Vol 5 | Issue 4 | 2015 | 228-231.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

International Journal of
Current Pharmaceutical & Clinical Research
www.ijcpcr.com

IMPORTANCE OF MICROSATELLITE MARKERS IN THE STUDY OF *PLASMODIUM FALCIPARUM* INFECTIONS

Myo Thura Zaw and Zaw Lin*

Department of Pathobiological and Molecular Diagnostics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah.

ABSTRACT

Microsatellites (MS) loci are abundant in the genome of the malaria parasite *Plasmodium falciparum* (*P.falciparum*). MS markers flanking *P. falciparum* chloroquine resistant (CQR) gene (*pfcrt*) are useful for mapping the origins of different *pfcrt* alleles as well as tracing CQR *P. falciparum* isolates. MS loci downstream of the K13 gene of *P.falciparum* propose the hypothesis that one of the commonest artemisinin resistant (AR) alleles has recent independent origins along the Thai-Cambodia and Thai-Myanmar borders. Twelve highly polymorphic MS loci in genetic structure of *P. falciparum* populations in patients with severe malaria were observed to be difference from that in patients with uncomplicated malaria. These loci might somehow influence the clinical outcome of malaria in Thai-Myanmar border region. In addition, multi-locus haplotypes were not matched among different populations in one study indicate that a fairly uniform malaria control strategy and drugs may be effective in this highly endemic region. In conclusion, in case of drug-resistant *P falciparum* infections, not only molecular determinants of related genes are necessary to be studied but also flanking MS markers should be assessed for the spread of drug resistance.

Key words: Microsatellites, *Plasmodium falciparum* infections, Chloroquine resistant gene, Clinical outcome, Malaria control strategy.

INTRODUCTION

Microsatellites are simple sequence tandem repeats (SSTRs). SSTRs are ubiquitous in eukaryotic genomes. MS loci are often polymorphic because there are differences in length of these simple sequences. MS loci are abundant in the genome of the malaria parasite *P.falciparum*. Genetic markers derived from polymorphic MS loci have been used to investigate parasite population genetics [1,2].

Twelve microsatellite markers were used to characterize *P. falciparum* samples obtained in Papua New Guinea and it was observed that MS could quantify multiple infections [3]. MS genotyping has revealed genetic diversity of parasites in some of the studies in which widely distributed parasite isolates were investigated. MS studies could differentiate population between areas of high and low transmission intensity [4]. In addition, predictions of spread of CQR have been made recently [5,6].

MS markers flanking *pfcrt* are useful for mapping the origins of different *pfcrt* alleles. When there is drug pressure on a parasitic population, mutations that lead to drug resistance are selected and propagated throughout the population. The MS markers flanking these resistance genes can also be propagated. The phenomenon is a form of genetic 'hitchhiking' [5]. This propagation results in lesser variation of allele frequency of the MS markers upstream and downstream of the gene which is under selection [7].

Corresponding Author :- **Zaw Lin** Email:- 56dr.zawlin@gmail.com

Analysis of microsatellite markers, the *pfcrt* alleles and genome-wide haplotypes for determination of origin of CQR *P. falciparum* isolates

Many alleles on chromosome 7 of CQR isolates from Africa and Asia are similar when compared with those from Chloroquine sensitive (CQS) isolates indicating common origins for CQR parasites. Therefore, analysis of the *pfcrt* alleles, flanking microsatellite markers and genome-wide haplotypes was undertaken [5].

Nearly all CQR isolates from both Papua New Guinea and the Brazilian/Peruvian Amazon have the same *pfcrt* allele, coding for five amino-acid substitutions. However, the *pfcrt*-flanking microsatellite haplotypes are notably different suggesting that this allele probably evolved independently in South America and Papua New Guinea [5]. In contrast, the genome wide allele sharing between Papua New Guinea and the Amazon is not significantly different in comparison with other Asian and American isolates [5]. The *pfcrt* alleles, the flanking microsatellite markers, and the lack of admixture shown by genome-wide haplotypes demonstrate that there were at least four independent CQR origins: one in Asia spreading to Africa, one in Papua New Guinea, and two in South America [5].

Microsatellite markers for the tracing of CQR *P. falciparum* isolates

In the study of CQR *P. falciparum* in Phillipines, for the tracing of CQR *P. falciparum* isolates, five MS markers named B5M77, 2E10, PE12A, 2H4, and PE14F were analysed [8]. The location of five MS markers in relationship with *pfcrt* gene are shown in Table 1. There were three alleles observed in parasites named P1a, P2a and P2b. Most of the isolates with the P2a allele had the identical size of all five MS markers. Some isolates with the P2b *pfcrt* allele also had identical MS markers while other had variations in MS markers [8]. The unique patterns of MS marker patterns of these two *pfcrt* allelic types indicate that these alleles evolved independently in the Philippines. P2b carried an extra mutation (C72S) suggest that the P2b allelic type was derived from P2a. In case of P1a *pfcrt* type of isolates, the MS marker patterns closely resembled that of the PNG and the Solomon Islands isolates [8].

Throughout the provinces of Solomon and Vanuatu Islands, genotyping of same five MS markers flanking *pfcrt* revealed six haplotypes H1-H6 [7]. In Temotu Province, Solomon Islands, 34 of the 50 samples were successfully typed at all five loci and resulted in three haplotypes H3, H4 and another haplotype which match with Southeast Asian *pfcrt* allelic type and a MS pattern of the Thailand C2B group at all three downstream MS loci. In Malaita Province, Solomon Islands, 28 of the 31 isolates were successfully typed at all five loci and revealed four MS haplotypes. H3 and H4, H1 and H6 haplotypes were present with dominance of H3 and H4 haplotype [7]. In Tanna Island, Vanuatu, eight isolates were successfully typed at all five loci resulting in two patterns. Of these 75% (6/8) were determined as H1 and the two remaining samples had unique MS sizes at three loci, were classified as H2 [7].

MS markers flanking K13 gene associated with Artemisinin Resistance in *P. falciparum* isolates

C580Y allele is the most common allele of K13 gene in *P. falciparum* associated with AR. C580Y and R539T were the only two mutations observed at the Thai-Cambodia border in the eastern Thailand. C580Y and R575K mutations have been reported near the Thai-Myanmar border while more than two mutations were observed in the western Thailand. Analysis of MS loci flanking two C580Y haplotypes revealed that alleles circulating in the east and west Thailand have two distinct patterns [9]. There were differences in the 8.6 kb and 31.0 kb and 31.5 kb loci downstream of the K13 gene suggesting the C580Y mutations may have arisen independently in the Thai-Cambodia (east) and Thai-Myanmar (west) borders [9]. Regarding downstream MS loci, C580Y alleles are more heterozygous to wild type than between each other, which suggest recent independent origins along the Thai-Cambodia and Thai-Myanmar borders (Table 2). The information which supports the hypothesis two different haplotypes of C580Y emerged independently is geographic distance in association with genetic difference [9].

MS loci and the clinical outcome of falciparum malaria

Ariey *et al* found that disease severity and certain alleles in the polymorphic MS loci of clinical isolates from French Guyana were associated [10]. However, Ferreira *et al* indicated from their findings that there was no evidence of correlation between the parasite genotypes of the isolates from malaria patients in Vietnam and disease severity [11]. These results demonstrate that evaluation of the role of parasite genetic factors in malaria pathogenesis is difficult. Two parasite populations collected from patients with either severe or uncomplicated malaria from the Thai-Myanmar border region were assessed for their genetic structures. This region is known to be endemic for multidrug-resistant malaria [12-14]. Twelve highly polymorphic MS loci were examined. The genetic structure of *P. falciparum* populations in patients with severe malaria was observed to be difference from that in patients with uncomplicated malaria [15]. The MS loci selected in this study were presumably unrelated to the antigenic characteristics of the parasites and it was suggested that the loci might somehow influence the clinical outcome of malaria [15].

MS diversity predicts malaria control strategy

Studies on genetic diversity in MS loci provide evidence of *P. falciparum* differentiation that could affect

fitness and adaptation to drugs. Ten neutral microsatellite loci were genotyped in *P. falciparum* infections from three localities: a rural community, an urban location and a peri-urban border settlement in southwestern Nigeria [16]. Analysis was undertaken on the genetic diversity, linkage disequilibrium (LD) and inter-population differentiation [16]. Allelic diversity values were similar across all populations. Multi-locus haplotypes were not matched and analysis of multi-locus LD showed no significant association [16]. The absence of detectable population structure of *P. falciparum* in southwestern Nigeria is evident [16]. This implies that a fairly uniform malaria control strategy and drugs may be effective in this highly endemic region [16].

Table 1. Name of Five MS markers, their distance from mutated *pfcrt* gene and size of MS markers in World reference samples

Samples	MS marker size (bp)		**PFCRT Mutations (amino acids)	MS marker size (bp)		
	B5M77 (-20 kb)	2E10 (-5kb)		PE12A (+6kb)	2H4 (+22kb)	PE14F (+106kb)
Thailand Dd2	149	170	C**IET**HAL**SESTI**	314	204	145
Thailand C2B	149	170	C**IET**HAL**SESTI**	314	204	145
Brazil 7G8	151	190	**S**MN**T**HAL**S**Q**DL**R	314	194	142
Philippines PH1	149	182	CMN**T**H**TY**AQ**DL**R	314	228	136
PNG AN001	149	174	**S**MN**T**HAL**S**Q**DL**R	328	184	142
PNG AN018	149	174	**S**MN**T**HAL**S**Q**DL**R	328	192	139
Solomon Island N18	149	174	**S**MN**T**HAL**S**Q**DL**R	328	184	142

**Mutations in nucleotide lead to change to amino acid change which makes the parasite resistant to CQ. Short terms of amino acids are shown in bold when there is change in amino acid.
Modified from Gresty *et al*, 2014 (7)

Table 2. MS downstream of K13 gene for Artemisinin Resistance in *Plasmodium falciparum*

K13 gene with C580Y mutation	+8.6 kb	+31.0 kb	+31.5 kb
Eastern Thailand alleles	-	*300-318bp (6 isoates)	-
Western Thailand alleles	*278bp (8 isolates)	*300bp (1 isolate)	*198bp (7 isolates)

*size of MS loci
Modified from Talundzic *et al*, 2015 [9]

CONCLUSIONS

In summary, the identification of polymorphic MS markers should enable investigators to address questions regarding the epidemiology and population structure of *Plasmodium* species and host-parasite interactions. The MS loci might somehow influence the clinical outcome of malaria. Moreover, the markers should improve assessments of therapeutic efficacy and ultimately contribute to development of more effective treatments needed for achieving control of a worsening trend in endemic malaria transmission [6]. In addition, in case of CQR *P. falciparum* and AR *P. falciparum* infections, not only molecular determinants of related genes were studied but also flanking MS markers should be assessed for the epidemiology purpose of drug resistance in this parasite.

ACKNOWLEDGEMENT

We would like to thank Professor Dr. Zainal Arifin Mustapha, Acting Dean, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah for the continuous support throughout the writing of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Su X, Ferdig MT, Huang Y, Huynh CQ, Liu A, You J, Wootton JC, Wellems TE. A genetic map and recombination parameters of the human malaria parasite *Plasmodium falciparum*. *Science*, 286, 1999, 1351–1353.
2. Ferdig MT, Su XZ. Microsatellite markers and genetic mapping in *Plasmodium falciparum*. *Parasitol Today* 16, 2000, 307–312.
3. Anderson TJ, Su XZ, Bockarie M, Lagog M, Day KP. Twelve microsatellite markers for characterization of *Plasmodium falciparum* from finger-prick blood samples. *Parasitology*, 119, 1999, 113–125.
4. Anderson TJ, Su XZ, Roddam A, Day KP. Complex mutations in a high proportion of microsatelliteloci from the protozoan parasite *Plasmodium falciparum*. *Mol Ecol*, 9, 2000, 1599–1608.

5. Wootton JC, Feng X, Ferdig MT, Cooper RA, Mu J, Baruch DI, Magill AJ, Su X. Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*. *Nature*, 418, 2002, 320–323.
6. Gomez JC, Mcnamara DT, Bockarie MJ, Baird JK, Carlton JM, Zimmerman PA. Identification of a polymorphic *Plasmodium vivax* microsatellite marker. *Am J Trop Med Hyg* , 69(4), 2003, 377–379.
7. Gresty KJ, Gray K, Bobogare A, Taleo G, Hii J, Wini L, Cheng Q, Waters NC. Genetic mutations in *pfcrt* and *pfmdr1* at the time of artemisinin combination therapy introduction in South Pacific islands of Vanuatu and Solomon Islands. *Malar J*, 13, 2014, 406.
8. Chen N, Wilson DW, Pasay C, Bell D, Martin LB, Kyle D, Cheng Q. Origin and dissemination of chloroquine-resistant *Plasmodium falciparum* with mutant *pfcrt* alleles in the Philippines. *Antimicrob Agents Chemother*, 49, 2005, 2102–2105.
9. Talundzic E, Okoth SA, Congpuong K, Plucinski MM, Morton L, Goldman IF, Kachur PS, Wongsrichanalai C, Satimai W, Barnwell JW, Udhayakumar V. Selection and Spread of Artemisinin-Resistant Alleles in Thailand Prior to the Global Artemisinin Resistance Containment Campaign. *PLOS Pathog*, 2015, 1-14.
10. Ariey F, Hommel D, Le Scanf C, Duchemin JB, Peneau C, Hulin A, Sarthou JL , Reynes JM, Fandeur T, Mercereau-Puijalon O. Association of severe malaria with a specific *Plasmodium falciparum* genotype in French Guiana. *J Infect Dis* 184, 2001, 237-241.
11. Ferreira MU, Nair S, Hyunh TV, Kawamoto F, Anderson TJ. Microsatellite characterization of *Plasmodium falciparum* from cerebral and uncomplicated malaria patients in southern Vietnam. *J Clin Microbiol*, 40, 2002, 1854-1857.
12. Congpuong K, Na Bangchang K, Mungthin M, Bualombai P, Wernsdorfer WH. Molecular epidemiology of drug resistance markers of *Plasmodium falciparum* malaria in Thailand. *Trop Med Int Health*, 10, 2005, 717-722.
13. Nosten F, ter Kuile F, Chongsuphajaisiddhi T, Luxemberger C, Webster HK, Edstein M, Phaipun L, Thew KL, White NJ. Mefloquine-resistant falciparum malaria on the Thai-Burmese border. *Lancet*, 337, 1991, 1140-1143.
14. Nelson AL, Purfield A, McDaniel P, Uthaimongkol N, Buathong N, Sriwichai S, Miller RS, Wongsrichanalai C, Meshnick SR. *pfmdr1* Genotyping and *in vivo* mefloquine resistance on the Thai-Myanmar border. *Am J Trop Med Hyg*, 72, 2005, 586-592.
15. Susomboon P, Iwagami M, Tangpukdee N, Krusood S, Looareesuwan S, Kano S. Differences in genetic population structures of *Plasmodium falciparum* isolates from patients along Thai-Myanmar border with severe or uncomplicated malaria. *Malar J*, 7, 2008, 212.
16. Oyebola MK, Idowu ET, Nyang H,. Olukosi YA, Otubanjo OA, Nwakanm DC, Awolola ST, Amambua-Ngwa A. Microsatellite markers reveal low levels of population sub-structuring of *Plasmodium falciparum* in southwestern Nigeria. *Malar J*, 13, 2014, 493.