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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 *msp1* and *msp2* 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 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 (inappropriate 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 on 3 different days were negative for malarial parasites. Computerized tomography and gadolinium-enhanced T_1- and T_2-weighted magnetic resonance imaging scans of the brain were also normal except for the presence of maxillary sinus exudate. On day 20, treatment with i.v. ceftriaxone (2,000 mg once a day) was begun. Analysis of a cerebrospinal fluid sample obtained by lumbar puncture revealed mild lymphocytic pleocytosis (45 lymphocytes/μl), a normal glucose concentration, and an elevated protein concentration of 1.29 g/liter (normal range, 0.2 to 0.4 g/liter). Pending herpes simplex virus testing, i.v. acyclovir (10 mg/kg three times a day) was empirically added to the treatment regimen on day 24 and stopped on day 28. Subsequent tests of cerebrospinal fluid for viral, bacterial, and fungal infections were all negative, including PCR analysis for herpesvirus types 1 and 2, human herpesvirus 6, cytomegalovirus, poliovirus, echoviruses, coxsackieviruses, and *Mycobacterium tuberculosis*. A PCR assay for *P. falciparum* in cerebrospinal fluid was also negative. A complete blood cell count with differential and a biochemical screening were also normal, including analyses of blood electrolyte and serum glucose, urea, and creatinine concentrations, liver function tests, and acid-base status. Serologic tests for herpes simplex virus types 1 and 2, cytomegalovirus, varicella-zoster virus, echoviruses, coxsackieviruses, poliovirus, *Trypanosoma brucei*, *Treponema pallidum*, and dengue virus were negative. Cultures of blood, urine, and stool samples were negative. The acute confusional state steadily improved over the course of a week, and the patient had no neurological symptoms on day 26. He remained afebrile from that day until discharge from the hospital on day 29.

On day 33, the patient complained again of fever and was admitted again to our hospital on day 35. *P. falciparum* malaria was diagnosed with an initial level of parasitemia of >100,000 parasites/μl; a complete blood cell count and a total bilirubin test were normal. He was treated with i.v. QN (20 mg of the hydrochloride salt per kg initially and then 10 mg/kg three times a day) plus clindamycin (600 mg three times daily) until day 42; on day 37, a single oral dose of pyrimethamine (PY; 75

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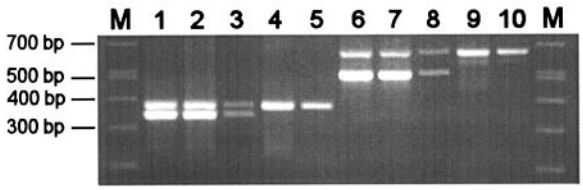

FIG. 1. Two percent agarose gel showing the products obtained by amplification with primers specific for the *msp1* (lanes 1 to 5) and *msp2* (lanes 6 to 10) genes. Lanes: M, Eurobioladder-L (Eurobio); 1 and 6, day 1; 2 and 7, day 2; 3 and 8, day 5; 4 and 9, day 35; 5 and 10, day 37. Lanes 1 to 3 and 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.

TABLE 1. Molecular marker codons in different isolates[a]

Isolate	*pfcrt* genotype		*pfmdr1* genotype					*dhfr* genotype		
	76	220	86	184	1034	1042	1246	51	59	108
Wild type	K	A	N	Y	S	N	D	N	C	S
Mutant type	T	S	Y	F	C	D	Y	I	R	N
Case report	T	S	Y	Y	S	N	D	N	C	S

[a] PCR primers specific for the *P. falciparum* CQ resistance transporter (*pfcrt*), multidrug resistance 1 (*pfmdr1*), and dihydrofolate reductase (*dhfr*) gene fragments containing the analyzed codons reported here: *pfcrt* 74 to 76, TCRP2Af (5′-CCCTTGTCGACCTTAACAGATGGCTCACG-3′) and TCRP2r (5′-CGGATGTTACAAAACTATAGTTACC-3′); *pfcrt* 220, CRT220f (5′-TCTCGGAGCAGTTATTATTGTTG-3′) and CRT220r (5′-CATGTTTGAAAAGCATACAGGC-3′); *pfmdr1* 86 to 184, A1f (5′-TGTTGAAAGATGGGTAAAGAGCAGAAAGAG-3′) and A3r (5′-TACTTTCTTATTACATATGACACCACAAACA-3′); *pfmdr1* 1034 to 1042, 1246f (5′-ATGATCACATTATATTAAAAATGATATGACAAAT-3′) and O2r (5′-ATGATTCGATAAATTCATCTATAGCAGCAA-3′); *dhfr* 51 to 108, AMP1f (5′-TTTATATTTTCTCCTTTTTA-3′) and AMP2 rev (5′-CATTTTATTATTCGTTTTCT-3′).

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. malariae*, *P. ovale*, and *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 Wooden 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 (*pfcrt*), 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*, *pfcrt*, and *dhfr* amplicons. We found a mutant-type codon profile for *pfcrt* (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 strains of *P. falciparum* with antigens in the CNS (11).

In our case report, the acute confusional state with fine tremors following the first in-hospital stay for *P. falciparum* malaria was consistent with a diagnosis of PMNS. This was also supported by the absence of laboratory evidence of coinfection with other agents causing encephalitis; moreover, the patient's

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 *pfcrt* 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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REFERENCES

1. **Duraisingh, M. T., P. Jones, I. Sambou, L. von Seidlein, M. Pinder, and D. C. Warhurst.** 2000. The tyrosine-86 allele of the *pfmdr1* gene of *Plasmodium falciparum* is associated with increased sensitivity to the anti-malarials mefloquine and artemisinin. Mol. Biochem. Parasitol. **108:**13–23.
2. **Falchook, G. S., C. M. Malone, S. Upton, and W. X. Shandera.** 2003. Post-malaria neurological syndrome after treatment of *Plasmodium falciparum* malaria in the United States. Clin. Infect. Dis. **37:**e22–e24.
3. **Jelinek, T., M. P. Grobush, and T. Loscher.** 2001. Pattern of *Plasmodium falciparum* drug resistance in nonimmune travellers to Africa. Eur. J. Clin. Microbiol. Infect. Dis. **20:**284–286.
4. **Kublin, J. G., K. F. Dzinjalamala, D. D. Kamwendo, E. M. Malkin, J. F. Cortese, L. M. Martino, R. A. G. Mukadam, S. J. Rogerson, A. G. Lescano, M. E. Molyneux, P. A. Winstanley, P. Chimpeni, T. E. Taylor, and C. V. Plowe.** 2002. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. J. Infect. Dis. **185:**380–388.
5. **Lawn, S. D., K. L. Flanagan, S. G. Wright, T. F. Doherty, and P. Godfrey-Faussett.** 2003. Postmalaria neurological syndrome: two cases from the Gambia. Clin. Infect. Dis. **36:**e29–e31.
6. **Makler, M. T., and D. J. Hinrichs.** 1993. Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia. Am. J. Trop. Med. Hyg. **48:**205–210.
7. **Ngo, T., M. Duraisingh, M. Reed, D. Hipgrave, B. Biggs, and A. F. Cowman.** 2003. Analysis of *pfcrt*, *pfmdr1*, *dhfr* and *dhps* mutations and drug sensitivities in *Plasmodium falciparum* isolates from patients in Vietnam before and after treatment with artemisinin. Am. J. Trop. Med. Hyg. **68:**350–356.
8. **Nguyen Mai, T. H., N. P. J. Day, L. V. Chuong, D. Waller, N. H. Phu, D. B. Bethell, T. T. Hien, and N. J. White.** 1996. Post-malaria neurological syndrome. Lancet **348:**917–921.
9. **Price, R. N., C. Cassar, A. Brockman, M. Duraisingh, M. van Vugt, N. J. White, F. Nosten, and S. Krishna.** 1999. The *pfmdr1* gene is associated with a multidrug-resistant phenotype in *Plasmodium falciparum* from the western border of Thailand. Antimicrob. Agents Chemother. **43:**2943–2949.
10. **Schnorf, H., K. Diserens, H. Schnyder, M. Chofflon, L. Loutan, V. Chaves, and T. Landis.** 1998. Corticosteroid-responsive postmalaria encephalopathy characterized by motor aphasia, myoclonus, and postural tremor. Arch. Neurol. **55:**417–420.
11. **Senanayake, N., and H. J. de Silva.** 1994. Delayed cerebellar ataxia complicating falciparum malaria: a clinical study of 74 patients. J. Neurol. **241:**456–459.
12. **Wongsrichanalai, C., A. L. Pickard, W. H. Wernsdorfer, and S. R. Meshnick.** 2002. Epidemiology of drug-resistant malaria. Lancet Infect. Dis. **2:**209–218.
13. **Wooden, J., S. Kyes, and C. H. Sibley.** 1993. PCR and strain identification in *Plasmodium falciparum*. Parasitol. Today **9:**303–305.