Genetic diversity of next generation antimalarial targets: A baseline for drug resistance surveillance programmes

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A B S T R A C T
Drug resistance is a recurrent problem in the fight against malaria. Genetic and epidemiological surveillance of antimalarial resistant parasite alleles is crucial to guide drug therapies and clinical management. New antimalarial compounds are currently at various stages of clinical trials and regulatory evaluation. Using ~2000 Plasmodium falciparum genome sequences, we investigated the genetic diversity of eleven gene-targets of promising antimalarial compounds and assessed their potential efficiency across malaria endemic regions. We determined if the loci are under selection prior to the introduction of new drugs and established a baseline of genetic variance, including potential resistant alleles, for future surveillance programmes.

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1. Introduction

The continuous emergence and spread of resistance to first line antimalarial treatments, including artemisinin and its derivatives, threatens global efforts to reduce the burden of malaria. The development of a fully effective vaccine has been hampered by the complex life cycle of the malaria parasite and the high genetic diversity of key parasite antigens. Thus, antimalarial drugs, particularly those targeting basic cellular machinery common to all stages of the parasite life cycle, are the most promising approaches to control malaria.

The pipeline of antimalarial drugs has greatly expanded over the past decade, particularly because of the strong public-private partnerships and significant investment in innovative technologies (Flannery et al., 2013; Wells et al., 2015). A set of next generation antimalarial compounds, for which the molecular targets are known or being investigated, are currently at various phases of preclinical and clinical assessment (Wells et al., 2015).

Knowledge of parasite molecular drug targets can be exploited to monitor the potential emergence and spread of resistant alleles, particularly from the introduction of a drug, and rapidly inform local policies to tailor interventions. Without knowledge of antimalarial gene targets, the identification and surveillance of resistant alleles needs to be based on accurate clinical drug efficacy trials and genome-wide population genetic studies of field collected samples (Anderson et al., 2011). This approach can be both costly and labour intensive. Alternatively, a powerful strategy to identify mutations linked to resistance, prior to the licensing of a drug, is the use of laboratory-adapted strains to induce selection in vitro with sub-lethal and increasing concentrations of drugs. This strategy has led to the identification of polymorphisms in the Plasmodium (P) falciparum kelch13 gene underlying resistance to artemisinin (Ariey et al., 2014). This gene was confirmed subsequently in association studies in field collected samples (Miotti et al., 2015) and using a reverse genetics approach (Ghorbal et al., 2014).

Here we consider eleven gene-targets of key investigated compounds that due to their efficiency might become the next antimalarial drugs, and for which mutations conferring resistance have been identified in in vitro studies (Baragaña et al., 2015; Dong et al., 2011; Flannery et al., 2015; Herman et al., 2015; Kato et al., 2016; LaMonte et al., 2016; Lim et al., 2016; McNamara et al., 2013; Ross et al., 2014). These 11 genes were also selected because they are gene-targets for a range of new antimalarial compounds already under evaluation in clinical trials. We present a survey of the natural genetic variation (SNPs, insertions and deletions) and sequence diversity of next generation antimalarial gene targets.

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deletions (indels), copy number variants (CNVs)) and diversity in these gene-targets using a publicly available global collection of ~2000 *P. falciparum* "field" parasite genomes from 18 countries. We use the variation to establish whether these areas are already under selective pressure, and report a baseline reference to assist future surveillance programmes with observing emergence of resistance mutations.

2. Materials and methods

Eleven gene-targets were analysed: PF3D7_1113300 (Pfugt), PF3D7_1036800 (Pfact), PF3D7_0109800 (PfPheRs), PF3D7_0603300 (Pfdhodh), PF3D7_0509800 (Pfp4k), PF3D7_0321900 (Pfcarl), PF3D7_1211900 (Pfatp4), PF3D7_1451100 (PfEE2), PF3D7_1320600 (Pfrab11A), PF3D7_1213800 (Pfprs) and Mal_Mito_3 (PFCYTB) (see Table 1). Genome variation data was analysed for isolates from East Africa (Kenya, Tanzania, n = 35), West Africa (Burkina Faso, The Gambia, Ghana, Guinea, Mali, Nigeria, n = 521), Central Africa (Democratic Republic of Congo (DRC), n = 56), South America (Colombia, Peru, n = 24), South Asia (Bangladesh, n = 53) and Southeast Asia (Cambodia, Laos, Myanmar, Papua New Guinea, Thailand, Vietnam, n = 1187).

Sequencing data was generated by the P3k project (www.malarianet.net/p3k), is open access and is described in (Miotto et al., 2015). Whole genome analysis of these data has also been recently described (Ravenhall et al., 2016) and we used a set of characterised high quality SNPs and indels identified in the 11 candidate target genes. In addition, larger structural variants (e.g. CNVs) in these regions were identified using Delly software (Rausch et al., 2012). Using the SNP variants, population genetic analyses were performed to establish if targeted coding regions are under selection. In particular, the Tajima’s D method was applied to detect regions under balancing selection (R package Pegas): extended haplotype homozygosity approaches (iHS, XP-EHH) were applied with proguanil in Malarone treatment of malaria. The PfCARL protein mediates GTP-dependent translocation of the ribosome along the mRNAs and is required during protein synthesis. The Rab11A protein is likely involved in cytokinesis and interacts with another antimalarial gene-target, the PfPheRs (McNamara et al., 2013). Only one non-synonymous SNP was detected for each of these two genes, supporting their likely essential function. A low number of non-synonymous SNPs (19.6%) was also detected for the mitochondrial cytochrome b (MtcytB) gene. This gene is the target for several antimalarial compounds under evaluation (Dong et al., 2011) and atrovucone, a longstanding antimalarial drug used in combination with proguanil in Malarone™ for the curative and prophylactic treatment of malaria. The PfPheRs gene has the highest percentage of non-synonymous changes (Ravenhall et al., 2016) for a detailed description of these methods.

3. Results

Across the eleven gene-targets, a total of 778 SNPs were identified, with half (n = 424, 54.5%) leading to non-synonymous changes (Table 1, Supplementary Table 1). The overall genetic diversity was low, with the majority of SNPs (75.1%) having minor allele frequencies of less than 5%. The SNP density (number of SNPs per kb) across genes was similar (~1 SNP per 33bp), except for those coding for the ras-related protein (Rab11A, 1 SNP per 258.6bp), elongation factor 2 (eEF2, 1 SNP per 73.4bp) and the acetyl-CoA transporter (ACT, 1 SNP per 64.2bp), all with lower density, suggesting greater gene conservatism. The PFACT gene was recently identified to be the target, together with the UDP-galactose transporter gene-target (Pfugt), of a variety of imidazolopiperazine compounds. One of these compounds (KAF156) has potent activity against gametocytes and parasite liver stages, and is currently in Phase II clinical trial (Lim et al., 2016). Rab11A is a molecular target for aminopyridine class compounds (McNamara et al., 2013), and eEF2 is the target for quinoline-4-carboxamide (DDD107498) compounds, both with activity against multiple lifecycle parasite stages (Baragana et al., 2015). The eEF2 protein mediates GTP-dependent translocation of the ribosome along the mRNA and is required during protein synthesis. The Rab11A protein is likely involved in cytokinesis and interacts with another antimalarial gene-target, the Pfp4k (McNamara et al., 2013). Only one non-synonymous SNP was detected for each of these two genes, supporting their likely essential function. A low number of non-synonymous SNPs (19.6%) was also detected for the mitochondrial cytochrome b (MtcytB) gene. This gene is the target for several antimalarial compounds under evaluation (Dong et al., 2011) and atrovucone, a longstanding antimalarial drug used in combination with proguanil in Malarone™ for the curative and prophylactic treatment of malaria.

### Table 1

| Gene-target ID | Gene | Active Compounds | Drug development stage | SNP (non-synonymous) | Non-reference allele frequency >5% (non-synonymous) | Known antimalarial resistant mutations |
|---------------|------|-----------------|------------------------|---------------------|----------------------------------------------|--------------------------------------|
| PfPheRs (PF3D7_0109800) | Phenylalanine-tRNA ligase alpha subunit | Bicyclic azetidine | Nonclinical development | 44 (28) | 5 (3) | L550V |
| Pfcarl (PF3D7_0321900) | Cyclic amine resistance locus protein Phosphatidylinositol-4 kinase | Imidazolopiperazines, benzimidazolyl piperidines | Clinical trials | 152 (90) | 34 (21) | 0 |
| PfPi4k (PF3D7_0509800) | Proline-tRNA | Aminopyridine class | Clinical trials | 182 (129) | 62 (49) | 0 |
| PfDhodh (PF3D7_0603300) | Dihydroorotate dehydrogenase | Triazoloazepiniminidine-based inhibitor, N-alkyl-5-thiophene-2-carboxamides | Clinical trials | 50 (26) | 8 (3) | 0 |
| Pfact (PF3D7_1036800) | Acetyl-CoA transporter | Imidazolopiperazines | Clinical trials | 46 (22) | 13 (6) | 0 |
| Pfugt (PF3D7_1113300) | UDP-galactose transporter | Acyl-CoA transporter | Clinical trials | 30 (12) | 10 (6) | 0 |
| Pfatp4 (PF3D7_1211900) | P-type cation transporter | Spiroindolones, sulfonamide, carboxamide, pyrazoles, dihydropyrimidines | Clinical trials | 123 (75) | 34 (23) | 0 |
| Pfprs (PF3D7_1213800) | Proline-tRNA synthetase | Aminopyridine class | Nonclinical development | 66 (31) | 16 (9) | 0 |
| Pfrab11A (PF3D7_1320600) | Ras-related protein Rab-11A | Februgine and derivatives | Clinical trials | 5 (1) | 0 (0) | 0 |
| PfEE2 (PF3D7_1451100) | Elongation factor 2 | Quinoline-4-carboxamide (DDD107498) | Preclinical development | 34 (1) | 4 (0) | 0 |
| PFCYTB (mal_mito_3) | Cytocrome b | Atrovucone, tetracyclic benzoiziazepine, benzylsulfonamide, deoxyquinate | Clinical drug trial | 46 (9) | 7 (2) | 0 |
Table 2
Genetic polymorphisms in target-genes at and surrounding resistant linked mutations.

| Gene          | Amino-acid substitution | Frequency (%) | Most frequent Population          |
|---------------|-------------------------|---------------|-----------------------------------|
| PfcPheRs (PF3D7_0109800) |                         |               |                                   |
|               | M116I                   | 0             | Bangladesh, Thailand              |
|               | T118A                   | 0.47          | Cambodia, Vietnam, Laos           |
|               | G512E                   | 0.76, 0.96, 2.68 | bacterial, viral                 |
|               | K519                    | 2.6, 5.5      | DRC, Kenya                        |
|               | V545                    | 1.79, 0.5     | DRC, Ghana                        |
|               | L552                    | 1.1           | Guinea                            |
| Pfdhodh (PF3D7_0603300)  |                         |               |                                   |
|               | M316I                   | 0             | Bangladesh, Thailand              |
|               | T318A                   | 0.47          | Cambodia, Vietnam, Laos           |
|               | G512E                   | 0.76, 0.96, 2.68 | bacterial, viral                 |
|               | K519                    | 2.6, 5.5      | DRC, Kenya                        |
|               | V545                    | 1.79, 0.5     | DRC, Ghana                        |
|               | L552                    | 1.1           | Guinea                            |
| Pfpi4k (PF3D7_0509800)   |                         |               |                                   |
|               | D1311                   | 0.26          | Guinea                            |
|               | S1329I                  | 0             | Guinea                            |
|               | E1355                   | 0.49          | Guinea                            |
|               | Y1356F                  | 0             | Guinea                            |
|               | L1479                   | 0.49          | Guinea                            |
| Pfcarl (PF3D7_0321900)   |                         |               |                                   |
|               | P122L                   | 0             | Guinea                            |
|               | L130V                   | 0             | Guinea                            |
|               | E834K                   | 0             | Guinea                            |
|               | S1076N1                 | 0             | Guinea                            |
|               | D1101G                  | 1.54          | Ghana                             |
|               | A1102                   | 0.24          | Ghana                             |
|               | V1103L                  | 0             | Ghana                             |
|               | A1135D                  | 0.49          | Ghana                             |
| Pfatp4 (PF3D7_1211900)   |                         |               |                                   |
|               | A185S                   | 0.49          | Ghana                             |
|               | V204L                   | 0             | Ghana                             |
|               | S132P                   | 0             | Ghana                             |
|               | L350H                   | 0.02          | Malawi                            |
|               | L379N                   | 0             | Malawi                            |
|               | L398F                   | 0             | Malawi                            |
|               | V400A                   | 0             | Malawi                            |
|               | V414D                   | 0             | Malawi                            |
|               | T416N                   | 0             | Malawi                            |
|               | T418N                   | 0             | Malawi                            |
|               | A421L                   | 0             | Malawi                            |
|               | P412L                   | 0             | Malawi                            |
|               | E895K                   | 0             | Malawi                            |
|               | F917L                   | 0             | Malawi                            |
|               | L938I                   | 0             | Malawi                            |
|               | P965A                   | 0             | Malawi                            |
|               | A967G                   | 0             | Malawi                            |
|               | K988R                   | 0.45          | Malawi                            |
|               | P999R                   | 0             | Malawi                            |
|               | A1158V                  | 0             | Malawi                            |
|               | A1207V                  | 0.18, 0.05, 0.5 | bacterial, viral                 |
|               | T1208S                  | 0.11          | Guinea                            |
|               | L1242                   | 2.67          | DRC                               |
| Pfreps (PF3D7_1213800)   |                         |               |                                   |
|               | D1247Y                  | 0             |                                   |
|               | L482H                   | 0             |                                   |
|               | T1445A                  | 0             |                                   |
|               | C1444T                  | 0             |                                   |
| Pfrub11A (PF3D7_1320600) |                         |               |                                   |
|               | E128                    | 0.96, 0.53    | Laos, Vietnam                     |
| PfeEF2 (PF3D7_151100)    |                         |               |                                   |
|               | D135Y                   | 0             |                                   |
|               | E134D                   | 0             |                                   |
|               | Y185N                   | 0             |                                   |
|               | L755F                   | 0             |                                   |
|               | T184I                   | 0             |                                   |
|               | H185T                   | 0             |                                   |
SNPs (71.4%), and is a lipid kinase that is a cellular target of imidazopyrazines and quinoxaline compounds (McNamara et al., 2013). This gene probably acts in the Golgi complex and regulates essential membrane trafficking events (McNamara et al., 2013). We also detected a high number of non-synonymous SNPs for the PfKPhers (62.7%), Pfapt4 (60.9%) and Pfctl (59.2%) genes. The Pfapt4 and Pfctl have been extensively studied as antimalarial targets. The Pfctl is an uncharacterized protein-coding gene that also localises in the Golgi apparatus of the parasite and the Pfapt4 locus probably functions as a Na⁺-efflux ATPase (Flannery et al., 2015; McNamara et al., 2013). We also reported antimalarial resistance mutations in the vicinity of the Pfctl and Pfapt4 genes. The Pfctl and Pfapt4 genes contributed the most to the observed regional clustering (Supplementary Fig. 1). The FST measure was used to identify SNPs with allele frequency differences between countries and continents. This analysis revealed nine SNPs with $F_{ST} > 0.45$, with clear geographic allelic frequency differences (Supplementary Table 2), particularly differentiating African from Asian origins. These SNPs were localized in the Pfctl, Pfapt4, Pfpi4k and the Pfcrs genes. The Pfcrs is a cytoplasmic prolyl-tRNA synthetase and a functional target of febrifugine and its synthetic derivatives with activity at erythrocytic and liver stages (Herman et al., 2015).

4. Discussion

Continuous monitoring of drug efficacy and genome selection pressure is crucial to ensure early detection and appropriate

| Table 2 (continued) |
|---------------------|
| **Gene** | **Amino-acid substitution** | **Frequency (%)** | **Most frequent Population** |
|---------|-----------------|-----------------|-----------------------------|
| Pfctl (Mal_Mito_3) | E336G | 0 | |
| | S473R | 0 | |
| | A481T | 0 | |
| | P756A | 0 | |
| | L757F | 0 | |
| | G33A | 0 | |
| | Y126C | 0 | |
| | G131S | 0 | |
| | M133I | 0 | |
| | L1445 | 0.22 | Ghana |
| | V150I | 0 | |
| | V234I | 0 | |
| | V248I | 0.1 | |
| | V248L | 12.5 | |
| | A94T | 0 | |
| | R108K | 0 | |
| | S110R | 0 | |
| | D167S | 0 | |
| | S110T | 0 | |
| | I190L | 2.9 | |
| | G131S | 4.7 | |
| | 6.7 | |
| | 3.2 | |
| | 2.9 | |
| | 6.7 | |
| **Pfctl (PF3D7_1036800)** | C193 | 0 | |
| | S242 | 0 | |
| | L253 | 0 | |
| | G559E | 0 | |
| | F267V | 0 | |
| | V284I | 0 | |
| | A94T | 0 | |
| | R108K | 0 | |
| | S110R | 0 | |
| | D167S | 0 | |
| | S110T | 0 | |
| | I190L | 2.9 | |
| | G131S | 4.7 | |
| | 6.7 | |
| | 3.2 | |
| | 2.9 | |
| | 6.7 | |

4. Discussion

Continuous monitoring of drug efficacy and genome selection pressure is crucial to ensure early detection and appropriate
Fig. 1. Geographic distribution of SNPs across eleven gene-targets. Distribution of genes across countries and continents showed that the majority of SNPs identified were found in only one country.

Fig. 2. Population structure at a continental level. Principal Components (PC) Analysis plot (x axis represents PC1 and y axis PC2) on the ~2000 Principal Components (PC) Analysis plot (x axis represents PC1, and y axis PC2) on the ~2000 P. falciparum field samples from 18 countries and using 778 SNPs identified in the eleven gene-targets.
response to the emergence of drug resistance. We assessed eleven potential antimalarial gene-targets of compounds that are at various stages of testing, and for which mutations linked to resistance are known. The availability of whole genome sequencing data for worldwide field isolates enables us to survey the genetic diversity in these targets. We identified one mutation associated with in vitro resistance to the antimalarial compounds in low frequency in two African countries. We also identified several amino-acid changes in close proximity to resistance-linked mutations (8 non-synonymous substitution detected <2 amino-acids away). These and other mutations detected in these genes may have a role in the development of resistance, highlighting the need for drug screening with field isolates in addition to laboratory adapted strains. The high divergence of *Plasmodium* biology and lack of crystallized protein structures hindered the assessment of the potential impact of the polymorphisms mutations detected in these genes.

The genetic diversity described here may have a role upon onset of selection, and should be taken into account by surveillance programmes. From these observations, we speculate that for new antigens of selection, and should be taken into account by surveillance genes.

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Glynne, R.J., Bodenreider, C., Fidock, D.A., Diagana, T.T., Winzeler, E.A., 2013. Targeting Plasmodium PI(4)K to eliminate malaria. Nature 504, 248–253. http://dx.doi.org/10.1038/nature12782.

Miotto, O., Amato, R., Ashley, E.A., Macninis, B., Almagro-Garcia, J., Amaratunga, C., Lim, P., Mead, D., Oyola, S.O., Dhorda, M., Imwong, M., Woodrow, C., Manske, M., Stalker, J., Drury, E., Campino, S., Amenga-Etego, L., Thanh, T.-N.N., Tran, H.T., Ringwald, P., Bethell, D., Nosten, F., Phyo, A.P., Pukrittayakamee, S., Chotivanich, K., Chuar, C.M., Nguen, C., Suon, S., Sreng, S., Newton, P.N., Mayxay, M., Khamthong, V., Hongvanthong, B., Htut, Y., Han, K.T., Kyaw, M.P., Faiz, M.A., Fanelio, C.I., Onyamboko, M., Mokuolu, O.A., Jacob, C.C., Takala-Harrison, S., Flowe, C.V., Day, N.P., Dondorp, A.M., Spencer, C.C.A., McVean, G., Fairhurst, R.M., White, N.J., Kwiatkowski, D.P., 2015. Genetic architecture of artemisinin-resistant Plasmodium falciparum. Nat. Genet. 47, 226–234. http://dx.doi.org/10.1038/ng.3189.

Rausch, T., Zichner, T., Schlattl, A., Stütz, A.M., Benes, V., Korbel, J.O., 2012. DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinformatics 28, i333–i339. http://dx.doi.org/10.1093/bioinformatics/bts378.

Ravenhall, M., Benavente, E.D., Mipando, M., Jensen, A.T.R., Sutherland, C.J., Roper, C., Sepulveda, N., Kwiatkowski, D.P., Montgomery, J., Phyo, A.P., Terlouw, A., Craig, A., Campino, S., Ocholla, H., Clark, T.G., 2016. Characterizing the impact of sustained sulfadoxine/pyrimethamine use upon the Plasmodium falciparum population in malawi. Malar. J. 15, 575. http://dx.doi.org/10.1186/s12936-016-1634-6.

Ross, L.S., Gamo, F.J., Lafuente-Monasterio, M.J., Singh, O.M.P., Rowland, P., Wiegand, R.C., Wirth, D.F., 2014. In Vitro resistance selections for Plasmodium falciparum dihydroorotate dehydrogenase inhibitors give mutants with multiple point mutations in the drug-binding site and altered growth. J. Biol. Chem. 289, 17980–17995. http://dx.doi.org/10.1074/jbc.M114.558353.

Wells, T.N.C., van Huijsduijnen, R.H., Van Voorhis, W.C., 2015. Malaria medicines: a glass half full? Nat. Rev. Drug Discov. 14, 424–442. http://dx.doi.org/10.1038/nrd4573.