure, a linear regression to determine the effect of SCT on 24-hour BP measures, while adjusting for age, sex and estimated glomerular filtration.
rate (eGFR). The literature suggests that the major consideration for sample size calculations in linear regression models is to ensure that there are at least 2-50 individuals per variable in the model\textsuperscript{20}, a requirement that would almost certainly be achieved if most of the assumptions stated above held true.

c) Quality control criteria for ABPM data

There are 2 internationally recognized quality control criteria used for ABPM data, which are based on completeness of observations. The International Database of Ambulatory blood pressure in relation to Cardiovascular Outcomes (IDACO) study\textsuperscript{21} defined ABPM data as acceptable if they include ≥ 10 daytime and ≥ 5 nighttime readings, where daytime is defined as 1000-2000 hrs and nighttime as 0000-0600 hrs.\textsuperscript{21} The guidelines from the European Society of Hypertension (ESH) are more stringent; they require ≥20 daytime and ≥7 nighttime readings where daytime is defined as 0900 to 2100 hrs and nighttime as 0100 to 0600 hours.\textsuperscript{22} It is important to note that these criteria were arbitrarily set by experts and were not based on outcome studies. As the ESH criteria are more stringent they are likely to lead to a greater loss of data and subsequent loss of power and precision. However, in order to reduce measurement bias and obtain as accurate an effect size as possible, an a priori decision was made to restrict our primary analysis to data that met the ESH criteria.

d) Primary and secondary outcome measures

The primary outcome measure was estimated using a linear regression model to determine the effect of SCT on 24-hour systolic blood pressure, after adjusting for age, sex and estimated glomerular filtration rate (eGFR). Blood pressures were obtained by ambulatory blood pressure monitoring using the Arteriograph\textsuperscript{24™}
Numerous studies have shown that the more accurate measurements resulting from repeated inflations and more standardized procedures in ABPM make it a much better predictor of cardiovascular events than other BP measurement methods. The justification for adjusting for age, sex and eGFR is given in the section below on confounders and model building.

Secondary outcome measures were defined as follows:

a) effect of \( \alpha \)-thalassaemia on 24-hour, daytime and nighttime systolic blood pressures, after adjusting for age, sex and estimated glomerular filtration rate

b) effect of SCT on 24-hour, daytime and nighttime diastolic blood pressures, after adjusting for age, sex and estimated glomerular filtration rate

c) effect of \( \alpha \)-thalassaemia on 24-hour, daytime and nighttime diastolic blood pressures, after adjusting for age, sex and estimated glomerular filtration rate

d) prevalence ratio for hypertension in those with and without SCT using log-binomial regression, adjusting for age, sex and estimated glomerular filtration rate

Hypertension was diagnosed by any one of the following criteria in individuals aged \( \geq 16 \) years: 

i) 24-hours systolic BP \( \geq 130 \) mmHg and/or 24-hour diastolic BP \( \geq 90 \) mm Hg

ii) Daytime systolic BP \( \geq 135 \) mm Hg and/or daytime diastolic BP \( \geq 85 \) mm Hg

iii) Nighttime systolic BP \( \geq 120 \) mm Hg and/or nighttime diastolic BP \( \geq 70 \) mm Hg
Adjustment for multiple testing was not considered necessary in this scenario of a limited number of clinically relevant pre-specified tests (e.g. compared to GWAS studies)\(^\text{24}\)

**e) Adjusting for confounders and model building**

The theoretical basis for the malaria-high blood pressure hypothesis has been published previously.\(^\text{25}\) Briefly, the primary hypothesis is that individuals in Kilifi who were exposed to more malaria disease in childhood (represented by those having haemoglobin AA) would have higher 24-hour systolic blood pressure than those who were exposed to less malaria disease (represented by those having haemoglobin AS [SCT]). The proposed causal diagram drawn purely for purposes of informing the analytical plan can be found in Figure S2.

For purposes of this analysis it is important to note that because malnutrition and stunting are on the causal pathway from malaria to the outcome, adjustment for body mass index (BMI) and other anthropometric indices (e.g. mid upper arm circumference) would be inappropriate.

**i) Confounders**

The principle of Mendelian randomization holds that because comparisons are based on genetic traits acquired at conception, any relationships between the genetic trait and the outcome are unlikely to be confounded by other exposures as these will be randomly distributed between carriers and non-carriers of the trait.\(^\text{26}\) However age, sex, and BMI are known to have a very strong influence on BP and other cardiovascular diseases\(^\text{27}\), and are usually adjusted for as ‘fixed covariates’ in MR/Genome wide association studies\(^\text{28-31}\). We have outlined above why it would be inappropriate to adjust for BMI.
Sickle cell trait has been associated with impaired kidney function as measured by decline in estimated glomerular filtration rate (eGFR) and albuminuria. This association is independent of blood pressure elevation. Impaired kidney function is associated with elevations in blood pressure as a result of sodium retention, increased activity of the renin-angiotensin system, increased sympathetic activity, secondary hyperparathyroidism, impaired nitric oxide synthesis and increased prevalence of nocturnal non-dipping BP pattern. It is also possible that kidney disease could arise from hypertension. The direction of the relationship between blood pressure and kidney function, has been the subject of debate. However, evidence from genetic studies suggests that the association between renal function and blood pressure is likely to be explained by decreased renal function giving rise to high blood pressure. In a large (n>200,000) genome wide association study (GWAS), loci that were associated with blood pressure elevation and cardiovascular disease showed no association with kidney disease or kidney function. If SCT compromises renal function and this in turn leads to elevated blood pressure, this would result in a bias toward a null result when using SCT as a proxy for testing the malaria-high blood pressure hypothesis. As can be seen in Figure S3, impaired kidney function (as measured by eGFR) is associated with both the exposure and the outcome, but is not on the causal pathway from malaria to the outcome. Kidney function is therefore considered a confounder and we adjusted for eGFR in all regression analyses. We also examined the effect of using urine albumin to creatinine ratio in place of eGFR in the regression models.
If, however, renal function lies on the causal pathway between malaria and high blood pressure it would be inappropriate to include it within the regression models. Severe malaria does occasionally present with acute renal failure and repeated episodes of malaria could result in chronic pyelonephritis and elevated BP. However, acute renal failure is a very rare complication of malaria in Kilifi, for example, it occurred in 2 out of 1844 children admitted with malaria.\textsuperscript{40} This suggests that renal failure is an unlikely mediator of the potential association between malaria and elevated BP.

We confirmed that each of the \textit{a priori} specified covariates (age, sex and eGFR) significantly improved the regression models using the likelihood ratio test.

Confounding due to pleiotropy

A special type of confounding can also occur if the genetic trait influences the outcome through a pathway that is independent of the exposure (pleiotropy)\textsuperscript{41} as illustrated in Figure S4.

In contrast with renal function, which is a known confounder and can be measured and adjusted for in regression analyses, confounding due to other (often unknown) causes can only be detected by examining the relationship between sickle cell trait and blood pressure in individuals who have not been exposed to malaria. The existence of pleiotropy can invalidate the use of the genetic trait as a marker for the infectious disease exposure. In order to exclude pleiotropy as a potential explanation for the association between SCT and BP, we studied lifelong residents of Nairobi, Kenya and Jackson, Mississippi, two sites where there is no malaria transmission. In addition, we conducted a pooled analysis incorporating data from the three study sites and conducted a linear regression with the previously specified covariates plus SCT and study site and their interaction. This increased the power to detect any
independent effect of SCT on BP while simultaneously checking for differential effect of SCT according to study site.

ii) Effect modifier: $\alpha^+\text{thalassemia}$

$\alpha^+\text{thalassemia}$, in which there is reduction in the amount of alpha hemoglobin, is common in regions where malaria transmission occurs. Williams et al.\textsuperscript{42} have demonstrated a negative epistatic effect when $\alpha^+\text{thalassemia}$ is coinherited with SCT. The effect of coinheritance of the mutations is to reduce the malaria protective effect of SCT to about 27% (from 50%) for uncomplicated malaria and to 44% (from 80%) for severe malaria.\textsuperscript{42} Put simply, the presence of $\alpha^+\text{thalassemia}$ reduces the protective effect of SCT against both uncomplicated and severe malaria by about half. We therefore included $\alpha^+\text{thalassemia}$ as an interaction term (interacting with SCT) in the main regression model and examined whether its inclusion changed the effect estimate for SCT in predicting blood pressure.

In a related analysis, we ran a linear regression model examining the effect of $\alpha^+\text{thalassemia}$ on blood pressure with the same covariates used in the main analysis for SCT. Because $\alpha^+\text{thalassemia}$ confers less protection against malaria than SCT, we expected that the effect estimate in this model would be lower than that of SCT.

f) Testing for cohort effects

Malaria incidence in Kilifi has been changing over time and we considered that this could influence results obtained. Data on the changing levels of transmission go back to 1990 and they show that a significant drop in transmission in Kilifi began around 1999-2000.\textsuperscript{43} In addition, because blood pressure rises with age, it is possible that the effects of malaria on outcome measures may be more apparent later in life. While it is not possible to determine the individual contributions of
changing malaria exposure and aging to any differences observed in outcome measures, we attempted to display these differences by performing comparisons of the outcomes by sickle trait in 3 age strata.
Table S1. Characteristics of those who consented to undergo ABPM versus those who declined.

| Characteristic | Kilifi N=2537 | Nairobi N=1119 | Jackson N=5306 |
|----------------|----------------|----------------|----------------|
|                | Consented n=2371 | Did not consent n=166 | Consented n=1026 | Did not consent n=93 | Consented n=1148 | Did not consent n=4158 |
| Female         | n (%)          | n (%)          | n (%)          | n (%)          | n (%)          | n (%)          |
|                | 1361 (54)      | 84 (51)        | 480 (47)       | 45 (48)        | 780 (68)       | 2591 (62)      |
| mean (SD)      | 39 (22)        | 48 (22)        | 22 (16)        | 25 (17)        | 59 (11)        | 54 (13)        |
| Mean Age, years | <0.001         |                | 0.073          |                | <0.001         |                |
Table S2. Characteristics of participants with and without good quality ABPM data.

| Characteristic               | Kilifi N=2371 | Nairobi N=1026 | Jackson N=1148 |
|------------------------------|---------------|----------------|----------------|
|                              | Met ESH criteria n=1140 | Did not meet ESH criteria n=1231 | p-value | Met ESH criteria n=542 | Did not meet ESH criteria n=484 | p-value | Met ESH criteria n=934 | Did not meet ESH criteria n=214 | p-value |
| Female                       | n (%)         | n (%)          | p-value       | n (%)         | n (%)          | p-value       | n (%)         | n (%)          | p-value       |
| Have sickle cell trait       | 660 (58)      | 520 (48)       | <0.001        | 290 (54)      | 237 (49)       | 0.300        | 103 (11)      | 40 (19)        | 0.001         |
| Smoker                       | 240 (21)      | 205 (19)       | 0.240         | 83 (15)       | 50 (14)        | 0.637        | 58 (8.9)      | 10 (7.5)       | 0.588         |
| Prev diagnosed hypertensive  | 96 (8)        | 87 (8)         | 0.761         | 11 (2)        | 12 (3)         | 0.198        | 610 (67)      | 150 (73)       | 0.134         |
| On anti-hypertensive medication | 165 (14)    | 133 (12)       | 0.140         | 62 (11)       | 23 (7)         | 0.017        | 122 (19)      | 59 (779)       | 0.779         |
| Age, years                   | 40 (22)       | 36 (21)        | <0.001        | 23 (17)       | 19 (15)        | 0.010        | 59 (11)       | 58 (12)        | 0.360         |
| BMI kg/m²                    | 20.6 (3.8)    | 20.6 (4.0)     | 0.689         | 20.4 (4.1)    | 19.7 (4.0)     | 0.010        | 30.9 (6.5)    | 33.1 (5.8)     | <0.001        |
| HbA1c, %                     | 5.1 (0.7)     | 5.1 (0.6)      | 0.134         | 5.3 (0.98)    | 5.4 (1.2)      | 0.043        | 6.0 (1.3)     | 6.1 (1.4)      | 0.776         |
| Hemoglobin, g/dl             | 12.6 (1.7)    | 12.9 (1.6)     | <0.001        | 13.3 (1.7)    | 13.4 (1.7)     | 0.421        | 13.0 (1.4)    | 13.0 (1.5)     | 0.661         |
| WBC count X10⁹/L             | 5.7 (1.4)     | 5.7 (1.6)      | 0.718         | 5.4 (1.6)     | 5.5 (1.4)      | 0.187        | 5.3 (1.6)     | 5.8 (1.9)      | 0.002         |
| Platelet count X10⁹/L        | 264 (86)      | 257 (81)       | 0.064         | 289 (100)     | 302 (88)       | 0.046        | 243 (62)      | 250 (69)       | 0.078         |
| eGFR, ml/min/1.73m²          | 113 (41)      | 119 (40)       | <0.001        | 116 (25)      | 119 (24)       | 0.090        | 86.6 (25)     | 87.8 (25)      | 0.540         |
| Log UACR, mg/g               | 1.2 (0.62)    | 1.2 (0.61)     | 0.674         | 1.3 (0.71)    | 1.40 (0.60)    | 0.345        | 0.90 (0.50)   | 0.90 (0.56)    | 0.860         |

ABPM: Ambulatory blood pressure monitoring; eGFR: Estimated glomerular filtration rate; ESH: European society of hypertension. UACR: urine albumin to creatinine ratio
ESH criteria require a minimum of 20 valid readings taken between 9 a.m. and 9 p.m. and a minimum of 7 valid readings taken between 1 a.m. and 6 a.m. in order for ABPM readings to be considered as complete.

§Answered "yes" to the question: Has a doctor or healthcare worker previously told you that you have high blood pressure?
Table S3. Effect of sickle cell trait on blood pressure in Nairobi and Jackson.

| ABPM measure   | Nairobi (N=516) | Jackson (N=651) | p-value for interaction |
|----------------|-----------------|-----------------|-------------------------|
|                | β (mm Hg)       | (95% CI)        | p value                 | β (mm Hg)       | (95% CI)        | p value     | β (mm Hg)       | (95% CI)        | p value     |
| 24 hour SBP    | 0.6             | (–2.5, 3.7)     | 0.722                   | 0.6             | (–3.0, 4.2)     | 0.732       | 0.944         |
| 24 hour DBP    | 0.5             | (–1.6, 2.6)     | 0.652                   | 0.8             | (–1.5, 3.1)     | 0.489       | 0.695         |
| Nighttime SBP  | 0.7             | (–2.5, 4.0)     | 0.669                   | –0.2            | (–4.3, 4.0)     | 0.938       | 0.766         |
| Nighttime DBP  | 0.6             | (–1.7, 2.9)     | 0.610                   | 0.8             | (–1.8, 3.3)     | 0.558       | 0.779         |
| Daytime SBP    | –0.1            | (–3.4, 3.2)     | 0.963                   | 1.3             | (–2.3, 4.9)     | 0.486       | 0.567         |
| Daytime DBP    | 0.1             | (–2.3, 2.4)     | 0.951                   | 1.2             | (–1.2, 3.6)     | 0.310       | 0.409         |

Linear regression models adjusted for age, sex and estimated glomerular filtration rate
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
p-value for interaction represents result of regression models testing for difference in effect of sickle cell trait on BP in Nairobi versus Jackson
### Table S4. Effects of sickle cell trait on blood pressure: effect of excluding participants taking anti-hypertensive medication.

#### All participants

| ABPM measure | Kilifi (N=1127) | Nairobi and Jackson (N=1166) |
|--------------|-----------------|-----------------------------|
|              | β (mm Hg)       | (95% CI) | p value | β (mm Hg) | (95% CI) | p value |
| 24 hour SBP  | -2.4            | (-4.7, -0.2) | 0.037 | 0.7       | (-1.6, 3.1) | 0.542 |
| 24 hour DBP  | -1.4            | (-2.8, 0.1)  | 0.068 | 0.1       | (-1.5, 1.8) | 0.860 |
| Nighttime SBP| -3.2            | (-5.7, -0.6) | 0.015 | 0.5       | (-2.2, 3.1) | 0.727 |
| Nighttime DBP| -3.2            | (-3.3, -0.2) | 0.026 | 0.3       | (-1.5, 2.0) | 0.773 |
| Daytime SBP  | -1.9            | (-4.2, 0.4)  | 0.113 | 0.7       | (-1.7, 3.1) | 0.566 |
| Daytime DBP  | -1.0            | (-2.6, 0.6)  | 0.223 | 0.1       | (-1.6, 1.8) | 0.889 |

#### Excluding participants on medication

| ABPM measure | Kilifi (N=1092) | Nairobi and Jackson (N=755) |
|--------------|-----------------|-----------------------------|
|              | β (mm Hg)       | (95% CI) | p value | β (mm Hg) | (95% CI) | p value |
| 24 hour SBP  | -2.5            | (-4.8, -0.2) | 0.034 | 0.2       | (-2.5, 2.8) | 0.896 |
| 24 hour DBP  | -1.4            | (-2.8, 0.1)  | 0.067 | -0.2      | (-2.0, 1.5) | 0.787 |
| Nighttime SBP| -3.1            | (-5.7, -0.6) | 0.017 | 0.8       | (-2.1, 3.6) | 0.597 |
| Nighttime DBP| -1.8            | (-3.3, -0.2) | 0.028 | 0.1       | (-1.8, 2.1) | 0.902 |
| Daytime SBP  | -2.0            | (-4.3, 0.4)  | 0.096 | -0.4      | (-3.1, 2.4) | 0.802 |
| Daytime DBP  | -1.0            | (-2.6, 0.6)  | 0.205 | -0.5      | (-2.4, 1.5) | 0.630 |

Linear regression models adjusted for age, sex and estimated glomerular filtration rate
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
Table S5. Effect of Sickle cell trait on blood pressure by sex and study site.

Kilifi

| ABPM measure | Women (N=659) | Men (N=473) | Interaction p-value |
|--------------|---------------|-------------|-------------------|
|              | β (95% CI) p value | β (95% CI) p value |                       |
| 24 hour SBP  | −3.7 (−7.1, −0.4) 0.028 | −0.7 (−3.7, 2.3) 0.645 | 0.218               |
| 24 hour DBP  | −1.9 (−2.8, 0.1) 0.079 | −0.7 (−2.7, 1.3) 0.493 | 0.398               |
| Nighttime SBP| −4.3 (−8.0, −0.6) 0.022 | −1.6 (−5.1, 1.8) 0.356 | 0.335               |
| Nighttime DBP| −2.0 (−4.2, 0.1) 0.067 | −1.4 (−3.6, 0.8) 0.217 | 0.661               |
| Daytime SBP  | −3.1 (−6.4, 0.2) 0.068 | −0.3 (−3.4, 2.9) 0.875 | 0.284               |
| Daytime DBP  | −1.6 (−3.9, 0.6) 0.154 | −0.2 (−2.4, 2) 0.867  | 0.131               |

Nairobi and Jackson

| ABPM measure | Women (N=705) | Men (N=461) | Interaction p-value |
|--------------|---------------|-------------|-------------------|
|              | β (95% CI) p value | β (95% CI) p value |                       |
| 24 hour SBP  | 0.9 (−2.2, 4) 0.574 | 0.7 (−2.9, 4.4) 0.700 | 0.800               |
| 24 hour DBP  | 1.2 (−0.9, 3.3) 0.272 | −0.4 (−2.9, 2.1) 0.737 | 0.106               |
| Nighttime SBP| 1.2 (−2.3, 4.7) 0.511 | −0.2 (−4.2, 3.8) 0.915 | 0.512               |
| Nighttime DBP| 1.7 (−0.6, 3.9) 0.139 | −0.8 (−3.5, 2.0) 0.586 | 0.046               |
| Daytime SBP  | 0.7 (−2.5, 3.9) 0.679 | 1.0 (−2.8, 4.8) 0.617  | 0.998               |
| Daytime DBP  | 0.8 (−1.4, 3.1) 0.464 | −0.1 (−2.7, 2.5) 0.936  | 0.319               |

Linear regression models adjusted for age, sex and estimated glomerular filtration rate
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
p-value for interaction represents result of regression models testing for difference in effect of sickle cell trait on BP in men versus women
Table S6. Results of interaction analysis comparing effect of sickle cell trait in Kilifi versus Nairobi/Jackson using log urine albumin to creatinine ratio as covariate instead of estimated Glomerular Filtration Rate.

|                  | All (N=1583) | Women N=958 | Men N=625 |
|------------------|--------------|-------------|-----------|
|                  | β (95% CI)   | p value     | β (95% CI) | p value     | β (95% CI) | p value |
| 24 hour SBP      | –5.2 (–9.5, –0.9) | 0.019       | –6.7 (–13, –0.6) | 0.030       | –3.5 (–9.5, 2.5) | 0.249   |
| 24 hour DBP      | –2.9 (–5.7, –0.1) | 0.040       | –4.6 (–8.5, –0.8) | 0.019       | –1.0 (–5.0, 3.1) | 0.635   |
| Nighttime SBP    | –5.5 (–10, –0.7) | 0.026       | –6.3 (–13, 0.5) | 0.067       | –4.7 (–12, 2.1) | 0.176   |
| Nighttime DBP    | –3.6 (–6.6, –0.6) | 0.018       | –5.1 (–9.1, –1.1) | 0.013       | –1.7 (–6.2, 2.8) | 0.456   |
| Daytime SBP      | –4.8 (–9.2, –0.4) | 0.032       | –6.5 (–13, –0.4) | 0.037       | –3.2 (–9.5, 3.0) | 0.308   |
| Daytime DBP      | –2.5 (–5.5, 0.5) | 0.106       | –4.1 (–8.3, 0.03) | 0.052       | –0.9 (–5.3, 3.4) | 0.673   |

SCT: sickle cell trait
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
Linear regression models tested for interaction in the effect of SCT by site. Other covariates were age, sex and log urine albumin to creatinine ratio.
Table S7. Results of pooled analysis comparing effect of sickle cell trait in Kilifi versus Nairobi/Jackson stratified by sex.

| ABPM measure | Women (N=1359) |  | Men (N=934) | Interaction p-value |
|---------------|----------------|---|-------------|-------------------|
| 24 hour SBP   | β (95% CI) p value | β (95% CI) p value | p-value |
| −5.0 (−9.7, −0.2) 0.039 | −1.9 (−6.6, 2.8) 0.431 | 0.419 |
| 24 hour DBP   | −3.2 (−6.2, −0.1) 0.041 | −0.3 (−3.5, 2.9) 0.871 | 0.110 |
| Nighttime SBP | −5.8 (−11, −0.6) 0.030 | −1.8 (−7.1, 3.5) 0.506 | 0.323 |
| Nighttime DBP | −3.9 (−7.1, −0.7) 0.017 | −0.5 (−4.0, 3.0) 0.782 | 0.086 |
| Daytime SBP   | −4.1 (−8.9, 0.7) 0.091 | −1.8 (−6.7, 3.2) 0.489 | 0.549 |
| Daytime DBP   | −2.5 (−5.8, 0.7) 0.126 | −0.2 (−3.6, 3.3) 0.925 | 0.207 |

Estimates were derived separately for each sex as the interaction term (malaria vs non-malaria sites) in a linear regression of blood pressure by sickle cell trait status after adjusting for age and estimated glomerular filtration rate. Interaction p-value is the result of 3-way interaction in regression models testing for difference in effect of sickle cell trait on BP in men versus women in Kilifi versus Nairobi and Jackson pooled together. DBP: diastolic blood pressure; SBP: systolic blood pressure.
Table S8. Effect of α+ thalassemia on ambulatory blood pressure by study site

**Kilifi (N=1125).**

| ABPM measure | No. of –3.7kb α+ thalassemia deletions |  |  |
|---------------|----------------------------------------|---|---|
|               | One deletion (Heterozygous)            | Two deletions (Homozygous) |   |
|               | β mm Hg (95% CI) | p value | β mm Hg (95% CI) | p value |
| 24 hour SBP   | –0.1 (–2.2, 2.0) | 0.921 | –1.1 (–3.8, 1.6) | 0.434 |
| 24 hour DBP   | –0.3 (–1.7, 1.0) | 0.651 | –0.4 (–2.2, 1.3) | 0.646 |
| Nighttime SBP | 0.2 (–2.2, 2.6) | 0.879 | –1.3 (–4.3, 1.8) | 0.416 |
| Nighttime DBP | –0.1 (–2.3, 1.4) | 0.919 | –0.5 (–2.4, 1.4) | 0.597 |
| Daytime SBP   | –0.2 (–2.3, 2.0) | 0.884 | –1.0 (–3.7, 1.8) | 0.500 |
| Daytime DBP   | –0.1 (–1.5, 1.4) | 0.930 | –0.3 (–2.2, 1.6) | 0.765 |

**Nairobi (N=514)**

| ABPM measure | No. of –3.7kb α+ thalassemia deletions |  |  |
|---------------|----------------------------------------|---|---|
|               | One deletion (Heterozygous)            | Two deletions (Homozygous) |   |
|               | β mm Hg (95% CI) | p value | β mm Hg (95% CI) | p value |
| 24 hour SBP   | 1.1 (–1.3, 3.5) | 0.373 | 3.9 (–0.5, 8.2) | 0.083 |
| 24 hour DBP   | 0.6 (–1.0, 2.3) | 0.440 | 2.6 (–0.4, 5.6) | 0.086 |
| Nighttime SBP | 1.9 (–0.6, 4.4) | 0.144 | 3.3 (–1.3, 7.9) | 0.157 |
| Nighttime DBP | 1.8 (0.05, 3.6) | 0.044 | 2.4 (–0.8, 5.6) | 0.138 |
| Daytime SBP   | 0.04 (–1.8, 2.6) | 0.974 | 2.9 (–1.8, 7.5) | 0.223 |
| Daytime DBP   | –0.3 (–2.1, 1.5) | 0.748 | 2.2 (–1.0, 5.5) | 0.181 |

SBP: Systolic blood pressure
DBP: Diastolic blood pressure
Linear regression models adjusted for age, sex and estimated glomerular filtration rate.
*No alpha thalassemia data were available for participants from Jackson.*
Results of analyses based on data meeting IDACO criteria for completeness

Table S9. Characteristics of study participants with and without sickle cell trait by study site (IDACO Criteria).

| Characteristic                      | Kilifi N=2048 |                  | Nairobi N=835 |                  | Jackson N=724 |                  |
|-------------------------------------|---------------|-----------------|---------------|-----------------|---------------|-----------------|
|                                    | SCT n=408     | Non-SCT n=1640  | SCT n=121     | Non-SCT n=714   | SCT n=63      | Non-SCT n=661   |
| Female                             | n=203 (50)    | n=884 (54)      | n=59 (49)     | n=369 (52)      | n=41 (65)     | n=439 (66)      |
| Smoker                             | n=34 (8)      | n=134 (8)       | n=5 (4)       | n=17 (2)        | n=9 (14)      | n=83 (13)       |
| Previously diagnosed hypertensive§  | n=59 (15)     | n=221 (14)      | n=9 (7)       | n=70 (10)       | n=33 (52)     | n=408 (62)      |
| Taking antihypertensive medication | n=11 (3)      | n=38 (2)        | n=2 (2)       | n=10 (1)        | n=27 (44)     | n=377 (61)      |
| Age, years                         | mean (SD)     | mean (SD)       | mean (SD)     | mean (SD)       | mean (SD)     | mean (SD)       |
| BMI kg/m²                           | 20.7 (3.8)    | 20.7 (3.9)      | 20.2 (4.0)    | 20.2 (4.1)      | 31.0 (6.9)    | 31.1 (6.4)      |
| HbA1c, %                           | 5.2 (0.6)     | 5.1 (0.7)       | 5.4 (1.3)     | 5.3 (1.0)       | 6.0 (1.2)     | 6.1 (1.3)       |
| Hemoglobin, g/dl                   | 12.7 (1.9)    | 12.7 (1.6)      | 13.4 (1.8)    | 13.3 (1.7)      | 12.8 (1.4)    | 13.0 (1.4)      |
| WBC count X10⁹/L                   | 5.8 (1.5)     | 6.5 (22.5)      | 5.4 (1.3)     | 5.4 (1.5)       | 4.9 (1.2)     | 5.3 (1.5)       |
| Platelet count X10⁹/L              | 261 (89)      | 259 (81)        | 289 (96)      | 294 (97)        | 229 (57)      | 243 (62)        |
| Plasma osmolality, mosm/Kg         | 290 (5.8)     | 290 (6.3)       | 291 (12)      | 291 (12)        | -             | -               |
| eGFR, ml/min/1.73m²                | 111 (39)      | 116 (40)        | 117 (27)      | 116 (24)        | 84 (27)       | 87 (26)         |
| Log UACR, mg/g                     | 1.3 (0.6)     | 1.2 (0.6)       | 1.5 (0.7)     | 1.3 (0.7)       | 1.1 (0.6)     | 0.9 (0.5)       |

BMI: Body mass index; eGFR: estimated glomerular filtration rate; HbA1c: glycosylated hemoglobin; SCT: Sickle cell trait; SD: standard deviation; UACR: urine albumin to creatinine ratio; WBC: white blood cell. Plasma osmolality measurements were not available for Jackson participants.
§Answered “yes” to the question: Has a doctor or healthcare worker previously told you that you have high blood pressure?
Table S10. Effect of sickle cell trait on blood pressure by malaria site (IDACO Criteria).

| ABPM measure  | Kilifi (N=1965) | Nairobi and Jackson (N=1500) | Nairobi/Jacks on Interaction p-value for interaction |
|---------------|----------------|-----------------------------|-----------------------------------------------------|
|               | β (mmHg) (95% CI) p value | β (mmHg) (95% CI) p value |                                                     |
| 24 hour SBP   | –1.4 (–3.1, 0.4) 0.128 | 0.4 (–1.6, 2.5) 0.697 | 0.557                                               |
| 24 hour DBP   | –0.7 (–1.8, 0.4) 0.226 | 0.1 (–1.3, 1.5) 0.910 | 0.394                                               |
| Nighttime SBP | –1.6 (–3.5, 0.4) 0.109 | –0.4 (–2.6, 1.9) 0.744 | 0.622                                               |
| Nighttime DBP | –0.9 (–2.1, 0.3) 0.146 | –0.3 (–1.8, 1.2) 0.685 | 0.273                                               |
| Daytime SBP   | –0.9 (–2.7, 0.9) 0.327 | 0.6 (–1.6, 2.7) 0.602 | 0.264                                               |
| Daytime DBP   | –0.6 (–1.8, 0.7) 0.367 | 0.1 (–1.4, 1.6) 0.858 | 0.237                                               |

Linear regression models adjusted for age, sex and estimated glomerular filtration rate
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
p-value for interaction represents result of regression models testing for difference in effect of sickle cell trait on BP in Nairobi versus Jackson
Table S11. Age specific effects of sickle cell trait on blood pressure by study site (IDACO Criteria).

### Kilifi

| Age, years | N  | SBP (95% CI)  | DBP (95% CI)  |
|------------|----|---------------|---------------|
| 10-29      | 917| -0.7 (-2.5, 1.1) | 0.2 (-1.0, 1.4) |
| 30-59      | 655| -3.0 (-6.3, 0.3) | -2.3 (-4.5, -0.1) |
| ≥60        | 393| -0.2 (-5.3, 4.9) | -0.4 (-3.1, 3.0) |

### Nairobi and Jackson pooled together

| Age, years | N  | SBP (95% CI)  | DBP (95% CI)  |
|------------|----|---------------|---------------|
| 10-29      | 620| -0.5 (-3.0, 1.9) | 0 (-1.5, 1.5) |
| 30-59      | 451| -0.4 (-4.6, 3.9) | -1.2 (-4.0, 1.6) |
| ≥60        | 429| 3.7 (-1.2, 8.6)  | 4.0 (0.9, 7.0) |

SBP: Systolic blood pressure  
DBP: Diastolic blood pressure  
Results of linear regression models adjusted for age, sex and estimated glomerular filtration rate  
*Participants in Jackson were aged 21 years and older
Table S12. Effect of Sickle cell trait on blood pressure by sex and study site (IDACO Criteria).

### Kilifi

| ABPM measure       | Women (N=1046) | Men (N=919) | Interaction p-value |
|--------------------|----------------|-------------|---------------------|
|                    | β   (95% CI)   | p value     | β   (95% CI)        | p value | p-value |
| 24 hour SBP        | -1.9 (-4.5, 0.6) | 0.141       | -0.8 (-3.2, 1.7) | 0.537 | 0.533 |
| 24 hour DBP        | -0.7 (-2.3, 0.9) | 0.396       | -0.7 (-2.3, 0.8) | 0.362 | 0.910 |
| Nighttime SBP      | -2.0 (-4.9, 0.8) | 0.160       | -1.1 (-3.7, 1.6) | 0.423 | 0.653 |
| Nighttime DBP      | -0.7 (-2.4, 1.0) | 0.417       | -1.1 (-2.8, 0.6) | 0.200 | 0.825 |
| Daytime SBP        | -1.4 (-3.9, 1.2) | 0.292       | -0.4 (-3.0, 2.2) | 0.759 | 0.612 |
| Daytime DBP        | -0.8 (-2.5, 0.9) | 0.353       | -0.4 (-2.1, 1.4) | 0.679 | 0.620 |

### Nairobi and Jackson

| ABPM measure       | Women (N=879) | Men (N=621) | Interaction p-value |
|--------------------|----------------|-------------|---------------------|
|                    | β   (95% CI)   | p value     | β   (95% CI)        | p value | p-value |
| 24 hour SBP        | 0.1 (-2.7, 2.8) | 0.962       | 1.0 (-2.1, 4.0) | 0.525 | 0.272 |
| 24 hour DBP        | 0.7 (-1.1, 2.6) | 0.429       | -0.3 (-2.3, 1.8) | 0.807 | 0.371 |
| Nighttime SBP      | -0.7 (-3.7, 2.4) | 0.674       | 0.1 (-3.1, 3.4) | 0.930 | 0.294 |
| Nighttime DBP      | 0.5 (-1.4, 2.5) | 0.604       | -0.8 (-3.1, 1.4) | 0.476 | 0.393 |
| Daytime SBP        | -0.3 (-3.1, 2.6) | 0.856       | 1.8 (-1.4, 5.0) | 0.275 | 0.096 |
| Daytime DBP        | 0.2 (-1.8, 2.1) | 0.881       | 0.6 (-1.7, 2.8) | 0.612 | 0.110 |

Linear regression models adjusted for age, sex and estimated glomerular filtration rate
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
p-value for interaction represents result of regression models testing for difference in effect of sickle cell trait on BP in men versus women
Table S13. Effects of sickle cell trait on blood pressure: effect of excluding participants taking anti-hypertensive medication (IDACO Criteria).

| ABPM measure | Kilifi (N=1918) | Nairobi and Jackson (N=1041) |
|--------------|-----------------|-----------------------------|
|              | β (mm Hg)       | (95% CI)        | p value | β (mm Hg)       | (95% CI)        | p value |
| 24 hour SBP  | -1.3            | (-3.1, 0.5)     | 0.157   | -0.3            | (-2.5, 1.9)     | 0.790   |
| 24 hour DBP  | -0.7            | (-1.8, 0.5)     | 0.254   | -0.4            | (-1.9, 1.1)     | 0.613   |
| Nighttime SBP| -1.5            | (-3.4, 0.5)     | 0.136   | -0.6            | (-2.9, 1.8)     | 0.639   |
| Nighttime DBP| -0.9            | (-2.0, 0.3)     | 0.162   | -0.7            | (-2.3, 0.8)     | 0.356   |
| Daytime SBP  | -0.9            | (-2.7, 1.0)     | 0.348   | -0.4            | (-2.8, 2.0)     | 0.729   |
| Daytime DBP  | -0.5            | (-1.8, 0.7)     | 0.382   | -0.4            | (-2.0, 1.3)     | 0.678   |
Table S14. Results of interaction analysis comparing effect of sickle cell trait in Kilifi versus Nairobi/Jackson (IDACO Criteria).

|                      | Model 1 (N=3465) |                      | Model 2 (N=2379) |                      |
|----------------------|-------------------|----------------------|-------------------|----------------------|
|                      | β (95% CI)        | p value              | β (95% CI)        | p value              |
| 24 hour SBP          | -2.1 (-5.0, 0.7)  | 0.146                | -3.3 (-7.0, 0.4)  | 0.078                |
| 24 hour DBP          | -1.0 (-2.9, 0.8)  | 0.275                | -2.3 (-4.7, 0.1)  | 0.057                |
| Nighttime SBP        | -1.5 (-4.6, 1.6)  | 0.339                | -3.1 (-7.2, 0.9)  | 0.133                |
| Nighttime DBP        | -0.8 (-2.8, 1.1)  | 0.337                | -2.7 (-5.3, -0.2) | 0.032                |
| Daytime SBP          | -1.8 (-4.8, 1.1)  | 0.225                | -3.1 (-6.8, 0.7)  | 0.112                |
| Daytime DBP          | -0.9 (-2.9, 1.1)  | 0.394                | -2.0 (-4.6, 0.6)  | 0.126                |

Estimates were derived as the interaction term (malaria vs non-malaria sites) in a linear regression of blood pressure by sickle cell trait status after adjusting for age, sex and renal function. Renal function was represented by estimated glomerular filtration rate in model 1 and by log urine albumin to creatinine ratio in model 2. SBP: Systolic blood pressure. DBP: Diastolic blood pressure.
Table S15. Effect of α⁺thalassemia on ambulatory blood pressure by study site (IDACO Criteria).

**Kilifi (N=1961)**

| ABPM measure | No. of −3.7kb α⁺ thalasemia deletions | | | |
|--------------|--------------------------------------|---|---|---|
|              | One deletion (Heterozygous) | Two deletions (Homozygous) | | | |
|              | β mm Hg (95% CI) p value | β mm Hg (95% CI) p value | | | |
| 24 hour SBP  | −0.4 (−1.9, 1.2) 0.637 | −1.9 (−3.9, 0.2) 0.073 | | | |
| 24 hour DBP  | −0.4 (−1.4, 0.7) 0.480 | −0.7 (−2.0, 0.6) 0.286 | | | |
| Nighttime SBP| −0.1 (−1.9, 1.6) 0.879 | −1.5 (−3.7, 0.8) 0.203 | | | |
| Nighttime DBP| −0.3 (−1.4, 0.8) 0.605 | −0.6 (−2.0, 0.8) 0.397 | | | |
| Daytime SBP  | −0.5 (−2.1, 1.2) 0.581 | −2.1 (−4.1, 0.1) 0.059 | | | |
| Daytime DBP  | −0.1 (−1.2, 1.0) 0.808 | −0.7 (−2.1, 0.8) 0.361 | | | |

**Nairobi (N=771)**

| ABPM measure | No. of −3.7kb α⁺ thalasemia deletions | | | |
|--------------|--------------------------------------|---|---|---|
|              | One deletion (Heterozygous) | Two deletions (Homozygous) | | | |
|              | β mm Hg (95% CI) p value | β mm Hg (95% CI) p value | | | |
| 24 hour SBP  | 0.7 (−1.3, 2.6) 0.494 | 1.6 (−2.0, 5.2) 0.390 | | | |
| 24 hour DBP  | 0.4 (−0.6, 1.6) 0.572 | 1.4 (−0.9, 3.7) 0.238 | | | |
| Nighttime SBP| 0.9 (−1.1, 3.0) 0.376 | 1.2 (−2.6, 4.9) 0.376 | | | |
| Nighttime DBP| 1.0 (−0.4, 2.3) 0.164 | 1.0 (−1.4, 3.5) 0.420 | | | |
| Daytime SBP  | 0.1 (−3.7, 4.1) 0.941 | 0.2 (−3.7, 4.1) 0.923 | | | |
| Daytime DBP  | −0.2 (−1.6, 1.3) 0.837 | 0.5 (−2.2, 3.1) 0.717 | | | |

SBP: Systolic blood pressure  
DBP: Diastolic blood pressure  
Linear regression models adjusted for age, sex and estimated glomerular filtration rate.  
*No α-thalassemia data were available for participants from Jackson.
Figure S1. Study locations.
Figure S2. Causal diagram for the malaria high blood pressure hypothesis.

Childhood Malaria (represented by SCT/alpha thalasemia) → Inflammation, stunting, malnutrition → High blood pressure
Figure S3. Illustrating confounding effect of kidney function (eGFR) in individuals with SCT.
Figure S4. Illustrating confounding due to pleiotropy.
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