LIMITED POLYMORPHISM IN PLASMODIUM FALCIPARUM
SEXUAL-STAGE ANTIGENS

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Abstract. In areas highly endemic for malaria, individuals are frequently found to be infected simultaneously with multiple Plasmodium falciparum clones. This raises the question of whether all parasite clones produce gametocytes equally or whether a dominant clone would suppress gametocytogenesis of the other clones. This information would have a large impact on our understanding of recombination rates, population structure, and dynamics of specific genetic traits such as drug resistance. These questions can be addressed by selecting polymorphic genes which are specifically expressed in sexual stages for analysis. Gametocytes can be detected by reverse transcribed polymerase chain reaction (RT-PCR) and different alleles of stage-specific expressed genes can be genotyped. Simultaneously, asexual stages can be genotyped for the same locus by genomic amplification. Such studies were not feasible until recently, because no robust RT-PCR-technique to amplify gametocyte-specific mRNAs was available. Additionally, little sequence information was available on gametocyte-specific genes. So far few variations are known in the sequences of Pfs16 and Pfs48/45; whereas greater polymorphism has been reported for Pfs230.

Babiker and others recently developed an RT-PCR-based detection for gametocytes using Pfs25, an antigen expressed late during gametocytogenesis. In a previous study we examined the expression of the gametocyte-specific genes Pfs16, Pfs48/45, and Pfs230 by reverse transcribed polymerase chain reaction amplifying expressed gametocyte-specific mRNAs was available. Additionally, little sequence information was available on gametocyte-specific genes. So far few variations are known in the sequences of Pfs16 and Pfs48/45, whereas greater polymorphism has been reported for Pfs230. Babiker and others recently developed an RT-PCR-based detection for gametocytes using Pfs25, an antigen expressed late during gametocytogenesis. In a previous study we examined the expression of the gametocyte-specific genes Pfs16, Pfs48/45, and Pfs230 by reverse transcribed polymerase chain reaction amplifying expressed gametocyte-specific mRNAs was available. Additionally, little sequence information was available on gametocyte-specific genes. So far few variations are known in the sequences of Pfs16 and Pfs48/45, whereas greater polymorphism has been reported for Pfs230.

Samples were analyzed in an automated sequencer (ABI PRISM® 310 Genetic Analyzer, Perkin Elmer, Rotkreuz, Switzerland). To establish a rapid typing scheme, 10 μl PCR product was digested with 5 U Nla III (New England Biolabs Inc., Beverly, MA) and run on 10% polyacrylamide gels in TBE buffer.

Pfs16 from 7G8 and four field isolates from Papua New Guinea (SN211, SN246, SN249, SN250) had new but identical nucleotide sequences (Genbank® accession number AF177633). The sequence differed from the published NF54 sequence (EMBL accession number M64705) by three nonsynonymous point mutations and a 6 base pair (bp) deletion. All mutations were found within recognition sequences of restriction enzymes and could be detected by restriction digests (Table 2).

Strains W2, SN211, SN246, and SN249 had an identical...
sequence of *Pfs48/45* (Genbank® accession number AF177636), whereas HB3 (AF177634) and blood sample P28 (AF177635) represented two different alleles. All three alleles differed from the published sequences (EMBL accession numbers Z22145 and X81648). Five non-synonymous and one silent mutation marked the differences. Four mutations were located within the recognition sites of restriction enzymes (Table 2). No additional polymorphism was seen with field samples.

A 2.4 kb PCR fragment of *Pfs230* from culture strains was digested by different restriction enzymes. Figure 1 shows *Nla* III digests separated on a 10% polyacrylamide gel, which revealed size and sequence polymorphism, such as an additional restriction site in the ItG2.F6 and SN246 sequence. Further size polymorphism was observed in a 210 bp restriction fragment of 7G8 (Figure 1). Sequencing of this 210 bp region from ItG2.F6 (AF177637) and MAD20 (AF177638) revealed that this size polymorphism derived from repeats of CAA (glutamine) and GAA (glutamate), differing in arrangement and copy number as shown in Figure 2. Amplification of this region from patient samples also revealed such size polymorphism (Figure 3). Further analysis by PCR-RFLP-analysis of an additional 2 kb fragment of *Pfs230* showed no further polymorphism.

In conclusion, polymorphism in gametocyte-specific expressed genes is larger than previously reported, but the degree of polymorphism in these genes is not comparable with polymorphism observed in erythrocyte-stage antigens of *P. falciparum*. It supports the hypothesis that polymorphism in the latter genes is driven by host responses, whereas gametocyte-specific antigens may not be selected by the human host or selection may only be very limited. Whilst the analysis of diversity of a single erythrocyte-stage antigen allows sufficient parasite clone discrimination, analysis of single gametocyte-specific antigens will not allow such powerful discrimination. Polymorphism of *Pfs16* is very limited and although several alleles could be identified for *Pfs48/45*, it will be difficult to determine these minute differences in field studies. *Pfs230* exhibits a reasonable degree of diversity and could be used as marker for genetic diversity of gametocytes. Since we analyzed only approximately 50% of the 10 kb coding region, it is possible that additional polymorphism...
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FIGURE 2. Alignment of four different amino-acid sequences of a polymorphic region in Pfs230. The fragment spans position 2408 to 2426 in the NF54 amino acid sequence. The sequences were imported from the EMBL database (accession numbers: 7G8: L22219, NF54: L08135) or translated from nucleotide sequences obtained by direct sequencing (GenBank® accession numbers: MAD20: AF177638, ItG2.F6: AF177637).

might exist within this gene. Further sequence data from field samples are needed to develop a typing scheme with a high discrimination power thus allowing the assessment of transmission dynamics and genetic diversity of gametocytes, and to elucidate the likelihood of recombination within a mosquito after a single blood meal.

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