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SNP Genotyping Identifies New Signatures of Selection in a Deep Sample of West African *Plasmodium falciparum* Malaria Parasites

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Abstract

We used a high-density single-nucleotide polymorphism array to genotype 75 *Plasmodium falciparum* isolates recently collected from Senegal and The Gambia to search for signals of selection in this malaria endemic region. We found little geographic or temporal stratification of the genetic diversity among the sampled parasites. Through application of the iHS and REHH haplotype-based tests for positive selection, we found evidence of recent selective sweeps at a known drug resistance locus, at several known antigenic loci, and at several genomic regions not previously identified as sites of recent selection. We discuss the value of deep population-specific genomic analyses for identifying selection signals within sampled endemic populations of parasites, which may correspond to local selection pressures such as distinctive therapeutic regimes or mosquito vectors.

Key words: *Plasmodium falciparum*, natural selection, SNP, genome, West Africa.
regions, indicative of a recent selective sweep. We detected 11 loci with genome-wide significance, including the well-characterized pfcrt locus (Fidock et al. 2000) and a large region on chromosome 6 (fig. 2, table 1; supplementary table S2 has detailed annotation, Supplementary Material online). Five of the loci overlap with previously reported signals (Mu et al. 2010; Van Tyne et al. 2011). The signals of selection appeared consistently in both our directly drawn and our culture-adapted parasite sets (supplementary fig. S4, Supplementary Material online) and were little changed when we restricted our analysis to a 2-year time period (supplementary fig. S5, Supplementary Material online) or used a uniform recombination map. After removing the 11 loci, the remainder of the genome roughly conforms to the null expectation for test scores (fig. 2c and d).

Senegal and The Gambia share a similar history of drug regimens, except for three years of amodiaquine use in Senegal but not in The Gambia (see supplementary note, Supplementary Material online). Given the role that loci such as pfcrt, with its large selective sweep, play in drug resistance (Fidock et al. 2000), it is reasonable to speculate that some of these novel sweeps also reflect adaptation to drug pressure. Although resistance alleles at loci such as pfcrt, pfmdr1, and dhfr contribute significantly to drug resistance in P. falciparum, it is known that other genes also affect resistance to drugs (Patel et al. 2010) or have undergone compensatory changes to offset the fitness cost of resistance alleles (Jiang et al. 2008).

Contrary to intuition, several of the loci with evidence of directional selection are known antigenic loci, which are thought to be subject primarily to balancing selection; these include ama1, trap/ssp2, clag2, and possibly PF13_0074 and PF14_0726. Similar findings have been noted previously (Mu et al. 2010). Genes under balancing selection typically exhibit short haplotypes, as their SNPs segregate for unusually long periods of time during which recombination breaks down haplotypes. If the selective sweeps do originate in these genes (and not at nearby variants absent from the array), they may reflect nonimmune pressures. Many highly polymorphic antigenic loci in P. falciparum have roles unrelated to immune evasion and presumably fix adaptive mutations related to these functions. For example, clag3 genes, in the same gene family as one of our sweep candidates (clag2), exhibit the high polymorphism typical of genes encoding surface-expressed cytoadherence proteins. Nevertheless, two clag3 genes were recently found to be associated with resistance to antimalarial drugs, suggesting that their products also mediate entry of drugs (Nguitragool et al. 2011).

Alternatively, we may be detecting positive selection at these loci as a variant unfamiliar to the host immune system rises from very low frequency. Previous studies of malaria antigenic genes (ama1 in particular) have shown complex patterns of selection (Cortes et al. 2003; Polley et al. 2003; Gunasekera et al. 2007), with evidence for both positive and balancing selection (Mu et al. 2010); a long-haplotype signal at ama1 has been detected in Asia (Mu et al. 2010). Sequencing-based study of these regions, preferably in multiple geographic regions, should provide better insight into the selective processes at work.

Another unexpected candidate locus codes for thrombospondin-related anonymous protein (TRAP), a conserved protein expressed in P. falciparum sporozoites. It contains extracellular domains associated with hepatocyte invasion (Muller et al. 1993; Wang et al. 2005) and has previously shown evidence of balancing selection (Weedall et al. 2007). TRAP has also been found to mediate the invasion of salivary glands in mosquitoes (Ghosh et al. 2009). It is possible that maintenance of long haplotypes reflects adaptation to divergent ligands in different mosquito species.

**Materials and Methods**

*Plasmodium falciparum* isolates were obtained in 2008 from three sites near Banjul in The Gambia and during the period 2001–2009 from three clinics in Senegal—in Thies (100 km from Dakar), in Pikine (10 km from Dakar), and in Vellingara (500 km from Dakar and on the other side of The Gambia). Gambian parasites were taken from clinical samples, whereas Senegal samples included both directly drawn and culture-adapted parasites; see supplementary note for details on DNA extraction, Supplementary Material online. Culture-adapted samples with evidence of multiple infection, based on polymorphism typing at the msp loci (Viriyakosol et al. 1995), were subcloned to isolate a single strain.

SNP calling was as described in Van Tyne et al. (2011): parasite DNA was hybridized to a *P. falciparum* Affymetrix array containing 74,656 SNP markers and genotypes called using the BRLMM-P algorithm. SNPs were validated by comparing array genotypes with Sanger sequencing genotypes for 17 reference strains (Van Tyne et al. 2011); perfect concordance was required, as was a minimum 80% call rate. Genomic positions and translations are based on the PlasmoDB v5.0 assembly and annotation (PlasmoDB.org). SNPs within var, rifin, or stever genes were dropped to reduce artifacts from duplicate sequence. After these filters,
Fig. 2. Signals of selective sweeps. Significance $-\log_{10}(P\text{-value})$ for all SNPs for (a) REHH and (b) iHS; QQ plots (observed vs. expected $P$ values) for (c) REHH and (d) iHS. Panels c and d also show the $P$ value distribution with sweep loci removed (teal). Dashed lines: Bonferroni significance (0.05 level).

Table 1. Genes in Regions with Signals of Selection.

| Chromosome | Genes          | Gene annotation                                                                 | Test |
|------------|----------------|---------------------------------------------------------------------------------|------|
| 2          | PFB0935w^1     | Cytoadherence-linked asexual protein 2                                           | iHS  |
| 4          | PFD0260c       | Sequestrin                                                                      | iHS  |
| 4          | PFD0735c^a     | Conserved *Plasmodium* protein, unknown function                                 | iHS  |
| 4          | Intergenic     |                                                                                  | REHH |
| 6          | PFF1335c       | 4-methyl-5-(B-hydroxyethyl)-thiazol monophosphate biosynthesis enzyme           | Both |
|            | PFF1350c       | Acetyl-coenzyme a synthetase                                                    |      |
|            | PFF1365c       | HECT-domain (ubiquitin transferase), putative                                   |      |
|            | PDD1460c       | Conserved *Plasmodium* protein, unknown function                                 |      |
|            | PFF1470c       | DNA polymerase epsilon, catalytic subunit a, putative                           |      |
| 7          | PF07_0035      | Cg1 protein                                                                      |      |
|            | PF07_0036      | Cg6 protein                                                                      |      |
|            | PF07_0038a     | Cg7 protein                                                                      |      |
|            | MAL7P1_28a     | pfcrt; chloroquine resistance transporter                                        |      |
|            | PF07_0042      | Conserved *Plasmodium* protein, unknown function                                 |      |
| 7          | PF07_0053      | Conserved *Plasmodium* protein, unknown function                                 | iHS  |
| 11         | PF11_0344      | Apical membrane antigen 1 precursor, AMA1                                       | iHS  |
| 13         | PF13_0074      | Surface-associated interspersed gene 13.1 (SURFIN13.1)                           | iHS  |
| 13         | PF13_0201a     | Sporozoite surface protein 2                                                    | iHS  |
| 14         | PF14_0726      | Conserved *Plasmodium* protein, unknown function                                 | iHS  |

^aGenes that are no longer significant if double Bonferroni correction applied.
missing SNP genotypes were imputed for use in the selective sweep search, with 12,885 SNPs successfully imputed.

\( F_{ST} \) was calculated using the method of Weir and Cockerham (1984); statistical significance was calculated by permuting population labels. iHS was computed according to the method of Voight et al. (2006) and REHH according to the method of Sabeti et al. (2002). Recombination maps for iHS were generated with the software package LDhat (McVean et al. 2002), using the program interval with a block penalty of 5.0. As both of these tests do not tolerate missing data, SNPs were imputed with PHASE 2.1.1 (Stephens et al. 2001; Stephens and Scheet 2005). A total of 7,486 fully imputed SNPs were polymorphic among the 70 individuals.

REHH and iHS scores were normalized in 20 equal-sized bins of derived allele frequency, with ancestral alleles inferred from a \( P. reichenowi \) hybridization (Van Tyne et al. 2011). Standardized iHS and REHH scores were converted to two-tailed \( P \) values using a normal distribution. Genome-wide significance was calculated 1) separately without correction for the two tests and 2) jointly across both tests. As the two tests are not independent, a Bonferroni correction for the two tests and 2) jointly across both tests. To test whether long haplotype signals could come from differences in sample preparation, iHS scores were calculated separately for direct and culture-adapted samples.

**Supplementary Material**

Supplementary methods, notes, figures S1–S5, and tables S1 and S2 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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**References**

Cortes A, Mellombo M, Mueller I, Benet A, Reeder JC, Anders RF. 2003. Geographical structure of diversity and differences between symptomatic and asymptomatic infections for \( Plasmodium falciparum \) vaccine candidate AMA1. Infect Immun. 71:1416–1426.

Fidock DA, Nomura T, Talley AK, et al. (14 co-authors). 2000. Mutations in the \( P. falciparum \) digestive vacuole transmembrane protein PCRT and evidence for their role in chloroquine resistance. Mol Cell. 6861–871.

Ghosh AK, Deavenport M, Jethwane Y, Kalume DE, Pandey A, Anderson VE, Sultan AA, Kumar N, Jacobs-Lorena M. 2009. Malaria parasite invasion of the mosquito salivary gland requires interaction between the \( Plasmodium \) TRAP and the Anopheles salgin proteins. PLoS Pathog. 5:e1000265.

Gunasekera AM, Wickaramachchi T, Neafsey DE, Ganguli I, Perera L, Premaratne PH, Harl D, Handunnetti SM, Udagama-Randeniyi PV, Wirth DF. 2007. Genetic diversity and selection at the \( Plasmodium vivax \) apical membrane antigen-1 (PvAMA-1) locus in a Sri Lankan population. Mol Biol Evol. 24:939–947.

Jiang H, Patel JJ, Yi M, Mu J, Ding J, Stephens R, Cooper RA, Ferdig MT, Su XZ. 2008. Genome-wide compensatory changes accompany drug-selected mutations in the \( Plasmodium falciparum \) crt gene. PLoS One 3:e2484.

McVean G, Awadalla P, Fearnhead P. 2002. A coalescent-based method for detecting and estimating recombination from gene sequences. Genetics 160:1231–1241.

Mu J, Awadalla P, Duan J, McGe KM, Keebler J, Seydel K, McVean GA, Su XZ. 2007. Genome-wide variation and identification of vaccine targets in the \( Plasmodium falciparum \) genome. Nat Genet. 39:126–130.

Mu J, Myers RA, Jiang H, et al. (27 co-authors). 2010. \( Plasmodium falciparum \) genome-wide scans for positive selection, recombination hot spots and resistance to antimalarial drugs. Nat Genet. 42:268–271.

Muller HM, Reckmann I, Hollingdale MR, Bujard H, Robson KJ, Crisanti A. 1993. Thrombospondin related anonymous protein (TRAP) of \( Plasmodium falciparum \) binds specifically to sulfated glycoconjugates and to HepG2 hepatoma cells suggesting a role for this molecule in sporozoite invasion of hepatocytes. EMBO J. 12:2881–2889.

Nguitragool W, Bokhari AA, Pillai AD, Rayavara K, Sharma P, Turpin B, Arawind L, Desai SA. 2011. Malaria parasite clag3 genes determine channel-mediated nutrient uptake by infected red blood cells. Cell 145:665–677.

Patel JJ, Thacker D, Tan JC, Pleeter P, Checkley L, Gonzales JM, Deng B, Roepe PD, Cooper RA, Ferdig MT. 2010. Chloroquine susceptibility and reversibility in a \( Plasmodium falciparum \) genetic cross. Mol Microbiol. 78:770–787.

Polley SD, Chokejindachai W, Conway DJ. 2003. Allele frequency-based analyses robustly map sequence sites under balancing selection in a malaria vaccine candidate antigen. Genetics 165:555–561.

Sabeti PC, Reich DE, Higgins JM, et al. (17 co-authors). 2002. Detecting recent positive selection in the human genome from haplotype structure. Nature 419:832–837.

Stephens M, Scheet P. 2005. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet. 76:449–462.

Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 68:978–989.

Van Tyne D, Park DJ, Schaffner SF, et al. (31 co-authors). 2011. Identification and functional validation of the novel antimalarial resistance locus Pf10_0355 in \( P. falciparum \). PLoS Genet. 7:e1001383.

Virjakosol S, Siripoon N, Petchparapat C, Petchparapat P, Jarra W, Thaithong S, Brown KN, Sounoung G. 1995. Genotyping of \( Plasmodium falciparum \) isolates by the polymerase chain reaction and potential uses in epidemiological studies. Bull World Health Organ. 73:85–95.

Voight BF, Kudaravalli S, Wen X, Pritchard JK. 2006. A map of recent positive selection in the human genome. PLoS Biol. 4:e72.

Wang X, Mu J, Li G, Chen P, Guo X, Fu L, Chen L, Su X, Wellems TE. 2005. Decreased prevalence of the \( Plasmodium falciparum \) chloroquine resistance transporter 76T marker associated with cessation of
 chloroquine use against *P. falciparum* malaria in Hainan, People’s Republic of China. *Am J Trop Med Hyg.* 72:410–414. Weedall GD, Preston BM, Thomas AW, Sutherland CJ, Conway DJ. 2007. Differential evidence of natural selection on two leading sporozoite stage malaria vaccine candidate antigens. *Int J Parasitol.* 37:77–85. Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370. WHO. 2010. World Health Organization World Malaria Report 2010 [cited 2012 June 15]. Available from: http://www.who.int/malaria/world_malaria_report_2010/en/index.html