



Emergence of Indigenous Artemisinin-Resistant Plasmodium falciparum in Africa

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CORRESPONDENCE

Emergence of Indigenous Artemisinin-Resistant *Plasmodium falciparum* in Africa

TO THE EDITOR: *Plasmodium falciparum* has developed resistance to artemisinin in many countries in Southeast Asia.^{1,2} Artemisinin combination therapy is the first-line treatment for malaria in the majority of countries in which the disease is endemic, and its efficacy is particularly important in Africa, where malaria is the most widespread.³ We report here an artemisinin-resistant strain of *P. falciparum* that was contracted in Africa.

On January 28, 2013, falciparum malaria was diagnosed in a 43-year-old man (identified here as CWX) at a hospital in Jiangsu Province, China. The patient had returned to China on December 3, 2012, after working for 20 months in Equatorial Guinea, where he had been treated for malaria six times. The date and therapeutic regimen associated with each episode are unknown, with the exception of the last episode, when the patient received parenteral artesunate monotherapy starting on November 20, 2012. Before his arrival in Equatorial Guinea, the patient had no history of malaria.

On the patient's presentation in Jiangsu Province, microscopy revealed *P. falciparum* parasites in peripheral blood, with an initial parasite density of 4221 per microliter. A monospecies infection was identified on polymerase-chain-reaction assay. The patient received a course of eight tablets of a combination of dihydroartemisinin (40 mg) and piperaquine (320 mg) under direct observation. The parasitemia declined over the next 3 days, but parasites were still detected on day 3 after treatment (Fig. 1A). By day 7, no parasites were detected. In contrast, three other isolates that originated from the same country were negative for asexual parasites as of day 3.

In vitro ring-stage survival assay³ revealed a 2.29% survival rate for the CWX isolate, which was substantially higher than the rate in control *P. falciparum* strains (including wild-type strain 3D7) and in another isolate from a Chinese worker (SBC) who had returned to China from Equatorial Guinea in 2013 but lower than the rate in an artemisinin-resistant parasite line with a C580Y kelch13 mutation (Fig. 1B, and the Methods section in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Polymorphisms in the *P. falciparum* gene encoding kelch13 (K13) have been linked to artemisinin resistance in Southeast Asia. Sequencing of K13 in the CWX isolate revealed a previously unreported nonsynonymous single-nucleotide polymorphism (SNP) that resulted in a switch from a methionine to an isoleucine at amino acid position 579 (M579I).

In order to determine whether the CWX strain was indigenous to Equatorial Guinea, we performed whole-genome sequencing (European Nucleotide Archive accession number, PRJEB18721), and compared the SNPs with those of 245 *P. falciparum* isolates collected worldwide.⁴ Principal component analysis showed that the CWX strain was of African origin and had not been recently imported from elsewhere in the world (Fig. 1C, and Fig. S2 in the Supplementary Appendix).

There is a perennially high rate of malaria transmission throughout Equatorial Guinea, and artemisinin combination therapies are commonly used for treatment.⁵ Awareness of artemisinin resistance is prudent in Equatorial Guinea and countries with similar malaria transmission dy-

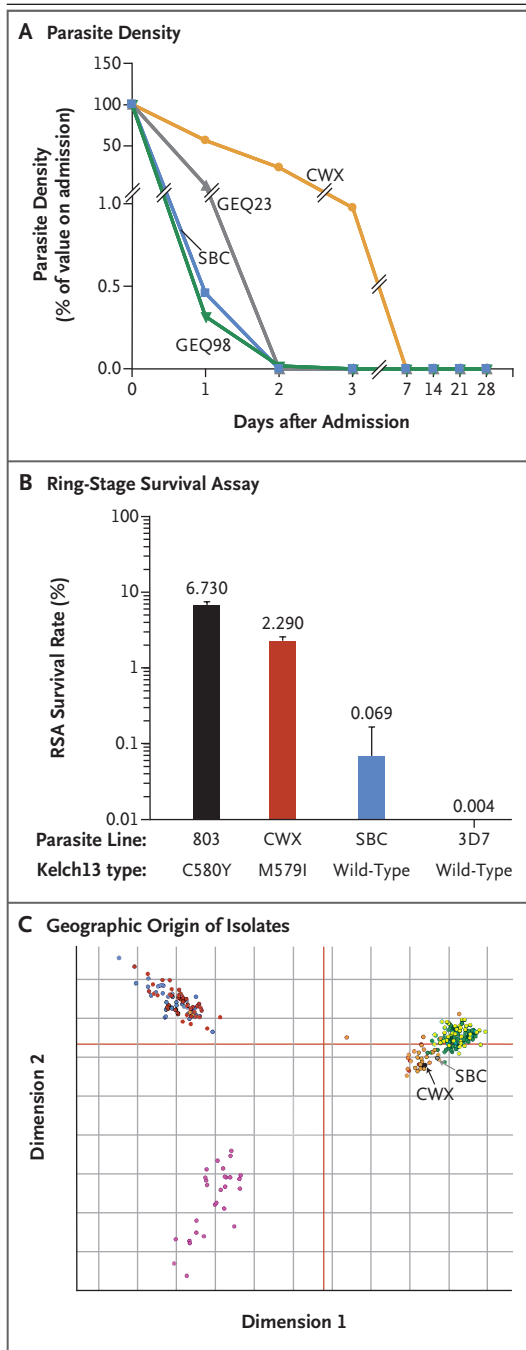


Figure 1. Artemisinin Resistance Associated with M579I Mutation in an Isolate of African Origin.

Panel A shows the rate of parasite clearance after treatment with artemisinin combination therapy in vivo in the CWX isolate and in three other isolates originating from the same country (including SBC, which was obtained from another Chinese man who had worked in Equatorial Guinea). The three other isolates were negative for asexual parasites as of day 3. Panel B shows the survival rate on ring-stage survival assay (RSA) for isolates with or without the M579I mutation. 3D7 is a wild-type strain, and 803 is an artemisinin-resistant parasite line with the C580Y kelch13 mutation. Panel C shows multidimensional scaling of CWX, SBC, and 245 other *P. falciparum* isolates with different geographic origins. Each dot represents an isolate and is spread in a three-dimensional space according to the similarities or dissimilarities among them. The distances between the isolates were inferred from 26,918 common genome-wide single-nucleotide polymorphisms and plotted with the use of a sequence variation analysis, maps, and phylogeny (SVAMP) program. The isolates are color-coded as follows: CWX, black; SBC, gray; Burkina Faso isolates, green; Mali isolates, yellow; Kenya isolates, gold; Papua New Guinea isolates, purple; Thailand isolates, red; and Cambodia isolates, blue. CWX and SBC cluster with other parasites from Africa.

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namics in order to monitor for the potential emergence of artemisinin resistance in Africa.

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1. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2009;361:455-67.
2. Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2014;371:411-23.
3. Witkowski B, Amaratunga C, Khim N, et al. 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* 2013;13:1043-9.
4. Manske M, Miotto O, Campino S, et al. Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing. *Nature* 2012;487:375-9.
5. World malaria report 2014. Geneva: World Health Organization, 2014 (http://www.who.int/malaria/publications/world_malaria_report_2014/en/).

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