

Malaria: a peek at the *var* variorum

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Geneticists encountering the diversity of the malaria parasite's *var* gene family for the first time often complain that its complexity is a nightmare. A new article by Barry *et al.* presents the latest and most systematic attempt to date to decipher the *var* variorum. This important work, combined with other recent articles on *var* global variation such as that by Kraemer *et al.*, suggests that only the tip of the *var* diversity iceberg is currently in view. In this article, we discuss these recent results and provide an overview of current understanding of *var* diversity.

The *var* gene family

Plasmodium falciparum is unique among the parasites that cause malaria in humans in its ability to induce cytoadhesion of the red blood cells that it infects. This property is a consequence of the placement of a protein called *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on the infected cell surface and the resulting adhesive properties are a major virulence determinant of *P. falciparum* infections. PfEMP1 is a large protein (200–350 kD) that spans the red blood cell membrane, with the extracellular portion typically consisting of multiple ligand-binding domains referred to as either Duffy binding-like domains (DBLs; see Glossary) or cysteine-rich interdomain regions (CIDRs). The position of PfEMP1 on the red blood cell surface enables the cell to interact with receptors on the vascular endothelium, thus mediating adhesion; however, this position also makes it particularly vulnerable to recognition by antibodies produced by the host. To avoid antibody-mediated destruction, *P. falciparum* has evolved a strategy of antigenic variation based on alternating expression of individual *var* genes, each of which encodes an antigenically distinct form of PfEMP1 [1]. The genome of each parasite contains ~60 *var* genes that are expressed in a strict, mutually exclusive fashion, with the expressed copy determining both the antigenic and cytoadhesive properties of the infected cells [2]. Further, comparison of the *var* gene repertoires of different parasite isolates indicates that each contains its own complement of PfEMP1 forms, thus exposure to multiple *var* gene products during one infection does not necessarily confer immunity to future malaria infections [3–5]. This highly effective strategy for avoiding the immune system of the human host is entirely dependent on the extremely high level of sequence diversity within the *var* gene family.

Big questions

High levels of *var* gene polymorphism observed in isolates from around the world provided the first suggestion of virtually unlimited *var* gene repertoires [3–5]. However, a more extensive and systematic analysis of parasites within a specific locality, as well as from around the world, was required to determine if there might be geographical or temporal patterns among *var* gene subtypes. The work of Barry *et al.* represents an extraordinary effort to gather the large amount of data needed to address these questions, and provides the information necessary to begin to understand how *var* diversity and selection pressure influence malaria pathogenesis [6].

Round up the usual suspects

Surveys of *var* gene sequences have generally relied on the use of DBL- α degenerate PCR primers [4]. These primers have been optimized to amplify sequences encoding the most highly conserved domain, called DBL- α , found in the vast majority of PfEMP1 proteins [7]. The use of this amplification strategy enables researchers to obtain a relatively unbiased sampling of *var* gene sequences even from small amounts of parasite material. It should be noted, however, that whereas these primers amplify DBL- α sequences from the vast majority of *var* genes, certain sequences, most notably that of the gene associated with pregnancy-associated malaria (*var2csa*), are not efficiently amplified using this strategy and thus must be considered separately [8].

To explore the complexity of the global *var* gene repertoire, Barry *et al.* obtained 895 DBL- α types culled from a global dataset of 1088 sequences (480 of the 895 DBL- α types were taken from the GenBank database), a much larger sample than available from previous studies [3–6]. Their parasite isolates differed not only geographically, but also temporally. The data constituting the global sample

Glossary

DNA shuffling: exchange between similar DNA sequences. The term usually refers to a recombination of domains to create novel functions.

Duffy binding-like domain α (DBL- α): an adhesive domain in *Plasmodium* proteins. PfEMP1 contains several DBL domains and they were classified into α , β , γ , δ and ϵ classes [23].

Gene conversion (duplicative transposition): a process in which a DNA double-stranded break is repaired using another similar sequence resulting in the generation of a new DNA copy of the template sequence.

Reciprocal recombination: a process in which two double-stranded DNA molecules undergo breakage and reunion.

were extracted from 59 field isolates and cultured lines collected as early as 1979 for the reference strain 3D7, and as recently as 2002 for some Indian strains [9]; this sample represents parasites from the equatorial regions in Africa, Asia, the Indian and Pacific Oceans, and Latin America. By contrast, the 460 DBL- α sequences isolated from the Amele population in Papua New Guinea (PNG) were collected from a limited geographical area of only a few square kilometers and in a matter of months rather than the usual years [10]. Thus, by comparing the local and global datasets, it was possible to investigate the possibility of underlying geographic structuring that had not previously been observed.

Tip of the iceberg

Barry *et al.* [6] applied a technique for estimating total *var* gene diversity based on the fraction of all the sequences represented multiple times in the sample. Sequences from various isolates were therefore compared, but after examining more than one thousand DBL- α sequences, the saturation point in diversity from the global samples could not be reached. This result indicates a vast amount of global *var* gene diversity. It confirms the complexity of the global *var* gene repertoire observed in studies with fewer samples. However, their data also suggest a limited amount of *var* gene diversity among isolates collected from PNG. In other words, *var* repertoires of parasites found within specific geographical regions show a greater degree of overlap among themselves than with the global samples, a finding that has important implications for the acquisition of long-term immunity by exposed individuals. Reduced intraregional variation seems to be the case here, but one might not be able to exclude the possibility of clonal infections among PNG isolates. The only way to address this point is to study *var* diversity from more regions around the world. Recently, Albrecht *et al.* reported overlapping *var* repertoires in Brazilian isolates [11]. This is consistent with Barry's hypothesis of local population structure.

Interestingly, *var1csa* seems to be rare among PNG isolates. The *var1csa* gene is a *var*-like gene that was originally identified as a *var* associated with chondroitin sulfate A (CSA) placenta binding [12]. The gene is highly conserved in several isolates; however, its function is unknown, and it seems to be aberrantly expressed and might, in fact, be a pseudogene [13,14]. The fact that *var1csa* is not common among PNG isolates suggests that it does not confer a selective advantage at least within this geographical region, and therefore the presence of this gene in parasites from other regions is of uncertain significance.

Diversity within large gene families can be generated through processes that involve DNA recombination, specifically through either reciprocal recombination or gene conversion (sometimes called duplicative transposition). Such recombination results in 'shuffling' of DNA sequences and, in the case of *var* genes, can lead to the rapid generation of new forms of PfEMP1 [15,16]. Analysis of the completed genome sequence of the 3D7 reference strain suggests that there is a hierarchy for determining which *var* genes recombine with one another based on

either chromosomal position or promoter type, leading to the evolution of distinct *var* gene subtypes [17,18]. A Gaussian (normal) distribution in DBL- α alignment was suggested by Barry *et al.* [6] as circumstantial evidence for the homogenizing effects of recombination. The extent to which reciprocal recombination and gene conversion drive the concerted evolution of multigene families depends on the rates of these processes relative to that of mutation. A formal population genetic theory of genetic diversity of the *var* gene family incorporating these processes has not been developed, and so it remains unclear how the shape of the distribution of pairwise alignment scores or its variance would depend on the parameters. Then, too, selection is expected to have a strong role in maintaining *var* gene diversity, and some DBL- α sequences are common among global isolates suggesting selection for these specific types. In a complementary study, Kraemer *et al.* analyzed fewer global isolates, but they also noticed low sequence identity among *var* alleles [19]. These authors pointed out similarities between *var* subtypes from different isolates, which might further implicate a hierarchy of recombination in which some subsets of sequences preferentially share sequence information with others or a functional constraint for each *var* subtype. It should prove interesting to study what influences the tentative hierarchy; possibilities include sequence homology, subtype-specific function, gene location or a recombination mechanism recognizing specific consensus sequences. In addition, it is not known whether *var* gene recombination occurs primarily during meiosis, mitosis or both, and whether it occurs spontaneously or in response to a specific stimulus.

Concluding remarks

The recent findings on *var* gene diversity will hopefully encourage broader analysis of *var* subpopulations from other geographical regions. It will be invaluable to analyze the temporal changes over time or as new *P. falciparum* strains invade endemic areas. It might seem counterintuitive to use the *var* gene family as the basis for vaccine or therapeutic targets owing to its extraordinarily high polymorphism. However, the well-studied *var2csa* is now regarded as a promising vaccine target for pregnancy-associated malaria [20–22]. With better understanding of *var* gene diversity and function, we might be able to take advantage of specific *var* subtypes beyond *var2csa*. After all, the *var* gene family remains the best defined factor contributing to malaria pathogenesis.

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Live immunisation against *Theileria parva*: containing or spreading the disease?

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Although a live vaccine against *Theileria parva* has been available for over 30 years, concerns that vaccine strains can become established in resident tick populations have impeded its uptake in endemic areas. Recently, Oura *et al.* have examined the persistence of vaccine strains in immunised cattle using polymorphic genomic markers. They confirm that elements of the vaccine establish a carrier state in vaccinated animals and present evidence that alleles associated with vaccine strains emerge in co-grazing non-vaccinated cattle. However, the epidemiological impact of these observations might be tempered by extensive recombination of co-ingested strains in the tick vector.

Vaccine-based control strategies for vector-borne diseases

Vector-borne diseases present several challenges for vaccine-based control strategies. These include variations

in challenge intensity associated with vector dynamics, emergence of novel pathogen variants in the vector population and the opportunities for immune evasion by the pathogen through its recombination in the vector. Such complexities often complicate the prediction of vaccine performance under field conditions, particularly when the vaccine itself gives rise to transient patent infection. Two recent papers by Oura and colleagues [1,2] provide a powerful insight into these issues with respect to *Theileria parva*, an apicomplexan parasite of cattle transmitted by *Rhipicephalus appendiculatus* ticks. This parasite invades and transforms lymphocytes, causing a severe lymphoproliferative disease known as East Coast fever (ECF) in eastern, central and southern Africa.

Theileria parva and ECF

The life cycle of *T. parva* is similar to that of malaria, with asexual expansion in the mammalian host and a brief sexual phase in the arthropod vector. The major replicating stage is the schizont, which transforms infected lymphocytes and divides in synchrony with them, ensuring

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