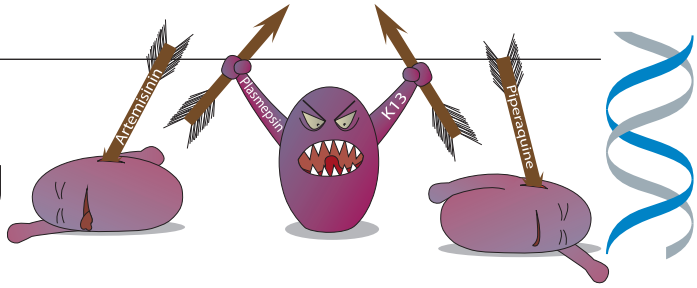
GENOME WATCH

Last parasite standing



Gavin G. Rutledge and Thomas D. Otto

This month's Genome Watch describes how whole-genome sequencing used for surveillance purposes has enabled the identification of new drug resistance markers in the malaria parasite.

Whole-genome sequencing has been used for diagnostic purposes and surveillance for a number of important diseases, including malaria. The systematic spatiotemporal sampling of genetic information provides an invaluable database to track changes in pathogen population genetics and to identify emerging resistance mutations early on. This approach has been successfully applied in malaria research, as the resistance markers for a number of antimalarial drugs have recently been identified using sequencing approaches.

Artemisinin combination therapies are the first-line drug regimes used to treat uncomplicated *Plasmodium falciparum* infections throughout all malaria-endemic regions. Owing to its short-acting half-life, artemisinin is administered with a long-acting partner drug such as piperaquine, which should theoretically slow down the evolution of antimalarial drug resistance, based on the assumption that parasites that survive the short-acting artemisinin would subsequently be 'cleaned up' by the longer-acting partner drug. However, despite these preventative measures, artemisinin resistance (identified by a reduced rate of clearance), started to emerge in 2009 (REF. 1) and is now highly prevalent in different regions of Southeast Asia. In addition, *Plasmodium* strains that have acquired a similar form of resistance (that is, exhibit a decreased clearance speed) to piperaquine have also emerged in recent years.

Two independent studies[2,3] performed genome-wide association studies (GWAS) using whole-genome sequences from routinely collected clinical isolates of *P. falciparum*, and identified a copy number amplification in two haemoglobin protease genes, plasmepsin 2 and plasmepsin 3, as the possible molecular marker for piperaquine resistance.

The first study[2] analysed 297 *P. falciparum* whole-genome sequences that were collected in Cambodia between 2010 and 2013, and described the rising incidence of piperaquine failure in regions with high levels of artemisinin resistance. Using a GWAS, with piperaquine IC_{50} (half-maximal inhibitory concentration) as the continuous dependent variable and correcting for confounders including artemisinin resistance and population structure, the authors identified both a nonsynonymous single-nucleotide polymorphism (SNP) in an exonuclease gene and the copy number amplification of the genes encoding plasmepsin 2 and plasmepsin 3 as genetic markers associated with piperaquine resistance. The extent of linkage disequilibrium between these markers seemed to be substantial. Using an *in vitro* survival assay of 12 clinical isolates, they showed that both markers are associated with higher piperaquine survival rates and that the presence of either of the two markers resulted in a ~40% treatment failure in 133 clinical samples.

In the second study[3], 725 patients from Cambodia were enrolled in a clinical study from 2009 to 2015 to assess the efficacy of dihydroartemisinin–piperaquine. The authors performed GWAS using 31 isolates that were identified to be artemisinin-resistant and either resistant or sensitive to piperaquine, and identified with high confidence the copy number amplification of the plasmepsin 2 and plasmepsin 3 gene cluster on chromosome 14. The amplification was present in 22 out of 23 piperaquine-resistant isolates. Using plasmepsin 2 as an amplicon reporter in 134 clinical isolates that were subjected to a piperaquine survival assay, isolates with two or more copies of plasmepsin 2 were shown to be significantly more resistant to piperaquine than those with only one copy. In addition, the authors[3] showed that multicopy plasmepsin 2 was significantly enriched in isolates from patients who exhibited higher recrudescence rates of *P. falciparum* malaria, and their analysis revealed a potential dose-dependent effect as the disease recurred more frequently in patients with isolates that contained a triplication than isolates with only a duplication.

The observation that piperaquine resistance is developing rapidly in regions with high levels of artemisinin resistance supports the idea that the higher residual parasite biomass following failed artemisinin treatment predisposes for the evolution of piperaquine resistance, essentially selecting for a multidrug-resistant 'last parasite standing'. This underscores the importance of surveillance and tracking of resistance to make informed decisions on drug administration. The identification of molecular markers such as kelch 13 for artemisinin resistance[4] and now the amplification of the plasmepsin 2 and plasmepsin 3 gene cluster for piperaquine resistance[2,3] will facilitate this, but only continued sampling of whole-genome sequences will enable the rapid identification of novel resistance markers when they arise[2,3] as well as shed light on the genomic context of these markers.

Gavin G. Rutledge and Thomas D. Otto are at the Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.
microbes@sanger.ac.uk

doi:10.1038/nrmicro.2016.181
Published online 9 Dec 2016

1. Dondorp, A. M. *et al.* Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **361**, 455–467 (2009).
2. Amato, R. *et al.* Genetic markers associated with dihydroartemisinin–piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype–phenotype association study. *Lancet Infect. Dis.* http://dx.doi.org/10.1016/S1473-3099(16)30409-1 (2016).
3. Witkowski, B. *et al.* A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype–genotype association study. *Lancet Infect. Dis.* http://dx.doi.org/10.1016/S1473-3099(16)30415-7 (2016).
4. Ariey, F. *et al.* A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* **505**, 50–55 (2014).

Competing interests statement
The authors declare no competing interests.