Genetic Confirmation of Quinine-Resistant *Plasmodium falciparum* Malaria Followed by Postmalaria Neurological Syndrome in a Traveler from Mozambique

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A case of quinine-resistant *Plasmodium falciparum* malaria, followed by a postmalaria neurological syndrome and a recurrence episode, is described. Genetic characterization of the *P. falciparum* isolate obtained by analysis of *msp*1 and *msp*2 amplicons revealed the coexistence of two genotypes causing the first malaria episode and the presence of a unique isolate responsible for the recurrence.

CASE REPORT

Fifteen days after returning from Bilene (Maputo Province, Mozambique), a previously healthy 42-year-old man was admitted to the National Institute for Infectious Diseases L. Spallanzani, Rome, Italy, with a 4-day history of headache and febrile illness. *Plasmodium falciparum* malaria was diagnosed on the basis of blood film examination; the initial level of parasitemia was >100,000 parasites/μl. The patient had not taken antimalarial prophylaxis during a business trip to Mozambique. On examination, the patient was fully conscious and had a temperature of 38°C. Hepatosplenomegaly was detected. Acute complications included hemolysis and severe thrombocytopenia. The patient was treated with intravenous (i.v.) quinine (QN; 20 mg of the hydrochloride salt per kg initially and then 10 mg/kg three times a day) plus oral doxycycline (100 mg two times a day). The parasitemia was cleared by day 4, whereupon the patient was treated with oral QN (8 mg of base/kg three times daily) plus oral doxycycline until day 8. He was discharged from the hospital on day 9.

Nine days later, however, the patient developed a low-grade fever with acute confusion (inaudible speech and markedly disturbed behavior), postural tremor, and nominal aphasia. He was readmitted to our hospital on day 20. On examination, the patient had a temperature of 38.2°C with no clinically detectable focus of infection and without meningism. He was in an acute confusional state with nominal aphasia and showed a fine postural tremor of the arms that worsened when he tried to move his arms. The lowest Glasgow coma score was 12. No abnormalities were found in the cardiovascular and respiratory systems. No previous history of neurological or psychiatric illness was ascertained. No medication had been taken by the patient at home. Simultaneous thick and thin blood film tests

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mg) and sulfadoxine (1,500 mg) was added. Serologic tests for human immunodeficiency virus types 1 and 2, Epstein-Barr virus, Toxoplasma gondii, Rickettsia spp., and Borrelia spp. were negative. There was no evidence of a transfusion-associated infection or an autoimmune process. The parasitemia cleared by day 38, and the patient remained afebrile from day 38 until he was discharged from the hospital on day 49, after a complete recovery. PCR analysis of his blood for P. falciparum, which remained positive during the three admissions, became negative on day 67. On the other hand, PCR analyses for P. ovale, P. vivax were negative during the three hospital stays.

**Genotyping of the P. falciparum isolate(s) and in vitro Plasmodium lactate dehydrogenase (pLDH) test.** We selected two polymorphic markers, the genes for merozoite surface protein 1 (MSP1) and MSP2, to genotype the P. falciparum isolates responsible for the patient’s infection. PCR amplification of these genes points out the presence of length polymorphism, allowing the detection of multiple infections by different P. falciparum genotypes (13). Total genomic DNAs were extracted from 1 ml of whole infected blood samples collected from the patient on the first and third hospital admittances with the QIAGEN Easy kit (QIAGEN) in accordance with the manufacturer’s instructions. Portions of about 300 and 500 nucleotides were PCR amplified for the genes for MSP1 and MSP2, respectively, as described by Wood et al. (13). The PCR result is shown in Fig. 1.

To identify the presence of point mutations in molecular markers of P. falciparum drug resistance, we performed a series of PCRs with specific primers that amplify informative regions of the P. falciparum multidrug resistance 1 (pfmdr1), P. falciparum chloroquine (CQ) resistance transporter (pfcr), and dihydrofolate reductase (dhfr) genes. We used as the template the genomic parasite DNA extracted from the blood samples containing the recurrent isolate. Molecular marker codons identified in the case report isolate are reported in Table 1, together with wild-type and mutant-type codons for comparison. The OMIGA2 program was used to compile and analyze the sequences obtained from the pfmdr1, pfcr, and dhfr amplicons. We found a mutant-type codon profile for pfcr (CQ) and pfmdr1 (CQ and QN) but a wild-type codon profile for the dhfr (PY) gene.

Finally, in vitro pLDH tests to evaluate the level of resistance of the recurrent isolate to QN and CQ were performed as described by Makler and Hinrichs (6). The results of pLDH tests (50% inhibitory concentration of CQ, 0.110 μg/ml; 50% inhibitory concentration of QN, 0.120 μg/ml) confirmed the full resistance of the isolate to CQ and QN, in accordance with the molecular marker analysis results.

The spread of resistance to the available antimalarials among malaria parasites represents a major worldwide health problem that seriously hampers efforts to control the disease. At present, clinical resistance to QN monotherapy occurs sporadically in Southeast Asia and western Oceania. From in vitro assays there is evidence of very little QN resistance in South America and Africa (3, 12).

We report here the first genetically characterized case of QN-resistant P. falciparum malaria acquired by a nonimmune traveler to Mozambique with neuropsychiatric manifestations of postmalaria neurological syndrome (PMNS).

**PMNS** is a self-limiting postinfective encephalopathy that occurs within 2 months after recovery from P. falciparum malaria whose neuropsychiatric manifestations are wide-ranging, including an acute confusional state or acute psychosis, cerebellar ataxia, generalized convulsions, motor aphasia, or fine tremor (2, 5, 8, 10, 11).

In a prospective study conducted in Vietnam, the overall incidence of PMNS after P. falciparum malaria was 1.2 per 1,000 cases and PMNS was associated with mefloquine treatment and with the severity of the preceding malaria infection (8). This syndrome has also been reported in nonimmune individuals (2, 5); this suggests that immunological mechanisms are implicated in PMNS. Indeed, the pathogenesis of PMNS is possibly mediated immunologically, caused by a cross-reaction of antibodies to antigens expressed by certain Plasmodium spp. strains of P. falciparum.

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![FIG. 1. Two percent agarose gel showing the products obtained by amplification with primers specific for the *msp1* (lanes 1 to 5) and *msp2* (lanes 6 to 10) genes. Lanes: M, Eurobioladder-L (Eurobio); 1 and 6, day 1; 2 and 7, day 2; 3 and 8, day 5; 4 and 9, day 35; 5 and 10, day 37. Lanes 1 to 3 and 6 to 8 (first hospital admittance) show two PCR bands, indicating the occurrence of at least two P. falciparum genotypes in the patient. The presence of a unique band, the upper one, in lanes 4 and 5 and lanes 9 and 10 (third hospital admittance) demonstrates the selection of one genotype. This genotype carries a mutant-type codon profile, as shown in Table 1.](http://jcm.asm.org/Downloaded_from)
symptoms began 10 days after the completion of antimalarial treatment, rendering unlikely a toxic effect due to the previous therapy.

Genetic characterization of the *P. falciparum* isolates obtained revealed the coexistence in the patient of two genotypes causing the first malaria episode and the presence of a unique isolate responsible for the recurrence episode (Fig. 1).

The PCR analysis we performed demonstrated the selective pressure exerted by QN in favor of the strain with the resistant genotype that was later responsible for the recrudescence episode. In vivo resistance to QN was confirmed by an in vitro test and by molecular identification of an *mdr1* Tyr-86 point mutation. The direct correlation between the presence of that mutation and QN resistance has been reported by Duraisingh et al. (1), although it is not consistently found (7, 9). As expected, in vitro testing showed CQ resistance, in line with the presence of mutated alleles at codons 76 and 220 of the *pfcr* gene. The complete parasite clearance and recovery from disease obtained after sulfadoxine-PY treatment suggested that the recurrent isolate was fully sensitive to the drug. Kublin et al. reported that a dihydrofolate reductase triple mutation is strongly associated with sulfadoxine-PY treatment failure; therefore, the absence in our case of the mutations at codons 51, 59, and 108 fully supports the finding of sulfadoxine-PY efficacy (4).

This study confirms the importance of a drug resistance surveillance system based on nonimmune travelers. The exclusion of a possible new infection gives the chance to make the best use of molecular approaches aimed at the genetic analysis of malaria parasites and permits correct interpretation of the outcome of antimalarial therapy.

We observed the coexistence of a PMNS and *P. falciparum* QN-resistant isolates, but we cannot speculate about any possible correlation in our case. If similar findings occur in the future, the possible link may deserve further investigation. Finally, these results further support the inclusion of PMNS in the differential diagnosis of patients with any neurological abnormality after recovery from *P. falciparum* malaria.

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