Polymorphisms in the K13-Propeller Gene in Artemisinin-Susceptible *Plasmodium falciparum* Parasites from Bougoula-Hameau and Bandiagara, Mali

Amed Ouattara, Aminatou Kone, Matthew Adams, Bakary Fofana, Amelia Walling Maiga, Shay Hampton, Drissa Coulibaly, Mahamadou A. Thera, Nouhoum Diallo, Antoine Dara, Issaka Sagara, Jose Pedro Gil, Anders Bjorkman, Shannon Takala-Harrison, Ogobara K. Doumbo, Christopher V. Plowe, and Abdoulaye A. Djimde*

Department of Epidemiology of Parasitic Diseases, University of Science, Techniques and Technologies of Bamako, Bamako, Mali; Vanderbilt University Medical Center, Nashville, Tennessee; Howard Hughes Medical Institute, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland; Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden; Department of Physiology and Pharmacology, Karolinska University Hospital/Karolinska Institutet, Stockholm, Sweden; Drug Resistance and Pharmacogenetics, Center for Biodiversity, Functional and Integrative Genomics, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal; The Harpur College of Arts and Sciences, Binghamton University, The State University of New York, Binghamton, New York

Abstract. Artemisinin-resistant *Plasmodium falciparum* malaria has been documented in southeast Asia and may already be spreading in that region. Molecular markers are important tools for monitoring the spread of antimalarial drug resistance. Recently, single-nucleotide polymorphisms (SNPs) in the PF3D7_1343700 kelch propeller (K13-propeller) domain were shown to be associated with artemisinin resistance *in vivo* and *in vitro*. The prevalence and role of K13-propeller mutations are poorly known in sub-Saharan Africa. K13-propeller mutations were genotyped by direct sequencing of nested polymerase chain reaction (PCR) amplicons from dried blood spots of pre-treatment falciparum malaria infections collected before and after the use of artemisinin-based combination therapy (ACT) as first-line therapy in Mali. Although K13-propeller mutations previously associated with delayed parasite clearance in Cambodia were not identified, 26 K13-propeller mutations were identified in both recent samples and pre-ACT infections. Parasite clearance time was comparable between infections with non-synonymous K13-propeller mutations and infections with the reference allele. These findings suggest that K13-propeller mutations are present in artemisinin-sensitive parasites and that they preceded the wide use of ACTs in Mali.

INTRODUCTION

In Sub-Saharan African countries, such as Mali, the first-line treatments for uncomplicated falciparum malaria are artemisinin-based combination therapies (ACTs).¹ The recent evidence of emerging resistance to artemisinin derivatives, including ACTs, in southeast Asia raises serious concern that such parasite populations may spread toward Africa, mirroring parasite resistance to chloroquine,² sulfadoxine, and pyrimethamine.³

Recently, mutations in the PF3D7_1343700 kelch propeller (K13-propeller) domain were shown to be useful markers of delayed parasite clearance in surveillance for artemisinin resistance in southeast Asia.⁴ The *Plasmodium falciparum* K13-propeller domain (PF3D7_1343700) is a 727-amino acid protein encoded by a gene located on chromosome 13.⁵ Kelch domain-containing proteins are observed in many cellular compartments of different organisms and have many functions involving protein–protein interactions.⁶ Little is known about the function of this protein in *P. falciparum*. Among 17 single K13-propeller mutations observed in Cambodia, 3 (C580Y, R539T, and Y493H) mutations were most associated with slow-clearing parasites in patients treated with ACTs.⁴

The prevalence of these molecular markers is poorly known in sub-Saharan Africa. Taking into consideration the critical importance of artemisinin-based combinations in the control and elimination of malaria in these regions, it is of urgent need to understand if K13-propeller mutations associated with delayed parasite clearance are already circulating. Previ-

ously, we reported that artesunate was highly efficacious in Bougoula-Hameau, Mali, with a median parasite clearance half-life of 2.07 hours; all study participants cleared their parasitemia by 72 hours.⁷ The main objective of this study was to measure the prevalence of *P. falciparum* K13-propeller polymorphisms in present-day artemisinin-sensitive parasite collected in Bougoula-Hameau and malaria infections that occurred before the scaling up of ACTs in Mali to detect any impact of the implementation of these therapies on the biodiversity of this gene.

MATERIALS AND METHODS

Samples origin. To measure the prevalence of K13-propeller mutations in Mali, we genotyped 90 samples collected before drug treatment on day 0 of a prospective artesunate monotherapy efficacy study conducted in Bougoula-Hameau, Mali from December of 2010 to February of 2011.⁷ Of these samples, 55 dried blood spots were also assessed for single-nucleotide polymorphisms (SNPs) at Mal10-688956 and Mal13-1718319 loci, which were previously suggested as candidate markers of artemisinin resistance⁸; 36 samples collected during malaria studies conducted between 2003 and 2005 in Bougoula-Hameau and 86 samples collected between 1999 and 2002 in Bandiagara were also used to measure the prevalence of K13-propeller mutations in the region before ACTs were recommended as the first-line treatment of uncomplicated malaria in Mali. In Bougoula-Hameau, parasite clearance time (PCT) was estimated as previously described.⁷ Briefly, thick and thin blood smears were examined for asexual and sexual parasites after staining with 5% Giemsa every 8 hours starting immediately before the first oral dose of artesunate monotherapy until three consecutive slides were negative for asexual parasites.

*Address correspondence to Abdoulaye A. Djimde, Department of Epidemiology of Parasitic Diseases, University of Science, Techniques and Technologies of Bamako, BP 1805, Bamako, Mali. E-mail: adjimde@icermali.org

Slides were also prepared on follow-up days 2, 3, 7, 14, 21, and 28. Blood smears were read by two trained microscopists according to World Health Organization (WHO) standard procedures. Species discrepancies were resolved by a third microscopist. Parasite density discrepancies of > 50% were resolved by averaging of a third microscopist's result and the closer of the two original results. Parasite density was estimated by counting the number of asexual parasites per 200 leukocytes and multiplying by 40 (assuming 8,000 leukocytes/ μ L).⁷

The study was approved by the Ethics Committee of the Faculty of Medicine and Odontostomatology and Faculty of Pharmacy of the University of Science, Techniques and Technologies of Bamako.

Procedures. Parasite DNA extracted from dried blood spots was subjected to nested polymerase chain reaction (PCR) followed by direct sequencing of the portion of K13 propeller encoding the propeller domain. DNA was extracted from dried blood spots using the Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. A nested PCR strategy based on previously published protocols⁴ was used to amplify a final 850-base pair product (primer sequences and conditions are in Supplemental Table 1). PCR products were purified using filter plates (Edge Biosystems, Gaithersburg, MD) and directly sequenced as described by Ouattara and others⁹ (Supplemental Table 1).

For genotyping Mal10-688956 and Mal13-1718319, DNA was extracted using the BloodPrep Chemistry on an ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems, Fresno, CA) according to an established protocol.¹⁰ SNPs were genotyped by pyrosequencing using a PyroMark ID according

to the recommendations of the manufacturer. Primer sequences and conditions are in Supplemental Table 1.

Statistical analysis. The output sequence data were assembled, edited, and aligned using the Sequencher v.5.0 suit (Gene Codes Corporation, Ann Arbor, MI). Mutations were assessed by comparing each sequence with the 3D7 reference (PF13_0238), with haplotypes defined as a combination of amino acids across 850 base pairs.

For the 2010–2011 study, parasite clearance slope half-life was calculated using the Worldwide Antimalarial Resistance Network parasite clearance estimator (www.wwarn.org). The time in hours for parasitemia to be reduced by 99% of its initial value (PCT99) was normally distributed, whereas the time in hours for parasitemia to be reduced by 50% of its initial value (PCT50) distribution was skewed. A parametric *t* test was used to compare the normally distributed variable (PC99), whereas a non-parametric Wilcoxon rank sum test was used to compare the wild type and the mutant parasites group for the non-normally distributed parasite clearance slope half-life.

RESULTS

K13-propeller sequences were generated from 87 samples (3 samples did not generate high-quality sequences that could be analyzed) collected in Bougoula-Hameau from 2010 to 2011, 36 samples from Bougoula-Hameau from 2003 to 2005, and 86 samples from Bandiagara (1999–2002). The K13-propeller sequences were deposited in GenBank (accession numbers AD1–AD209 and KM359177–KM359385). Based on all 26 SNPs distributed across 850 base pairs of K13

FIGURE 1. Haplotypes of K13 propeller observed in Bougoula-Hameau and Bandiagara, Mali. Sequence alignment of *P. falciparum* 3D7 and 25 haplotypes (blue) showing polymorphisms in the K13-propeller gene. Numbers in the top row are amino acid positions. Amino acids positions highlighted in yellow have been described by Ariey and others.⁴ Amino acids in green were identified in this study dataset. *A mutation in Mali at the same position as a mutation described in the work by Ariey and others⁴ but with a different amino acid present.

FIGURE 2. Prevalence of K13-propeller mutations at two sites before the use of ACTs and one site after the introduction of ACTs. Prevalence of K13 mutations may not add up to 100%, because infections may have more than one K13 mutation present in one sample.

propeller, we identified 26 haplotypes (Figure 1). Other than the 3D7 reference, all but one of the remaining 25 haplotypes were observed only one time in the dataset (the haplotype containing the F446I mutation was observed in two different samples from Bandiagara). Two samples contained more than one K13-propeller mutation, likely representing multiple parasite clones within the infection; all other infections contained one or no K13-propeller mutations. The 3D7 haplotype prevalence was 89.7% ($N = 87$) in Bougoula-Hameau in 2011, 77.8% ($N = 36$) in Bougoula-Hameau in 2003–2005 ($P = 0.08$), and 94.2% in Bandiagara in 1999–2002 (Figure 2). Apart from the reference 3D7, none of the haplotypes observed during the pre-ACT years were found in 2011 samples.

Slope half-lives and PC99 were only available for 87 samples from the Bougoula-Hameau 2010 and 2011 study, where parasitemia was measured every 8 hours.⁷ Nine of these samples had a non-synonymous SNP in the K13-propeller domain; 78 were wild type. The means of slope half-lives of the mutant and wild-type infections were similar (Wilcoxon rank sum test, $P = 0.32$) (Figure 3A). The mean of PC99 was 20.6 in the infections with mutant K13 propeller and 17.9 in those with the wild-type allele (t test, $P = 0.09$) (Figure 3B).

The analysis of the 55 DBS for the Mal10-688956 and Mal13-1718319 SNPs showed that all infections were wild type (3D7) for Mal10-688956 (T) and Mal13-1718319 (A) alleles.

DISCUSSION

We showed a low prevalence of several novel K13-propeller mutations in Mali, where delayed parasite clearance caused by artemisinin resistance has not been reported. A recent study in Cambodia reported three K13-propeller alleles that were associated with increase malaria parasite resistance to artemisinin-based drugs.⁴ Furthermore, Ashley and others¹¹ showed that

artemisinin resistance has now reached eastern Myanmar, western Cambodia and Thailand, and southern Vietnam and is emerging in southern Laos and northeastern Cambodia. Multiple K13-propeller SNPs have been shown to be predictive of delayed parasite clearance,^{4,12} but none of these alleles were found in samples collected in this high malaria transmission region of Mali. Mean slope half-life of parasites carrying K13-propeller SNPs was comparable with that of parasites with the reference allele. However, there was a trend toward longer PC99 in parasites with K13-propeller mutations versus those with the reference allele, although the difference did not reach statistical significance. Similar analyses involving larger sample sizes are required to further clarify the role of these novel mutations in *in vivo* parasite clearance in sub-Saharan Africa.

All mutations observed in this study were found within six kelch domains of the protein (K13 propeller), which is hypothesized to be the region of the protein associated with prolonged parasite clearance.¹¹ Interestingly, two haplotypes contained mutations in the same position as mutations observed in Cambodia but had different amino acids present (i.e., G449S and D584N). In addition, mutations at positions 578 and 581 were also observed in Mali and were very close to the C580Y mutation, a key mutation associated with artemisinin resistance in Cambodia.^{4,12} A578S was also observed in Faladje, another site in western Mali, as well as four other sub-Saharan countries (i.e., Kenya; Democratic Republic of Congo; Gabon, Ghana [Kamau E and others, unpublished data]; and Uganda).¹³

A578S represents the change from a non-polar toward a polar amino acid, whereas in the V581F SNP, albeit not registering a change in the non-polar (hydrophobic) nature, the presence of phenylalanine implicates the introduction of a markedly different side chain (benzene ring group). Such (simultaneous) changes might significantly alter the shape of the protein in this region.

FIGURE 3. (A) Dot plot of the median slope half-lives of parasites in Bougoula-Hameau in 2010 and 2011. (B) Dot plot of mean PC99 of parasites in Bougoula-Hameau in 2010 and 2011. A parametric *t* test was used to compare PC99, whereas a Wilcoxon rank sum test was used to compare parasite clearance slope half-life.

The SNPs described in this setting could be relevant as markers associated with artemisinin resistance. *In vitro* assessment of artemisinin resistance through the ring-stage survival assay¹⁴ will be required to rule out resistance in these K13-propeller mutants from these high transmission areas of Mali, where resistant parasites can be cleared by the immune system. It is also possible that resistance requires the presence of additional mutations at secondary loci that are not yet present in Africa but that are present in southeast Asia (i.e., K13-propeller mutations are necessary but not sufficient for the manifestation of resistance). A number of K13-propeller mutations were also found in parasites collected before the introduction of ACTs in the study area, indicating that these haplotypes existed before the widespread use of artemisinins in Mali and that they may have neither been imported from Asia nor selected by artemisinin pressure in Africa. Although we cannot completely rule out the possibility of local *de novo* emergence of artemisinin resistance in Africa, which has been observed in some regions of southeast Asia,¹² the results of this study indicate a lack of association between K13 mutations and parasite resistance to artemisinin-based drugs in Mali. Similar observations have been made by a recent K13

genotyping study conducted in 14 African countries.¹⁵ However, recent studies have shown a few examples of median parasite clearance half-life > 5 hours in Democratic Republic of Congo and Nigeria.¹¹ Although factors other than parasite resistance could account for these observations, vigilant surveillance for initially low-prevalence resistance provides an important early-warning system for emerging or spreading resistance.

Our findings suggest that K13-propeller mutations may occur at low frequency, irrespective of drug selection by artemisinin treatment or possibly, on selection pressure by other drugs (e.g., chloroquine). The K13-propeller proteins have been associated with the cell's reduction and oxidation (REDOX) stress management,^{16,17} a type of effect characteristic of the action of most antimalarials on *P. falciparum*, including chloroquine.¹⁸ Stress management or the effect of other drugs in selecting for K13-propeller mutations and the relevance, if any, of these K13-propeller mutations to artemisinin efficacy will require additional investigation, including genetic transformation studies.

Received September 25, 2014. Accepted for publication November 9, 2014.

Published online April 27, 2015.

Note: Supplemental table appears at www.ajtmh.org.

Acknowledgments: The authors thank the Malaria Research and Training Center research team and the communities of Bougoula-Hameau and Bandiagara, Mali.

Financial support: This work was supported by European and Developing Countries Clinical Trials Partnership EDCTP IP_07_31060_002, the West African Network for Clinical Trials of Antimalarial Drugs (WANECAM), and the Howard Hughes Medical Institute (sequencing and genetic analysis). S.T.-H. is supported by National Institutes of Health Grant R01AI101713.

Authors' addresses: Amed Ouattara, Department of Epidemiology of Parasitic Diseases, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, and Howard Hughes Medical Institute, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, E-mail: aouattara@medicine.umaryland.edu. Aminatou Kone, Bakary Fofana, Drissa Coulibaly, Mahamadou A. Thera, Nouhoum Diallo, Antoine Dara, Issaka Sagara, Ogobara K. Doumbo, and Abdoulaye A. Djimde, Department of Epidemiology of Parasitic Diseases, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, E-mails: aouattara@medicine.umaryland.edu, amina@icermali.org, bfofana@icermali.org, coulibalyd@icermali.org, mthera@icermali.org, nouhoumd@icermali.org, tonydara@icermali.org, isagara@icermali.org, okd@icermali.org, and adjimde@icermali.org. Matthew Adams, Shay Hampton, Shannon Takala-Harrison, and Christopher V. Plowe, Howard Hughes Medical Institute, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, E-mails: madams@medicine.umaryland.edu, shampton@medicine.umaryland.edu, stakala@medicine.umaryland.edu, and cplowe@medicine.umaryland.edu. Amelia Walling Maiga, Vanderbilt University Medical Center, Nashville, TN, E-mail: kadia.ongoiba@gmail.com. Jose Pedro Gil, Department of Physiology and Pharmacology, Karolinska University Hospital/Karolinska Institutet, Stockholm, Sweden, Drug Resistance and Pharmacogenetics, Center for Biodiversity, Functional and Integrative Genomics, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal, and The Harpur College of Arts and Sciences, Binghamton University, The State University of New York, Binghamton, NY, E-mail: jose.pedro.gil@ki.se. Anders Bjorkman, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, E-mail: anders.bjorkman@karolinska.se.

REFERENCES

- Djimde AA, Fofana B, Sagara I, Sidibe B, Toure S, Demele D, Dama S, Ouologuem D, Dicko A, Doumbo OK, 2008.

- Efficacy, safety, and selection of molecular markers of drug resistance by two ACTs in Mali. *Am J Trop Med Hyg* 78: 455–461.
2. Wellemes TE, Hayton K, Fairhurst RM, 2009. The impact of malaria parasitism: from corpuscles to communities. *J Clin Invest* 119: 2496–2505.
 3. Mita T, Tanabe K, 2012. Evolution of *Plasmodium falciparum* drug resistance: implications for the development and containment of artemisinin resistance. *Jpn J Infect Dis* 65: 465–475.
 4. Arie F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier C, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chuor CM, Bout DM, Ménard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Le Bras J, Berry A, Barale JC, Fairhurst RM, Benoit-Vical F, Mercereau-Puijalon O, Ménard D, 2014. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 505: 50–55.
 5. Hall N, Pain A, Berriman M, Churcher C, Harris B, Harris D, Mungall K, Bowman S, Atkin R, Baker S, Barron A, Brooks K, Buckee CO, Burrows C, Cherevach I, Chillingworth C, Chillingworth T, Christodoulou Z, Clark L, Clark R, Corton C, Cronin A, Davies R, Davis P, Dear P, Dearden F, Doggett J, Feltwell T, Goble A, Goodhead I, Gwilliam R, Hamlin N, Hance Z, Harper D, Hauser H, Hornsby T, Holroyd S, Horrocks P, Humphray S, Jagels K, James KD, Johnson D, Kerhornou A, Knights A, Konfortov B, Kyes S, Larke N, Lawson D, Lennard N, Line A, Maddison M, McLean J, Mooney P, Moule S, Murphy L, Oliver K, Ormond D, Price C, Quail MA, Rabinowitz E, Rajandream MA, Rutter S, Rutherford KM, Sanders M, Simmonds M, Seeger K, Sharp S, Smith R, Squares R, Squares S, Stevens K, Taylor K, Tivey A, Unwin L, Whitehead S, Woodward J, Sulston JE, Craig A, Newbold C, Barrell BG, 2002. Sequence of *Plasmodium falciparum* chromosomes 1, 3–9 and 13. *Nature* 419: 527–531.
 6. Adams J, Kelso R, Cooley L, 2000. The kelch repeat superfamily of proteins: propellers of cell function. *Trends Cell Biol* 10: 17–24.
 7. Maiga AW, Fofana B, Sagara I, Dembele D, Dara A, Traore OB, Toure S, Sanogo K, Dama S, Sidibe B, Kone A, Thera MA, Plowe CV, Doumbo OK, Djimde AA, 2012. No evidence of delayed parasite clearance after oral artesunate treatment of uncomplicated falciparum malaria in Mali. *Am J Trop Med Hyg* 87: 23–28.
 8. Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, Fukuda MM, Nosten F, Noedl H, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Socheat D, Arie F, Phyto AP, Starzengruber P, Fuehrer HP, Swoboda P, Stepniewska K, Flegg J, Arze C, Cerqueira GC, Silva JC, Ricklefs SM, Porcella SF, Stephens RM, Adams M, Kenefic LJ, Campino S, Auburn S, MacInnis B, Kwiatkowski DP, Su XZ, White NJ, Ringwald P, Plowe CV, 2013. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia. *Proc Natl Acad Sci USA* 110: 240–245.
 9. Ouattara A, Takala-Harrison S, Thera MA, Coulibaly D, Niangaly A, Saye R, Tolo Y, Dutta S, Heppner DG, Soisson L, Diggs CL, Vekemans J, Cohen J, Blackwelder WC, Dube T, Laurens MB, Doumbo OK, Plowe CV, 2013. Molecular basis of allele-specific efficacy of a blood-stage malaria vaccine: vaccine development implications. *J Infect Dis* 207: 511–519.
 10. Dahlstrom S, Veiga MI, Ferreira P, Martensson A, Kaneko A, Andersson B, Bjorkman A, Gil JP, 2008. Diversity of the sarco/endoplasmic reticulum Ca(2+)-ATPase orthologue of *Plasmodium falciparum* (PfATP6). *Infect Genet Evol* 8: 340–345.
 11. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroeth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyto AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ, Tracking Resistance to Artemisinin Collaboration (TRAC), 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 371: 411–423.
 12. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, Fukuda MM, Hien TT, Mayxay M, Noedl H, Nosten F, Kyaw MP, Nhien NT, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Arie F, Mercereau-Puijalon O, Menard D, Newton PN, Khanthavong M, Hongvanthong B, Starzengruber P, Fuehrer HP, Swoboda P, Khan WA, Phyto AP, Nyunt MM, Nyunt MH, Brown TS, Adams M, Pepin CS, Bailey J, Tan JC, Ferdig MT, Clark TG, Miotto O, MacInnis B, Kwiatkowski DP, White NJ, Ringwald P, Plowe CV, 2014. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. *J Infect Dis*. September 1, 2014. doi:10.1093/infdis/jiu491.
 13. Conrad MD, Bigira V, Kapisi J, Muhindo M, Kanya MR, Havlir DV, Dorsey G, Rosenthal PJ, 2014. Polymorphisms in K13 and falcipain-2 associated with artemisinin resistance are not prevalent in *Plasmodium falciparum* isolated from Ugandan children. *PLoS ONE* 9: e105690.
 14. Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, Lim P, Mao S, Sopha C, Sam B, Anderson JM, Duong S, Chuor CM, Taylor WR, Suon S, Mercereau-Puijalon O, Fairhurst RM, Menard D, 2013. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: *in-vitro* and *ex-vivo* drug-response studies. *Lancet Infect Dis* 13: 1043–1049.
 15. Taylor SM, Parobek CM, DeConti DK, Kayentao K, Coulibaly SO, Greenwood BM, Tagbor H, Williams J, Bojang K, Njie F, Desai M, Kariuki S, Gutman J, Mathanga DP, Mårtensson A, Ngasala B, Conrad MD, Rosenthal PJ, Tshefu AK, Moormann AM, Vulule JM, Doumbo OK, ter Kuile FO, Meshnick SR, Bailey JA, Juliano JJ, 2014. Absence of putative artemisinin resistance mutations among *Plasmodium falciparum* in Sub-Saharan Africa: a molecular epidemiologic study. *J Infect Dis*. September 1, 2014 doi:10.1093/infdis/jiu467.
 16. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M, 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 13: 76–86.
 17. Niforou K, Cheimonidou C, Trougakos IP, 2014. Molecular chaperones and proteostasis regulation during redox imbalance. *Redox Biol* 2: 323–332.
 18. Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H, 2004. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *Int J Parasitol* 34: 163–189.