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Full Length Research Paper

In-silico studies of multi drug resistance (MDR) genetic markers of *Plasmodium* species

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Multi-drug resistance malaria species has been and still is the cause of much morbidity and mortality of malaria throughout the tropics. This epidemic has devastated large populations likewise posed a serious barrier to economic growth in developing countries. The major obstacles however, is it prevention and treatment due to emerging multi-drug resistant (MDR) species. Therefore, anti-malarial drug development needs to continue so that novel and highly effective anti-malarial can be plugged into recommended strategies and vaccine development. The sequencing of the various MDR genes of *Plasmodium* has contributed tremendously to the understanding of the MDR malaria parasites. The current research therefore, engaged the use of an in-silico approach to seek the strategies in analyzing as well as offering some likely solutions to malaria therapies. Four *Plasmodium* species: 2 from rodents (*Plasmodium chabaudi* and *Plasmodium yoelii*) and 2 from human (*Plasmodium vivax* and *Plasmodium falciparum*) multi drug resistance genes were compared using bioinformatics tools. The phylogenetic relationships and species identification of the MDR genes of the parasites were downloaded from web base resources and performed as confirmed by the ClustalX programs. The results showed a variation in the up/down stream algorithms alignment of their phylogenetic relationships. This therefore, showed that some resistance genes within a population may vary within the same drug. The results showed a significant difference of $p < 0.001$ with a 95% CI. Through these efforts, our goal was to better understand how drug resistance occurs. This knowledge therefore, will facilitate the rationale to design new effective as well as check the emerging of multi-drug resistant *Plasmodium* strains.

Key words: In-silico, comparison, multi drug, resistance genes, *Plasmodium*.

INTRODUCTION

Multi-drug-resistant *Plasmodium* species is a major public health problem worldwide (Rijken et al., 2008; Karyana et al., 2008). However, substantial progress has been made in the last decade to reveal the underlying mechanisms of drug action and resistance. The current emergence of multi-drug-resistant (MDR) plasmodia species menace threatens to reverse these efforts. This is due to an increased exposure of *Plasmodium* species to sub-lethal doses of anti-malarial drugs. Apart from that it has placed the populations that are routinely in contact with malaria-transmitting mosquitoes and those with limited access to health services at risk to malaria attack (Rockman and Kruglyak, 2006; Emiliana et al., 2008). If steps are not taken to address the root causes of drug resistance, the drugs will also lose their effectiveness in the near future.

However, according to Carlton et al. (2004) the arrival of the genomics and bioinformatics revolution, comparative genomics has scaled up the whole genome comparisons which are now used to describe relative genome composition, genome organization, identify orthologous and paralogous genes. This has given a clear picture of classifying species-specific genes and the evolution of organisms, in all three domains of life: bacterial (Fraser et al., 200), archaeal (Nelson et al., 2000) and eukaryotic (Rubin et al., 2000). However, the comparative genomics of plasmodia genomes still is a discipline in its understudy (Carlton et al., 2004). The

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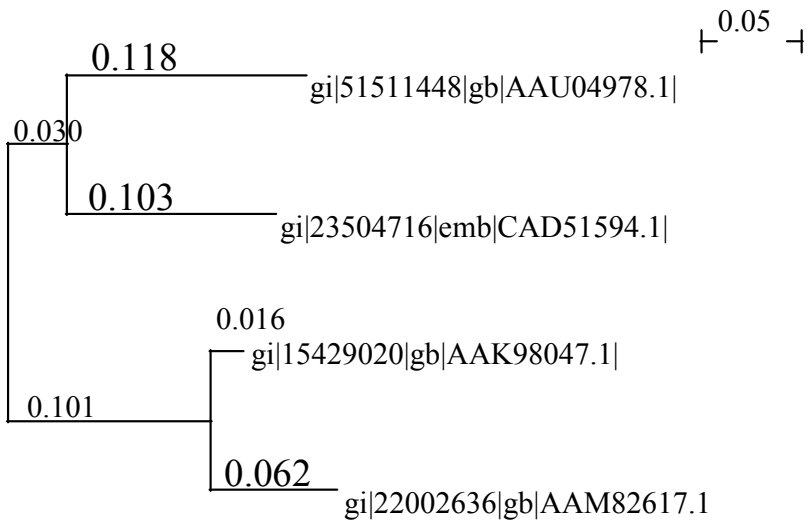

Figure 1. Phylogenetic tree of the MDR gene sequences of the 4 *Plasmodium* species from GenBank using similarities.

current research therefore, engaged the use of in-silico approach to compare MDR genes of *Plasmodium* species downloaded from various resourced data bases. The work will focus primarily on the MDR genes of rodent malaria parasites (*P. yeolii* and *P. chabaudi*) and comparative MDR genomic studies with the human malaria species (*P. falciparum and P. vivax)*, since these are the most advanced. This will give us the insight of the sequence analyses which might be of help to malaria therapies.

The human MDR (P-glycoprotein) gene family is known to include two members, MDR1 and MDR2. The product of the MDR1 gene which is responsible for resistance to different cytotoxic drugs (multi drug resistance) appears to serve as an energy dependent efflux pump for various lipophilic compounds (Chin et al., 1989). Multi drug resistance in human and rodent cell lines is associated with decreased intracellular drug accumulation and correlates with the increased expression of MDR genes which encode membrane glycoprotein (P-glycoprotein) of approximately 170 kilodaltons. Expression of a single human gene designated MDR1 or its rodent counterpart is sufficient to confer multi drug resistance phenotype to drug- sensitive cells. However, the molecular bases for multi drug resistance in plasmodia are still not fully understood. An association between *P. falciparum* pfmdr1, which encodes a trans-membrane glycoprotein (Pgh1, for Pglycoprotein homologue 1) and the multi drug- resistant (MDR) phenotype was first reported in 1989 (Foote et al., 1989). Point mutations in pfmdr1, most notably at codon 86, have been associated with decreased chloroquine sensitivity (Price et al., 1999; Agbonlahor et al., 2008). Furthermore, in a study involving genetic cross, chloroquine resistance was found to segregate with cg2 (located on chromosome 7) rather than with pfmdr1 (located on chromosome 11) (Su et al., 1997). Amplification of the pfmdr1 gene copy number is associated with resistance to mefloquine and halofantrine, both in laboratory and field isolates (Wilson et al., 1993). In *Plasmodium,* mdr homologues encoding P-glycoprotein–like molecules have been proposed as determinants of malaria drug resistance and associations have been reported between chloroquine resistance and amplification or mutation of the mdr -like gene pfmdr 1, which encodes Pgh1 (Reed et al., 2000)

MATERIALS AND METHODS

Sequence encoding for multi drug resistances in 4 *Plasmodium* species: 2 from rodents (*Plasmodium chabaudi* and *Plasmodium yoelii*) and 2 from human (*Plasmodium vivax* and *Plasmodium falciparum*) were downloaded from GenBank (USA) and ExPASy (Swiss). The algorithms of nucleotide-nucleotide BLAST and protein-protein BLAST of protein sequences were obtained from the above databases and done using the fasta format. A comparative homology study of the 2 MDR genes of rodent malaria parasite sequences and 2 from human were done. The multiple sequence alignment algorithms of the multidrug resistance genes of these plasmodia were done using ClustalX (ver 2.0.9). The plot of trees were obtained from amino acid sequences of the multi drug resistance genes of the malaria parasites with the help of "drawgram" program of the PHYLIP package (ver. 3.6) which was downloaded from http://evolution.genetics.washington.edu/phylip.html. All the databases and software used in the studies were supplied by Wellcome Trust, UK and are currently available on the world-wide web.

RESULTS

Multiple sequence algorithms alignment of the 4 MDR resistance genes has depicted the presence of the conserved sequence regions and their relationship as shown in Figures 1 and 2. The results showed a significant difference of ($p < 0.001$) with a 95%

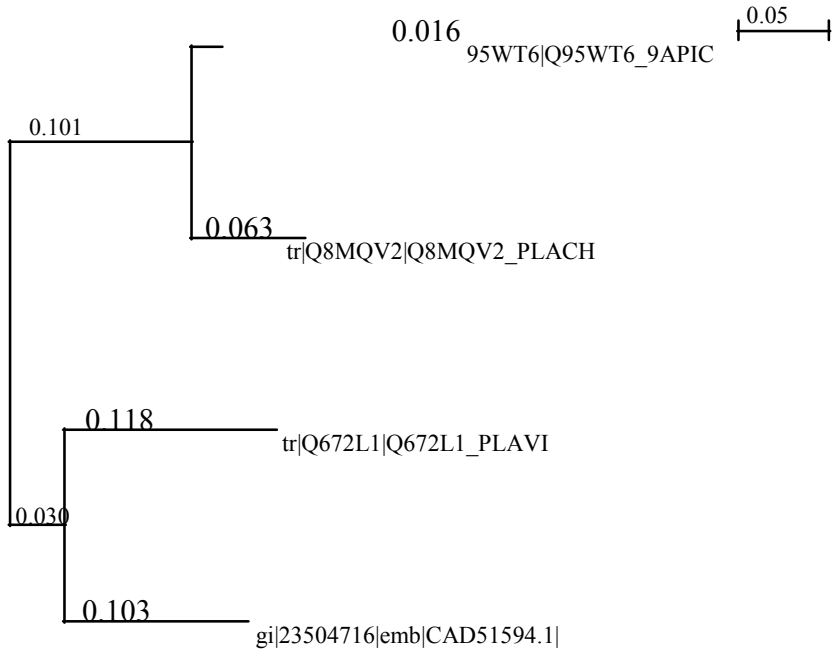

Figure 2. Phylogenetic tree of the MDR gene sequences of the 4 *Plasmodium* species from ExPASy using similarities.

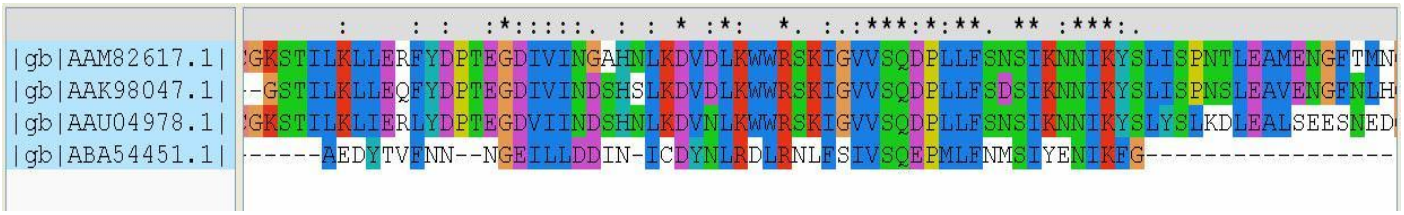

Figure 3. Multiple sequence alignment of MDR genes encoding nucleotide of the 4 *Plasmodium* species obtained from GenBank.

confidence limit (CI) among all the MDR genes studied. The results in Figure 1 showed that the genealogy of the *Plasmodium* species MDR orthologue gene sequences from the GenBank were variable. There was no total homology in the encoding genes as can be seen in their branch lengths ($p < 0.001$). The branch lengths of the human's parasites were 0.103 (*P. vivax*) and 0.118 (*P. falciparum*) respectively while the one from rodent was 0.016 (*P. yoelii*) and 0.062 (*P. chabaudi*) respectively. There was a similarity between the two *Plasmodium* rodent species as well as between the two *Plasmodium* human parasites (Figure 1).

The results in Figure 2 show the 4 *Plasmodium* species MDR gene sequences from ExPASy resourced web base. The branch lengths for the human *Plasmodium* species were the same as those obtained from GenBank (0.103 and 0.118 respectively) while the one from rodent parasites were 0.016 (*P. yoelii*) and 0.063 (*P. chabaudi*) respectively (Figure 2) . The results also showed a great similarity between the 2 *Plasmodium* rodent species as against the 2 *Plasmodium* human parasites (Figure 2).

The phylogenetic tree showed that the 4 *Plasmodium* parasites were from the same genus as shown from their branch lengths of 0.030 and 0.101 (Figures 1 and 2) indicating that great sequence homology.

The results of Figure 3 showed the multiple sequence alignments encoding the amino sequences of the 4 *Plasmodium* parasites from GenBank. Almost the entire sequence from the 4 *Plasmodium* species could be found aligned. The residues in the sequences also showed a slight varied homology with close variation as shown in the phylogeny results. The same results were obtained from ExPASy as shown in Figure 4. The multiple sequence alignment encoding the amino sequences of the 4 *Plasmodium* parasites showed their high level genus sequence homology (Figures 3 and 4).

DISCUSSION

Plasmodia parasites isolated from vertebrates have been shown to exhibit a wide range of phenotypic variations,

Figure 4. Multiple sequence alignment of MDR genes encoding nucleotide of the 4 *Plasmodium* species obtained from ExPASy.

including drug responses, growth rates and virulence factors. These inter-strain variations occurred primarily at the DNA sequences resulting into phenotype variations (Gonzales et al., 2008). Therefore, with the fully sequenced *P. falciparum* genome (Rockman and Kruglyak, 2006; Carlton et al., 2004; Gardner et al., 2002), several large-scale gene expression have been studied (Le Roch et al., 2003; Llinas et al., 2006). Also with the discovery of the gene functions of the malaria life cycle this however, have provided us with detailed insights into gene expression (Gonzales et al., 2008; Le Roch et al., 2003). In this study, the sequences from the 2 websites were shown to have varied similarities: the 4 *Plasmodium* species; two from rodents (*P. chabaudi* and *P. yoelii*) and two from human (*P. vivax* and *P. falciparum*). Earlier studies by Pedro et al. (2003) showed that amplification of, or mutations in, the multi drug resistance gene of pfmdr1 in *P. falciparum* may be involved. Using the rodent malaria model *P. chabaudi*, Petro et al (2003) found that pfmdr1 gene exists as a single copy gene on chromosome and the sensitive clone can undergo duplication. Other reports however, have shown that not all mefloquine-resistant progeny contained the duplicated gene, but at least one of the duplicated genes have been involved in drug resistance (Rockman and Kruglyak, 2008; Pedro et al., 2003). Although, reports have shown that genetic determinants of resistance to anti-malarial drugs of malaria parasites are unclear (Rockman and Kruglyak, 2008).

The present studies also showed the possible existence of additional genes for a drug resistant phenotype. This may be due to specific loci that may mutate drug resistance genes and the parasite can respond to the pressure and further develop resistance to the anti-malaria drug. With this inline, the genetic mutations in drug resistance genes can contribute to worldwide drug resistance. This gives an insight to the identification of novel markers of anti-malaria drugs which can enhance malaria treatment. According to reports of Rohrbach et al. (2006) this however, has limited the widely used anti-malarial agents likewise their efficacy. Based on the alteration of these mutant genes, this can be extended to other anti-malaria drugs. This therefore, had shown to reduce the susceptibility of 4-aminoquinoline drug chloroquine, the folate antagonist's pyrimethamine and sulfadoxine quinine, mefloquine and possibly artemether (Baird, 2005; Jambou et al., 2005).

The chloroquine and sulfadoxine-pyrimethamine have been shown to be the most widely used anti-malaria drugs, but the parasites have evolved resistance leading to the drugs' failure (Tatfeng et al., 2008; Fatumo and Yah, 2009). Base on the available information, there's no magic bullet for cleaning the disease, but this study offers some suggestions that can check the evolution of the resistance among the species. According to this in-silico study, the MDR genes among the *Plasmodium* species have some correlation with the transporter superfamily that transport a great variety of substrates modulating a host transport systems (Rohrbach et al., 2006; Borges et al., 2003). According to Babiker et al. (2001), this great transfer of events across the membrane can lead to resistances of the anti-malaria agent. This has made it easier for drug-resistant genes to evolve and spread throughout the parasite population. Therefore, anti-malarial drug development needs to continue so that we have novel and highly effective anti-malarial agents that can be plugged into the recommended strategy.

Our results showed that comparison of identical orthologous genes is paving a way for the identification of genes under selection pressure which are useful for vaccine design. This stimulation of comparative genomics of the *Plasmodium* parasites, can promise to produce a useful results for the development of anti-malarial drugs and vaccines. This was similar to the findings of Carlton et al. (2004) which showed that non-synonymous substitution rates between orthologous genes can provide useful techniques for the identification of genes under selection pressure as well as vaccine develop-ment. Through these efforts our goal was to better understand how drug resistance occurs. This knowledge therefore, will facilitate the progressive rationale to design new effective and tolerated anti-malarial drugs.

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REFERENCES

Agbonlahor DE, Tatfeng YM, Oviasogie F, Omolu PI, Tchounga KS, Yah CS (2008). High prevalence of *Plasmodium falciparum pfmdr1* 86Y mutant gene in sickle disease in Nigeria. J. Microbiol. Immunol. Infect. 41: 393-396.

Babiker HA, Pringle SJ, Abdel-Mushin A, Mackinnon M, Hunt P, Walliker DC (2001). High-level chloroquine resistance in Sudanese isolates of *P. falciparum* is associated with mutations in the chloroquine resistance transporter gene *pfcrt* and the multidrug-resistance gene *pfmdr1*. J. Infect. Dis. 183: 1535–1538.

Baird JK (2005). Effectiveness of antimalarial drugs. N. Engl. J. Med. 352: 1565–1577.

Borges-Walmsley MI, McKeegan KS, Walmsley AR (2003) Structure and function of efflux pumps that confer resistance to drugs. Biochem. J. 376: 313–338.

Carlton1 J, Silva J, Hall N (2004). The Genome of Model Malaria Parasites and Comparative Genomics. Curr. Issues Mol. Biol. 7: 23-38. Online journal at www.cimb.org.

Chin JE, Soffir R, Noonan KE, Choi K, Roninson IB (1989). Structure and expression of the human MDR (P-glycoprotein) gene family, Mol. Cell Biol. 9(9): 3808–3820.

Emiliana T, Nicholas MA, Paulus S, Noah W, Enny K, Muhammad K, Daniel AL, Ric N (2008). Price. Multidrug-Resistant *Plasmodium vivax* Associated with Severe and Fatal Malaria: A Prospective Study in Papua, Indonesia PLoS Medicine 5(6)| e128: 0890-0899.

Fatumo SA, Yah CS (2009). *In-Silico* Detection of Chokepoints Enzymes In Four *Plasmodium* Species. J. Pharm. Allied Sci. 6 (3): 632-640.

Foote SJ, Thompson JK, Cowman AF, Kemp DJ (1989). Amplification of the multidrug resistance gene in some chloroquine-resistant isolates of *P. falciparum*. Cell 57: 921–930.

Fraser CM, Eisen J, Fleischmann, RD, Ketchum, KA, Peterson S (2000). Comparative genomics and understanding of microbial biology. Emerg. Infect. Dis. 6: 505-512.

Gardner MJ, Hall N, Fung E, White O, Berriman M (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419: 498–511.

Gonzales JM, Patel JJ, Ponmee N, Jiang L, Tan A (2008). Regulatory Hotspots in the Malaria Parasite Genome Dictate Transcriptional Variation. PLoS Biol 6(9): e238 doi:10.1371/journal.pbio.0060238.

Jambou R , Legrand E, Niang M, Khim N, Lim P, Volney B, Ekala MT, Bouchier C, Esterre P, Fandeur T (2005). Mercereau-Puijalon O. Resistance of *Plasmodium falciparum* field isolates to *in-vitro* artemether and point mutations of the SERCA-type PfATPase6. *Lancet* 366: 1960–1963.

Karyana M, Burdarm L, Shunmay Y, Kenangalem K, Wariker N, Maristela R, Gde KU, Vemuri R, Okoseray MJ, Penttinen PM, Ebsworth P, Sugiarto P, Anstey MN, Emiliana T, Richard NP (2008). Malaria morbidity in Papua Indonesia, an area with multidrug resistant *Plasmodium vivax* and *Plasmodium falciparum.* Malaria J. 7:148 doi:10.1186/1475-2875-7-148.

Le Roch KG, Zhou Y, Blair PL, Grainger M, Moch JK (2003). Discovery of gene function by expression profiling of the malaria parasite life cycle. Science 301: 1503–1508.

Llinas M, Bozdech Z, Wong ED, Adai AT, DeRisi JL (2006). Comparative whole genome transcriptome analysis of three *Plasmodium falciparum* strains. Nucleic Acids Res. 34: 1166–1173.

Nelson KE, Paulsen IT, Heidelberg JF, Fraser CM (2000). Status of genome projects for nonpathogenic bacteria and archaea. Nat. Biotechnol. 18: 1049-1054.

Pedro VL, Cravo Jane MR, Carlton., Paul H, Laura B, Rose AP, David W (2003). Genetics of Mefloquine Resistance in the Rodent Malaria Parasite *Plasmodium chabaudi;* Antimicrob. Agents Chemother. 47(2): 709–718.

Price RN, Cassar C, Brockman A, Duraisingh M, Van Vugt M, White NJ, Nosten F, Krishna S (1999). The *pfmdr1* Gene Is Associated with a Multidrug-Resistant Phenotype in *Plasmodium falciparum* from the Western Border of Thailand Antimicrobial Agents and Chemotherapy 43(12): 2943–2949.

Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF (2000). Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. Nature 403: 906–909.

Rijken MJ, McGready R, Machteld EB, Barends M, Proux S, Mupawjay P, Pratap S, Nosten F (2008). Short Report: Dihydroartemisinin-Piperaquine Rescue Treatment of Multidrug-resistant *Plasmodium falciparum* Malaria in Pregnancy: A Preliminary Report. Am. J. Trop. Med. Hyg. 78(4): 543–545.

Rockman MV, Kruglyak L (2006). Genetics of global gene expression. Nat. Rev. Genet. 7: 862–872.

Rohrbach P, Sanchez CP, Karen H, Friedrich O, Jigar P, Amar BSS, Michael TF, Fidock DA, Lanzer M (2006). Genetic linkage of *pfmdr1* with food vacuolar solute import in *Plasmodium falciparum;* The EMBO J. 25: 3000–3011, doi:10.1038/sj.emboj.7601203.

Rubin GM, Yandell MD, Wortman JR, Gabor Miklos, G.L, Nelson CR, Hariharan I.K, Fortini ME, Li PW, Apweiler R, Fleischmann W (2000). Comparative genomics of the eukaryotes. Science 287: 2204-2215.

Su XZ, Kirkman LA, Fujioka H, Wellems TE (1997). Complex polymorphisms in an; 330 kDa protein are linked to chloroquine-resistant *P. falciparum* in Southeast Asia and Africa. Cell 91: 593–603.

Tatfeng YM, Agbonlahor DE, Tchounga KS, Omolu PI, Okodua M, Yah CS, Adeolu A (2008). Chloroquine prophylaxis associated with high prevalence of *Plasmodium falciparum Pfcrt* K76T mutation in people with sickle-cell disease in Benin City, Nigeria. J. Vector Borne Dis. 45: 51–55.

Wilson CM, Volkman S, Thaithong SK, Martin RK, Kyle DE, Milhous WK, Wirth DF (1993). Amplification of *pfmdr1* associated with mefloquine and halofantrine resistance in *Plasmodium falciparum* from Thailand. Mol. Biol. Parasitol. 57: 151–160.