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Frequency of the FC 27 allelic family of merozoite surface protein 2 gene signifies highly virulent strain of *Plasmodium falciparum* in Jos, Nigeria

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Abstract: **Understanding the genetic diversity in *Plasmodium falciparum* populations is of major importance in the outcome of antimalarial drug trials and vaccine design. Merozoite surface protein-2 (msp 2) is a well known antigenic marker for distinguishing infections with *P. falciparum*. Parasite DNA was extracted from 127 blood samples collected from patients confirmed by microscopy to be *P. falciparum*-positive followed by PCR-genotyping for msp 2 (block2) allelic family. Out of the 127 positive samples, only 12 were confirmed so by PCR. Six samples were positive for the FC 27 family of msp 2. The FC27 allele sequence gave high frequency of 50% (6/12). The *P. falciparum* FC 27 allele sequence analyses presented *P. falciparum* MSA-2 gene having high identity to the query sequence although the nucleotide alignment of the sequence demonstrated several polymorphisms. The 349bp *Plasmodium falciparum* FC 27 allele of msp2 gene obtained exhibited polymorphisms. The frequency of occurrence of this molecular marker of malaria virulence was high suggesting Jos is endemic to highly virulent *Plasmodium falciparum* strains.**

Keywords: Plasmodium falciparum, **FC 27 allele, merozoite surface protein 2 gene malaria.**

I. INTRODUCTION

Malaria remains an important public health concern in the tropical parts of the world especially in the African continent as a whole and Nigeria in particular. Understanding the parasites is very significant in clinical diagnosis and malaria management (Branch et al., 2001). The process of identifying *Plasmodium* species, house-keeping genes are chosen as molecular targets in PCR assays (Miller et al., 1993).

As one of the targets, merozoite surface protein 2 gene *(msp2)* has been shown to be a useful marker for strain identification and differentiation (Mwingira et al., 2011). Studies have shown that anti- *msp2* antibodies inhibit merozoite invasion and parasite growth (Richards and Beeson, 2009). Therefore, *msp2* is involved in erythrocyte invasion more so that it has been demonstrated that synthetic *msp2* peptides bind with high affinity to RBCs, and can also inhibit parasite invasion (Branch et al., 2001). *Msp2* has also been implicated in naturally acquired clinical immunity to malaria (Osier et al., 2010) and so it has been studied as a candidate malaria vaccine antigen (McCarthy et al., 2011). *Msp2* has been linked to malaria parasite virulence. Report shows that it is so because of its pathogenicity and genetic diversity, with a high degree of both length and sequence polymorphism (Ferreira et al., 2006). Its chemistry reveals that it is organized in domains of blocks (block 1 to 5). Sequences in block 2 and block 4 are dimorphic and used as the basis to differentiate *msp2* alleles into two families which are FC27 and IC-1/3D7. Studies have shown mixed results with both the FC27 and 3D7 families connected to virulence in *Plasmodium* strains (Mohammed et al., 2018). This work was therefore designed to study the FC 227 allele in *Plasmodium falciparum* causing infection in Jos, Nigeria.

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II. MATERIALS AND METHODS

A. Ethical Approval

Ethical Clearance (Reference Number: PSSH/ADM/ETH.CO/2018/005) was obtained from the Health Research Ethics Committee of the Plateau State Ministry of Health and the Local Health Research Ethics Committee of the health facilities.

B. Sample Collection

All the blood samples clinically screened and ascertained to be infected by malaria parasites and archived between February and May, 2019 in the health centers were collected. Clinical identification was done using microscopy and Rapid Diagnostic Test (RDT) methods. Microscopy was by examination of rapidly stained thick and thin film for the presence of at least one Plasmodium parasite. The RDT used is based on lateral flow immunochromatographic technique.

C. DNA Extraction

Total DNA was extracted from whole blood samples collected using the ZR Quick-gDNA™ Miniprep Extraction Kit (ZYMO RESEARCH USA) according to the manufacturer's instructions. Briefly, 400 µl of Genomic Lysis Buffer was added to 100 µl of whole blood and mixed completely by vortex mixing for 6 seconds and was then allowed to stand for 10 minutes at room temperature. The mixture was transferred into a Zymo-spin™ IIC Column in a collection tube and centrifuged at 10, 000 x g for 1 minute. The flow through in the collection tube was discarded with the collection tube. Next, the Zymo-spin™ IIC Column was then transferred into a new collection tube. 200 µl of DNA Pre-wash Buffer was added into the spin column and then centrifuged at 10, 000 g for 1 minute. Then, 500 µl of g-DNA Wash Buffer was added in to the spin column and then centrifuged at 10, 000 g for 1 minute. The spin column was transferred into a clean 1.5 ml microcentrifuge tube and 50 µl DNA Elution Buffer was added into spin column and was then incubated for 5 minutes at room temperature and then centrifuged at 14, 000 x g for 30 seconds. The eluted DNA was then stored at -70 ^{0}C for further analysis.

D. PCR amplification of msp2 gene and FC 27 allele

The nested PCR assay were carried out according to the modified method of Krishna et al., (2015) using oligonucleotide primers designed based on the Plasmodium small subunit ribosomal RNA (ssrRNA) genes. One set of primers coded PspF (F:TTAAAATTGTTGCGTTAAAAG) and PspR (R:CCTGTTGTTGCCTTAAACTTC) were designed to amplify a primary product of 1200bp which served as a template for species- specific amplification of *Plasmodium falciparum*. The species specific primers were (F:TTAAATGGTTTGGGAAAACCAAATATATT; R:ACACAATGAACTCAATCA TGACTACCCGTA) synthesized in Inqaba Biotech West Africa Limited. In theprocedure, a 25µl reaction mixture for nest-1 amplification contained 3 µl of genomic DNA extract, 0.5 µl of each 20µM primer (PspF and PspR), 12.5µl of One Taq® Quick-Load® 2x Master Mix with Standard Buffer(New England BioLabs) and the volume made up with 8.5µl Nuclease Free Water. Nest-1 amplification conditions were as follows: initial denaturation at 940C for 1 min; denaturation at 94^{0}C for 1 min; annealing at 58^{0}C for 2 min; extension at 72^{0}C for 2 min for 25 cycles. Final extension was at 72^{0}C for 4 min and rest at 4^{0}C. Two microliters(2 µl) of the nest 1 amplification products served as the DNA template for each of the 25µl nest -2 amplifications. The concentration of the nest 2 primers and volume of One Taq® Quick-Load® 2x Master Mix were same as nest-1amplifications. Nest 2 amplification conditions were as nest -1.

E. Gel Electrophoresis

The PCR products were analyzed in ethidium bromide stained 2% agarose gel prepared by suspending 2.0 g of agarose powder in 100 ml 1X TBE buffer and boiling to dissolve the agarose. The agarose gel was cast pouring the solution into appropriate tanks, inserting the combs and allowing the solution to gel after adding 5 µlethidium bromide (Fermentas,® USA). A 10 µl PCR product was then mixed uniformly and loaded into the wells. A 10 µl PCR ladder marker (Fermentas®, USA) was loaded into the first well and the PCR products into the subsequent wells. Electrophoresis separation was at 100V and set for ~40 minutes and image capture and gel documentation was with ChemiGenuis® Gel Documentation System (Synegene Tokyo Japan).

F. Bioinformatics Analyses

The Finch TV® programmes (GeoPiza) was used to access the FC 27 nucleotide sequence while NCBI BLASTN programme and CLUSTAL O software were used to analysed the gene sequence.

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III. RESULTS

The PCR amplification analysed on 2% agarose gel gave product bands of *Plasmodium falciparum* FC 27 allele of msp2 gene. The sequence obtained is 349bp of partial length *FC 27* gene of *falciparum* presented in Figure 1.

The 127 samples were clinically diagnosed for malaria with microscopy only. Only 12 were found to be truly positive using PCR technique. A total of six FC 27 alleles were identified from the *msp*-2. The frequency of the total true positive samples with FC27 allele was 50% as shown in Table 1.The NCBI BLAST tool analysis of *P. falciparum* FC 27allele sequence gave Blast Hits presenting sequences producing

g significant alignments. Among the hits were 9 *Plasmodium falciparum* subject sequences all having 95% query cover and 90.48% identity to the query sequence. The first four hit sequences are those of *P. falciparum* genome assembly, chromosome: 2 (LR131355.1); *P. falciparum* isolate 229 MSP-2 gene, partial cds (HM568659.1); *P. falciparum* isolate Mimia FC27-D msp 2 gene, partial cds (GU075713.1) and *P. falciparum* MSA-2 gene, partial cds (L19049.1). This result is in Table 2 showing that the FC 27 allelic family of merozoite surface protein 2 gene of *Plasmodium falciparum* in Jos, Nigeria is very similar to the sequences of the human malaria parasite *P. falciparum* strains already characterized.

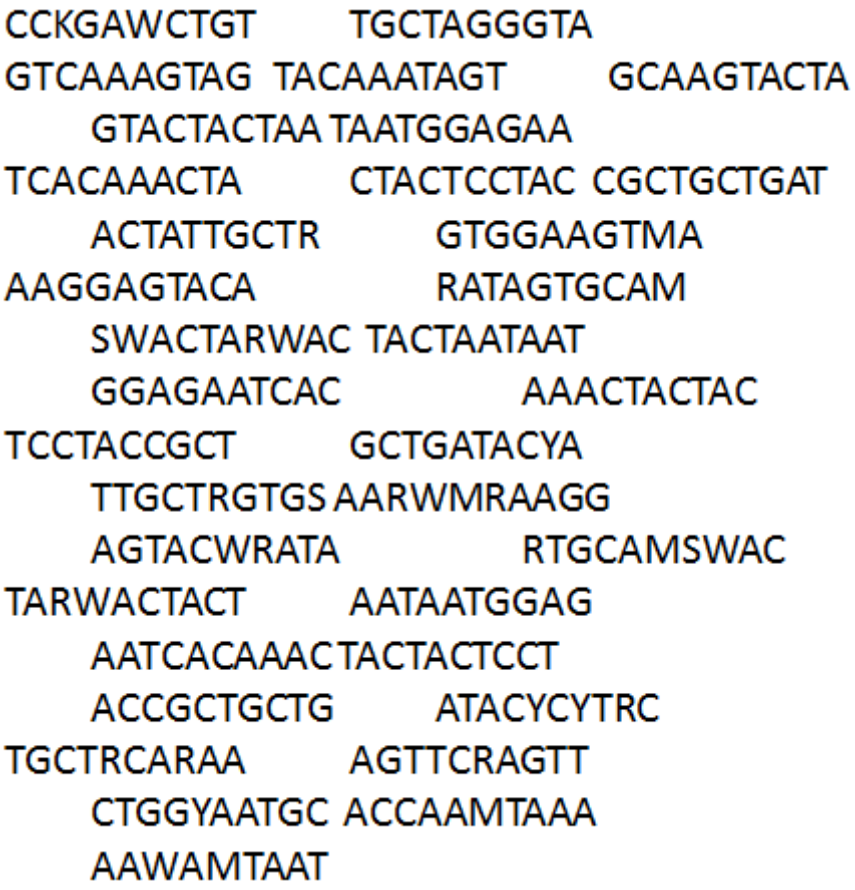

Figure 1: Primary Nucleotide sequence of FC 27 gene from *Plasmodium falciparum* (FinchTV (GeoPiza)

Table 1: Distribution of samples collected from the health centers and their correspondence PCR positive samples and frequencies of FC 27 allele detected *Plasmodium falciparum.*

Hospital	Detected by Microscopy	RDT detected	PCR detected	True positive	FC27 alleles detected	% frequency
1	40	0	6	6	4	33.33
2	60	0	2	2	1	0.08
3	27	0	6	6	1	0.08
TOTAL	127	0	12	12	6	50.00

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Table 2: Distribution of the Blast Hits on top 4 FC27 gene sequences from *Plasmodium falciparum* producing significant alignments compared with the query sequence

Accession No	Description	Max score	Total score	Query cover	% identity
LR131355.1	*P. falciparum* genome assembly, chromosome: 2	499	1333	95%	90.48%
HM568659.1	*P. falciparum* isolate 229 MSP-2 gene, partial cds	499	1333	95%	90.48%
GU075713.1	*P. falciparum* isolate Mimia FC27-D msp 2 gene, partial cds	499	1333	95%	90.48%
L19049.1	*P. falciparum* MSA-2 gene, partial cds	499	1333	95%	90.48%

IV. DISCUSSION

The PCR product of *Plasmodium falciparum* FC 27 allele of msp2 gene obtained was a 349bp sequence. This falls within a size range from (250 to 700 bp) for FC27 allelic families earlier reported (Mohammed et al., 2018).

Out of the 127 clinically diagnosed samples only 12 were truly positive based on PCR technique and six FC 27 alleles were identified making a frequency of 50%. This report is similar to some earlier reports; that the frequency of FC27 was 77% (57/74) in Northwest Ethiopia (Mohammed et al., 2018); of 277 *msp2* sequences from single-clone infections, 114 (41.2%) belonged to the FC27 family (Chaorattanakawee et al., 2018). However, some regions have recorded lower frequencies. For instance, Kidima and Nkwengulila (2015) reported that the *msp2* FC 27 frequency was FC27 (27.3%) in Kibaha, Tanzania. The presence of the FC 27 in malaria infection has severe implications. The *msp2* FC27 block 2-8N allele has been associated with severe malaria in Thailand (Chaorattanakawee et al., 2018) and Uganda (Kiwuwa et al., 2013).

The *P. falciparum* FC 27 allele sequence bioinformatics analyses presented *P. falciparum* MSA-2 gene Hits having high identity to the query sequence although the nucleotide alignment of the sequence demonstrated several polymorphisms. This polymorphic feature is similar to findings reported by a study in Cambodia (Gosi et al., 2013).

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Query            7   CTGTTGCTAGGGTA-GTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG  65
LR131355.1  157617   CTGTTGCTAGGGTAAGTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG  157558
GU075713.1      56   CTGTTGCTAGGGTAAGTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG  115
L19049.1        71   CTGTTGCTAGGGTAAGTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG  130

Query           66   GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTRGTGGAAGTMAAAGGA  125
LR131355.1  157557   GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGA  157498
GU075713.1     116   GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGA  175
L19049.1       131   GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGA  190

Query          126   GTACARATAGTGCAMSWACTAR.ACTACTAATAATGGAGAATCACAAACTACTACTCCTA  185
LR131355.1  157497   GTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACTACTCCTA  157438
GU075713.1     176   GTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACTACTCCTA  235
L19049.1       191   GTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACTACTCCTA  250

Query          186   CCGCTGCTGATACYATTGCTRGTGsaarwmraaGGAGTACWRATARTGCAMSWACTARWA  245
LR131355.1  157437   CCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTA  157378
GU075713.1     236   CCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTA  295
L19049.1       251   CCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTA  310

Query          246   CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACYCYTRCTGCTR  305
LR131355.1  157377   CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACCCCTACTGCTA  157318
GU075713.1     296   CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACCCCTACTGCTA  355
L19049.1       311   CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACCCCTACTGCTA  370

Query          306   CARAAAGTTCRAGTTCTGGYAATGCACCAAMTaaaa  341
LR131355.1  157317   CAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAA  157282
GU075713.1     356   CAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAA  391
L19049.1       371   CAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAA  406
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Query 7 CTGTTGCTAGGGTA-
GTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG 65
LR131355.1 157617
CTGTTGCTAGGGTAAGTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG
157558
GU075713.1 56
CTGTTGCTAGGGTAAGTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG 115
L19049.1 71
CTGTTGCTAGGGTAAGTCAAAGTAGTACAAATAGTGCAAGTACTAGTACTACTAATAATG 130
Query 66
GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTRGTGGAAGTMAAAGGA 125
LR131355.1 157557
GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGA
157498
GU075713.1 116
GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGA 175
L19049.1 131
GAGAATCACAAACTACTACTCCTACCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGA 190
Query 126
GTACARATAGTGCAMSWACTAR.ACTACTAATAATGGAGAATCACAAACTACTACTCCTA 185
LR131355.1 157497
GTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACTACTCCTA
157438
GU075713.1 176
GTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACTACTCCTA 235
L19049.1 191
GTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACTACTCCTA 250
Query 186
CCGCTGCTGATACYATTGCTRGTGsaarwmraaGGAGTACWRATARTGCAMSWACTARWA 245
LR131355.1 157437
CCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTA
157378
GU075713.1 236
CCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTA 295
L19049.1 251
CCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTA 310
Query 246
CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACYCYTRCTGCTR 305
LR131355.1 157379
CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACCCCTACTGCTA 157318
GU075713.1 296
CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACCCCTACTGCTA 355
L19049.1 311
CTACTAATAATGGAGAATCACAAACTACTACTCCTACCGCTGCTGATACCCCTACTGCTA 370
Query 306 CARAAAGTTCRAGTTCTGGYAATGCACCAAMTaaaa 341
LR131355.1 157317 CAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAA 157282
GU075713.1 356 CAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAA 391
L19049.1 371 CAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAA 406

Figure 3: Multiple sequence alignment of FC 27 gene sequence from *P. falciparum* sample with those from some *P. falciparum* strains deposited in the gene bank data base

V. CONCLUSION

The 349bp *Plasmodium falciparum* FC 27 allele of msp2 gene obtained exhibited polymorphism, was highly identical to those of already characterized *P.falciparum*. The frequency of occurrence of this molecular marker of malaria virulence was high signaling Jos is endemic to virulent *Plasmodium falciparum* strains

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