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<th>Title</th>
<th>Short report: polymorphisms in the chloroquine resistance transporter gene in Plasmodium falciparum isolates from Lombok, Indonesia.</th>
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The polymorphisms in the Plasmodium falciparum multidrug resistance 1 (pfmdr1) and P. falciparum chloroquine resistance transporter (pfcrt) genes, which are associated with chloroquine resistance, were examined in 48 P. falciparum isolates from uncomplicated malaria patients from the West Lombok District in Indonesia. The point mutation N86Y in pfmdr1 was present in 35.4% of the isolates and mutation K76T in pfcrt was found in all but one of the samples studied. Identified pfcrt haplotypes were mainly identical to the Papua New Guinea type S_{sup}VMNT (42 of 48, 87.5%), and a few isolates had the Southeast Asia type CVIET (5 of 48, 10.4%). Moreover, one P. falciparum isolate harbored the K76N mutation, giving rise to the haplotype CVMMN, which was not previously reported in field isolates. Our findings suggest that chloroquine resistance in this area might have the same origin as in Papua New Guinea.

In Indonesia, the first cases of resistance were reported in the early 1970s from Kalimantan and Irian Jaya. Although resistance has been reported on several islands in Indonesia, with resistance as high as 95% for P. falciparum isolates from the West Lombok District in Indonesia. The point mutation N86Y in pfmdr1 was present in 35.4% of the isolates and mutation K76T in pfcrt was found in all but one of the samples studied. Identified pfcrt haplotypes were mainly identical to the Papua New Guinea type S_{sup}VMNT (42 of 48, 87.5%), and a few isolates had the Southeast Asia type CVIET (5 of 48, 10.4%). Moreover, one P. falciparum isolate harbored the K76N mutation, giving rise to the haplotype CVMMN, which was not previously reported in field isolates. Our findings suggest that chloroquine resistance in this area might have the same origin as in Papua New Guinea.

Abstract. The polymorphisms in the Plasmodium falciparum multidrug resistance 1 (pfmdr1) and P. falciparum chloroquine resistance transporter (pfcrt) genes, which are associated with chloroquine resistance, were examined in 48 P. falciparum isolates from uncomplicated malaria patients from the West Lombok District in Indonesia. The point mutation N86Y in pfmdr1 was present in 35.4% of the isolates and mutation K76T in pfcrt was found in all but one of the samples studied. Identified pfcrt haplotypes were mainly identical to the Papua New Guinea type S_{sup}VMNT (42 of 48, 87.5%), and a few isolates had the Southeast Asia type CVIET (5 of 48, 10.4%). Moreover, one P. falciparum isolate harbored the K76N mutation, giving rise to the haplotype CVMMN, which was not previously reported in field isolates. Our findings suggest that chloroquine resistance in this area might have the same origin as in Papua New Guinea.

The DNA was extracted from filter paper by cutting the blood spot into pieces and soaking them in 0.5% saponin in HBS buffer (140 mM NaCl, 10 mM KCl, 10 mM HEPES, pH 7.2). Thereafter, the QIAamp DNA Kit (Qiagen, Valencia, CA) was used according to the manufacturer’s instructions. The parasite lines FCR3 and K1 were used as controls for the PCR amplification products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). The DNA extracts were amplified with primers specific for the pfcrt and pfmdr1 genes.

Molecular Applications, Rockland, ME). For pfcrt gene analysis, a first amplification was carried out with previously designed primers, and the products obtained were used as a template in a nested PCR encompassing the polymorphic codons 72-76 and 97 in exon 2 as previously reported. The PCR amplification products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA).
PCR purification kit (Qiagen) and directly sequenced on an ABI310 automated sequencer using ABI PRISM Big Dye Terminator Cycle kit (Applied Biosystems, Foster City, CA) following the manufacturer’s instructions.

The glurp gene, located on chromosome 11, which has a high degree of polymorphism, was assessed for evaluation of diversity of the *P. falciparum* isolates population in the region. The amplification products were resolved by electrophoresis on a 1% agarose gel and stained with ethidium bromide. The glurp amplification product sizes were estimated using DNAfrag version 3.03 Software (John Nash, Institute for Biologic Sciences, National Research Council of Canada, Ottawa, Ontario, Canada). For comparisons, Fisher’s exact test was used.

Seventeen isolates (35.4%) had the 86Y mutation in the *pfmdr1* gene, 26 had wild type N86, and 5 carried both alleles (Table 1). In previous studies in Irian Jaya and West Papua, N86Y was found to show a correlation with CQ-resistant *P. falciparum* parasites. The mutation K76T in the *pfcr* gene was found in all but one (47 of 48, 97.9%) of the isolates studied. A previous report showed that the K76T mutation showed a correlation with clinical resistance to CQ and as a molecular marker had a sensitivity of 93% and a specificity of 82%. Both point mutations in the *pfmdr1* and *pfcr* genes have been proposed to indicate a tendency toward reduced susceptibility to CQ. Thus, our results suggest potential CQ resistance in the region, although other factors may influence the final treatment outcome. The combination of geographic remoteness to health facilities and lack of interest in seeking medical attention driven by both financial reasons and lack of knowledge resulted in an overall follow-up rate of 23% (11 of 48). After 14 days of CQ treatment, 5 of 11 patients were not clear the parasites, and had tendency to harbor 86Y in the *pfmdr1* gene (Table 1). However, a larger sample size is required to obtain conclusive results.

A new mutation, K76N, which substitutes asparagine for lysine, was found in one isolate from the sub-village Pusuk, generating the haplotype CVMNN. For confirmation of this finding, independent PCR amplifications and at least three repetitions of sequencing were carried out. In all cases, unambiguous electropherograms were obtained, showing AAT codons for asparagine at position 76. The K76N mutation could be misidentified as a K76T substitution by a PCR-restriction enzyme protocol. To our knowledge, this is the first time that K76N has been reported in a field study. However, it has been reported in laboratory experiments after exposure of parasites to lethal concentrations of CQ. In those experiments, Cooper and others demonstrated that the K76N mutation confers the verapamil-reversible CQ-resistance phenotype associated with greatly reduced accumulation of the drug. Contrary to those in *in vitro* experiments, the patient possessing this rare *pfcr* haplotype cleared parasites after treatment with CQ. Since other factors participate in the clinical outcome, it would be interesting to look for more isolates with CVMNN and carry out the *in vitro* susceptibility test.

The sequence analysis of codons 72-76 in the *pfcr* gene (Table 1) allowed identification of previously reported haplotypes S*, *SVMNT (42 of 48, 87.5%) and CVIET (5 of 48, 10.4%). The *pfcr* SVMNT haplotype with serine coded by AGT has been found in Bougainville, Papua New Guinea.16

![Map of Indonesia showing the location of Lombok.](image)
the main island of Papua New Guinea, and East Timor. The haplotype CVIET has been reported in countries of Southeast Asia. Since Lombok, Indonesia is located near Papua New Guinea (Figure 1), it is not unexpected that both the SVMNT and CVIET haplotypes were detected. Furthermore, in our attempt to evaluate the diversity among the isolates studied, we assessed the glurp gene and 11 glurp genotypes were found in West Lombok, ranging from 450 to 1,100 basepairs. The West Lombok District, despite its small area, shows a high degree of diversity in the P. falciparum population that might be a product of high rate of transmission of malaria or human transmigration. Upon examination for any linkage among the alleles studied in the pfmdr1, pfcrt, and glurp genes, significant associations were found between the pfcrt CVIET haplotype and pfmdr1 86Y (P = 0.0193), the CVIET haplotype and glurp 450 (P < 0.001), and pfmdr1 86Y and glurp 450 (P = 0.0057). Our findings showed that the majority of isolates have pfcrt haplotype SVMNT, pfmdr1 86N, and glurp with molecular masses greater than 450 homogeneously distributed in all the villages from West Lombok, indicating that these might be indigenous in the area. A few isolates harboring pfcrt haplotype CVIET, pfmdr1 86Y, and glurp 450, found mainly in Kedongd Angas village, were most likely introduced recently. Therefore, CQ resistance in Lombok might have the same origin as the Papua New Guinea strains, and the Southeast Asian pfcrt haplotype CVIET might have been introduced only recently in a particular region. Further studies are being carried out in isolates from Lombok and other Indonesian islands to determine the prevalence of the novel K76N mutation and its association with clinical outcome/in vivo susceptibility to CQ. Received December 16, 2003. Accepted for publication February 4, 2004.

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