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Short communication

Near-fixation of a *Pfmsp1* block 2 allelic variant in genetically diverse *Plasmodium falciparum* populations across Western Colombia☆

Judy Natalia Jiménez[a,b], Georges Snounou[c,d,e,*], Frank Letourneur[f,g], Laurent Rénia[f,g,h], Iván D. Vélez[a], Carlos E. Muskus[a,**]

[a] *Programa de Estudio y Control de Enfermedades Tropicales, PECET, Facultad de Medicina, Universidad de Antioquia, Sede de Investigación Universitaria, Carrera 53 No 61-30, Laboratorio 632, Medellín, Colombia*
[b] *Grupo de Microbiología Molecular, Escuela de Microbiología, Universidad de Antioquia, Apartado Aéreo 1226 Medellín, Colombia*
[c] *INSERM UMR S 945, Département de Parasitologie, Hôpital Pitié-Salpêtrière, Paris, France*
[d] *Université Pierre et Marie Curie, Paris, France*
[e] *Laboratory of Molecular and Cellular Parasitology, Department of Microbiology, Faculty of Medicine, National University of Singapore, Singapore*
[f] *Institut Cochin, Université Paris Descartes, CNRS (UMR 8104), Paris, France*
[g] *INSERM, U567, Paris, France*
[h] *Singapore Immunology Network, Agency for Science, Technology and Research and, Technology (A*STAR), Biopolis, 138648, Singapore*

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ABSTRACT

Assessment of the genetic diversity of *Plasmodium falciparum* in 110 Colombian isolates revealed that nearly all the parasites in the 97 isolates collected in endemic regions west of the Andes shared the same *Pfmsp1* block 2 MAD20-type allelic variant, despite showing high diversity for other genetical markers. Analysis of published data indicated that the prevalence of this allelic variant of a major vaccine candidate antigen was already dominant since 1998. This phenomenon, which had not been hitherto recorded for a malaria blood stage antigen, is of biological and immunological interest but remains unexplained.

1. Introduction

Malaria remains a serious problem in Colombia, where many of the inhabitants reside in areas where malaria transmission occurs. The incidence of malaria in Colombia is high relative to that observed in other endemic countries in the Americas. Incidence fluctuated around 100,000–200,000 cases per year since 1990 (http://www.who.int/countries/col/en/; http://www.paho.org/home.htm). In Colombia, the majority of the clinical cases of malaria are caused by *Plasmodium vivax* (65%), while *P. falciparum* is responsible for the remaining 35% (PAHO, 2003). The recommended first line treatment for *P. falciparum*, amodiaquine (AQ) combined to sulfadoxine-pyrimethamine (SP), remains effective, however, resistance to each of these two drugs is on the rise (Blair et al., 2006). Artemisinin-based combination therapies (ACT) are under investigation (Osorio et al., 2007a) as a future first line treatment.

Monitoring of drug efficacy is best performed through in vivo trials in endemic countries, where the risk of misclassifying re-infections during the follow-up as treatment failure can be minimized by PCR genotyping (Snounou and Beck, 1998), which helps distinguish recrudescences (true treatment failures) from re-infections (treatment success). The validity of PCR-corrected results relies on the use of genetic molecular markers with allelic variants that are diverse and with an unbiased frequency distribution. In areas where transmission intensity is low to moderate, as is the case in Colombia, the genetic diversity of *P. falciparum* populations might be low, which will limit the usefulness of PCR genotyping for in vivo drug efficacy trials.

☆ *Note:* Nucleotide sequence data reported in this paper are available in the GenBank™ database under the accession numbers FJ999664, FJ999665 and FJ999666.

* Corresponding author at: INSERM UMR S 945, Département de Parasitologie, Hôpital Pitié-Salpêtrière, 91 Boulevard de l'Hôpital, Paris 75013, France. Tel.: +33 1 40 77 97 36; fax: +33 1 45 82 28 59.
** Corresponding author. Tel.: +57 4 2196501; fax: +57 4 2196511.
E-mail addresses: georges.snounou@upmc.fr (G. Snounou), carmusk@yahoo.com (C.E. Muskus).

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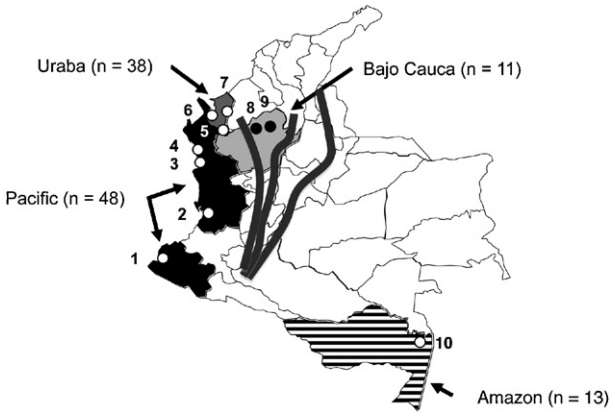

Fig. 1. Samples were collected from four regions with the higher *P. falciparum* prevalences in Colombia. The sites from which samples (numbers in parentheses) were collected in each region are as follows: Pacific Region (black shading), 1 = Tumaco ($n = 5$), 2 = Buenaventura ($n = 15$), 3 = Pangui ($n = 14$), 4 = Nuqui ($n = 14$); Uraba region (dark grey shading) 5 = Carepa ($n = 11$), 6 = Turbo ($n = 20$), 7 = San Pedro de Uraba ($n = 7$); Bajo Cauca Region (light grey shading) 8 = Caceres ($n = 6$), 9 = Caucasia ($n = 5$); and Amazon region (dark grey shading) 10 = La Pedrera ($n = 13$). The dark grey broad lines indicate the position of the Andean mountain ranges that run the length of Colombia.

2. Materials and methods

In order to obtain a comprehensive snapshot of *P. falciparum* genetic diversity across Colombia, we collected 128 admission blood samples (November 2002 to June 2003) after informed consent from patients with microscopically diagnosed *P. falciparum* malaria presenting in clinics from 10 localities in four regions (Pacific, Uraba, Bajo Cauca and Amazon) (Fig. 1). The four regions included in this study contribute more than 80% of the total recorded cases of *P. falciparum* in the country. The pacific coast and the Amazon regions are characterized by humid tropical forest with moderate transmission of *P. falciparum*. This contrasts from the Uraba and Bajo Cauca regions that are characterized by dry tropical forest with low transmission. The inhabitants of the Pacific, Uraba and Bajo Cauca regions are a mixture of indigenous, Hispanic and African backgrounds, while those of the Amazon are predominantly of indigenous background.

DNA was extracted from blood samples dried on Whatman # 3 filter paper, and the presence of *P. falciparum* parasites was confirmed by PCR (Snounou and Singh, 2002) for 110 of the samples, which were then used to genotype *P. falciparum* with respect to the polymorphic regions of three genetic markers, *P. falciparum* merozoite surface protein 1 (*Pfmsp1*) (block 2), *P. falciparum* merozoite surface protein 2 (*Pfmsp2*) (block 3) and *P. falciparum* glutamate rich protein *Pfglurp* (repeat region RII) using a previously described nested PCR protocol (Snounou et al., 1999).

3. Results

A summary of the frequency data obtained is presented (Fig. 2 and Table 1). The markers *Pfglurp* and *Pfmsp2* were diverse in that one could discern 11 and 9 distinct allelic variants, respectively. There were four *Pfmsp2* variants with the FC27-repeat family (300–450 bp) and five with the IC/3D7-repeat family (450–650 bp). The FC27 variants were found in only 28% of the samples. The frequency distribution for the *Pfglurp* (600–1150 bp) and the *Pfmsp2* variants was relatively unbiased. In stark contrast, the diversity of the *Pfmsp* marker was quite limited, with only a single variant observed per allelic families, whose frequency distribution was highly biased. All RO33 variants and most K1 variants were observed in the Amazon region samples, whereas all the parasites from the Uraba–Bajo Cauca regions (50/50) and nearly all those from the Pacific region (45/48) carried a single *Pfmsp1*-MAD20 allelic variant of a unique size. Direct sequencing of this amplified fragment purified from 16 distinct samples (7 from Pacific, 6 from Uraba, and 3 from Bajo Cauca) revealed that the sequence bounded by the amplification primers was the same in all cases (GenBank accession no. FJ999664). Variants with the same or very similar sequence to the one we obtained had been observed in parasites from endemic areas in Africa and Asia. The C-terminal domain (MSP_{19}) of *Pfmsp1*, a conserved region on which many experimental vaccine formulations are based and where non-synonymous mutations nonetheless occur (Kang and Long, 1995) was amplified and directly sequenced from 38 isolates harbouring single genotype infections. Of these, 34 were of the MAD20-type (14 isolates originated from the Pacific, 15 from the Uraba, and 5 from the Bajo Cauca regions), 2 were of the K1-type and 2 of the RO33-type (these 4 were from the Amazon region). Care was taken to select samples where the parasites differed with respect to their *Pfmsp2* and *Pfglurp* genotype patterns. Two distinct sequences were found; the first (GenBank accession no. FJ999665) was shared by the parasites from 37 samples, while the second was present in the 2

Table 1
Allelic frequencies of the three *P. falciparum* genetic markers for Colombian parasites.

Year	Samples per region[a]					*Pfmsp1*[b]			*Pfmsp2*[b]		*Pfglurp*[b]	Ref.
	Am	BC	Pa	Ur	Ot	K1	MAD20	RO33	FC27	IC/3D7		
1989–1991[c]		6	24		1	3	15	26 (1)	7	6		Snewin et al. (1991)
1997			53			12 (1)	66 (2)	27 (1)				Gómez et al. (2002)
1998–2001			46			0	46 (1)	0				Terrientes et al. (2005)
2000–2001		50				0	50 (1)	0	12 (1)	40 (2)	50 (2)	Montoya et al. (2003)
				50		0	50 (1)	0	0	10 (2)	50 (4)	
2001			441			41	416	0				Osorio et al. (2007a,b)
			402						34	390		
2002–2004				105		6 (1)	101 (1)	2 (1)				Guerra et al. (2006)
					18	2 (1)	17 (1)	1 (1)				
2003		11	48	38		3 (1)	94 (1)	0	45 (4)	52 (5)	97 (11)	This study
	13					8 (1)	0	6 (1)	7 (3)	7 (3)	13 (6)	

[a] The region in Colombia from which the samples were collected are abbreviated as follows: Am = Amazon, BC = Bajo-Cauca, Pa = Pacific, Ur = Uraba, Ot = other regions.
[b] The number of different allelic variants observed, when provided, is presented in parenthesis.
[c] The authors did not provide a detailed dataset, thus, it was not possible to extract the allelic frequencies for samples collected from a specific region. It should be noted that the numbers for each genetic marker often add up to more that the total number of samples, this is simply because mixed genotype infections were observed in some samples (this applies for all the studies presented in this table).