The drug transporter \textit{ABCB1} c.3435C\textgreater T SNP influences artemether–lumefantrine treatment outcome

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**Abstract**

Malaria treatment performance is potentially influenced by pharmacogenetic factors. This study reports an association study between the \textit{ABCB1} c.3435C\textgreater T, \textit{CYP3A4*1B} (g.-392A\textgreater G), \textit{CYP3A5*3} (g.6986A\textgreater G) SNPs and artemether + lumefantrine treatment outcome in 103 uncomplicated malaria patients from Angola. No significant associations with the \textit{CYP3A4*1B} and \textit{CYP3A5*3} were observed, while a significant predominance of the \textit{ABCB1} c.3435CC genotype was found among the recurrent infection-free patients (p < 0.01), suggesting a role for this transporter in AL inter-individual performance.

**Keywords:** Human polymorphism, \textit{CYP450}, \textit{MDR1}, Artemether–lumefantrine, Angola

**Background**

Artemisinin combination therapy (ACT) has contributed to the remarkable decline by 48% in the malaria mortality rate between 2000 and 2015 [1]. The disease remains a major public health challenge, causing over 400,000 deaths annually, partly due to underperforming treatments. The success of malaria treatment depends on many factors, not least inter-individual pharmacokinetic differences, which are potentially influenced by patient pharmacogenetic background [2].

Artemether–lumefantrine (AL) is the most adopted antimalarial by national malaria control programs worldwide. In Angola, it represents the first-line treatment of choice for uncomplicated malaria. Upon AL oral administration, artemether shows an elimination half-life of 1–3 h, CYP3A4 being the main enzyme involved in its conversion towards the (also active) dihydroartemisinin (DHA) metabolite [3]. Both artemether and DHA act rapidly to clear malaria parasites from circulation, reducing asexual parasite mass [4]. The lumefantrine partner has a half-life of 3–6 days and is responsible for the elimination of parasites remaining from the artemisinin ‘first impact’ action, while preventing recurrent malaria parasitaemia [5]. Only <10% of the absorbed LUM is biotransformed towards the active desbutyl-benflumetol (DBB) metabolite, mainly by CYP3A enzymes [6].

Both lumefantrine and DHA are essentially eliminated through the bile. In the apical biliocanalicular membrane of the hepatocyte, the ABCB1 (MDR1/Pgp) ATP-binding cassette (ABC) transporter is a major biliary efflux pump, particularly for lipophilic substrates, as lumefantrine [7, 8]. Significant inter-individual variation in drug exposure is known for both artemisinin and lumefantrine, suggesting the potential importance of \textit{CYP3A4} and \textit{ABCB1} pharmacogenetic characteristics influencing AL performance.

A previous attempt to correlate lumefantrine pharmacokinetic (PK) parameters with \textit{CYP3A4} and \textit{ABCB1} tag SNPs, particularly the promoter located g.-392A\textgreater G in the former (\textit{CYP3A4*1B}) and the synonymous c.3435C\textgreater T in the latter, did not yield significant associations [9], having prompted the natural conclusion that such variation had a negligible effect [9]. Possible positive associations were anyway recently suggested for \textit{ABCB1} c.3435C\textgreater T with altered LUM exposure among HIV positive subjects under Efavirenz based therapy.

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In the present work, we hypothesized that small pharmacokinetic differences might have observable pharmacodynamic consequences, the parasite reaction being a more sensitive parameter of the individual pharmacogenetic influence. Parasite clearance on day 3 post-treatment, recurrent infection prevalence and the 28-day cure rate endpoint of adequate clinical and parasitologic response (ACPR) were herein used as parameters to assess the effect of the patient pharmacogenetic status on AL in vivo anti-parasite performance. To test this hypothesis, a previously performed AL efficacy trial was analysed.

**Methods**

**Patients**

One-hundred and three unrelated patients with microscopy confirmed (1000–100,000 asexual parasites/μL) uncomplicated *Plasmodium falciparum* malaria involved in an AL (Coartem®, Novartis AG, Basel) efficacy trial in the Luanda region, Angola, conducted during the 2011–2013 period [10]. Briefly, upon informed written consent by the participant or their guardians, patients were treated with weight-adjusted, six-dose AL in 3 days, in accordance with national guidelines [11, 12]. Clinical assessment was performed at D2y, D3, D7, D14, D21 and D28. At each time-point, thick blood films were examined for the presence of parasites and a capillary blood sample obtained for PCR analysis.

Ethical approval was obtained from the Angolan National Public Health Institute/Ministry of Health Ethics Committee. All procedures followed the latest version of the Declaration of Helsinki.

**Molecular genotyping**

Capillary blood sample were collected on filter paper (FTA® Classic Card, Whatman). DNA extraction was done by phenol–chloroform methods. The *ABCB1* c.3435C>T, *CYP3A4* g.-392C>G and *CYP3A5* g.6986A>G SNPs were analysed by established PCR–RFLP protocols [13]. Presence of parasitaemia was further tested through the PCR. Ninety-eight patients were successfully analysed for the *pfmsp2* SNP. The genotype frequency in this Angolan population was 0.112 (0.060–0.196, 11/98) for the wild type (g.-392AA), 0.541 (0.438–0.641, 53/98) minor allele homozygous (g.-392GG) and 0.347 (0.438–0.641, 34/98) for the heterozygous (g.-392AG). The population was found in Hardy–Weinberg equilibrium for this locus (p > 0.05).

The patient *CYP3A4*4*1B* genotype was not found to be significantly associated with either D3 parasite positivity (χ² = 4.07, df = 1, p = 0.0391) or treatment outcome upon the 28-day follow up (χ² = 2.378, df = 1, p = 0.123) (Table 2).

Concerning the *ABCB1* c.3435C>T SNP, 101 patients were successfully tested. Genotype frequencies were 0.762 (0.667–0.841, 77/101) for the wild type (c.3435CC), 0.079 (0.035–0.150, 8/101) for the minor allele homozygous (c.3435TT) and 0.158 (0.093–0.244, 16/101) for the heterozygous (c.3435CT). The population was found in Hardy–Weinberg equilibrium for this locus (p > 0.05).

The c.3435C>T SNP was also not significantly associated with the D3 parasite PCR positivity (χ² = 0.09, df = 1, p = 0.767) (Table 1). On the other hand, c.3435TT genotypes were found to be significantly more frequent among patients experiencing recurrent events during follow-up (χ² = 6.9693, df = 1, p = 0.008) (Table 3, Fig. 1).

**Results**

The 28-day PCR-corrected cure rate was 91.3%. On D3, 46.6% (n = 48) had positive PCR. During the 28-day follow up, 29/103 patients experienced recurrent parasitaemia, as detected through PCR. Ninety-eight patients were successfully analysed for the *CYP3A4* -392A>G SNP. The genotype frequency in this Angolan population was 0.122 (0.060–0.196, 11/98) for the wild type (g.-392AA), 0.883 (0.438–0.641, 87/103) minor allele homozygous (g.-392GG) and 0.079 (0.035–0.150, 8/103) for the heterozygous (g.-392AG). The population was found in Hardy–Weinberg equilibrium for this locus (p > 0.05).

The patient *CYP3A4*4*1B* genotype was not found to be significantly associated with either D3 parasite positivity (χ² = 5.493, df = 1, p = 0.0191) (Table 1) or treatment outcome upon the 28-day follow up (χ² = 2.378, df = 1, p = 0.123) (Table 2).

Concerning the *ABCB1* c.3435C>T SNP, 101 patients were successfully tested. Genotype frequencies were 0.762 (0.667–0.841, 77/101) for the wild type (c.3435CC), 0.079 (0.035–0.150, 8/101) for the minor allele homozygous (c.3435TT) and 0.158 (0.093–0.244, 16/101) for the heterozygous (c.3435CT). The population was found in Hardy–Weinberg equilibrium for this locus (p > 0.05).

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These changes were further reflected on a significant increase in recurrence risk (OR = 10.59, 1.96–57.30, z-score = 2.739, p = 0.006) in this subgroup.

During the completion of the present work, a new report has suggested CYP3A5 as a contributor to lumefantrine metabolism [16]. Following this lead, we have successfully analyzed CYP3A5*3 (c.6986A>G) in 84 samples, as this is the allele is the most investigated as having a robust deleterious effect in the expression of the gene [17]. Genotype frequencies were 0.750 (0.644–0.838, 63/84) for the wild type (c.6986AA), 0.048, (0.013–0.117, 4/84) for the heterozygous (c.6986AG) and 0.202 (0.123–0.304, 17/84) for the minor allele homozygous (c.6986GG). The sample population was found in Hardy–Weinberg equilibrium for this locus (p > 0.05).

As with CYP3A4*1B, no significant association was observed between the patient status for carrying a CYP3A5*3 alleles and the parasitological outcome during the 28 day follow up (χ² = 0.932, df = 1, p = 0.335) (Table 2).

Due to the size limitations of the study, multivariate analysis was limited to variables expected to interact concerning the clinical outcome under focus (D28 follow up positivity), namely the ABCB1 c.3435C>T patient status and the pfmdr1 N86Y status. A subset of 92 cases was available with complete data for these three variables (Table 4).

Upon the assumption of a false discovery rate of (q) of 10% for Benjamini–Hochberg multiple test correction, only two associations stood out as near the threshold of significance: the overall interaction between the three analysed variables (G² = 10.84, df = 4, p = 0.0284 vs p (corrected) = 0.0286), and the association between recurrence during follow-up and the ABCB1 c.3435C>T status.

### Table 1  CYP3A4-392A>G and ABCB1 3435C>T genotype frequencies and D₃ PCR positivity (IC95%)

| Gene/SNP               | Genotype frequencies (IC 95%)                  |
|------------------------|-----------------------------------------------|
|                        | D3 positive                  | D3 negative                  |
| CYP3A4*1B              |                               |                               |
| AA                     | 3/48 (0.062; 0.016–0.182)      | 14/48 (0.292; 0.174–0.443)   |
| AG                     | 31/48 (0.646; 0.440–0.775)     | 8/49 (0.163; 0.078–0.302)    |
| GG                     | 8/49 (0.163; 0.078–0.302)      | 21/49 (0.429; 0.291–0.577)   |
| MDR1, c.3435C>T CC     |                               |                               |
| AG                     | 34/47 (0.723; 0.571–0.839)     | 4/47 (0.085; 0.028–0.213)    |
| AG                     | 4/47 (0.085; 0.028–0.213)      | 7/51 (0.137; 0.062–0.269)    |
| TT                     | 27/51 (0.519; 0.358–0.664)     | 31/51 (0.599; 0.358–0.813)   |
| NO significant associations were                                           |
| D3 positive positive PCR by day 3; D3 negative negative PCR by day 3 |

### Table 2  CYP3A4-392A>G and ABCB1 3435C>T genotype frequencies and risk of recurrence during the 28-day follow-up (IC95%)

| Gene/SNP               | Genotype frequencies (IC 95%)                  |
|------------------------|-----------------------------------------------|
|                        | Recurrence-free group                  | Recurrence group                  |
| CYP3A4*1B              |                               |                               |
| AA                     | 8/71 (0.113; 0.053–0.215)               | 26/71 (0.366; 0.258–0.490)      |
| AG                     | 37/71 (0.521; 0.400–0.640)              | 3/28 (0.107; 0.028–0.294)       |
| GG                     | 26/71 (0.366; 0.258–0.490)              | 10/28 (0.357; 0.193–0.559)      |
| ABCB1 3435C>T CC       |                               |                               |
| AA                     | 60/72* (0.833; 0.723–0.907)             | 17/29* (0.586; 0.391–0.760)     |
| AG                     | 10/72* (0.077; 0.072–0.245)             | 6/29* (0.207; 0.087–0.403)      |
| GG                     | 2/72* (0.028; 0.005–0.106)              | 6/29* (0.207; 0.087–0.403)      |
| CYP3A5*3               |                               |                               |
| AA                     | 46/59 (0.780; 0.653–0.877)              | 17/25 (0.680; 0.465–0.851)     |
| AG                     | 11/59 (0.186; 0.097–0.309)              | 6/25 (0.240; 0.094–0.451)      |
| GG                     | 2/59 (0.034; 0.004–0.117)               | 2/25 (0.080; 0.010–0.260)      |

* Significant (p < 0.01)

Recurrence-free group without positive PCR during the follow-up days

Recurrence group with positive PCR during the follow-up days

### Table 3 Analysis of the association between ABCB1 c.3435C>T patient status and treatment outcome (χ² = 6.9693, df = 1, p = 0.008)

| ABCB1 c.3435C>T Recurrence status | Total |
|-----------------------------------|-------|
| Positive                         | 17    |
| ACPR                              | 60    |
| Total                             | 77    |

| CC | 12 |
| CT | 29 |
| Total | 71 |
then the final phase II glucuronidated DHA extracted from the liver.

The CYP3A4*1B and CYP3A4*3 status were not seen to influence the risk of malaria recurrence. The AL post-treatment protective effect is essentially related with the action of lumefantrine, the long half-life partner. In regular conditions, it is expected that the large majority of lumefantrine is eliminated unchanged [18], a result supported by the previously observed modest effect of ketoconazole in interaction studies [6]. This means that any role of CYP3A4 and/or CYP3A5 will be limited to variations in this remain biotransformed fraction, which expected small size might have precluded its detection during the present works. One cannot nevertheless rule out the possibility that small changes in the concentrations of the resulting DBB metabolite might influence the treatment, in particular because of its higher anti-parasitic potency, as previously suggested [19]. Also, it is likely that scenarios of long-term CYP3A induction might increase the fraction of LUM metabolism-as potentially observed among patients under Efavirenz therapy [20]-and as such the role of this cytochrome P450s on lumefantrine elimination. Nevertheless, inside its size limitations and in this specific population, our study suggests a likely minor contribution of the CYP3A4*1B and CYP3A5*3 SNPs in modulating AL post-treatment prophylactic action.

A significant increase in the frequency of the ABCB1 c.3435TT genotype was found among patients suffering recurrent infections during the 28-day follow up, suggesting a role of the encoded P-glycoprotein. The synonymous c.3435C>T polymorphism has been proposed to be linked with altered rates of protein synthesis, leading to proteins that albeit having the same primary sequence, emerge from the process of translation with different tertiary conformations [21]. The functional effect of such changes in the P-glycoprotein seems to depend on the drug under consideration. In the present studies, a substantial predominance was found of the ABCB1 c.3435CC genotype among the recurrence-free patients, signalling an increased lumefantrine exposure associated with this genotype, which better shielded the recovering patients from new infections.

A shortcoming of the present study is the unavailability of pharmacokinetic data, namely D7 LUM levels, in order to have a complete pharmacokinetic/pharmacogenetic picture. Nevertheless, it is interesting to note that the present results are in agreement with recent data from Maganda et al. [20], where the ABCB1 c.3435TT genotype, was suggested to be associated with a significantly decreased D7 lumefantrine levels among patients undergoing malaria treatment with AL. Such an effect in drug exposure can explain the increase risk of these patients towards recurrent infection.
These data suggest lumefantrine as part of the group of ABCB1 substrates where this genotype is associated with increased drug exposure, probably due to a less efficient efflux. Other examples include tacrolimus [22, 23], silibinin [24], amlodipine [25], or in some studies, digoxine [26].

**Conclusion**

By exploring potential pharmacodynamics/pharmacogenetic associations in anti-malarial therapy, this report shows a non-negligible influence of the host ABCB1 c.3435C>T SNP in the performance of artemether–lumefantrine. The present observations join other recent reports pointing for the importance drug transporter pharmacogenetics in ACT pharmacokinetics and pharmacodynamics [20, 27].

**Authors’ contributions**

KK participated in the design and implementation of the study in Angola, molecular laboratory work and analysis, data analysis and drafting the manuscript; JPG participated in the analysis of the data, manuscript writing and review; VR and AR reviewed the manuscript, and DL conceived, coordinated the study. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Ethical approval was obtained from the Angolan National Public Health Institute/Ministry of Health Ethics Committee. All procedures followed the latest version of the Declaration of Helsinki.

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