During a half-century of global efforts to conquer malaria, scientists have developed a series of anti-malarial drugs, only to see them defanged, one by one, by the shape-shifting parasite’s ability to rapidly evolve drug-resistant variants.

Today, with massive new malaria eradication campaigns launched against the disease that kills nearly a million people every year in Africa, drug resistance remains a critical problem—and one whose molecular underpinnings are poorly understood.

The antimalarial medicine chest has dwindled to a single reliably effective compound, artemisinin, which became available in 2005. And now doctors in Southeast Asia are seeing signs that artemisinin may be next on the parasite’s hit list.

“If resistance to artemisinins develops and spreads to other large geographical areas, as has happened before with chloroquine and sulfadoxine-pyrimethamine (SP),” the World Health Organization warned in 2009, “the public health consequences could be dire, as no alternative antimalarial medicines will be available in the near future.”

**EARLY WARNINGS KEY**

Today, these rogue strains are usually recognized only when patients stop responding to a drug, says Dyann Wirth, chair of the Department of Immunology and Infectious Diseases at Harvard School of Public Health and a co-director of the Infectious Disease Initiative, a collaboration that involves Harvard University and the Broad Institute of MIT and Harvard. “By the time you find a very resistant parasite,” she says, “you’ve already lost the battle.”
With this threat always at hand, new research at HSPH is addressing an urgent need: early warning methods to detect the first signs of drug-resistant malaria strains, so that a swift response might keep them from gaining a foothold.

**WATCHING RESISTANCE UNFOLD**
In April, Wirth and other leaders of the Initiative reported on a powerful combination of genome search methods that enabled them to discover new resistance genes in *Plasmodium falciparum*, the malaria parasite. They even used one of these genes to convert a docile, easily killed parasite into a resistant one.

“We really didn’t know what to expect,” says Daria Van Tyne, a graduate student in Wirth’s lab and co-first author of the study, which appeared in *PLoS Genetics*. “This is the first time anyone has observed resistance as it’s happening.”

At the molecular level, little is known about the pathways leading to resistant phenotypes. At HSPH, Wirth and her colleagues are looking for clues by comparing the genomes of resistant parasites with “sensitive” or nonresistant organisms.

“Our approach is to develop a tool that will enable us to observe a tendency toward loss of drug sensitivity in a population before the problem is well established,” explains Wirth. “The goal is to make the discovery early so you can use that knowledge to focus efforts,” such as enforcing appropriate drug-use guidelines.

**FIVE FIRST AUTHORS**
The *PLoS Genetics* paper is unusual in having five co-first authors (including Van Tyne) and three senior authors: Wirth, HSPH research scientist Sarah Volkman, and Pardis Sabeti of the Broad Institute and Harvard.

“This is a very strong collaboration,” Wirth notes. The research drew on HSPH expertise in parasite biology and drug resistance mechanisms, the Broad Institute’s resources in sequencing, genotyping, and computational methods, and field researchers in Senegal who track the spread of resistance. Contributions also came from investigators at other U.S. universities and at universities in Senegal and Nigeria.

**SEARCHING FOR RESISTANCE GENES**
The National Institutes of Health maintains a depository of malaria parasites from around the world collected over decades. Tapping this resource and the labs of HSPH partners in Africa, the researchers collected 57 parasites from three continents—some of them sensitive to antimalarial drugs, and others resistant to one or more. Though the first *P. falciparum* genome was sequenced in 2002, only recently have malaria scientists begun genomic searches for resistance genes.

For their study, the HSPH and Broad investigators began by scanning the parasites’ DNA for regions that had undergone recent evolutionary changes, some of which might reflect adaptation under selective pressure from malaria drugs. With this method, the scientists identified 15 genes showing signs of recent selection.

Determining which of these genes were implicated in resistance required a second step—a genome-wide association study, or GWAS. The GWAS search drew on HSPH researchers’ expertise in molecular biology and drug resistance, combined with the Broad’s prowess in gene sequencing and computational biology.

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The GWAS was designed to home in on genetic variants present in parasites resistant to 13 antimalarial drugs, but not present in drug-sensitive ones. The scientists constructed a fine-resolution map of more than 17,000 points of reference, called SNPs, spaced regularly throughout the genomes; it was the densest SNP array yet applied to the malaria parasite.

“By the time you find a very resistant parasite, you’ve already lost the battle.”

—Dyann Wirth, chair, HSPH Department of Immunology and Infectious Diseases

“We had no idea if it was going to work or not,” Van Tyne said of the two-pronged method. Much to the scientists’ relief, the searches immediately detected a gene for chloroquine resistance that had been discovered previously with older techniques. This validation that they were on the right track “made us very happy when we saw it,” recalls Van Tyne. “It gave us a green light for the rest of the study.”

GENES TO THE GLOBE

Wirth and others involved in the project say the new genomic search tool opens up “endless possibilities” that could lead to improvements in detecting and combating antimalarial resistance.

“The paper we published is just the tip of the iceberg,” explains Van Tyne. With these new genomic tools, researchers can search not only for resistance genes, but also other mutated or variable genes that govern parasitic traits and the different outcomes that infected people experience.

Their greatest hope is that distinctive resistance-related mutations could become the basis of simple blood tests, able to be performed in community clinics in Africa, that could determine when a drug like artemisinin is losing its effectiveness in a certain population or geographic area. According to Wirth, “That would tell us that we need to use other drug combinations and implement focused intervention strategies to prevent the spread of resistant parasites.”

And, Van Tyne says, “this would all feed into research that could yield better antimalarial drugs.”

It would be hard to find a better story illustrating Dean Julio Frenk’s “genes to the globe” concept, adds Wirth. “Harvard has the ability to approach a problem like malaria from a fundamental understanding of the biology all the way to global issues like policy, finance, and all the pieces needed to address public health problems.”

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