GENOME WATCH

Juggling resistance mutations

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This month's Genome Watch discusses how a better understanding of the genetic mechanisms of antimalarial drug resistance can maximize the utility of genomic surveillance programs in informing the most effective drug policy.

A multidrug-resistant malaria parasite strain is currently spreading throughout the Greater Mekong subregion in Southeast Asia¹, resulting in the failure of first-line artemisinin combination therapies in clearing malaria infections. Genome analysis of more than 1000 *Plasmodium falciparum* samples from Southeast Asia revealed that this particular strain seems to have become susceptible to mefloquine², an antimalarial drug that was not in use at the time but has now been reintroduced. This information is vital, as the antimalarial drug-development pipeline is thin and mostly includes derivatives of existing drugs, to which resistance has often already spread. A better understanding of the different drug resistance profiles that parasites

can develop, such as cross-resistance or mutually exclusive resistance phenotypes, is crucial to most effectively use the limited antimalarial drugs that are already approved. This would enable a more tailored approach to drug deployment in the field.

Recently, Cowell et al.³ developed an in vitro evolution system

using three isogenic *P. falciparum* parasites that were exposed to more than thirty small molecules, some of which were previously identified to have potent antimalarial activity in vitro. The authors sequenced the entire genome of the strains that acquired resistance to a specific compound and compared them with those of the sensitive parental strains. This data revealed both unknown as well as known genes that were previously shown to be involved in drug resistance. Examples of the latter include the *pfcr* and *pfmdr1* genes, which have been implicated in resistance to the established antimalarial drugs chloroquine and mefloquine, respectively. The authors showed that these two genes confer resistance to one-quarter of the compounds tested in this study. Variants in genes not previously linked to drug resistance, such as a gene encoding an AP2 transcription factor, also seemed to provide cross-resistance to multiple compounds. Although the loss of efficacy of most compounds seemed to be associated with changes in a specific gene, some compounds, such as GNF179, became ineffective possibly owing to mutations in several genes, which suggests that multiple resistance mechanisms to this compound can develop.

It is an important insight that many of the genes implicated in conferring resistance to the compounds used in this study have previously been associated with resistance to established drugs. The molecular mechanism of resistance is well understood for these genes, shedding light on the potential mode of action of the new compounds. By contrast, this finding also raises questions in terms of cross-resistance. A new compound may be ineffective on arrival in the field if resistance to it is already widespread as a result of another drug having been deployed previously. Understanding the natural genetic variation of the local parasite population can inform us in that regard. At the same time, multiple drug resistance phenotypes resulting from changes in the same gene may open

up the possibility of uncovering compounds that result in mutually exclusive changes. These drug resistance genes often encode proteins that perform important biological functions within the parasite, and, indeed, drug resistance mutations frequently reduce the biological fitness of the parasites that harbour them. Compounds that select for different mutations in the same gene could result in combinations of resistance mutations that yield a non-viable protein. Like juggling balls, if you have too many in the air, eventually they might all come falling down. This in turn would mean that resistance to those compounds cannot arise unless (or until) the parasites develop completely different resistance mechanisms. Uncovering such a scenario would open up the possibility of developing resistance-proof drug treatments.

The methodology developed by Cowell et al.³ now enables us to explore these questions about mutually exclusive drug resistance mutations, as well as compensatory mutations, and the functional basis of cross-resistance. Combining this understanding of the genetic mechanisms of drug resistance evolution with ongoing genomic surveillance in the field will enable the identification of rapidly emerging resistance and enable informed choices about the antimalarial treatment strategy.

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Competing interests

The authors declare no competing interests.