Short Report: No Association of the p53 Codon 72 Polymorphism with Malaria in Ghanaian Primiparae and Rwandan Children

Prabhanjan P. Gai, Stefanie Meese, George Bedu-Addo, Jean Bosco Gahutu, and Frank P. Mockenhaupt*

Institute of Tropical Medicine and International Health, Charité–Universitätsmedizin Berlin, Berlin, Germany; Department of Medicine, Komfo Anoyke Teaching Hospital, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; University Teaching Hospital of Butare, School of Medicine, University of Rwanda, Huye, Rwanda

Abstract. The p53 protein is a key cell-signaling mediator integrating host responses to various types of stress. A common polymorphism of the encoding *TP53* gene (codon 72, Pro > Arg, rs1042522) is associated with susceptibility to virus-related and other cancers. The p53 has also been shown to be central for successful *Plasmodium* liver stage infection. We examined whether the polymorphism is associated with *P. falciparum* infection in Ghanaian primiparae and Rwandan children. The allele frequency of *TP53* codon 72 Arg was 0.30 among 314 Ghanaian primiparae and 0.31 among 545 Rwandan children, respectively, and it was not associated with infection prevalence or parasite density. This does not exclude p53 to be of pathophysiological relevance in malaria but argues against a major respective role of the *TP53* codon 72 polymorphism.

In view of the ongoing burden of malaria, improved knowledge of pathophysiological mechanisms may provide novel targets for prevention and treatment.¹ Recently, the p53 tumor suppressor protein, a central host cell-signaling factor, has been shown to be critical for successful *Plasmodium* liver stage infection.² As a transcription factor, p53 responds to various stimuli to induce apoptosis or cell cycle arrest, or to integrate a variety of other host responses.^{3,4} *Plasmodium* liver stage parasites suppress p53 thereby promoting survival of the infected hepatocyte. Conversely, increased levels of p53 counterbalance this suppression and reduce liver stage parasite burden.²

The *TP53* gene, encoding p53, shows a common single nucleotide polymorphism at codon 72 (proline to arginine, Pro > Arg, rs1042522), conferring various functional consequences including the Arg allele being more effective at inducing apoptosis.⁵ This allele has been associated with altered susceptibility to several virus-related and other cancers.^{4,6} Notably, indirect evidence for a malaria-protective role of the *TP53* codon 72 Arg allele is derived from a small study in Sardinia.⁷

We, therefore, examined the distribution of the *TP53* codon 72 alleles in two African populations with or without *P. falciparum* infection, i.e., placental *P. falciparum* infection in Ghanaian primiparae, and largely asymptomatic *P. falciparum* infection among Rwandan children.

Socio-demographic, clinical, and parasitological characteristics of the two cross-sectional studies and the study groups have been reported elsewhere.^{8,9} In brief, 314 primiparous pregnant women were recruited in southern Ghana of whom two-thirds (by polymerase chain reaction [PCR]) had largely asymptomatic placental *P. falciparum* infection (i.e., only 6.3% of these were febrile), which nevertheless was associated with maternal anemia, low birth weight, and preterm delivery.⁸ The second group involved 545 children < 5 years of age randomly selected from 24 rural villages in southern highland Rwanda of whom 16.1% were *P. falciparum* infected (by PCR; 2.9% categorized as symptomatic malaria defined as a positive blood film plus fever, or a history of fever within

the preceding 48 hours).⁹ All participants (or legal guardians) gave informed consent, and the study protocols were approved by the Committee on Human Research Publications and Ethics, School of Medical Sciences, University of Science and Technology, Kumasi, Ghana, and the National Ethics Committee, Republic of Rwanda, respectively. The DNA was extracted from stabilized blood samples (AS1 and QIAmp DNA Blood Mini Kit; Qiagen, Hilden, Germany). The Arg allele was differentiated from the Pro allele at codon 72 of *TP53* by restriction fragment length polymorphism of PCR-generated amplicons using primers and the restriction enzyme *Bst*U1 as published elsewhere.¹⁰ Data were analyzed with Statview 5.0 (SAS Institute Inc., Cary, NC). Allele frequencies and genotypes were compared by the χ^2 test, and continuous variables by the Mann Whitney *U* test or Student's *t* test as applicable.

The distribution of *TP53* codon 72 genotypes according to *P. falciparum* infection among Ghanaian primiparous women and Rwandan children is presented in Table 1. Genotypes were in Hardy–Weinberg equilibrium among Rwandan children ($P = 0.51$) but not among Ghanaian women ($P = 0.03$). Arg allele frequencies (Ghana, 0.30; Rwanda, 0.31) and genotypes did not differ between *P. falciparum* infected and non-infected subjects. Adjusting for the age difference between infected and non-infected individuals and for further associated factors,^{8,9} the lack of association between *TP53* genotypes and *P. falciparum* infection remained (data not shown). Likewise, there was no association between genotypes and peripheral blood geometric mean parasite density (μL , 95% confidence interval [CI]), in either pregnant women (Pro/Pro, 1,096 [513–2,346]; Pro/Arg, 675 [390–1,168]; Arg/Arg, 1,449 [134–15,690]; $P = 0.52$) or in children [1,837 (978–3,449); 968 [404–2,322]; 6,792 [615–75,062]; $P = 0.12$). In pregnant women, the *TP53* codon 72 allele had no influence on maternal anemia, birth weight, or preterm delivery, irrespective of placental malaria (data not shown), and in Rwandan children, the prevalence of symptomatic malaria did not differ significantly with genotype (Pro/Pro, 3.1% [8 of 260]; Pro/Arg, 1.8% [4 of 228]; Arg/Arg, 7.1% [4 of 57]; $P = 0.11$).

Findings from murine models indicate a critical role of p53 in susceptibility to *Plasmodium* infection in that increased levels reduce parasite liver stage burden.² The Arg allele of the *TP53*

* Address correspondence to Frank P. Mockenhaupt, Institute of Tropical Medicine and International Health, Charité–Universitätsmedizin Berlin, Spandauer Damm 130, 14050 Berlin, Germany. E-mail: frank.mockenhaupt@charite.de

TABLE 1

Distribution of *TP53* genotypes according to *Plasmodium falciparum* infection in Ghanaian primiparae and Rwandan children

Parameter	<i>P. falciparum</i> infection*		<i>P</i>
	None	Present	
Ghanaian primiparae			
No.	107	207	
Age (years; median, range)	21.0 (16–36)	20.0 (15–33)	0.04
<i>TP53</i> codon 72 genotypes			
Pro/Pro	49.5 (53)	44.9 (93)	
Pro/Arg	44.9 (48)	48.3 (100)	
Arg/Arg	5.6 (6)	6.8 (14)	0.72
<i>TP53</i> codon 72 Arg allele frequency	0.28	0.31	0.46
Rwandan children			
No.	457	88	
Age (months; median, range)	31 (1–60)	35.5 (4–60)	0.02
<i>TP53</i> codon 72 genotypes			
Pro/Pro	47.9 (219)	46.6 (41)	
Pro/Arg	41.6 (190)	43.2 (38)	
Arg/Arg	10.5 (48)	10.2 (9)	0.96
<i>TP53</i> codon 72 Arg allele frequency	0.31	0.32	0.89

* In pregnant women and children, *P. falciparum* was detected in placental and peripheral blood samples, respectively.

codon 72 variant is pro-apoptotic, potentially protective with respect to virus-related and other cancers,^{4–6} and suggested to be subject to selection because of protection against malaria.⁷ In this study, we are unable to show a role of the *TP53* codon 72 allele in malaria. As a matter of fact, this study was not *a priori* designed to assess an association between *TP53* codon 72 alleles and *P. falciparum* infection. Considering the given prevalence, the sub-studies were powered (80%) to detect only substantial reductions of infection caused by the Arg allele (Ghana, OR, ≤ 0.60 ; Rwanda, OR, ≤ 0.50). Smaller effects may thus be discernible at considerably larger sample sizes. Nevertheless, allele frequencies in our African study populations were considerably lower than in Sardinia or other Caucasian populations,^{7,11,12} and a declining Arg allele frequency toward the equator argues against selection by malaria.^{11,12} Notably, respective genetic data from Africans are comparatively scarce. Genotype frequencies deviated from Hardy–Weinberg equilibrium in the Ghanaian subgroup. One reason for this might be a small sample size. Interestingly, the *TP53* codon 72 Pro/Pro genotype has been associated with reduced pregnancy rates,¹³ and in fact, this genotype tended to be underrepresented among the Ghanaian primiparae. The present findings of lacking genetic association do not exclude p53 to be pathophysiologically relevant in malaria,² but argue against a major respective role of the *TP53* codon 72 allele. The p53 is activated by various stress signals and can induce a variety of host responses^{3,4}; possibly, the biological effects conferred by this allele are not those essential in antiparasmodial defense. Alternatively, the variant may not influence *P. falciparum* infection per se, as examined in this study, but rather specific malaria entities, e.g., severe malaria, or infection risk in other populations.

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Authors' addresses: Prabhanjan P. Gai, Stefanie Meese, and Frank P. Mockenhaupt, Institute of Tropical Medicine and International Health, Charité–Universitätsmedizin Berlin, Berlin, Germany, E-mails: prabhanjan.gai@charite.de, stefanie.messe@charite.de, and frank.mockenhaupt@charite.de. George Bedu-Addo, Department of Medicine, Komfo Anoyke Teaching Hospital, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, E-mail: gbeduaddo@gmail.com. Jean Bosco Gahutu, Butare University Teaching Hospital, School of Medicine, University of Rwanda, Huye, Rwanda, E-mail: jgahutu@nur.ac.rw.

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