Published in final edited form as:
*Am J Trop Med Hyg*. 2007 May ; 76(5): 855–859.

# SHORT REPORT: RARE *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN 1 19-KDA ($MSP\text{-}1_{19}$) HAPLOTYPES IDENTIFIED IN MALI USING HIGH-THROUGHPUT GENOTYPING METHODS

**SHANNON L. TAKALA**, **DAVID L. SMITH**, **MAHAMADOU A. THERA**, **DRISSA COULIBALY**, **OGOBARA K. DOUMBO**, and **CHRISTOPHER V. PLOWE***
Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland; Fogarty International Center, National Institutes of Health, Bethesda, Maryland; Malaria Research and Training Center, University of Bamako, Bamako, Mali

## Abstract

Genetic diversity in malaria vaccine antigens may compromise malaria vaccine efficacy, so it is important to understand this diversity and the processes that generate it. By applying new high-throughput genotyping methods to a large sample of infections from Mali ($N$ = 1369), seven new 19-kDa merozoite surface protein 1 ($MSP\text{-}1_{19}$) haplotypes were identified. Herein we report the sequences of these new haplotypes and discuss their possible origins. Although they are present in < 1% of the samples examined, the existence of these rare haplotypes reveals a greater degree of diversity at this locus than previously reported and highlights the potential for *Plasmodium* to evolve under selective pressure from the immune system and from such interventions as vaccines and drugs.

## INTRODUCTION

Progress toward a malaria vaccine has been slow due in part to the extensive genetic variability in *Plasmodium*. Such genetic variability is generated through mutation under selective pressure from the human immune system and sexual recombination in the mosquito vector, and is particularly prevalent in surface antigens being targeted for malaria vaccines.[1] Merozoite surface protein 1 is a candidate antigen for a blood-stage malaria vaccine. The 195-kDa precursor of this protein undergoes two rounds of proteolytic cleavage, leaving only the C-terminal 19 kDa on the surface of the merozoite as it invades the erythrocyte.[2] $MSP\text{-}1_{19}$ contains two epidermal growth factor (EGF)-like motifs that are thought to play a role in erythrocyte invasion.[3] Antibodies to this region can block erythrocyte invasion in vitro[2] and are associated with protection from clinical malaria in field studies.[4–9] The sequence of $MSP\text{-}1_{19}$ is highly conserved[10]; however, six non-synonymous single nucleotide polymorphisms (SNPs) have been documented at amino-acid positions 1644, 1691, 1699, 1700, 1701, and 1716,[10–14] and it is unclear how these polymorphisms affect immunity. Intragenic recombination has been proposed as an important mechanism for generating novel

*Address correspondence to Christopher V. Plowe, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD 21201. E-mail: cplowe@medicine.umaryland.edu.
Authors' addresses: Shannon L. Takala and Christopher V. Plowe, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, Telephone: +1 (410) 706-3082, Fax: +1 (410) 706-6205, E-mail: cplowe@medicine.umaryland.edu. David L. Smith, Fogarty International Center, National Institutes of Health, Bethesda, MD. Mahamadou A. Thera, Drissa Coulibaly, and Ogobara K. Doumbo, Malaria Research and Training Center, University of Bamako, Bamako, Mali.

genetic variants in MSP-$1_{19}$[13]; however, new variants can also be derived from single-nucleotide mutations that are maintained by positive natural selection.[1]

If vaccine efficacy is allele-specific, then vaccination against polymorphic antigens could lead to selection for nontarget alleles in the parasite population, compromising vaccine efficacy. Such vaccine-induced selection has been suggested by theoretical studies[15–19] and has been observed in a clinical trial of a blood-stage vaccine.[20] We report the sequence of seven rare MSP-$1_{19}$ haplotypes identified at a malaria vaccine-testing site in Mali and discuss the possible origins of these new haplotypes and the potential implications of genetic diversity for the efficacy of MSP-1–based vaccines.

New MSP-$1_{19}$ haplotypes were identified from samples collected in a cohort study conducted in Bandiagara, Mali, during the years 1999–2001.[21] From July to January of each year, individuals were visited weekly and contributed a filter paper blood sample at least monthly and at every clinical malaria episode. Among 629 study participants, 100 who had at least 2 years of follow up were randomly selected within three age strata: 30 children of age $\leq$ 5 years, 32 children of age 6–10 years, and 38 children of age $\geq$ 11 years.[21] Samples were collected under protocols reviewed and approved by Institutional Review Boards of the University of Maryland School of Medicine and the University of Bamako Faculty of Medicine. Informed consent was obtained from all study participants or their guardians.

MSP-$1_{19}$ was amplified from samples collected at monthly surveys and clinical episodes occurring during the transmission season in the 3 years of the incidence study. A single PCR was used to amplify MSP-$1_{19}$ from samples with parasitemia > 1,000 parasites/μL, and a nested PCR was used to amplify MSP-$1_{19}$ from samples with parasitemia < 1,000 parasites/μL and microscopy-negative samples. Of the 2309 samples that underwent PCR (including microscopy-negative samples), 1375 were parasite-positive (by PCR).[21]

All PCR-positive samples underwent Pyrosequencing to determine allele frequencies at each of the six SNPs in MSP-$1_{19}$.[22] Pyrosequencing (Biotage, Charlottesville, VA) is a high-throughput method that allows quantification of the proportions of alternative nucleotides at each SNP. Of the 1,375 PCR-positive samples, 1,369 gave successful MSP-$1_{19}$ genotyping results.[21]

A mathematical model was used to estimate the frequency of 14 confirmed MSP-$1_{19}$ haplotypes in each genotyped sample.[21,22] The haplotype-estimating algorithm uses maximum likelihood methods to determine the most probable combination of haplotypes given the allele frequencies for an infection, the haplotypes known to be circulating in the population, and a probability distribution of the measurement errors. Three of the 14 haplotypes included in the haplotype-estimating algorithm had not been observed previously and were confirmed by reamplification of MSP-$1_{19}$ followed by PCR cloning.[22] When applied to the 1,369 genotyped samples, the algorithm was able to resolve haplotype frequencies for all but six samples given the list of 14 haplotypes.[21]

The six samples that were unable to be resolved by the algorithm had allele frequencies consistent with the presence of additional new MSP-$1_{19}$ haplotypes. The PCR and Pyrosequencing were repeated to rule out genotyping error as an explanation for the observed allele frequencies. Upon obtaining the same results, MSP-$1_{19}$ was reamplified from these six samples, using non-biotinylated MSP-$1_{19}$ primers, and cloned. Twelve clones were picked from each transformation. Pyrosequencing and direct sequencing of MSP-$1_{19}$ clones from the six samples revealed four additional new haplotypes: QKSNRF, QKSSGF, EKSNRL, and EKNNGF. Nucleotide and amino-acid alignments of the seven new haplotypes (three from the previous study[22] and four from the current study) are shown in Figure 1, as well as haplotypes with substitutions at positions other than those at the six known SNPs (sequences available in

GenBank, accession numbers DQ677569–DQ677579). Sequences for the 3D7 and FVO *P. falciparum* strains are also included for reference. As indicated in the figure, the QKSSGF haplotype also has nonsynonymous substitutions at positions 1674 (N→D) and 1690 (A→V). Additional non-synonymous substitutions were observed in other clones at residues 1647 (G→R, clones 0818c1–6 and 1–8), 1673 (E→G, clone 0818c1–6), and 1677 (P→L, clone 0818c1–7). The QKSSRL haplotype has a synonymous substitution at codon 1667. Synonymous substitutions were also observed in other clones at codons 1659 (0818c1–6), 1666 (0818c4–1), 1698 (0818c1–7, 4-1), and 1699 (0818c1–6, 1–8). It is possible that other polymorphic sites exist in the parasites infecting the cohort; however, because Pyrosequencing™ genotypes the short regions surrounding known SNPs, only those samples giving unusual Pyrosequencing™ results were flagged for cloning and direct sequencing.

Using the haplotype-estimating algorithm, QKSSGL, QKSSRL, and QTSSGL had prevalences of 0.07%, 1.3%, and 0.51%, respectively, in the cohort. QKSNRF, QKSSGF, EKSNRL, and EKNNGF were not included in the haplotype-estimating algorithm and were found in one sample each.

Table 1 contains a comprehensive list of MSP-$1_{19}$ haplotypes reported in the literature. Including the seven new haplotypes identified in Mali, 22 haplotypes have been documented, including one isolate from India that contained a Y allele at position 1700.[23] All but four of the reported haplotypes (EKSSGL, QTSSRF, ETSSRF, and EKSYGF) have been observed in the samples from Mali.

The role of recombination in the generation of genetic diversity in *Plasmodium* has been debated,[24,25] but intragenic recombination has been implicated as a factor in generating diversity in MSP-1.[13,14] Three of the haplotypes observed in this study (QKSSRL, QKSSGL, and EKSNRL) were predicted to exist based on single and double crossover events between previously identified alleles[13]; however, until now they had not been identified in field isolates. Table 2 shows how the seven new haplotypes identified in this study could have arisen via recombination events. As indicated in the table, all but one of the seven new haplotypes (QKSNRF) could have arisen from single crossover events between haplotypes observed in Mali. If all known haplotypes are considered (including those not observed at the site), then all seven haplotypes could have been generated via single crossovers.

Although reshuffling of known polymorphisms via recombination could be responsible for the observed haplotypes, these new haplotypes could also be the result of convergence (i.e., selection for mutations that are identical but that do not share common ancestry).[1,26] For example, the haplotype QKSSRL could have arisen from a single nucleotide change from GAA to CAA in codon 1644 of the EKSSRL haplotype or from a single nucleotide change from ACA to AAA in codon 1691 of the QTSSRL haplotype. However, without additional sequence information from adjacent regions (e.g., neighboring microsatellites), it is difficult to distinguish recombination from convergent point mutations in this context.[1]

In conclusion, by applying new high-throughput genotyping methods to a large sample of infections from Mali, seven new MSP-$1_{19}$ haplotypes have been identified, representing a 50% increase in the number of haplotypes previously reported in the literature. Few studies have examined MSP-$1_{19}$ genetic diversity in Africa,[13] even though Africa carries the heaviest malaria burden and most MSP-1–based vaccines currently being developed and tested target this region of the protein.[27,28] Additional MSP-$1_{19}$ diversity may continue to be discovered as high-throughput methods are used to conduct large molecular epidemiology studies of this locus in other malaria endemic areas of Sub-Saharan Africa.

Although the impact of genetic diversity in MSP-$1_{19}$ on immunity and efficacy of MSP-1–based vaccines is not clear, it is possible that vaccination with one of the common MSP-$1_{19}$

haplotypes could give a competitive advantage to rare haplotypes such as those observed in this study, allowing them to increase in frequency in the parasite population.[16,17,19] Understanding the mechanisms by which diverse MSP-$1_{19}$ haplotypes arise may improve our ability to predict how *Plasmodium* will evolve in response to interventions such as vaccines and drugs.

## Acknowledgments

The authors thank the population of Bandiagara, Mali, for their continued participation in our studies, as well as the regional and district health authorities of Bandiagara, Mali for their continued support. We also thank Dr. Alan Shuldiner and the Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, for use of the Pyrosequencer. In addition, we acknowledge Dr. Ananias Escalante for helpful comments on the manuscript.

Financial support: This study was funded by NIAID (N01AI85346) and the USAID Malaria Vaccine Program.

## References

1. Escalante AA, Lal AA, Ayala FJ. Genetic polymorphism and natural selection in the malaria parasite *Plasmodium falciparum*. Genetics 1998;149:189–202. [PubMed: 9584096]
2. Blackman MJ, Heidrich HG, Donachie S, McBride JS, Holder AA. A single fragment of a malaria merozoite surface protein remains on the parasite during red cell invasion and is the target of invasion-inhibiting antibodies. J Exp Med 1990;172:379–382. [PubMed: 1694225]
3. Holder AA, Blackman MJ, Burghaus PA, Chappel JA, Ling IT, McCallum-Deighton N, Shai S. A malaria merozoite surface protein (MSP1)—structure, processing and function. Mem Inst Oswaldo Cruz 1992;87:37–42. [PubMed: 1343716]
4. Branch OH, Udhayakumar V, Hightower AW, Oloo AJ, Hawley WA, Nahlen BL, Bloland PB, Kaslow DC, Lal AA. A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-1 19-kiloDalton domain of *Plasmodium falciparum* in pregnant women and infants: associations with febrile illness, parasitemia, and anemia. Am J Trop Med Hyg 1998;58:211–219. [PubMed: 9502606]
5. Branch OH, Oloo AJ, Nahlen BL, Kaslow D, Lal AA. Anti-merozoite surface protein-1 19-kDa IgG in mother-infant pairs naturally exposed to *Plasmodium falciparum*: subclass analysis with age, exposure to asexual parasitemia, and protection against malaria. V. The Asembo Bay Cohort Project. J Infect Dis 2000;181:1746–1752. [PubMed: 10823777]
6. Egan AF, Morris J, Barnish G, Allen S, Greenwood BM, Kaslow DC, Holder AA, Riley EM. Clinical immunity to *Plasmodium falciparum* malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1. J Infect Dis 1996;173:765–769. [PubMed: 8627050]
7. John CC, O'Donnell RA, Sumba PO, Moormann AM, Koning-Ward TF, King CL, Kazura JW, Crabb BS. Evidence that invasion-inhibitory antibodies specific for the 19-kDa fragment of merozoite surface protein-1 (MSP-$1_{19}$) can play a protective role against blood-stage *Plasmodium falciparum* infection in individuals in a malaria endemic area of Africa. J Immunol 2004;173:666–672. [PubMed: 15210830]
8. Okech BA, Corran PH, Todd J, Joynson-Hicks A, Uthaipibull C, Egwan TG, Holder AA, Riley EM. Fine specificity of serum antibodies to *Plasmodium falciparum* merozoite surface protein, PfMSP-1 (19), predicts protection from malaria infection and high-density parasitemia. Infect Immun 2004;72:1557–1567. [PubMed: 14977962]
9. Riley EM, Allen SJ, Wheeler JG, Blackman MJ, Bennett S, Takacs B, Schonfeld HJ, Holder AA, Greenwood BM. Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen (PfMSP1) of *Plasmodium falciparum* are associated with reduced malaria morbidity. Parasite Immunol 1992;14:321–337. [PubMed: 1625908]
10. Miller LH, Roberts T, Shahabuddin M, McCutchan TF. Analysis of sequence diversity in the *Plasmodium falciparum* merozoite surface protein-1 (MSP-1). Mol Biochem Parasitol 1993;59:1–14. [PubMed: 8515771]

11. Da Silveira LA, Ribeiro WL, Kirchgatter K, Wunderlich G, Matsuoka H, Tanabe K, Ferreira MU. Sequence diversity and linkage disequilibrium within the Merozoite Surface Protein-1 (MSP-1) locus of *Plasmodium falciparum*: a longitudinal study in Brazil. J Eukaryot Microbiol 2001;48:433–439. [PubMed: 11456319]
12. Ferreira MU, Ribeiro WL, Tonon AP, Kawamoto F, Rich SM. Sequence diversity and evolution of the malaria vaccine candidate merozoite surface protein-1 (MSP-1) of *Plasmodium falciparum*. Gene 2003;304:65–75. [PubMed: 12568716]
13. Qari SH, Shi YP, Goldman IF, Nahlen BL, Tibayrenc M, Lal AA. Predicted and observed alleles of *Plasmodium falciparum* merozoite surface protein-1 (MSP-1), a potential malaria vaccine antigen. Mol Biochem Parasitol 1998;92:241–252. [PubMed: 9657329]
14. Sakihama N, Kimura M, Hirayama K, Kanda T, Na-Bangchang K, Jongwutiwes S, Conway D, Tanabe K. Allelic recombination and linkage disequilibrium within MSP-1 of *Plasmodium falciparum*, the malignant human malaria parasite. Gene 1999;230:47–54. [PubMed: 10196473]
15. Gupta S, Swinton J, Anderson RM. Theoretical studies of the effects of heterogeneity in the parasite population on the transmission dynamics of malaria. Proc R Soc Lond B Biol Sci 1994;256:231–238.
16. Gupta S, Ferguson NM, Anderson RM. Vaccination and the population structure of antigenically diverse pathogens that exchange genetic material. Proc R Soc Lond B Biol Sci 1997;264:1435–1443.
17. Lipsitch M. Vaccination against colonizing bacteria with multiple serotypes. Proc Natl Acad Sci USA 1997;94:6571–6576. [PubMed: 9177259]
18. Lythgoe KA. Effects of acquired immunity and mating strategy on the genetic structure of parasite populations. Am Nat 2002;159:519–529. [PubMed: 18707434]
19. McLean AR. Vaccination, evolution and changes in the efficacy of vaccines: a theoretical framework. Proc Biol Sci 1995;261:389–393. [PubMed: 8587880]
20. Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, Rare L, Baisor M, Lorry K, Brown GV, Pye D, Irving DO, Smith TA, Beck HP, Alpers MP. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1–2b trial in Papua New Guinea. J Infect Dis 2002;185:820–827. [PubMed: 11920300]
21. Takala SL, Coulibaly D, Thera MA, Dicko A, Smith DL, Guindo AB, Kone AK, Traore K, Ouattara A, Djimde AA, Sehdev PS, Lyke KE, Diallo DA, Doumbo OK, Plowe CV. Dynamics of polymorphism in a malaria vaccine antigen at a vaccine-testing site in Mali. PLoS Med 2007;4:e93. [PubMed: 17355170]
22. Takala SL, Smith DL, Stine OC, Coulibaly D, Thera MA, Doumbo OK, Plowe CV. A high-throughput method for quantifying alleles and haplotypes of the malaria vaccine candidate *Plasmodium falciparum* merozoite surface protein-1 19kDa. Malar J 2006;5:31. [PubMed: 16626494]
23. Vijay KS, Ranjan S, Saxena V, Rajesh V, Roy SK, Kochar D, Ranjan A, Das A. *Plasmodium falciparum*: genetic diversity of C-terminal region of MSP-1 in isolates from Indian sub-continent. Exp Parasitol 2005;110:384–388. [PubMed: 15907839]
24. Conway DJ, Roper C, Oduola AM, Arnot DE, Kremsner PG, Grobusch MP, Curtis CF, Greenwood BM. High recombination rate in natural populations of *Plasmodium falciparum.* Proc Natl Acad Sci USA 1999;96:4506–4511. [PubMed: 10200292]
25. Tibayrenc M, Kjellberg F, Arnaud J, Oury B, Breniere SF, Darde ML, Ayala FJ. Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. Proc Natl Acad Sci USA 1991;88:5129–5133. [PubMed: 1675793]
26. McCutchan TF, Lal AA, do Rosario V, Waters AP. Two types of sequence polymorphism in the circumsporozoite gene of *Plasmodium falciparum*. Mol Biochem Parasitol 1992;50:37–45. [PubMed: 1542315]
27. Chang SP, Case SE, Gosnell WL, Hashimoto A, Kramer KJ, Tam LQ, Hashiro CQ, Nikaido CM, Gibson HL, Lee-Ng CT, Barr PJ, Yokota BT, Hut GS. A recombinant baculovirus 42-kilodalton C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. Infect Immun 1996;64:253–261. [PubMed: 8557348]
28. Stoute JA, Gombe J, Withers MR, Siangla J, McKinney D, Onyango M, Cummings JF, Milman J, Tucker K, Soisson L, Stewart VA, Lyon JA, Angov E, Leach A, Cohen J, Kester KE, Ockenhouse CF, Holland CA, Diggs CL, Wittes J, Gray HD Jr. Phase 1 randomized double-blind safety and

immunogenicity trial of Plasmodium falciparum malaria merozoite surface protein FMP1 vaccine, adjuvanted with AS02A, in adults in western Kenya 1. Vaccine 2007;25(1):176–184. [PubMed: 16388879]Epub 2005 Dec 7

29. Holder AA, Lockyer MJ, Odink KG, Sandhu JS, Riveros-Moreno V, Nicholls SC, Hillman Y, Davey LS, Tizard ML, Schwarz RT. Primary structure of the precursor to the three major surface antigens of *Plasmodium falciparum* merozoites. Nature 1985;317:270–273. [PubMed: 2995820]
30. Tanabe K, Mackay M, Goman M, Scaife JG. Allelic dimorphism in a surface antigen gene of the malaria parasite *Plasmodium falciparum*. J Mol Biol 1987;195:273–287. [PubMed: 3079521]
31. Chang SP, Kramer KJ, Yamaga KM, Kato A, Case SE, Siddiqui WA. *Plasmodium falciparum*: gene structure and hydropathy profile of the major merozoite surface antigen (gp195) of the Uganda-Palo Alto isolate. Exp Parasitol 1988;67:1–11. [PubMed: 3049134]
32. Jongwutiwes S, Tanabe K, Kanbara H. Sequence conservation in the C-terminal part of the precursor to the major merozoite proteins (MSP-1) of *Plasmodium falciparum* from field isolates. Mol Biochem Parasitol 1993;59:95–100. [PubMed: 8515786]
33. Kang Y, Long CA. Sequence heterogeneity of the C-terminal, Cysrich region of the merozoite surface protein-1 (MSP-1) in field samples of *Plasmodium falciparum*. Mol Biochem Parasitol 1995;73:103–110. [PubMed: 8577318]
34. Lalitha PV, Malhotra P, Chattopadhyay R, Chauhan VS. *Plasmodium falciparum*: variations in the C-terminal cysteine-rich region of the merozoite surface protein-1 in field samples among Indian isolates. Exp Parasitol 1999;92:12–18. [PubMed: 10329360]

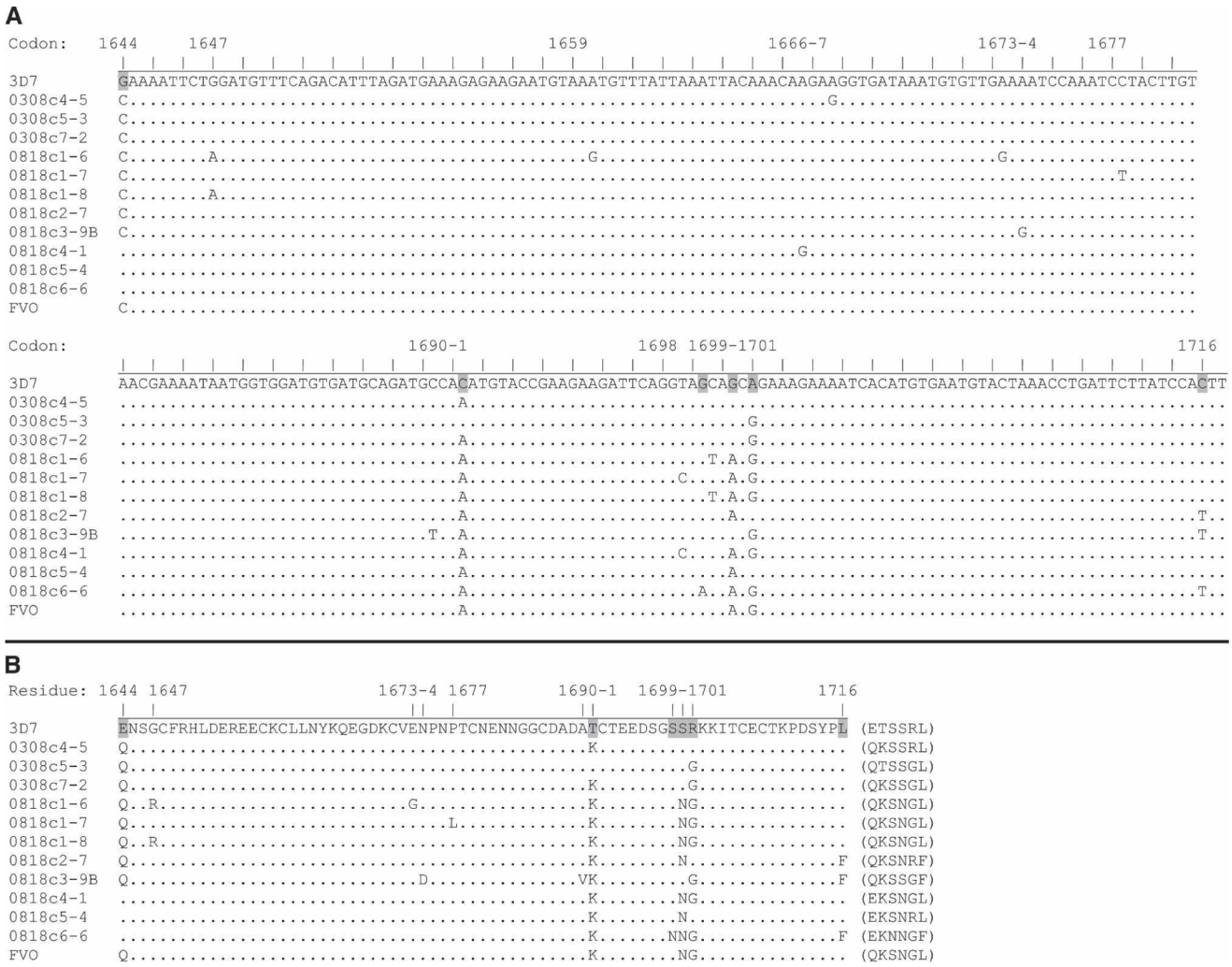


**Figure 1.**

Alignment of novel MSP-$1_{19}$ haplotypes observed in Mali: (A) nucleotide alignment; (B) amino-acid alignment. Shaded text indicates SNPs at positions 1644, 1691, 1699, 1700, 1701, and 1716. Haplotypes based on these six positions are in parentheses.

**Table 1**

Comprehensive list of MSP-$1_{19}$ haplotypes reported in the literature to date

| Haplotype | Amino acid position | | | | | | Isolates/country | Reference no. |
|---|---|---|---|---|---|---|---|---|
| | 1644 (E/Q) | 1691 (T/K) | 1699 (S/N) | 1700 (S/N) | 1701 (R/G) | 1716 (L/F) | | |
| 1 | Q | K | S | N | G | L | FVO, Wellcome | 29 |
| 2 | E | T | S | S | R | L | 3D7, MAD20 | 30 |
| 3 | E | K | S | N | G | L | FUP, Uganda-PA | 31 |
| 4 | Q | K | S | N | G | F | T807 (Thai) | 32 |
| 5 | Q | T | S | S | R | L | Indo | 33 |
| 6 | E | K | S | S | R | L | Kenya-2 | 13 |
| 7 | E | K | N | N | G | L | Kenya | 13 |
| 8* | E | K | S | S | G | L | Kenya-1 | 13 |
| 9 | E | K | S | N | G | F | Kenya-3 | 13 |
| 10 | Q | K | N | N | G | L | Thai-Variant 2 | 14 |
| 11 | E | T | S | S | G | L | India | 34 |
| 12* | E | T | S | S | R | F | Brazil-1 | 11 |
| 13* | Q | T | S | S | R | F | Brazil-2 | 11 |
| 14 | E | T | S | N | G | L | Vietnam | 12 |
| 15* | E | K | S | Y† | G | F | India | 23 |
| 16 | Q | K | S | S | G | L | Mali-1 | |
| 17 | Q | T | S | S | G | L | Mali-2 | |
| 18 | Q | K | S | S | R | L | Mali-3 | |
| 19 | Q | K | S | N | R | F | Mali-4 | |
| 20 | Q | K | S | S | G | F | Mali-5 | |
| 21 | E | K | S | N | R | L | Mali-6 | |
| 22 | E | K | N | N | G | F | Mali-7 | |

*Haplotypes not observed in Bandiagara, Mali.

†The Y allele at position 1700 was recently reported in one isolate from India.

**Table 2**

Possible recombination events that could have generated new MSP-$1_{19}$ haplotypes

| Recombination events | Progeny |
|---|---|
| Single crossover | |
| Q* KNNGL × E* TSSGL | EKNNGL, **QTSSGL** |
| Q* K^ NNGL × E* K^ SSRL | EKNNGL, **QKSSRL** |
| Q* KSNGL × E* TSSGL | EKSNGL, **QTSSGL** |
| QK* SNGL × ET* SSGL | ETSNGL, **QKSSGL** |
| Q* T^ SSRL × E* T^ SSGL | ETSSRL, **QTSSGL** |
| Q* K^ SNGL × E* K^ SSRL | EKSNGL, **QKSSRL** |
| Q* TSSRL × E* KSSRL | ETSSRL, **QKSSRL** |
| QKSNG* F × EKNNG* L | QKSNGL, **EKNNGF** |
| QKSSG* L × EKSNG* F | EKSNGL, **QKSSGF** |
| QKSSG* L × QKSNG* F | QKSNGL, **QKSSGF** |
| EKSN* GL × EKSS* RL | EKSSGL, **EKSNRL** |
| Q* K^ SNGL × E* K^ SSGL | EKSNGL, **QKSSGL** |
| Q* TSSRL × E* KSSGL | ETSSRL, **QKSSGL** |
| Q* K^ NNGL × E* K^ SSGL | EKNNGL, **QKSSGL** |
| QT* SSRL × EK* SSGL | EKSSRL, **QTSSGL** |
| QKSN* GL × ETSS* RF | ETSSGL, **QKSNRF** |
| QKSN* GL × QTSS* RF | **QTSSGL, QKSNRF** |
| QTSSR* F × EKNNG* L | QTSSRL, **EKNNGF** |
| Double crossover | |
| $Q^{1}$ KSNG$^{2}$ F × $E^{1}$ KSNR$^{2}$ L | EKSNGL, **QKSNRF** |
| QKSN$^{1}$ G$^{2}$ F × ETSS$^{1}$ R$^{2}$ L | ETSSGL, **QKSNRF** |
| QK$^{1}$ SNG$^{2}$ F × ET$^{1}$ SSG$^{2}$ L | ETSNGL, **QKSSGF** |
| $Q^{1}$ KSN$^{2}$ GL × $E^{1}$ KSS$^{2}$ RL | **QKSSGL, EKSNRL** |
| $Q^{1}$ KNNG$^{2}$ L × $E^{1}$ KSNG$^{2}$ F | QKSNGL, **EKNNGF** |

*Position of crossover.

^Alternative position for the crossover.

[1,2]Crossover positions in a double-crossover event. Haplotypes in bold text indicate new haplotypes identified in this study. Haplotypes in underlined text indicate haplotypes that have not been observed at the Bandiagara, Mali site and therefore represent events that are less likely to have occurred in Bandiagara but that could have occurred elsewhere and been imported.