Pharmacogenetic assessment of tafenoquine efficacy in patients with *Plasmodium vivax* malaria

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*Plasmodium vivax* has the largest geographic range of human malaria species and is challenging to manage and eradicate due to its ability to establish a dormant liver stage, the hypnozoite, which can reactivate leading to relapse. Until recently, the only treatment approved to kill hypnozoites was the 8-aminoquinoline, primaquine, requiring daily treatment for 14 days. Tafenoquine, an 8-aminoquinoline single-dose treatment with activity against *P. vivax* hypnozoites, has recently been approved by the US Food and Drug Administration and Australian Therapeutic Goods Administration for the radical cure of *P. vivax* malaria in patients 16 years and older. We conducted an exploratory pharmacogenetic analysis (GSK Study 208099) to assess the role of host genome-wide variation on tafenoquine efficacy in patients with *P. vivax* malaria using data from three GSK clinical trials, GATHER and DETECTIVE Part 1 and Part 2. Recurrence-free efficacy at 6 and 4 months and time to recurrence up to 6 months postdosing were analyzed in 438 *P. vivax* malaria patients treated with tafenoquine. Among the approximately 10.6 million host genetic variants analyzed, two signals reached genome-wide significance (*P* value ≤ 5 x 10^-8), rs62103056, and variants in a chromosome 12 intergenic region, were associated with recurrence-free efficacy at 6 and 4 months, respectively. Neither of the signals has an obvious biological rationale and would need replication in an independent population. This is the first genome-wide association study to evaluate genetic influence on response to tafenoquine in *P. vivax* malaria. *Pharmacogenetics and Genomics* XXX: 000–000 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.

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**Introduction**

*Plasmodium vivax*, a major cause of malaria in the Americas, Central and Southeast Asia, and Eastern parts of Africa, can result in severe and fatal illness [1]. The *P. vivax* lifecycle includes a dormant liver stage, the hypnozoite, activation of which causes relapses. The WHO recommends treatment with a schizonticide to treat the blood stage, combined with hypnozoite clearance [2]. Until recently, the only option available to kill hypnozoites was primaquine, first registered in the 1950s, and often associated with poor compliance because it requires administration once daily for 14 days [3]. Tafenoquine is an 8-aminoquinoline with longer-acting antihypnozoite activity. Tafenoquine is a single-dose treatment, reducing the risk of noncompliance which is expected to result in improved individual and public health outcomes. Phase 2 and 3 trial results of recurrence prevention have consistently shown that tafenoquine given with chloroquine was superior to chloroquine alone [4,5] and is similar in efficacy and safety to primaquine given with chloroquine [6]. Approximately, 15% of effective drugs are estimated to have robust genetic predictors of efficacy with roughly
half of these being of clinical relevance [7]. Therefore, GlaxoSmithKline routinely screens for genetic variants that may predict efficacy as part of clinical development. While there has been candidate gene research into chloroquine and primaquine efficacy in *P. vivax* treatment [8–10] including the effect of CYP2D6 on primaquine efficacy [8,10], this is the first exploration to examine genome-wide variation and tafenoquine treatment response.

**Methods**

**Participants**

Participants in this exploratory, retrospective pharmacogenetics study were enrolled in GATHER or DETECTIVE Parts 1 and 2 (ClinicalTrials.gov numbers NCT02216123 and NCT01376167, respectively). All patients were in the intent-to-treat (ITT) population and all but one patient, in DETECTIVE Part 1, had microscopically confirmed *P. vivax* microscopically confirmed ITT (mITT). Details on the studies can be found in the Supplementary material, Supplemental digital content 1, http://links.lww.com/FPC/B371. Participants were drawn from the DETECTIVE and GATHER mITT populations, provided written informed consent for genetics research, a DNA sample, and were successfully genotyped. All patients were treated with chloroquine on days 1–3 (600, 600, and 300 mg) to treat the blood stage of *P. vivax* malaria. The main pharmacogenetic (PGx) analysis group, PGx TQ, included patients on 300 or 600 mg of tafenoquine in combination with chloroquine; patients on 50 and 100 mg tafenoquine doses were not analyzed as these doses were found to not be efficacious [4]. Two additional pharmacogenetic analysis groups were analyzed to aid in the interpretation of the PGx TQ results; the PGx CQ group consisting of patients treated with chloroquine alone and the PGx PQ group, patients treated with primaquine and chloroquine.

**Statistical analysis**

Definition of outcomes, genotyping and imputation details are summarized in the Supplementary materials, Supplemental digital content 1, http://links.lww.com/FPC/B371. Analysis was conducted using an additive genetic model on genome-wide variants with a minor allele frequency ≥0.01 and an imputation quality score ≥0.30. The conventional *P* ≤ 5 × 10<sup>−8</sup> threshold for declaring genome-wide significance for common variants was used [12]. No multiplicity adjustment was made for multiple outcomes or analysis subgroups.

**Results**

**Patients analyzed**

The pharmacogenetic sample was comprised of 900 patients of whom 529, 221, and 150 were treated with tafenoquine, primaquine, or chloroquine alone, respectively. From this, 73 of the 529 tafenoquine patients were excluded from analysis as they had been treated with 50 or 100 mg doses of tafenoquine. A further 44 patients were excluded due to close relatedness (third degree or closer) resulting in pharmacogenetic analysis groups of 438, 206, and 139 for tafenoquine, primaquine, and chloroquine, respectively. Demographic and efficacy information were similar across the pharmacogenetic analysis groups and their constituent mITT populations from which they were drawn (Table 1).

**Genome-wide association results in the PGx TQ analysis group**

The results for the three outcomes appear well calibrated as shown in the Manhattan and QQ plots (Supplementary Fig. 1a–f, Supplemental digital content 1, http://links.lww.com/FPC/B371). No significant association was observed for time to recurrence. Recurrence-free efficacy at 6 months was significantly associated with an intergenic variant, rs62103056 (hg19 position 18:41424097, imputation quality score of 1.0) where the A allele, with a frequency of 0.25, was associated with increased recurrence [OR = 2.98, 95% confidence interval (CI) 1.99–4.46, *P* = 3.79 × 10<sup>−8</sup>] (Fig. 1, Supplementary Fig. 2, Supplemental digital content 1, http://links.lww.com/FPC/B371). A similar trend was seen in the PGx TQ South American (OR = 3.39, 95% CI 2.07–5.56, *P* = 5.64 × 10<sup>−7</sup>) and PGx TQ Asian subgroups (OR = 3.10, 95% CI 1.33–7.25, *P* = 0.013). Results for recurrence-free efficacy at 4 months and time to recurrence show a similar trend in PGx TQ (Supplementary Fig. 3, Supplemental digital content 1, http://links.lww.com/FPC/B371). No significant association was seen for this variant in either PGx PQ (OR = 1.03, 95% CI 0.57–1.89, *P* = 0.92) or PGx CQ (OR = 0.46, 95% CI 0.20–1.05, *P* = 0.08). rs62103056 is approximately 12.5 kb 5′ of RNU6-443P (RNA, U6,
small nuclear 443, pseudogene) (Supplementary Fig. 2, Supplemental digital content 1, http://links.lww.com/FPC/B371). A forest plot for the rs62103056 results for all three outcomes and all pharmacogenetic analysis groups is shown in Supplementary Fig. 4, Supplemental digital content 1, http://links.lww.com/FPC/B371.

Recurrence-free efficacy at 4 months revealed a significant association with the allele frequencies of ~2% were associated with improved efficacy. For purposes of illustration, results for rs11104986 will be discussed as this variant had the smallest P value. The minor G allele was associated with increased recurrence (OR = 42.30, 95% CI 7.95–225.10, P = 4.18 × 10−9) (Fig. 2, Supplemental digital content 1, http://links.lww.com/FPC/B371). The frequency of the G allele was ~2% in both the South American and Asian subgroups and the direction of effect was the same as in the overall PGx TQ group. Results for recurrence-free efficacy at 6 months and time to recurrence show a similar trend in PGx TQ, while no significant association was seen for this variant in either the PGx PQ (OR = 1.22, 95% CI 0.28–5.35, P = 0.81) or PGx CQ (OR = 0.57, 95% CI 0.06–5.59, P = 0.64) groups (Supplementary Fig. 6, Supplemental digital content 1, http://links.lww.com/FPC/B371). This region is approximately 22 and 52kb 3′ of RNU1−117P and KITLG, respectively (Supplementary Fig. 5, Supplemental digital content 1, http://links.lww.com/FPC/B371). RNU1−117P is a small, nuclear pseudogene. KITLG (KIT ligand) is associated with the skin, hair, and eye pigmentation, and GTEx RNA-seq [13] expression data show the highest median expression in transformed fibroblasts.

### Discussion

Previous assessments of *P. vivax* drug response have focused on candidate gene host effects on chloroquine and primaquine treatment. This is the first pharmacogenetic study to assess the influence of host genome-wide variation on response to tafenoquine in the radical cure for *P. vivax* malaria and is powered at 80% to rule out large genetic effects with minor allele frequencies ≥10% and per-allele OR ≥4. Two signals passed the statistical threshold for genome-wide significance, but neither has been seen with the current pharmacogenetic analysis groups.

Table 1 Demographic and efficacy information across treatment arms in the pharmacogenetic analysis groups

| Treatment | mITT TQ | PGx mITT TQ | mITT PQ | PGx mITT PQ | mITT CQ | PGx mITT CQ |
|-----------|---------|-------------|---------|-------------|---------|-------------|
| Study, N (%): | | | | | | |
| DETECTIVE Part 1 | 109 (21%) | 69 (16%) | 45 (18%) | 30 (15%) | 51 (29%) | 28 (20%) |
| DETECTIVE Part 2 | 253 (49%) | 236 (54%) | 126 (51%) | 110 (53%) | 125 (71%) | 111 (80%) |
| GATHER | 159 (31%) | 133 (30%) | 78 (31%) | 66 (32%) | 0 (0%) | 0 (0%) |
| Sex, F:M, N (%F): | | | | | | |
| Censored prior to 4 months | 31 (6%) | 25 (6%) | 20 (8%) | 13 (6%) | 10 (6%) | 9 (6%) |
| Recurrence-free | 389 (75%) | 324 (74%) | 175 (70%) | 146 (71%) | 64 (36%) | 44 (32%) |
| Recurrence | 101 (19%) | 89 (20%) | 54 (22%) | 47 (23%) | 102 (58%) | 86 (62%) |
| Censored prior to 6 months | 31 (8%) | 26 (8%) | 18 (7%) | 11 (5%) | 12 (7%) | 10 (7%) |
| Recurrence-free | 347 (67%) | 285 (65%) | 167 (67%) | 139 (67%) | 50 (28%) | 35 (25%) |
| Recurrence | 134 (26%) | 118 (27%) | 64 (22%) | 52 (25%) | 114 (65%) | 94 (68%) |
| Censored prior to 6 months | 31 (6%) | 25 (6%) | 20 (8%) | 13 (6%) | 10 (6%) | 9 (6%) |
| Recurrence-free | 389 (75%) | 324 (74%) | 175 (70%) | 146 (71%) | 64 (36%) | 44 (32%) |

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a strong biological rationale after reviewing information from GTEx release 7 and the Ensembl Variant Effect Predictor CRCh37 release 95 [14] and replication in an independent population is needed. The chromosome 12 signal, even if it were to replicate, would not provide much use in a clinical setting due to the low frequency of the minor allele. The chromosome 18 signal, rs62103056, if replicated, may be of clinical relevance in guiding treatment decisions as patients treated with tafenoquine and carrying the AA genotype show reduced efficacy; however, this replication would need to be done in a real-world setting that considers noncompliance with treatment.

In areas where malaria transmission is high, it is difficult to distinguish relapse, due to hypnozoite activation, from recurrence which includes hypnozoite activation and reinfection. A limitation of this study is that we cannot distinguish relapse from reinfection. Additional studies in areas of low or without malaria transmission would be needed to confirm that these results are related to relapse. This study is important in providing an initial view into the pharmacogenetics of tafenoquine efficacy in the treatment of *P. vivax* malaria. Further investigation into the role of host genetics and the interaction between pathogen and host may help elucidate the role of host genetics on tafenoquine efficacy.

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Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com

**Conflicts of interest**

The views expressed here are solely those of the authors and do not reflect the views, policies or positions of the U.S. Government or Department of Defense. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the

**Fig. 1**

Proportion, with 95% confidence intervals, of patients who were recurrence-free at 6 months by rs62103056 genotype in PGx TQ and its Asian and South American subgroups. *N* represents the sample size in the entire PGx TQ group. The number of patients with AA, AG, and GG was 15, 39, and 51 in Asians and 15, 101, and 164 in South Americans.
Fig. 2

Proportion, with 95% confidence intervals, of patients who were recurrence-free at 4 months by rs11104986 genotype in PGx TQ and its Asian and South American subgroups. N represents the sample size in the entire PGx TQ group. The one patient in PGx TQ with genotype GG was in the African region and had a recurrence within 4 months. The patient numbers with AA and AG genotypes were 103 and 5 in Asians and 279 and 8 in South Americans, respectively.

Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human participants as prescribed in AR 70–25.

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