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Neither the African-Centric S47 Nor P72 Variant of *TP53* Is Associated With Reduced Risk of Febrile Malaria in a Malian Cohort Study

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Background. *TP53* has been shown to play a role in inflammatory processes, including malaria. We previously found that p53 attenuates parasite-induced inflammation and predicts clinical protection to *Plasmodium falciparum* infection in Malian children. Here, we investigated whether p53 codon 47 and 72 polymorphisms are associated with differential risk of *P. falciparum* infection and uncomplicated malaria in a prospective cohort study of malaria immunity.

Methods. p53 codon 47 and 72 polymorphisms were determined by sequencing *TP53* exon 4 in 631 Malian children and adults enrolled in the Kalifabougou cohort study. The effects of these polymorphisms on the prospective risk of febrile malaria, incident parasitemia, and time to fever after incident parasitemia over 6 months of intense malaria transmission were assessed using Cox proportional hazards models.

Results. Confounders of malaria risk, including age and hemoglobin S or C, were similar between individuals with or without p53 S47 and R72 polymorphisms. Relative to their respective common variants, neither S47 nor R72 was associated with differences in prospective risk of febrile malaria, incident parasitemia, or febrile malaria after parasitemia.

Conclusions. These findings indicate that p53 codon 47 and 72 polymorphisms are not associated with protection against incident *P. falciparum* parasitemia or uncomplicated febrile malaria.

Keywords. malaria; P47S; p53 polymorphisms; P72R; prospective cohort study.

The most clinically significant agent of malaria in Africa, *Plasmodium falciparum*, has infected humans for nearly 10 000 years [1]. Over this time, the high mortality rate of severe falciparum malaria has imposed strong selective pressure on the human genome [2]. As such, specific erythrocyte polymorphisms that may otherwise be detrimental to the host (eg, sickle hemoglobin [HbS] mutation responsible for sickle cell disease) have been maintained in malaria-endemic populations as that these mutations confer malaria resistance by rendering the erythrocyte less hospitable for the invading parasite [3–5]. Genome-wide association studies have identified additional polymorphisms not obviously linked to erythrocyte function as protective against severe malaria, but as much as 89% of susceptibility to severe malaria has yet to be attributed to specific genomic loci [6–9]. Moreover, evidence for nonerythrocyte host polymorphisms that may be protective against uncomplicated (nonsevere) malaria has been lacking.

Previous studies have implicated the tumor suppressor p53 in the control of malaria infection. Increased host p53 has been shown to reduce *Plasmodium* liver-stage burden in mice [10]. Our group previously showed increased expression of *TP53* and p53 target genes in the blood of uninfected Malian children who would later present with asymptomatic *P. falciparum* parasitemia relative to those who later presented with febrile malaria [11]. These observations have led to the question of whether *TP53* polymorphisms could account for these differences in clinical phenotype.

TP53 is the most frequently mutated gene in human cancers, with the majority of missense mutations occurring in hot spots within either the proline-rich domain (amino acids 55–100) or the DNA binding domain (amino acids 102–192) [12, 13]. The most common naturally occurring mutation occurs at codon 72 (rs1042522), arising from a single-nucleotide polymorphism (SNP) in exon 4 [14, 15]. A proline residue at this position (P72), considered the ancestral form, is preferentially found in individuals of African descent, whereas an arginine residue (R72) more commonly occurs in non-Hispanic white

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Americans and Europeans, with frequency increasing linearly with latitude [16, 17]. Although a substitution of a proline with arginine results in significant structural and functional changes [18], both residues are believed to function sufficiently to protect against the different cancers that individuals may encounter at their respective latitudes (reviewed in [13]).

Notably, the R72 variant has been shown to be associated with cold temperatures and low UV intensity [19], but studies evaluating the association of codon 72 polymorphisms with skin cancer have yielded inconsistent findings [20–23]. Despite the increased frequency of P72 among Africans relative to Europeans [17], it has been proposed by others that the R72 variant may be protective against malaria infection in humans [24], based on evidence that R72 has increased apoptotic potential relative to P72 [25] and that apoptosis is a mechanism by which the host eliminates parasitized hepatocytes in the liver [10].

The second most frequent p53 coding SNP also occurs in exon 4 and is localized within the region encoding the N-terminal transactivation domain at codon 47 (rs1800371) [26], where the more common proline (P47) can be replaced by serine (S47) [27]. In contrast to the very common polymorphism at codon 72, the S47 variant is relatively rare in humans, with an allele frequency of 2%–4% in African populations [26, 27]. As S47 has not yet been detected in Americans of non-African origin [28], this polymorphism has been referred as the African-centric variant in the literature. The S47 variant can confer resistance to iron-dependent programmed cell death (ferroptosis), leading to increased risk of spontaneous cancers [27, 29]. In S47 mice, this defective ferroptosis can result in iron accumulation and an anti-inflammatory response to the malarial pigment hemozoin, leading to speculation that the S47 variant may limit malaria-induced inflammation and thus improve survival in individuals living in areas with intense malaria transmission [30].

Studies on p53 polymorphisms and malaria susceptibility in humans have been limited to date. Two studies have examined the association between codon 72 variants and malaria risk in humans with conflicting results [24, 31]. However, to our knowledge, the association between p53 codon 47 polymorphisms and malaria risk has yet to be investigated in humans. In the current study, we examined whether p53 codon 47 and 72 polymorphisms affect the risk of *P. falciparum* infection and clinical malaria in a prospective cohort of Malian children and adults.

METHODS

Study Design and Participants

The study site and study population have been described elsewhere [32]. Briefly, the study was conducted in the village of Kalifabougou, Mali, where *P. falciparum* malaria transmission is intense and seasonal, occurring June through December [32]. In May 2011, we enrolled 695 healthy children and adults, aged 3 months to 25 years, into a longitudinal observational cohort study to investigate malaria immunity, in which biweekly active malaria surveillance was conducted with interval weekly home check-ups and passive surveillance by self-referral. Exclusion criteria at enrollment included hemoglobin level <7 g/dL, axillary temperature >37.5°C, acute systemic illness, underlying chronic disease, use of antimalarial or immunosuppressive medications in the past 30 days, and pregnancy.

Malaria episodes were defined as parasitemia of >2500 parasites per microliter, an axillary temperature of ≥37.5°C within 24 hours, and no other cause of fever discernible by physical examination. Episodes were detected prospectively by self-referral to the study clinic and weekly active clinical surveillance visits. All individuals with signs and symptoms of malaria and any level of parasitemia detected by microscopy were treated according to the Malian National Malaria Control Program guidelines. During the scheduled clinic visits, blood was collected by finger prick every 2 weeks for blood smears and dried blood spots (DBSs) on filter paper.

Asymptomatic *P. falciparum* infections were detected by microscopic examination of blood smears and polymerase chain reaction (PCR) analysis of blood spots at the end of the surveillance period. First *P. falciparum* infections were detected retrospectively with PCR of longitudinally collected DBSs, as described elsewhere [32]. First malaria episodes were determined from the clinical visit data. Hemoglobin values, measured by a HemoCue analyzer, were used to determine anemia status, based on World Health Organization criteria. Hemoglobin typing for HbS was performed with a D-10 Hemoglobin Testing System (Bio-Rad). Three clinical classes with different levels of clinical immunity were described elsewhere [11].

DNA Isolation and Sequencing

Genomic DNA was extracted from whole-blood samples using 3 methods: (1) from whole-blood pellets (100 µL) using a QIAamp 96 DNA Blood Kit (Qiagen), per the manufacturer's protocol; (2) from DBSs using the QIAamp DNA Mini Kit (Qiagen), per the manufacturer's protocol; or (3) from DBSs using a custom high-throughput extraction method, as described elsewhere [33]. Purified genomic DNA was used to amplify exon 4 of *TP53* by PCR using the following previously published primers [34]: 5′-TGAGGACCTGGTCCTCTGAC-3′ (forward) and reverse 5′-AGAGGAATCCCAAAGTTC CA-3′ (reverse). PCR amplification was performed using the HotStarTaq Plus Master Mix Kit (Qiagen) at an annealing temperature of 60°C, per the manufacturer's recommended protocol. Amplified PCR products were purified and sequenced by the chain-termination method at Quintara Biosciences. Sequence files received were analyzed using Benchling software

(2022.2.3 release; https://benchling.com) and aligned to the *TP53* reference sequence (gene identifier 7157; National Center for Biotechnology Information). Base calls reported by the software were manually checked against the chromatograms. Generated sequences will be available on GenBank (accession nos. OP593553–OP594185).

Statistical Analysis

Kaplan-Meier curves were used to estimate the respective probabilities of remaining free of (1) clinical malaria, (2) *P. falciparum* parasitemia, or (3) febrile malaria once parasitemic. For time to febrile malaria once parasitemic, the time of incident parasitemia, estimated as the midpoint between the last negative *P. falciparum* PCR result and the first positive *P. falciparum* PCR result, was used as the start time. Cox proportional hazards models were used to estimate the Wald statistic for testing the significance of differences in time to first event between *TP53* variants at codon 47 or 72 in either univariate analyses or analyses that included covariates as indicated in the tables. Kruskal-Wallis rank sum test was used to compare the differences between groups for continuous outcomes. For categorical variables, group comparisons were performed using χ^2 tests. Statistical significance was defined as a 2-tailed P value <.05. All analyses were performed using R software (version 4.2.0).

Ethical Approval

The Kalifabougou cohort study was approved by the Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry at the University of Sciences, Technique and Technology of Bamako, and the Institutional Review Board of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Written informed consent was obtained from adult participants and from the parents or guardians of participating children. This study was approved as exempt human subjects research by the Indiana University Institutional Review Board (protocol 2005922267).

RESULTS

Frequency of *TP53* Polymorphisms Among Study Participants

Of the 695 individuals initially enrolled in the Kalifabougou cohort, 631 had sufficient genomic DNA for *TP53* exon 4 sequencing. Sequence alignments revealed 40 individuals (6.3%) with S47 polymorphisms, of which 33 were heterozygous and 7 were homozygous (Table 1). R72 polymorphisms were observed in 327 individuals (51.8%), of which 206 (32.6%) were heterozygous and 121 (19.2%) were homozygous (Table 1). Neither codon 47 nor codon 72 polymorphisms were in Hardy-Weinberg equilibrium (Haldane exact test, $P = 6.5 \times 10^{-6}$ and $P = 1.0 \times 10^{-12}$, respectively). The distribution of potential modifiers of malaria risk (age, sex, HbS or hemoglobin, existing *P. falciparum* parasitemia, and anemia) did not differ significantly among the *TP53* variant groups for either S47 or R72 (Table 1). All 6 individuals who were heterozygous for both the S47 and R72 alleles were male and PCR positive for *P. falciparum* at enrollment (Table 1).

No Association of *TP53* Codon 47 and Codon 72 Polymorphisms With Reduced Risk of Clinical Malaria Episodes or Incident *P. falciparum* Parasitemia

Time-to-event analyses were performed to examine the association between S47 and P72 variants and the risk of febrile malaria. The initial analysis between P47/P47, P47/S47, and S47/S47 showed no significant difference in malaria risk (data not shown), presumably owing to the small sample sizes for the

Table 1. Potential Confounders of Malaria Risk and *TP53* Genotypes

	Patients, No. (%)[a]							
			Codon 72 Polymorphisms		Codon 47 Polymorphisms			
Variable	Overall (N = 631)	Wild Type P47/P47 P72/P72 (n = 270)	P47/P47 P72/R72 (n = 200)	P47/P47 R72/R72 (n = 121)	P47/S47 P72/P72 (n = 27)	S47/S47 P72/P72 (n = 7)	Heterozygous at 47 and 72 P47/S47 P72/R72 (n = 6)	*P* Value
Age, median years (IQR)	7.5 (4.5)	7.5 (4.7)	7.7 (4.4)	7.4 (4.4)	7.3 (4.7)	7.6 (1.8)	9.0 (1.3)	.95[b]
Male sex	319 (50.6)	143 (53.0)	100 (50.0)	54 (44.6)	13 (48.1)	3 (42.9)	6 (100.0)	.13[c]
HbS	65 (10.3)	28 (10.4)	19 (9.5)	15 (12.4)	1 (3.7)	1 (14.3)	1 (16.7)	.80[c]
HbC	65 (10.3)	32 (11.9)	21 (10.5)	9 (7.4)	2 (7.4)	0 (0.0)	1 (16.7)	.68[c]
Asymptomatic *Plasmodium falciparum* parasitemia at enrollment[d]	294 (46.6)	113 (41.9)	100 (50.0)	60 (49.6)	11 (40.7)	4 (57.1)	6 (100.0)	.045[c]
Presence of anemia at enrollment	188 (29.8)	80 (29.6)	62 (31.0)	34 (28.1)	6 (22.2)	3 (42.9)	3 (50.0)	.73[c]

Abbreviations: HbC, hemoglobin C; HbS, sickle hemoglobin; IQR, interquartile range.

[a]Data represent no. (%) of patients unless otherwise specified.

[b]Significance determined using 1-way analysis of variance.

[c]Significance determined using χ^2 tests.

[d]Determined with polymerase chain reaction of dried blood spots.

heterozygous P47/S47 and homozygous S47/S47 genotypes. Thus, these genotypes were collapsed into a single stratum. For codon 72, the heterozygous and homozygous genotypes were maintained as 3 separate strata. There were no significant differences in the time to first clinical malaria episode between P47/P47 (median [95% confidence interval], 184 [165–210] days) and the S47 variant (151 [133–210] days) genotypes or between P72/P72 (183 [59–210] days) and either P72/R72 (210 [160–210] days) or R72/R72 (159 [142–210] days) genotypes in either the univariate analyses (Figure 1*A* and 1*B*) or the adjusted analyses that included age, sex, baseline parasitemia, anemia, and presence of HbS as covariates (Supplementary Table 1). Individuals who were P47/S47 and P72/R72 demonstrated a similar risk of clinical malaria as P47/P47 and P72/P72 homozygous individuals (Supplementary Figure 1).

Increased p53 has been shown to reduce *Plasmodium* liver-stage burden in mouse models [10]. We therefore determined whether *TP53* polymorphisms were associated with differential risk of incident *P. falciparum* infections as determined by intensive, active PCR surveillance in individuals who began the study negative for *P. falciparum* by PCR (n = 330). We did not detect a significant difference in the time to first PCR-detectable *P. falciparum* infection between P47/P47 (median [95% confidence interval], 85 [81–91] days) and S47 variant (85 [58–124] days) genotypes or between P72/P72 (88 [80–95] days) and either P72/R72 (81 [72–91] days) or R72/R72 (91 [80–116] days) genotypes in either univariate analyses (Figure 1*C* and 1*D*) or adjusted analyses that included relevant covariates (Supplementary Table 2).

No Association of *TP53* Polymorphisms With Protection From Febrile Malaria After Incident *P. falciparum* Parasitemia or Differences in Gene Expression

Our group previously identified a subset of children within the Kalifabougou cohort who were afebrile at the time of incident, PCR-confirmed *P. falciparum* parasitemia [11]. These children were classified as “delayed fever” or “immune” depending on whether they had progressed to fever after 2–14 days or remained asymptomatic for the remainder of the malaria season, respectively. These 2 subsets demonstrated increased expression of *TP53* and *TP53* target genes at the preinfection baseline relative to children who were febrile at the time of incident *P. falciparum* parasitemia (“early fever”) [11]. To determine whether *TP53* polymorphisms were associated with either the delayed fever or immune phenotypes, we examined the distribution of S47 and R72 polymorphisms in these subsets but did not observe any significant differences between the 3 clinical phenotypes (Table 2). Reexamination of *TP53* expression in the RNA-seq transcriptomes obtained at each child’s healthy baseline (n = 80) [11] by codon 47 and 72 polymorphisms revealed no differences between the common polymorphisms and their respective variants (Figure 2).

To determine whether S47 or P72 variants are associated with protection from malaria fever once parasitemic in the parent cohort, we determined the risk of febrile malaria, using the time of first parasitemia as the start time. Here, we included all individuals who began the study *P. falciparum* negative by PCR and subsequently had PCR-confirmed *P. falciparum* parasitemia (n = 292). No significant differences in the time to first fever once parasitemic were observed in subgroups with S47 or P72 polymorphisms relative to their respective common genotypes by univariate analyses (Figure 3*A* and 3*B*). Although there was a trend toward increased risk of febrile malaria once parasitemic for S47 compared with P47/P47 (Figure 3*A*), this difference became more statistically insignificant when adjusted for relevant covariates such as age and HbS (Supplementary Table 3).

DISCUSSION

In contrast to severe malaria, host genetic polymorphisms that associate with resistance to uncomplicated malaria have primarily involved erythrocyte gene variants [2, 5]. Whereas several nonerythrocyte genes have been associated with protection against severe malaria [6, 7, 9], only a handful of genes involved in the inflammatory response, namely *NOS2* and *TNF*, have been specifically associated with differential risk of uncomplicated or mild malaria [2, 35–38].

The transcription factor p53 maintains cellular homeostasis in response to stress signals by regulating a broad range of downstream targets, including inflammatory pathways, that together suppress oncogenic processes [39]. The oncogenic potential of chronic inflammation is well established [40], and there is increasing evidence that p53 can modulate host inflammation in the context of pathogens [41, 42]. In support of this, our group previously observed that increased expression of *TP53* and its downstream targets was associated with protection from fever during incident *P. falciparum* parasitemia, suggesting that p53 could initially dampen the febrile response during malaria infection [11]. In an earlier study, mice with the p53 S47 variant exhibited an anti-inflammatory response to the malarial pigment hemozoin, suggesting that specific p53 polymorphisms could limit inflammation during malaria infections in humans [30]. In the context of inflammatory challenge with lipopolysaccharide, mice with the p53 R72 polymorphism demonstrated increased survival relative to P72 mice, implying that the R72 variant may protect against pathological responses to gram-negative bacteria [43].

Here, we evaluated whether codon 47 or 72 polymorphisms affect the risk of *P. falciparum* malaria in a prospective longitudinal cohort and showed that neither of these polymorphisms are associated with differential risk of incident parasitemia or

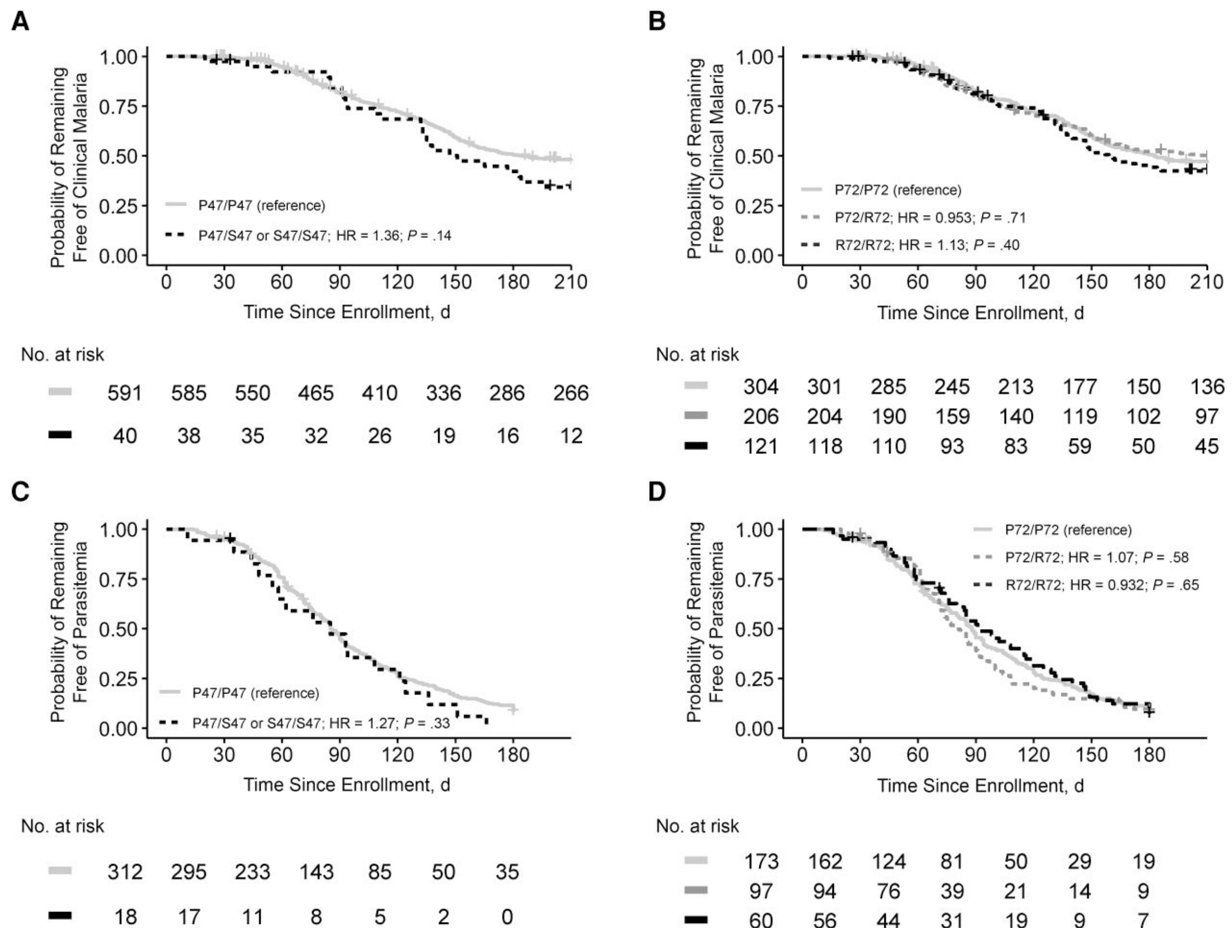

Figure 1. p53 Codon 47 and 72 polymorphisms and malaria risk in the Kalifabougou cohort during the 2011 malaria season. Kaplan-Meier curves of time to first febrile malaria episode (defined as >2500 parasites per microliter by blood smear and axillary temperature >37.5°C) (*A*, *B*) and time to first polymerase chain reaction (PCR)–confirmed *Plasmodium falciparum* parasitemia (*C*, *D*), stratified by the indicated p53 variant genotypes. For *C* and *D,* the analysis included only individuals who started the malaria season negative for *P. falciparum* by PCR. Significance was determined by Cox proportional hazards, using the Wald test statistic. Tables show the number of individuals at risk at the indicated days since enrollment. Abbreviation: HR, hazard ratio.

incident febrile malaria. Moreover, neither of these *TP53* polymorphisms were overrepresented among the subset of children who were protected from malarial fever and who demonstrated increased expression of *TP53* and its targets at the preinfection baseline in our group's prior study [11]. Our findings are consistent with cross-sectional studies of Ghanaian primiparous women (n = 314) and Rwandan children (n = 545), which showed no association between the R72 polymorphism and *P. falciparum* infection prevalence or parasite intensity [31]. The only other published investigation of p53 polymorphisms in malaria was a study of newborns in Sardinia, which found a greater frequency of the presumed malaria-protective R72 homozygous genotype in an area with previously higher malaria endemicity (n = 46) relative to an area with previously lower malaria endemicity (n = 47) [24]. Based on the premise that the R72 variant of p53 has enhanced apoptotic potential [25] and thus may prevent liver-stage infection via apoptosis of parasitized hepatocytes [10], the authors suggested that malaria endemicity may have positively selected for the R72 polymorphism [24]. However, this hypothesis is not supported by our data showing no difference in the time to incident PCR-confirmed parasitemia among the codon 72 genotypes.

In addition, a malaria-protective R72 polymorphism could not explain why the P72 allele is more prevalent in Africans than in Europeans [17], an observation that would imply P72 as the allele selected to be maintained in malaria-endemic populations according to the "malaria hypothesis" [8]. It may be that the functional activity conferred by R72 is not relevant to the risk of febrile malaria. As stated above, although the R72 variant may protect mice against lipopolysaccharide challenge [43], it has been also shown to drive inflammation in the context of a high-fat diet [44] or breast cancer [45] in mouse studies. Thus, how the R72 polymorphism affects the inflammatory response may depend on both the type and acuity of the perturbation.

Table 2. Distribution of TP53 Genotypes Among Children With Differential Susceptibility Febrile Malaria After Initial *Plasmodium falciparum* Parasitemia

		Children, No. (%)[a]				
Variable	Genotype	Overall (n = 80)[c]	Immune (n = 20)	Delayed Fever (n = 34)	Early Fever (n = 26)	*P* Value[b]
Codon 47	P47/P47	74 (96.1)	19 (100.0)	29 (90.6)	26 (100.0)	.36
	P47/S47	2 (2.6)	0 (0.0)	2 (6.2)	0 (0.0)	
	S47/S47	1 (1.3)	0 (0.0)	1 (3.1)	0 (0.0)	
Codon 72	P72/P72	42 (54.5)	9 (47.4)	20 (62.5)	13 (50.0)	.59
	P72/R72	25 (32.5)	7 (36.8)	10 (31.2)	8 (30.8)	
	R72/R72	10 (13.0)	3 (15.8)	2 (6.2)	5 (19.2)	

[a]Immune was defined as infection without progression to fever during the entire malaria season; delayed fever, as infection with a delay of 2–14 days before progression to fever; and early fever, as infection with concurrent fever.
[b]Significance was determined using χ^2 tests to evaluate the distribution of each genotype across the 3 clinical classes.
[c]Genotyping was performed only for children for whom DNA samples were available and passed quality control (19 in Immune; 32 in Delayed Fever; 26 in Early Fever).

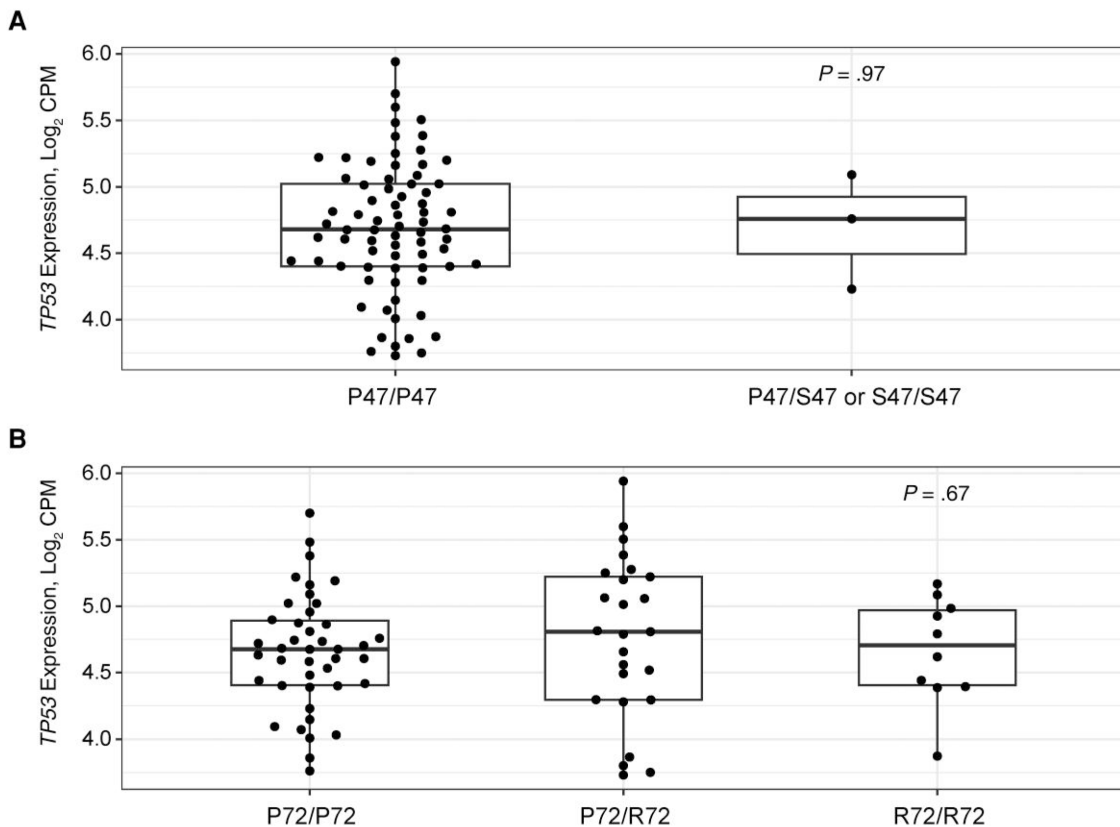

Figure 2. *TP53* expression by codon 47 and 72 polymorphisms. Expression of *TP53* by indicated p53 codon 47 (*A*) and 72 (*B*) polymorphisms in whole-blood samples from 80 Malian children at their healthy, uninfected baseline in May 2011, before the malaria season [11]. Significance was determined by means of Wilcoxon test (*A*) or analysis of variance (*B*). Gene expression is reported as $\log_2$ counts per million (CPM).

The lack of association between either codon 47 or codon 72 variants and protection from malaria could also be explained by their lack of effect on p53 expression as evident in our data (Figure 2) and prior studies [27, 46]. One main finding of our group's prior study was that expression of p53 and its downstream targets were increased in children who were protected from early fever during incident parasitemia [11], suggesting that induction of p53 expression may be required to control inflammation. Although the S47 variant decreases the ability to induce apoptosis relative to P47, p53 protein expression within the cell is still maintained at similar levels [27]. This, combined with the observation that none of the children in the most protected "immune" group harbored the S47 variant, suggests that reduction of apoptosis through this polymorphism is unlikely the mechanism by which p53 affects malarial fever. Similarly, the R72 polymorphism is functionally

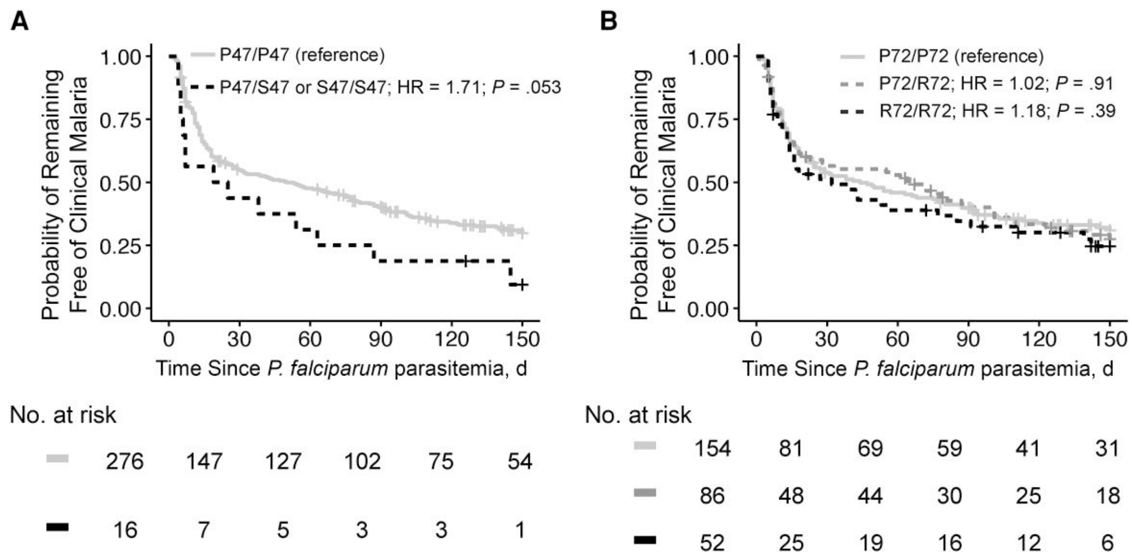

Figure 3. p53 Codon 47 and 72 polymorphisms and risk of febrile malaria after incident parasitemia. Kaplan-Meier plots of time to first febrile malaria using first polymerase chain reaction–confirmed *Plasmodium falciparum* blood-stage infection as the start time stratified by p53 codon 47 (*A*) or codon 72 (*B*) variant polymorphisms. Significance was determined by Cox proportional hazards using the Wald test statistic. Tables show the number of individuals at risk at the indicated time since enrollment. Abbreviation: HR, hazard ratio.

significant but does not necessarily result in increased p53 expression [25, 46].

Selective forces can increase the frequency of favored alleles over time. The S47 allele was present in 6.3% of the participants evaluated, with 1.1% being homozygous, giving an allele frequency of 3.7%, which is consistent with the 2%–4% previously reported for Africans from sub-Saharan Africa [28]. Conversely, the R72 allele was present in 51.8% of participants (19.2% homozygous), giving an allele frequency of 35.5% in this population. The predominance of the P72 allele in the Malian cohort is consistent with the increased frequency of this allele at latitudes closer to the equator, a finding that has been attributed to natural selection [17]. It is tempting to speculate why the S47 allele is maintained at a such a low rate among Africans. Given that S47 has decreased apoptotic potential relative to P47 [27], the low frequency of the S47 allele could be explained by the fitness cost incurred by its maintenance owing to selective disadvantages unrelated to pathological inflammation during malaria, such as increased premenopausal breast cancer risk [47]. However, the S47 variant, by limiting the hemozoin-induced inflammatory response [30], could still be protective against more severe malaria. Because the current study evaluated only uncomplicated *P. falciparum* malaria, it did not address whether either of these polymorphisms were associated with a reduction in severe malaria risk.

The modestly significant association between double heterozygosity (P47/S47 and P72/R72) and asymptomatic *P. falciparum* parasitemia at May enrollment is worth noting (Table 1). In areas of intense, seasonal malaria, chronic asymptomatic *P. falciparum* infection at the end of the dry season reflects higher cumulative malaria immunity [48]. Because these 6 double heterozygous individuals have a higher median age relative to the other groups, the overrepresentation of asymptomatic parasitemia within this genotype may simply be a chance function of past malaria exposure rather than a consequence of the p53 polymorphisms. Additional cohort studies of codon 47 and 72 p53 polymorphisms and malaria risk would be needed to confirm the significance of the double heterozygote genotype.

More limitations of this study must be noted. Although our study is one of the larger studies on p53 polymorphisms and malaria risk to date, only 7 of the 631 participants were homozygous for S47, limiting our power to detect an association. In addition, we evaluated only p53 polymorphisms within exon 4 and thus may have missed the impact of other potentially relevant mutations, including the 3KR mutations (K117R + K161R + K162R) involving p53 acetylation sites [49]. Of note, we did evaluate codon 117, which is located on exon 4, and found only lysine to be present. We also did not evaluate polymorphisms in *TP53* noncoding regions or in p53 regulators that may affect p53 at the protein level, such as MDM2 [28, 50]. Next-generation sequencing approaches that comprehensively assess polymorphisms across 5′ and 3′ untranslated regions as well as all coding regions for *TP53* and p53 regulators could address these limitations. Finally, we did not determine whether the p53 genotypes differed in their expression of p53 at the cellular level, which may have provided important insights as to

whether the codon 47 and 72 polymorphism could affect p53-regulated pathways.

In summary, the current study provides evidence that neither codon 47 nor codon 72 variants of p53 are protective against malaria as measured by time to incident *P. falciparum* parasitemia and uncomplicated febrile malaria. Given the lower frequencies of the S47 allele in this population, larger studies would be needed to confirm the lack of association of the S47 variant with malaria risk.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. P. D. C. and T. M. T. conceived the project. S. D., K. K., A. O., B. T., P. D. C., and T. M. T. designed the original cohort study, and S. D., K. K., A. O., and B. T. implemented it. E. L. G. performed the experiments. J. B., A. U., E. L. G., and T. M. T. performed the data analysis. P. D. C. contributed additional resources. J. B., P. D. C., and T. M. T. wrote the manuscript. All authors read and approved the manuscript.

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