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Human Malaria Parasite: Are We Ready for a New Species?

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There are four well-established human malaria parasites, although a nonhuman primate malaria parasite, *Plasmodium knowlesi*, can also infect humans [1]. *Plasmodium ovale* is one of the human malaria parasites, but it traditionally receives little attention because of its mild disease and relatively low infection rate. An intriguing and provocative report in this issue of *the Journal* by Sutherland et al. raises the possibility of two distinct parasite species under the name *P. ovale*.

P. ovale was established as a species by Stephens in 1922 after observing oval-shaped infected erythrocytes in the blood of East African patients [2]. Malaria parasites are small protozoan organisms living within human blood cells, and are commonly recognized by microscopic observation of Giemsa-stained blood smears from patients. Various morphologic and developmental characteristics have been employed to distinguish the parasite, These include the size and shape of infected red blood cells and parasite stages, the time required to complete their life cycles, their host preference and specificity, and the disease symptoms they cause. *P. ovale* is morphologically similar to another human malaria parasite, *Plasmodium vivax*, and it can be difficult to tell them apart in blood samples in regions where both parasites are present [3]; however, they can be distinguished using established differences in morphology and other characteristics [4,5].

The introduction of molecular techniques has greatly improved the diagnosis of malaria parasites. Genus- and species-specific PCR methods have been developed to assist parasite identification [6,7]. *P. ovale* is differentiated from other malaria species using species-specific PCR primers designed based on sequences encoding the parasite's small subunit ribosomal RNA (SSU rRNA) and other genes. Applying species-specific amplification method and DNA sequencing, it has been shown that *P. ovale* parasites worldwide belong to two genetic haplotypes: “classic” and “variant” [8,9]. Application of molecular techniques has also led to discovery of the parasites in many regions not previously known to have *P. ovale*, particularly in many Asian countries [5]. In the current report, Sutherland et al. examined genetic polymorphisms in 55 *Plasmodium ovale* isolates obtained from 12 African and 3 Asian countries. They confirmed the presence of complete dimorphism in five out of six loci located on different chromosomes. One isolate from Papua New Guinea had eight classic residues and 17 variant residues in the SSU rRNA gene, but the classic residues appeared to be derived from nucleotide substitution or mutiple recombination events that were unlikely. They wondered why they did not see genetic exchanges between the two forms of parasites in their samples. Because the variant and classic forms occured in sympatry, the genetic differentiation between them cannot be explained by present day geographic isolation [9], although historical allopatry cannot be ruled out. They found both variant and classic forms in five African countries, and yet no evidence of inter- or intragenic recombination between the classic and variant forms was observed in their samples. The observations suggest independent segregation multigenic haplotypes and the existence of a species barrier, leading to a conclusion that the classic and variant *P. ovale* are, in fact, two distinct, non-recombining species. The authors, therefore, named the species *Plasmodium ovale curtisi* (classic type) and *Plasmodium ovale wallikeri* (variant type) in

honor of two outstanding malariologists: Drs. Christopher F. Curtis (1939–2008) and David Walliker (1940–2007), respectively.

Further evidence supporting their conclusion is the relatively large genetic distance between the two parasite forms, similar to or greater than that seen between the pair of *Plasmodium fieldi*/ *Plasmodium simiovale* or the pair of *P. vivax*/*Plasmodium simium* (**Figure 2** in the report). Additionally, the variant-type *P. ovale* has been associated with higher levels of parasitemia in humans [10,11], suggesting that more dramatic biologic and clinical differences between these two types of parasites may exist. Further studies are necessary to investigate whether the two parasites differ in disease manifestations, including the pattern of relapse.

Although the evidence from this and other studies strongly suggest that the two types of *P. ovale* are two distinct species, many questions remain regarding the actual mechanism of speciation before a definitive conclusion can be reached. For example, how have these parasites evolved independently if they can infect the same host species and have coexisted in the same geographic locations? Potential explanation for the observed dimorphisms include geographic isolation in the past or two distinct host switches from primates to humans, separated by sufficient time to allow divergence between lineages. Host switching between nonhuman and human hominids has been suspected in other malaria parasites [12,13]. As to why the dimorphism persists in the present, there exit several potential explanations in addition to the suggested species barrier. One possibility, as the authors point out, is that the two parasite types may have mutually exclusive mosquito specificities. Some mutations that allow one parasite to adapt to a new mosquito species may explain the differentiation; however, we do not yet have any evidence to support this hypothesis. It is also possible that the observed genetic dimorphism is perpetuated by a low transmission rate and/or clonal infection. Because *P. ovale* can only invade young erythrocytes, the infections usually result in low parasitemia [3]. Additionally, it has been shown that infection with *P. ovale* can generate strong and relatively long lasting (partial protection after six years) protection against re-infection, even with heterologous strains [14]. Acquired immunity will prevent a secondary infection and greatly reduce the chance of an individual carrying parasites with different genotypes, and the chance of genetic recombination in a mosquito.

To address these issues, it is important to estimate the transmission rates, the frequencies of natural recombination and the `neutral' genetic distances of the parasites, using more parasite samples. To definitively confirm that a reproductive barrier exists between the two *P. ovale* types, one can perform a genetic cross by feeding mosquitoes with blood samples infected with the variant and classic types. Sporozoites can be isolated from the mosquito and injected into a chimpanzee (or infected mosquitoes can be allowed to bite a chimpanzee). Parasites can be cloned from the chimpanzee blood, and DNA from individual parasites can be typed with genetic markers to determine the haplotypes of the cloned parasites. Unfortunately, without in vitro culture for the parasites, cloning parasites could be challenging. Alternatively, DNA can be isolated from a single sporozoite and genotyped with multiple genetic markers from different chromosomes to look for recombinant parasites. Regardless of whether we accept the authors' claim of two species based on the genetic evidence presented, this report certainly raises many interesting questions and opens up a new field of investigation.

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