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Characterization of *Plasmodium vivax pvmdr1* Polymorphisms in Isolates from Mangaluru, India

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Abstract. India accounts for approximately half of the global *Plasmodium vivax* cases, but information as to the presence of chloroquine (CQ) resistance is scarce. In an observational study in Mangaluru, south-western India, of 116 vivax malaria patients analyzed, 89.5% (102/114) had cleared parasitemia on days two or three of CQ treatment. Two remaining patients presented on days four and five without parasitemia. One hundred eight isolates of these 116 patients were successfully sequenced for *pvmdr1* polymorphisms. Eight non-synonymous polymorphisms but no wild-type isolate were detected. Ten *pvmdr1* haplotypes were observed with mutations T958M and F1076L occurring in all isolates, whereas the candidate CQ resistance marker Y976F was present in one isolate only. *Pvmdr1* polymorphisms were not associated with early parasite clearance. The high proportion of early parasite clearance and the virtual absence of *pvmdr1* Y976F and of sextuple *pvmdr1* mutants suggest that CQ in the study area is still sufficiently effective. However, the abundance of *pvmdr1* mutations in the local parasite population warrants monitoring.

India accounts for approximately half of the global *Plasmodium vivax* malaria cases.[1] The city of Mangaluru, located at the Arabian Sea in south-western India, shows a peculiar pattern of urban malaria with importation of plasmodia particularly from the north-eastern parts of the country.[2] Chloroquine (CQ, plus primaquine) still is the mainstay of treating vivax malaria, even though treatment failures have been reported in several Asian countries including India.[3] Chloroquine resistance has been linked to polymorphisms in the *P. vivax* multidrug resistance gene *pvmdr1*, orthologue to *Plasmodium falciparum pfmdr1*. Particularly, the substitution Y976F in *pvmdr1* gene has been associated with a reduced CQ sensitivity in few studies in Southeast Asia, especially in Thailand, Myanmar, and Indonesia.[4–6] Furthermore, *P. vivax* isolates carrying the Y976F mutation reportedly show significantly increased IC_{50} values for CQ in vitro.[7] In Madagascar, all CQ treatment failures occurred in infections with sextuple *pvmdr1* mutant parasites (S513R-G698S-M908L-T958M-Y976F-F1076L).[8] However, present knowledge on the distribution of these mutations and of the respective haplotypes remains scarce, especially in India.

A recent study from Mangaluru,[9] southern India, reported *pvmdr1* mutations including Y976F, which might reflect a trend toward emerging drug resistance. Here, we aimed at further investigating these polymorphisms to achieve a more thorough understanding of CQ resistance in the area.

Plasmodium isolates were obtained between June and December 2015 from 909 malaria outpatients attending Wenlock Hospital, the largest governmental health facility of Mangaluru. Recruitment procedures and patient characteristics have been detailed elsewhere.[10]

Six hundred thirty-three patients had *P. vivax* mono-infections and were treated with CQ for 3 days plus primaquine (0.25 mg/kg body weight) for 14 days. Patients investigated were mostly young (median age, 25 years) males (93%) with a geometric mean parasite density of 2,999 parasites/μL (95% CI, 2,660–3,382). Chloroquine intake within the 4 weeks preceding presentation was stated by < 1% of patients.[10]

Study participants were asked to return to the hospital on day 2 (48 hours) or day 3 (72 hours) of CQ treatment to evaluate parasite clearance by thick blood film microscopy. Among 633 vivax malaria patients, 114 returned for the recommended control on day 2 (81) or on day 3 (33). Two additional patients presented at days 4 and 5.

For *pvmdr1* typing, DNA was extracted from blood samples obtained from these 116 patients at initial presentation, *pvmdr1* was amplified as published elsewhere,[11] and polymerase chain reaction (PCR) products were bidirectionally sequenced (Eurofins Genomics, Berlin, Germany). Multiple sequence alignment was performed using SnapGene v. 3.1 (GSL Biotech, Chicago, IL) software and the *pvmdr1* Sal-1 strain sequence (GenBank: AY618622.1) as the reference. Data analysis was performed using SPSS v. 22 (IBM Corp., Armonk, NY).

On day 2 of CQ treatment, 87.7% (71/81) of patients presenting for a checkup had cleared parasitemia, and this figure was 93.9% (31/33) on day 3. Two further patients were free of malaria parasites when presenting on days four and five of treatment. *Pvmdr1* sequencing was successful for 108 isolates (93.1%, 108/116). Four synonymous (T529T, A970A, S1358S, and R1422R) and eight non-synonymous (S513R, T958M, Y976F, F1076L, Y1028C, L1393N, L1425R, and T1269S) point mutations were identified. All 108 *P. vivax* isolates presented the synonymous single-nucleotide polymorphism (ssSNP) T529T (A970A, 1.9% [2/108], S1358S, 8.4% [9/108], R1422R, 0.9% [1/108]) and the non-synonymous (ns) SNP T958M. Of these, 87.0% (94/108) additionally had nsSNP F1076L. The prevalence of the other nsSNPs was S513R (9.6%, 10/108), Y976F (0.9%, 1/108), Y1028C (2.8%, 3/108), L1393N (24.0%, 26/108), L1425R (0.9%, 1/108), and T1269S (3.7%, 4/108). Of note, F1076L isolates did not carry mutations T1269S and L1393N in an almost mutually exclusive manner ($P < 0.001$). Vice versa, S513R did only occur among F1076L parasites.

Ten *pvmdr1* haplotypes were recognized (Table 1), including T958M-Y976F-F1076L in one isolate (0.9%). None of

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TABLE 1

Prevalence of *pvmdr1* haplotypes and proportion of parasitemic patients on follow-up

Pvmdr1 haplotype	No.	%	Proportion of patients parasitemic on day 2 or day 3 of chloroquine treatment
S513R-T958M-Y976F-F1076L	1	0.9	1/1 (100%)
S513R-T958M-Y1028C-F1076L	3	2.8	0/3 (0%)
S513R-T958M-F1076L-L1393N	2	1.9	0/2 (0%)
S513R-T958M-F1076L-L1425R	1	0.9	0/1 (0%)
T958M-F1076L-T1269S-L1393N	1	0.9	1/1 (100%)
T958M-F1076L-T1269S	3	2.8	1/3 (33.3%)
S513R-T958M-F1076L	3	2.8	0/3 (0%)
T958M-F1076L-L1393N	9	8.3	0/9 (0%)
T958M-L1393N	14	13	1/14 (7.2%)
T958M-F1076L	71	65.7	6/71 (8.5%)

the individual polymorphisms (data not shown) or haplotypes (Table 1) were associated with day 2 or day 3 positivity.

In this study from coastal, south-western India, CQ was successful in eliminating *P. vivax* malaria in 88% and 94% of patients on days 2 and 3, respectively. In a meta-analysis of *P. vivax* CQ resistance, the earliest treatment failure occurred at a median of 14 days (range 3–28 days), and early parasite clearance correlated with treatment outcome as assessed on day 28. Of note, parasite clearance in 95% or 100% of patients by day 2 or day 3, respectively, was found to be 100% predictive of CQ sensitivity as defined by the day 28 outcome.[3] The present study was not designed as a treatment trial, but against this background, it seems justifiable to state that CQ in the study area is sufficiently effective. This is supported by the virtual absence of the candidate CQ resistance marker *pvmdr1* Y976F, the lacking association of the detected polymorphisms with follow-up positivity and the absence of sextuple *pvmdr1* mutants carrying mutation S513R and Y976F.

The high prevalence of *pvmdr1* T958M and F1076L in our study is in accordance with the genotype pattern previously reported at this location.[9,12] However, whereas the candidate marker Y976F occurred only once (0.9%) in the present study, the figure was almost 8-fold higher in a previous report.[9] The abundance of *pvmdr1* F1076L in isolates from Mangaluru has been considered an indication of emerging CQ resistance.[9,12] However, as with most previous investigations, the present data do not support a predictive role of that polymorphism. Ultimately, prolonged monitoring of treated patients is required to elucidate the role of *pvmdr1* variants in recrudescence and to enable the prompt detection of CQ resistance in south-western India.

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REFERENCES

1. World Health Organization, 2017. *WHO Malaria Report*. Available at: https://apps.who.int/iris/bitstream/10665/259492/1/9789241565523-eng.pdf/. Accessed March 1, 2019.
2. Anvikar AR, Shah N, Dhariwal AC, Singh G, 2016. Epidemiology of *Plasmodium vivax* malaria in India. *Am J Trop Med Hyg 95:* 108–120.
3. Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ, 2014. Global extent of chloroquine-resistant *Plasmodium vivax*: a systematic review and meta-analysis. *Lancet Infect Dis 14:* 982–991.
4. Suwanarusk R et al., 2008. Amplification of *pvmdr1* associated with multidrug-resistant *Plasmodium vivax*. *J Infect Dis 198:* 1558–1564.
5. Nyunt MH, Han JH, Wang B, Aye KM, Aye KH, Lee SK, Htut Y, Kyaw MP, Han KT, Han ET, 2017. Clinical and molecular surveillance of drug resistant vivax malaria in Myanmar (2009–2016). *Malar J 16:* 117.
6. Imwong M, Pukrittayakamee S, Rénia L, Letourneur F, Charlieu JP, Leartsakulpanich U, Looareesuwan S, White NJ, Snounou G, 2003. Novel point mutations in the dihydrofolate reductase gene of *Plasmodium vivax*: evidence for sequential selection by drug pressure. *Antimicrob Agents Chemother 47:* 1514–1521.
7. Suwanarusk R et al., 2007. Chloroquine resistant *Plasmodium vivax*: in vitro characterization and association with molecular polymorphisms. *PLoS One 2:* e1089.
8. Barnadas C, Ratsimbasoa A, Tichit M, Bouchier C, Jahevitra M, Picot S, Menard D, 2008. *Plasmodium vivax* resistance to chloroquine in Madagascar: clinical efficacy and polymorphisms in *pvmdr1* and *pvcrt-o* genes. *Antimicrob Agents Chemother 52:* 4233–4240.
9. Joy S, Mukhi B, Ghosh SK, Achur RN, Gowda DC, Surolia N, 2018. Drug resistance genes: *pvcrt-o* and *pvmdr-1* polymorphism in patients from malaria endemic south western coastal region of India. *Malar J 17:* 40.
10. Gai PP et al., 2018. Manifestation of malaria in Mangaluru, southern India. *Malar J 17:* 313.
11. Lu F et al., 2011. Genetic polymorphism in *pvmdr1* and *pvcrt-o* genes in relation to in vitro drug susceptibility of *Plasmodium vivax* isolates from malaria-endemic countries. *Acta Trop 117:* 69–75.
12. Anantabotla VM, Antony HA, Parija SC, Rajkumari N, Kini JR, Manipura R, Nag VL, Gadepalli R, Chayani N, Patro S, 2019. Polymorphisms in genes associated with drug resistance of *Plasmodium vivax* in India. *Parasitol Int 70:* 92–97.