Annals of Parasitology 2017, 63(2), 111–116
doi: 10.17420/ap6302.93

Original papers

High concordance of *Pfdhfr* and *Pfdhps* genotypes between matched peripheral and placental isolates of delivered women in Bobo-Dioulasso, Burkina Faso

Mamoudou Cissé[1,2], Gordon A. Awandare[3], Fabrice A. Somé[4], Marie-Pierre Hayette[5], Robert T. Guiguemdé[1,2]

[1]Laboratory of Parasitology and Entomology, Centre MURAZ, Bobo-Dioulasso, Burkina Faso
[2]Laboratory of Parasitology and Mycology, Université Polytechnique de Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso
[3]College of Basic and Applied Sciences, West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Legon, Acra, Ghana
[4]Department of Parasitology, Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso
[5]Laboratory of Clinical Microbiology, University Hospital of Liège, Belgium

Corresponding Author: Mamoudou Cissé; email: mamoudou.cisse@centre-muraz.bf

ABSTRACT. Whether maternal peripheral parasites constitute a representative sample of the overall population infecting the individual, remains unknown in Burkina Faso. We therefore compared *Pfdhfr* and *Pfdhps* genotypes between matched peripheral and placental isolates. PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of polymorphic codons of the *Pfdhfr* gene (51, 59, 108 and 164) and the *Pfdhps* gene (437 and 540) was performed in 18 matched peripheral and placental dried blood spots of delivered women in Bobo-Dioulasso. Both *Pfdhfr* and *Pfdhps* genes were successfully genotyped in 94.4% (17/18) of the matched samples. Only 8.8% (3/34) of genotypes were of the wild type, while 20.6% (7/34), 20.6% (7/34), 23.5% (8/34) and 26.5% (9/34) comprised one, two, three and four mutations, respectively. None of the samples carried both *Pfdhfr* I164L and *Pfdhps* K540E mutations. A concordance of 82.4% was observed in matched samples for both the *Pfdhfr* and *Pfdhps* genes. Setting placental alleles as the reference, a concordance of 100% was obtained with *Pfdhfr* mutation S108N, *Pfdhfr* mutation C59R+S108N, and *Pfdhfr* mutation N51I+C59R +S108N, respectively. Likewise, a concordance of 85.7% was observed with the *Pfdhps* mutation A437G. For epidemiological purposes, peripheral blood *Pfdhfr* and *Pfdhps* genotyping is sufficient for monitoring SP resistant molecular markers in pregnant women.

Key words: malaria, pregnancy, sulfadoxine-pyrimethamine, resistance, concordance

Introduction

Malaria during pregnancy remains a major public health challenge in sub-Saharan Africa, with adverse consequences for both the pregnant woman and the developing foetus [1,2]. The World Health Organization (WHO) has recommended since 2004, the administration of intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy (IPTp-SP) as one of the most relevant strategies to reduce the burden of malaria and improve pregnancy outcomes [3]. This has prompted adoption of IPTp-SP policies in many African countries such as Burkina Faso. Current policy dictates that SP should be provided to mothers at each scheduled focused antenatal care (ANC) visit in the second and third trimesters [4], and IPTp-SP has shown considerable benefits to the mother and the foetus in the field settings [5,6]. However, there is increasing concern that this strategy is losing effectiveness in areas of existing high grade SP resistance [7,8]. Resistance to SP has been linked to point mutations at codons 51, 59, 108 and 164 in *Plasmodium falciparum dihydrofolate*

reductase (Pfdhfr) gene [9,10] and at codons 437 and 540 in *P. falciparum dihydropteroate synthase (Pfdhps)* gene [11].

P. falciparum sequesters in the placental intervillous space and complicates investigation of the molecular characteristics of *P. falciparum* such as drug resistance profiles and parasites polymorphism [12]. Discordance between placental and peripheral polymorphic merozoite surface protein (MSP) genotypes has been previously reported with a rate ranging from 32% to 69.4% [12–14]. However, whether and to what extent *Pfdhfr* and *Pfdhps* genotypes discordance in pregnant women affects the value of SP resistance genotyping has been poorly investigated in the World [15–17].

In Burkina Faso, *P. falciparum* resistance to SP in pregnancy has been poorly reported since the introduction of IPTp-SP policy in 2005 [18,19] and those studies were based on analysis of the sole peripheral blood stages. Yet, whether maternal peripheral parasites constitute a representative sample of the overall population infecting the individual, remains unknown. The present study sought to fill this knowledge gap by comparing *Pfdhfr* and *Pfdhps* genotypes between matched peripheral and placental isolates of post-partum women in Bobo-Dioulasso, Burkina Faso.

Materials and Methods

Study design and sample collection. Paired peripheral and placental dried blood spots were collected from 320 post-partum women during a cross sectional study carried out from September to December 2010 in two primary health care facilities in Bobo-Dioulasso [20]. The study area has been already described elsewhere [21]. Briefly, delivering women who provided signed informed consent were tested for both *P. falciparum* peripheral and placental blood infection using microscopic examination of Giemsa-stained thick and thin blood smears. Overall, 26 paired peripheral and placental dried blood spots (DBS) were collected from *P. falciparum*-infected women during our previous study [20]. For the current study purpose, only 18 available paired DSB were used for molecular analysis of SP resistance.

Ethical aspects and informed consent. The study protocol was approved by the National Ethics Committee for Health Research of Burkina Faso, (No 2010-054). Participants were only included after obtaining their written informed consent.

Analysis of *Pfdhfr* and *Pfdhps* genes. *P. falciparum* DNA was extracted from dried blood spots using QIAamp DNA Mini Kit (QIAgen, USA) according to the manufacturer's recommendations. SP resistance-mediating single nucleotide polymorphisms were analyzed in both *Pfdhfr* and *Pfdhps* genes using polymerase chain reaction followed by restriction enzyme digestion as previously described [22]. Polymorphisms investigated were as follows: N51I, C59R, S108N and I164L for *Pfdhfr* gene and A437G and K540E for *Pfdhps* gene. Nested PCR products were resolved by 2.5% gel electrophoresis and results classified as wild type, pure mutant and mixed infection (presence of both wild type and pure mutant in the same sample) on the basis of migration pattern.

Table 1. Distribution of *Pfdhfr* and *Pfdhps* genotypes in the isolates (n = 34)

Mutated codons						Number of positive isolates (%)
N51I	C59R	S108N	I164L	A473G	K540E	
+	+	+	–	+	–	9 (26.5)
+	+	+	–	–	–	1 (2.9)
+	–	+	–	+	–	1 (2.9)
–	+	+	–	+	–	6 (17.6)
+	–	–	–	+	–	2 (5.9)
–	+	+	–	–	–	2 (5.9)
–	–	+	–	+	–	3 (8.8)
+	–	–	–	–	–	1 (2.9)
–	–	–	–	+	–	6 (17.6)
–	–	–	–	–	–	3 (8.8)

+ Mutant; - Wild-type

Table 2. Comparison of peripheral and placental blood *Pfdhfr* genotypes

Placental blood *Pfdhfr* genotype of sample*	Peripheral blood *Pfdhfr* genotype of sample*					Total (%)
	5159108	5159**108**	**51**59108	51**59108**	**5159108**	
5159108	4		1			5 (29.4)
5159**108**		1				1 (5.9)
5159108		1				1 (5.9)
5159**108**			1			1 (5.9)
51**59108**				4		4 (23.5)
5159108					5	5 (29.4)
Total (%)	4 (23.5)	2 (11.8)	2 (11.8)	4 (23.5)	5 (29.4)	

*Mutated codons are displayed in bold

Statistical analysis. Data were entered and cleaned using Excel 2013 then transferred into Stata 12 software and analyzed. In this analysis, mixed genotypes (wild or mutant) were considered as mutants, and the prevalence of each type of allele (wild or mutant) were calculated.

Results

Prevalence of *Pfdhfr* and *Pfdhps* mutations in all isolates. A total of 36 samples (18 pairs of matched peripheral and placental blood samples) were genotyped. Both *Pfdhfr* and *Pfdhps* genes were successfully genotyped in 94.4% (17/18) peripheral and placental matched samples. *Pfdhfr* and *Pfdhps* mutations were frequent. Indeed, only 8.8% (3/34) of genotypes were of the wild type, while 20.6% (7/34), 20.6% (7/34), 23.5% (8/34) and 26.5% (9/34) comprised one, two, three and four mutations, respectively (Table 1).

None of the samples carried both *Pfdhfr* I164L and *Pfdhps* K540E mutations.

Genotypes comparison between the matched peripheral and placental isolates. Comparing the 17 peripheral genotypes to those of placental, complete concordance was observed in 82.4% (14/17) of the matched samples for both *Pfdhfr* and *Pfdhps* genes. Setting placental alleles as the reference, peripheral genotyping was able to correctly identify 80% of isolates with *Pfdhfr* wild type (4/4), and 100% of isolates with *Pfdhfr* mutation S108N (1/1), *Pfdhfr* double mutation C59R+S108N (4/4), and *Pfdhfr* triple mutation N51I+C59R+S108N (5/5), respectively (Table 2). Likewise, concordances of 66.7% (2/3) and 85.7% (12/14) were observed with *Pfdhps* wild type and *Pfdhps* mutation A437G, respectively (Table 3).

Discussion

The prevalence of quadruple mutation (triple *Pfdhfr* mutation (N51I+C59R+S108N) + *Pfdhps* mutation A437G) reported in our study (26.5%) although high was lower than those published in Gabon (53%) [16] and in Benin (70%) [23]. Such variation may be due to differences in drug pressure [16,19]. Furthermore, the absence of the *Pfdhfr* I164L and the *Pfdhps* K540E mutations in our study is consistent with previous findings reported in Burkina Faso [18,19,24–27] as well as in other settings in West Africa [15,28,29] and suggests that SP may still be efficacious when used as IPTp in Burkina Faso.

To our knowledge, this is the first study comparing peripheral and placental *Pfdhfr* and *Pfdhps* genotypes in Burkina Faso. We used PCR-RFLP for that purpose. This technique is highly specific but its sensitivity might drop at very low parasitaemia [30]. In our study, we failed to genotype all the points of mutation in 2 samples. A very low parasitaemia (160 parasites/μL) found in those samples could be a plausible explanation.

Table 3. Comparison of peripheral and placental blood *Pfdhps* genotypes

Placental blood *Pfdhps* genotype of sample*	Peripheral blood *Pfdhps*		Total (%)
	437+540	**437**+540	
437+540	2	1	3 (17.6)
437+540	2	12	14 (82.4)
Total (%)	4 (23.5)	13 (76.5)	

*Mutated codons are displayed in bold

Despite our small sample size, we found a high concordance for the *Pfdhfr* gene similar to that reported in Ghana (83.2%) [15] with 297 paired peripheral and placental samples. Bouyou-Akotet et al. [16] reported a full concordance of 100% in Gabon using 22 matched peripheral and placental samples. Nevertheless, we observed concordances of 80% and 100% with both wild type and mutated parasites, respectively. These findings were higher than those for both groups of parasites reported in Ghana [15]. In addition, the concordance for the *Pfdhps* genotypes found in our study was lower than the reported concordance of 91% (20/22) in Gabon [16].

The concordance of placental and peripheral *Pfdhfr* and *Pfdhps* genotypes found in our study was higher than those reported for MSP genotypes ranging from to 10% to 57% [12–14]. A plausible explanation is less polymorphism in *Pfdhfr* and *Pfdhps* genotypes as previously shown [15].

Altogether, our data confirm previous reports from Ghana [15], Gabon [16], and Kenya [17], namely that for epidemiological purposes, maternal peripheral blood genotyping is sufficient for monitoring SP resistant molecular markers in pregnant women.

This study provides an update on the prevalence of mutations conferring SP resistance during pregnancy in Burkina Faso. A high concordance was observed for both *Pfdhfr* and *Pfdhps* genes when comparing peripheral genotypes to placental alleles. Therefore, for epidemiological purposes peripheral blood *Pfdhfr* and *Pfdhps* genotyping is sufficient to monitor molecular markers of SP resistance in pregnant women.

Acknowledgements

We are very grateful to Prof. Kirk Deitsch (Weill Medical College of Cornell University, USA) who kindly provided us primers. We thank all midwives and pregnant women of both Kua and Lafiabougou health centers.

References

[1] Steketee R.W., Nahlen B.L., Parise M.E., Menendez C. 2001. The burden of malaria in pregnancy in malaria-endemic areas. *American Journal of Tropical Medicine and Hygiene* 64: 28-35.

[2] Umbers A.J., Aitken E.H., Rogerson S.J. 2011. Malaria in pregnancy: small babies, big problem. *Trends in Parasitology* 27: 168-175. doi:10.1016/j.pt.2011.01.007

[3] A strategic framework for malaria prevention and control during pregnancy in the African region. 2004. WHO. http//www.who.int/malaria/publications/atoz/afr_mal_04_01/en

[4] Updated WHO Policy Recommendation (October 2012): Intermittent preventative treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP). 2012. WHO. http//www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012. pdf

[5] Gies S., Coulibaly S.O., Ouattara F.T., D'Alessandro U. 2009. Individual efficacy of intermittent preventive treatment with sulfadoxine-pyrimethamine in primi- and secundigravidae in rural Burkina Faso: impact on parasitaemia, anaemia and birth weight. *Tropical Medicine and International Health* 14: 174-182. doi:10.1111/j.1365-3156.2008.02215.x

[6] Valea I., Tinto H., Drabo M.K., Huybregts L., Henry M.C., Roberfroid D., Guiguemde R.T., Kolsteren P., D'Alessandro U., FSP/MISAME study Group. 2010. Intermittent preventive treatment of malaria with sulphadoxine-pyrimethamine during pregnancy in Burkina Faso: effect of adding a third dose to the standard two-dose regimen on low birth weight, anaemia and pregnancy outcomes. *Malaria Journal* 9: 324. doi:10.1186/1475-2875-9-324

[7] Ter Kuile F.O., van Eijk A.M., Filler S.J. 2007. Effect of sulfadoxine-pyrimethamine resistance on the efficacy of intermittent preventive therapy for malaria control during pregnancy: a systematic review. *JAMA* 297: 2603-2616. doi:10.1001/jama.297.23.2603

[8] Harrington W.E., Mutabingwa T.K., Kabyemela E., Fried M., Duffy P.E. 2011. Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance. *Clinical Infectious Diseases* 53: 224-230. doi:10.1093/cid/cir376

[9] Bzik D.J., Li W.B., Horii T., Inselburg J. 1987. Molecular cloning and sequence analysis of the *Plasmodium falciparum* dihydrofolate reductase – thymidylate synthase gene. *Proceedings of the National Academy of Sciences of the United States of America* 84: 8360-8364.

[10] Hamel M.J., Poe A., Bloland P., McCollum A., Zhou Z., Shi Y.P., Ouma P., Otieno K., Vulule J., Escalante A., Udhayakumar V., Slutsker L. 2008. Dihydrofolate reductase I164L mutations in *Plasmodium falciparum* isolates: clinical outcome of 14 Kenyan adults infected with parasites harbouring the I164L mutation. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 102: 338-345. doi:10.1016/j.trstmh.2008.01.018

[11] Triglia T., Cowman A.F. 1994. Primary structure and expression of the dihydropteroate synthetase gene of *Plasmodium falciparum*. *Proceedings of the National*

Academy of Sciences of the United States of America 91: 7149-7153.

[12] Schleiermacher D., Le Hesran J.Y., Ndiaye J.L., Perraut R., Gaye A., Mercereau-Puijalon O. 2002. Hidden *Plasmodium falciparum* parasites in human infection. *Infection, Genetics and Evolution* 2: 97-105.

[13] Kamwendo D., Snounou G., Mhango C.G., Rogerson S.J., Kanjala C.C. 2002. *Plasmodium falciparum*: PCR detection and genotyping of isolates from peripheral, placental, and cord blood of pregnant Malawian women and their infants. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 96:145-149.

[14] Mayengue P.I., Rieth H., Khattab A., Issifou S., Kremsner P.G. 2004. Submicroscopic *Plasmodium falciparum* infections and multiplicity of infection in matched peripheral, placental and umbilical cord blood samples from Gabonese women. *Tropical Medicine and International Health* 9: 949-958. https://doi.org/10.1111/j.1365-3156.2004.01294.x

[15] Mockenhaupt F.P., Bedu-Addo G., Junge C., Hommerich L., Eggelte T.A., Bienzle U. 2007. Markers of sulfadoxine-pyrimethamine-resistant *Plasmodium falciparum* in placenta and circulation of pregnant women. *Antimicrobial Agents and Chemotherapy* 51: 332-334. https://doi.org/10.1128/AAC.00856-06

[16] Bouyou-Akotet M.K., Mawili-Mboumba D.P., Tchantchou T., Kombila M. 2010. High prevalence of sulfadoxine/pyrimethamine-resistant alleles of *Plasmodium falciparum* isolates in pregnant women at the time of introduction of intermittent preventive treatment with sulfadoxine/pyrimethamine in Gabon. *Journal of Antimicrobial Chemotherapy* 65: 438-441. doi:10.1093/jac/dkp467

[17] Iriemenam N.C., Shah M., Gatei W., van Eijk A.M., Ayisi J., Kariuki S., Vanden Eng J., Owino S.O., Lal A.A., Omosun Y.O., Otieno K., Desai M., ter Kuile F.O., Nahlen B., Moore J., Hamel M.J., Ouma P., Slutsker L., Shi Y.P. 2012. Temporal trends of sulphadoxine-pyrimethamine (SP) drug-resistance molecular markers in *Plasmodium falciparum* parasites from pregnant women in western Kenya. *Malaria Journal* 11: 134. doi:10.1186/1475-2875-11-134

[18] Coulibaly S.O., Kayentao K., Taylor S., Guirou E.A., Khairallah C., Guindo N., Djimde M., Bationo R., Soulama A., Dabira E., Barry B., Niangaly M., Diakite H., Konate S., Keita M., Traore B., Meshnick S.R., Magnussen P., Doumbo O.K., ter Kuile, F.O. 2014. Parasite clearance following treatment with sulphadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in vivo follow-up study. *Malaria Journal* 13: 41. doi:10.1186/1475-2875-13-41

[19] Tahita M.C., Tinto H., Erhart A., Kazienga A., Fitzhenry R., VanOvermeir C., Rosanas-Urgell A., Ouedraogo J.B., Guiguemde R.T., Van Geertruyden J.P., D'Alessandro U. 2015. Prevalence of the dhfr and dhps mutations among pregnant women in rural Burkina Faso five years after the introduction of intermittent preventive treatment with sulfadoxine-pyrimethamine. *PloS One* 10: e0137440. doi:10.1371/journal.pone.0137440

[20] Cisse M., Diallo A.H., Somé D.A., Poda A., Awandare A.G., Guiguemdé T.R. 2016. Association of placental *Plasmodium falciparum* parasitaemia with maternal and newborn outcomes in the periurban area of Bobo-Dioulasso, Burkina Faso. *Parasitology Open* 2: e15. doi:10.1017/pao.2016.12

[21] Cisse M., Sangare I., Lougue G., Bamba S., Bayane D., Guiguemde R.T. 2014. Prevalence and risk factors for *Plasmodium falciparum* malaria in pregnant women attending antenatal clinic in Bobo-Dioulasso (Burkina Faso). *BMC Infectious Diseases* 14: 631. doi:10.1186/s12879-014-0631-z

[22] Vlahos J. 2004. Protocols for detecting mutations conferring resistance to the antifolate class of antimalarial drugs. In: Molecular markers antifolate resistance dhfr, dhps. http://www.muucsf.org

[23] Bertin G., Briand V., Bonaventure D., Carrieu A., Massougbodji A., Cot M., Deloron P. 2011. Molecular markers of resistance to sulphadoxine-pyrimethamine during intermittent preventive treatment of pregnant women in Benin. *Malaria Journal* 10: 196. doi:10.1186/1475-2875-10-196

[24] Dokomajilar C., Lankoande Z.M., Dorsey G., Zongo I., Ouedraogo J.B., Rosenthal P.J. 2006. Roles of specific *Plasmodium falciparum* mutations in resistance to amodiaquine and sulfadoxine-pyrimethamine in Burkina Faso. *American Journal of Tropical Medicine and Hygiene* 75: 162-165.

[25] Tinto H., Ouédraogo J.B., Zongo I., van Overmeir C., van Marck E., Guiguemdé T.R., D'Alessandro U. 2007. Sulfadoxine-pyrimethamine efficacy and selection of *Plasmodium falciparum* DHFR mutations in Burkina Faso before its introduction as intermittent preventive treatment for pregnant women. *American Journal of Tropical Medicine and Hygiene* 76: 608-613.

[26] Somé A.F., Séré Y.Y., Dokomajilar C., Zongo I., Rouamba N., Greenhouse B., Ouédraogo J.B., Rosenthal P.J. 2010. Selection of known *Plasmodium falciparum* resistance-mediating polymorphisms by artemether-lumefantrine and amodiaquine-sulfadoxine-pyrimethamine but not dihydro-artemisinin-piperaquine in Burkina Faso. *Antimicrobial Agents and Chemotherapy* 54: 1949-1954. doi:10.1128/AAC.01413-09

[27] Geiger C., Compaore G., Coulibaly B., Sie A., Dittmer M., Sanchez C., Lanzer M., Jänisch T. 2014. Substantial increase in mutations in the genes pfdhfr and pfdhps puts sulphadoxine-pyrimethamine-based

intermittent preventive treatment for malaria at risk in Burkina Faso. *Tropical Medicine and International Health* 19: 690-697. doi:10.1111/tmi.12305

[28] Mockenhaupt F.P., Eggelte T.A., Böhme T., Thompson W.N., Bienzle U. 2001. *Plasmodium falciparum* dihydrofolate reductase alleles and pyrimethamine use in pregnant ghanaian women. *American Journal of Tropical Medicine and Hygiene* 65: 21-26.

[29] Mockenhaupt F.P., Bedu-Addo G., Eggelte T.A., Hommerich L., Holmberg V. von Oertzen C., Bienzle U. 2008. Rapid increase in the prevalence of sulfadoxine-pyrimethamine resistance among *Plasmodium falciparum* isolated from pregnant women in Ghana. *Journal of Infectious Diseases* 198: 1545-1549. doi:10.1086/592455

[30] Ranford-Cartwright L.C., Johnston K.L., Abdel-Muhsin A.M., Khan H.A., Babiker B.K.

2002. Critical comparison of molecular genotyping methods for detection of drug-resistant *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 96: 568-572.

Received 15 December 2016
Accepted 4 April 2017