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BRIEF REPORT

Circulation of an Artemisinin-Resistant Malaria Lineage in a Traveler Returning from East Africa to France

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A returned traveler to Uganda presented with a *Plasmodium falciparum kelch13* A675V mutant infection that exhibited delayed clearance under artesunate therapy. Parasites were genetically related to recently reported Ugandan artemisinin-resistant A675V parasites. Adequate malaria prevention measures and clinical and genotypic surveillance are important tools to avoid and track artemisinin resistance.

Keywords. *Plasmodium falciparum*; kelch13; artemisinin resistance; East Africa; whole-genome sequencing

Artemisinin (ART)-based combination therapies (ACTs) are the current first-line treatments for uncomplicated *Plasmodium falciparum* malaria [1]. However, ART-resistant *P. falciparum* parasites that display delayed clearance have emerged and largely spread in the Greater Mekong Subregion of Southeast Asia during the past 15 years [2, 3]. ART resistance is mainly conferred by nonsynonymous mutations in the *kelch13* gene of the parasite (PlasmoDB accession: PF3D7_1343700) [4, 5], and about 10 of those mutations are validated markers of clinical ART resistance in Southeast Asia [6, 7].

ART-resistant parasites harboring the *kelch13* mutations R561H, A675V, and C469Y are now independently emerging in Rwanda and Northern Uganda, East Africa [8, 9]. Because no alternative to ART derivatives is currently available, the spread of ART-resistant parasites in African settings could threaten the long-term use of ACTs to treat malaria. A continual screening of *kelch13* gene diversity in Africa is mandatory to track the presence of ART-resistant parasites lineages.

Here we surveyed *kelch13* mutations in *P. falciparum* isolates from 125 malaria cases imported in France after a travel to Eastern Africa (Burundi, Djibouti, Eritrea, Ethiopia, Kenya, Rwanda, Sudan, Tanzania, and Uganda) between January 2006 and December 2020 (Supplementary Figure 1). Malaria was diagnosed in French metropolitan hospitals participating to the national malaria survey network (French National Malaria Reference Center, France) using thin or thick blood smear or both. Thick blood smears were considered positive if at least 1 trophozoite was visualized after examination of 1 000 leukocytes. The *Plasmodium* species identification was confirmed microscopically or with polymerase chain reaction (PCR) in case of low parasitemia. Patients were treated according to French recommendations [9].

Using this biobank, we identified by Sanger sequencing 1 adult patient with parasites carrying the *kelch13* A675V mutation (Supplementary Figure 1). The patient first traveled to Northern Thailand (Southeast Asia) and, then, about 1 month later, visited several regions of Uganda (East Africa) in December 2019/January 2020. The patient reported to sleep under insecticide-treated bed net but not to take antimalarial medicines for prophylaxis. At hospital admission in France, 2 weeks after the return from Uganda, *P. falciparum* malaria was diagnosed with hyperparasitemia (765 000 parasites/μL) and other biological criteria of severity. Four intravenous artesunate doses (2.4 mg per kilogram of body weight) were administered at hours 0, 12, 24, and 48, followed by a full course of dihydroartemisinin-piperaquine on day 3 [10]. Although a day-3 follow-up parasitemia is recommended, a day-2 plus 8 hours (56 hours) was done instead for logistical reasons within the intensive care unit. At 56 hours post-treatment, the parasitemia was still positive (20 250 parasites/μL; 37.8-fold reduction compared to pretreatment). No other follow-up parasitemia was available between day 0 and day 7. On days 7 and 36 follow-ups, blood smears were negative, indicating that the overall parasitological response was adequate. As the positive parasitemia at 56 hours was suggestive of a delayed parasite clearance in the traveler, we looked for follow-up parasitemia at a similar timepoint in a reference set of Ugandan infections (n = 229) similarly treated and in which the *kelch13* A675V mutation was frequent [9]. In

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this trial in Uganda, artesunate was administered intravenously at a dose of 3 mg or 2.4 mg per kilogram of body weight (in patients weighing <20 kg or ≥20 kg, respectively) at 0, 12, and 24 hours, followed by a standard 3-day course of artemether-lumefantrine. If parasites were still observed after the initial 3 doses, additional doses of intravenous artesunate were administered every 24 hours until parasite clearance was observed. Follow-up parasitemia were collected at multiple timepoints: 0, 4, and 6 hours and then at 6-hour intervals until 2 consecutive negative blood smears had been obtained. Therefore, we extracted the parasitemia collected at 54 hours in the trial in Uganda as the closest timepoint to the one collected at 56 hours in the traveler. Of the 28 Ugandan patients carrying parasites with the *kelch13* A675V mutation, 10 (35.7%) showed parasite positive at 54 hours, whereas of the 201 patients with the *kelch13* wild-type allele, one only (0.5%) showed parasite positive at 54 hours ($P < .001$ by the Fisher exact test). In this *kelch13* A675V population (n = 28), the largest ratio of parasitemia at 54 hours to pretreatment parasitemia was 0.0098, compared to a ratio of 0.0260 at 56 hours for the *kelch13* A675V from the traveler (Figure 1A). Altogether, despite different epidemiological and demographic contexts, the data are suggesting a delayed parasite clearance in the traveler under artesunate therapy.

We determined the whole genome of the *kelch13* A675V mutant parasites from the traveler by Illumina sequencing using the selective whole-genome amplification strategy (Supplementary Method 1 and Supplementary Tables 1 and 2; next-generation sequence files are accessible on the European Nucleotide Archive under the accession number ERS7266441). This new *kelch13* A675V genome was compared with 131 published African and Asian parasite genomes, including 14 ART-resistant A675V mutants from Uganda recently reported (Supplementary Table 3) [9, 11–13]. Using 20 266 genome-wide, high-quality single-nucleotide polymorphisms (SNPs) (Supplementary Method 2), the phylogenetic tree showed that the parasite isolate from the traveler was genetically highly related to parasites from Uganda but not from Southeast Asia (Figure 1B). This indicated that the traveler got infected in Uganda, rather than during the recent previous trip in Thailand. Also, the A675V mutant from the traveler formed a monophyletic group with previously reported Ugandan A675V mutants, within the East African clade (Figure 1B). The high genetic relatedness of all Ugandan A675V mutants was also evidenced at the *kelch13* locus using a subset of 238 SNPs flanking the *kelch13* gene within ±100 kb (Supplementary Figure 2). We conclude that the traveler got infected by ART-resistant *kelch13* A675V parasites related to those already circulating in Uganda [9].

In conclusion, this study shows that people traveling to East Africa are at risk of being infected with ART-resistant malaria parasites associated with delayed parasite clearance under

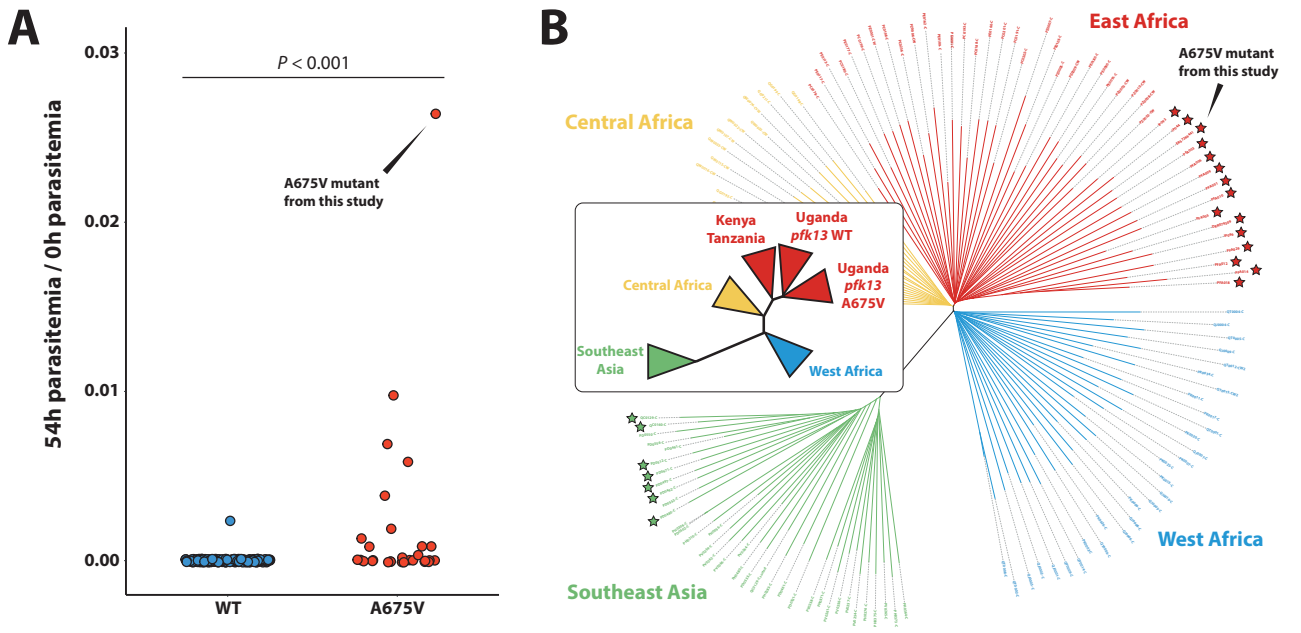

Figure 1. In vivo clearance phenotype and geographic origin of the *kelch13* A675V mutant. *A*, Ratio of parasitemia at 54 hours post-treatment to pretreatment parasitemia by *kelch13* genotype (*blue*: wild-type [WT], *red*: A675V mutation). Data are from Ugandan patients reported in [9]. For the A675V mutant isolate from this study, we estimated the ratio of parasitemia at 56 hours post-treatment to pretreatment parasitemia. Statistical comparison was done using Mann-Whitney *U* test and did not include the data from the traveler. *B*, Genome-wide phylogenetic tree of the 15 *kelch13* A675V parasite isolates (one from this study, and 14 from [9] and [11]) and 117 parasite isolates collected in Sub-Saharan Africa and Southeast Asia [12, 13]. Countries of collection of the samples are Benin, Burkina-Faso, Nigeria for West Africa (*blue*); Congo and Gabon for Central Africa (*yellow*); Kenya, Tanzania, and Uganda for East Africa (*red*); and Cambodia, Myanmar, Thailand, and Vietnam for Southeast Asia (*green*). Tree was inferred by neighbor-joining using 20 266 SNPs. Isolates marked with a green or red star are A675V mutants from Southeast Asia or Uganda, respectively. Abbreviation: SNP, single-nucleotide polymorphism.

treatment with artemisinin derivatives. As highly mobile individuals, travelers may also participate in spreading drug resistance [14]. Here, the traveler visited several regions of Uganda, although we do not know the precise itinerary, and exhibited malaria symptoms (which appear when parasites are circulating in blood) only after returning to France. The traveler therefore likely did not spread this A675V resistant strain in some other parts of Uganda. However, vigilance is mandatory to avoid such a scenario in the future, especially with mid- or long-term travelers. Adequate malaria prevention measures are warranted in travelers to protect from both the disease and drug resistance spread. Also, monitoring of ACT therapeutic efficacy and genotypic surveillance of drug resistance should be carefully conducted for imported malaria.

Supplementary Data

Supplementary materials are available at *Clinical 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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Ethics statement. Samples received at the French National Malaria Reference Center were registered and declared for research purposes as a biobank for both the Assistance Publique des Hôpitaux de Paris and the French National Institute of Health Survey. No institutional review board approval was required according to French legislation (article L. 1111-7 du Code de la Santé Publique, article L. 1211-2 du Code de Santé Publique, articles 39 et suivants de la loi 78-17 du 6 janvier 1978 modifiée en 2004 relative à l'informatique, aux fichiers, et aux libertés). The online reporting system for malaria cases of the French National Malaria Reference Center has been declared to the "Commission Nationale de l'Informatique et des Libertés" (CNIL) under number 1223103.

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