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Parasite Genetic Factors Implicated in

Cerebral Malaria

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Summary

*Plasmodium falciparum* is the most deadly species, causing broad spectrum of disease manifestations ranging from asymptomatic infection to symptomatic outcomes, including uncomplicated and severe malaria. This latter comprises various clinical forms, notably cerebral malaria. The biological processes involved in the heterogeneity of these clinical outcomes are not well understood. We hypothesized that differential gene expressions contribute to phenotypic variation of parasites leading to specific interaction with the host which subsequently induces several clinical categories of malaria. In order to verify this hypothesis, we investigated the transcriptomes of parasites isolated from Cameroonian children with asymptomatic (AM), uncomplicated (UM), and cerebral malaria (CM). We also analyzed the transcriptomes of the 3D7 line and the selected 3D7-Lib line. The latter has been selected by several rounds of incubation with anti-IgG of Liberian hyper-immune plasma. It has the ability to express group A var and neighboring rif genes, which have been described in several studies to be associated with severe disease and cerebral malaria.

Our results of transcriptomic study revealed the up-regulation of several genes in CM-associated isolates and the 3D7-Lib line compared to AM-associated isolates, and the 3D7 line. Gene Ontology term analysis shows that a majority of differentially expressed genes are mainly implicated in pathogenesis, cytoadherence to host endothelial tissues, and erythrocyte aggregation. Among up-regulated genes in CM-associated isolates compared to AM-associated isolates, there were genes encoding for surface antigens, Maurer’s cleft proteins, transcriptional factors, and antigens involved in protein transport to the host cell surface. In addition, an important variation in the expression of variant surface polymorphic families like var, rif and phist genes has been observed. The most remarkable outcomes were the up-regulation of UPS A and UPS B var genes containing architectural Domains Cassettes DC4, DC5, DC13, and DC8 and their neighboring rif genes in isolates from CM and in the 3D7-Lib line compared with isolates from AM and the unselected 3D7 line, respectively.

Several studies have shown that group A and B var genes containing DC13 and DC8 are implicated in severe malaria, including cerebral malaria. However, the transcriptome profiles were similar in CM and UM isolates, and between AM and UM parasites. The lack of differences in genes expression between CM and UM parasites may be due to similar transcription patterns between isolates of these two clinical groups, or to the inability of microarrays to detect trivial variations. Therefore, in order to confirm the specific transcription profiles of these genes in CM isolates, we examined by RT-qPCR the expression abundance of UPS A1, A3, and B1 var groups found to be highly up-regulated in the CM group, using new samples collected from Beninese children with CM or UM. These genes
were significantly highly transcribed in CM compared to UM group. The involvement of these genes in the parasite virulence rises from the ability of their encoded proteins to mediate the cytoadherence of infected erythrocytes to post capillary endothelial cells. Cytoadherence is essential for parasite life to evade spleen-dependent clearance. However, the clinical consequences of this cytoadherence differ among infected individuals. Several endothelial receptors that are involved in the cytoadherence of IEs to venular endothelia have been identified, and few studies attempted to correlate a particular binding phenotype of parasites with a given clinical presentation of malaria. Of these receptors, CD36 and Inter Cellular Adhesion Molecule-1 (ICAM-1) were found as the most commonly used by patient parasites. The implication of these two receptors carried out along with PfEMP-1 ligands in the pathogenesis of cerebral malaria needs to be more elucidated. We examined the adhesive phenotype and the transcription patterns of Pfemp-1 variants of fresh isolates from Beninese children with cerebral malaria and uncomplicated malaria by static binding assay and RT-qPCR respectively, to identify a relationship between the Pfemp-1 variants expressed by the parasites and their cytoadherence phenotype to a specific receptor. The binding levels of IEs were investigated to CD36, ICAM-1 and CSPG (CSA) and the transcription level was assessed for groups A, B, var2, var3, DC8, and DC13, up-regulated in CM isolates in our transcriptomic study.

Our findings reported for the first time that isolates from CM patients have a significant preference for CD36-binding compared with those from UM cases. No differences were observed in binding levels to ICAM-1 or CSPG between these two groups. Furthermore, cerebral malaria isolates transcribed groups A, B, var2, var3, DC8 and DC13 of var genes to higher levels than UM isolates. Interestingly, the high transcription levels of group B in CM parasites correlated with their raised level of binding to CD36, supporting the implication of this receptor along with group B in cerebral malaria pathogenesis. In contrary, the expression profiles of a specific var group and the binding phenotype of isolates to ICAM-1 and to CSPG were not correlated.

The molecular basis of interaction between CD36 and PfEMP-1 variants of group B need to be further investigated for better understanding of cerebral malaria pathogenesis. This interaction could be targeted by specific antibodies or suitable agonist molecules to prevent *P. falciparum* severe complicated malaria as cerebral malaria.

In addition to PfEMP1 group A1, A3 and B1 subset, other genes encoding exported proteins as well as non-exported proteins, were up-regulated in CM-associated parasites. The potential role of these genes in CM pathogenesis deserves to be investigated and may allow the identification of new targets for malaria control.
Chapter 1

1. Introduction

1.1. Malaria History at a glance

Malaria or mal’aria “spoiled air” is caused by parasites which have existed with us since the dawn of time. The earliest Plasmodium falciparum DNA has been detected in two Pharaonic mummies aged more than 3200 years B.C.

Hippocrates was one of the first to notice and describe malaria symptoms such as poor health, fever and enlarged spleens in the 5th century B.C.

The first documented treatment against malaria was brought from Peru in the 1600s A.C by the Jesuits. The natives at that time were using the Cinchona barks as a traditional treatment which then had been transported to Europe under the name of “Jesuit’s powder” to treat patients experiencing fever “agues” (Fig. 1).

Fig. 1: Rawhide Bag of Cinchona bark, namely “Seron” used to store and transport Cinchona barks, brought from Peru in 1777 by Hipolito Ruiz Lopez and Antonio Pavon y Jimienz who had been sent by Spanish monarch Charles III to discover the region. Source: Science and society, 2014. (http://www.scienceandsociety.co.uk)

In 1820, Pelletier and Caventou, French pharmacists, were able to successfully isolate the quinine and the quinidine from Cinchona barks that were used later by French workers in Senegal and in the United States of America.

The discovery of malaria parasite was made in 1880 by Alphonse Laveran, a French surgeon in the French army posted in Constantine city, Algeria. He observed parasites in blood samples of patients with fever that were absent in healthy individual blood. He also noticed the disappearance of parasites after administration of quinine. These observations
encouraged Laveran to identify and describe the causative agent of malaria, “the parasite”. For this precious finding, Laveran has been attributed Nobel prize of medicine in 1907.

In 1885, Marchiafava, an Italian physician, named the genus of malaria parasites “Plasmodium”, and this was followed by the identification of P. vivax and P. malariae by Camillo Golgi and P. falciparum by Marchiafava. The exflagellation property of male gametocytes and the ability to enter inside female gametocyte of P. falciparum parasites has been recognized by MacCallum in 1897. The fourth species of human malaria parasites P. ovale had been identified in 1922 by Stephens.

The incrimination of mosquito in malaria transmission was made in 1897, when Ronald Ross in India observed different stages of development such as oocytes in the stomach wall as well as sporozoites in the salivary glands inside mosquitoes that had fed on infected birds, and independently by Grassi in Italy who incriminated Anopheles for the transmission of P. falciparum. Ross was was awarded the Nobel Prize of medicine in 1902.

The sporogonic cycle of Plasmodium parasites inside the female mosquito was observed in 1899 by Grassi, Bastianelli and Bignami. Using blood samples from naïve volunteers infected with mosquito fed on malarial patients, they were able to describe the life cycle inside the anopheles and the biological cycle of P. falciparum, P. vivax and P. malariae. In 1948, Shortt and Garnham discovered the pre-erythrocytic stage of Plasmodium parasites, which takes place inside the human host. Finally, in 1982, Krotoski was able to characterize the hypnozoite or the latent phase of P. cynomolgi and then of P. vivax (Krotoski et al. 1982).

The discovery of natural infections by a 5th species, Plasmodium knowlesi, came late in 2007 after cross infection from Macaca monkey to human being. During the past years, hundreds of cases had been diagnosed with malaria caused by P. knowlesi infection. This latter morphologically resembles to P. malariae, However, P. knowlesi infections, unlike those of P. malariae, can be fatal to humans (Cox et al. 2010).

1.2. Malaria burden worldwide

Malaria, the implacable enemy, is one of the most serious health problems in the world (Fig. 2). In 2010, the World health organization estimated 216 million acute cases of malaria in 106 endemic countries and up to 655,000 deaths annually (WHO 2011). The highest rate of malaria cases (81%) and deaths (91%) took place in sub-Saharan Africa, though other continents including Latin America, the Middle East and Asia are at risk as well (Murray et al. 2012). Children and pregnant women in endemic regions bear the brunt. While the majority of deaths occur among children under five years of age, most surviving patients suffer from grave complications, mainly severe anemia. Pregnancy associated malaria (PAM) has undesirable birth consequences such as premature delivery, spontaneous abortion, still birth and low birth weight (Steketee et al. 2001). This disease affects negatively on the economic growth rate of endemic areas, where it is estimated to decrease by 1.3% annually compared
to that in malaria-free countries (Sachs and Malaney 2002). Thus, malaria has social and economic impact on needy people and indigent populations who are not able to afford treatment costs or have restrained access to public health care (Orem et al. 2012). Insecticidal bed nets and indoor residual spraying (pyrethroids-vector control) and anti malarial drugs against parasites (notably artemisinins) have significantly favored the reduction of malaria cases and death rate (WHO, 2011). However, these strategies have been thwarted by the emergence of resistance in both mosquitoes and parasites, and by human migration and the recent financial crisis (Leach-Kemon et al. 2012). All these causes are imminent threats to individuals living in endemic regions (WHO 2010). Another strategy to fight against malaria is a vaccine, which, if available, could decrease the malaria incidence by protecting the population at high risks (children and women) from the serious complications of disease; thereby it would reduce the medical expenses and support the reconstructive and developmental projects in the affected countries. However, all the vaccine candidates tested during the last decade were not sufficiently efficient (Snounou and Renia 2007).

**Fig. 2:** *P. falciparum* malaria risk defined by Annual Parasite Incidence in 2010. Carmine color refers to stable-transmission geographical zone where annual parasite incidence $PfAPI \geq 0.1\%$, whereas pink color represents unstable-transmission zone with $PfAPI < 0.1\%$. Light grey mentions to free malarial areas. Source: Malaria Atlas Projects (Map), 2010.

### 1.2.1. Malaria burden in Benin

The republic of Benin is localized in West Africa, bordered by Nigeria on the East, Togo on the West, Burkina Faso and Niger on the north and the Atlantic Ocean on the south (Fig. 3). The surface area of Benin is 112,600 km² with a population density estimated to 10, 100,000 habitants (WHO, 2013). The climate is tropical in the south and soudanian in the north with
two distinct dry and rainy seasons. The rainfall is not stable; it varies between regions and over the years with a range between 1400 mm in the south and around 800 mm in the north (Fink et al, (9) MPETUS Atlas du Bénin; http://www.impetus.uni-koeln.de/fr/impetus-atlas/impetus-atlas-benin).

In Benin, 100% of people are at risk of malaria (WHO, 2013). In 2006, the estimated death rate in infants under 5 years was 125 deaths/1000 live births (Demographic and Health Survey, 2006; http://dhsprogram.com/pubs/pdf/FR197/FR197.pdf).

*Plasmodium falciparum* is the principal cause of malaria, transmitted by three major mosquito vectors: *An. gambiae* and *An. melas* and *An. funestus* (Akogbeto and Romano 1999; Moiroux et al. 2012). The epidemiological studies have been concentrated in the coastal areas south of Benin, where the economic and the administrative capitals are situated. Cotonou is the economic capital, and it is characterized by two rainy seasons from April to July and from September to November during which malaria infection is exclusively caused by *P. falciparum* parasites with approximately 33 infected bites per individual annually (Akogbeto, 1995) (Fig. 3). In this city, the average parasite density in children is from 3000 to 6000 parasites/mm$^3$ and less than 1000 parasites/mm$^3$ in adults (Chippaux et al. 1991). A study conducted at CNHU hospital in Cotonou, showed that 20% of medical visits was due to malaria and 44% of cases were children between 6-23 months (Boulard et al. 1990). Another study reported that the mean number of fever episodes occurred every year in Beninese children under 3 years was 2.4 per children and 33% of those cases were infected with *P. falciparum* with an estimated mortality rate of 8/1000 cases yearly (Velema et al. 1991).

Part of my thesis work was carried out in Cotonou where field isolates had been collected from three hospitals located in this city, as it is described in the Materials and Methods in the second manuscript.
1.3. Human *Plasmodium* species

The etiological agent of malaria is a eukaryotic protozoan parasite of the genus *Plasmodium* belonging to the apicomplexan family. Parasites are transmitted to human beings by *Anopheles* mosquitoes’ bites. To date, more than 400 species of anopheline mosquitoes have been distinguished, but only 40 are recognized as malaria vectors (Service 1993). Some of these mosquitoes e.g. *Anopheles gambiae*, prefer human being to animals so-called “anthropophilic”, making them highly active malaria transmitters to human hosts.

There are five common species of *Plasmodium* that could infect humans: *P. falciparum* (Welch 1887) (Schaudin 1802), *P. vivax* (Grassi and Feletti 1890), *P. malariae* (Grassi and Feletti 1892), *P. ovale* (Stephens 1922) and *P. knowlesi* which has recently been identified in Malaysia as the 5th human infecting species after cross infection from Macaca monkey (Sabbatani et al. 2010).

Among these species, *P. falciparum* is the most virulent species since it is associated with severe malaria and high mortality in sub-Saharan Africa.

1.3.1. *Plasmodium falciparum* life cycle

The *Plasmodium falciparum* life cycle is complex, and the parasite needs to develop in two different hosts to complete its cycle. The infection is initiated when infected female mosquito bites an individual and inoculates sporozoites with the saliva into the skin. Within
one hour the parasites move to the liver and invade hepatocytes. The sporozoites spend 6-10 days inside hepatocytes during which they differentiate and undergo asexual replication yielding upto ~40,000 merozoites that then burst from the infected liver cell into the blood stream. The merozoites invade erythrocytes and go through several morphological stages, beginning with the ring and followed with the trophozoite stages, where the parasites ingest part of erythrocyte cytoplasm and degrade its hemoglobin into amino acids to be used for protein synthesis. Schizonts are produced after multiple round of nuclear multiplication resulting in 4-32 daughter merozoites that are released into the circulation after the infected erythrocyte (IE) bursts. Each merozoite is able to infect a new erythrocyte within 30 seconds and reinitiates the cycle. The duration of the intra-erythrocytic stage of *P. falciparum* is 48 hours, and clinical symptoms of malaria, fever and chills correlate with IEs bursting. After 10-11 days, another form of parasites can be found in the peripheral blood, namely gametocytes. In fact, some of erythrocytic parasites do not develop into schizonts and instead they differentiate into sexual forms, male and female gametocytes, which are not harmful to human host, but are infectious to mosquitoes (Mouchet et al. 2004; Tilley et al. 2011). Once in the mosquito midgut, following a blood meal, the parasites encounter environmental change, low temperature, modified pH and blood digestive factors such as chymotrypsins and serine proteases. After 30 minutes, the gametocytes mature, where the males transform into eight-motile microgametes and the females into round shaped macrogametes that then emerge from IEs and fertilize to produce a zygote. At this stage the genome of the parasite is diploid and recombination events can lead to an extensive genetic diversity. The sexual life cycle continues inside the mosquito and the zygote pursues its development, as it becomes motile ookinete that enters the stomach wall and resides between basal lamina and epithelial cells. This is followed by the differentiation into an oocyte containing thousands of parasites harboring haploid genomes. The mature oocyte bursts and releases thousand of sporozoites that then migrate to the mosquito salivary glands where they are ready to be injected into a human host (Cox et al. 2010; Vizioli et al. 2001).
Fig. 3: Life cycle of *Plasmodium falciparum*. (1) Sporozoites injected by mosquito, migrate through the circulatory system to the liver, where they invade hepatocytes, multiply then released to blood stream as merozoites which are able to invade erythrocytes. Parasites enter asexual intraerythrocytic multiplications (2), which take 48 hours for *P. falciparum*. Each schizont gives birth to 30 daughter merozoites. Once erythrocyte ruptures, these merozoites infect other erythrocytes. Some merozoites differentiate to male and female gametocytes, which are taken by mosquito with blood meal, to be fertilized, differentiate and replicate resulting in sporozoites stages ready to infect human host. Source: Modified from (Bousema and Drakeley 2011).
1.4. Selected clinical outcomes of P. falciparum malaria infection

The clinical manifestations of P. falciparum malaria vary from asymptomatic infection to uncomplicated malaria and severe malaria including cerebral malaria. The disease symptoms range from fever to severe complications.

Mild form of malaria is mainly manifested as fever and sometimes is accompanied with gastro-intestinal troubles, while severe or complicated malaria consists of life threatening disease. The most vulnerable populations are individuals with non-developed immunity such as children and travelers in endemic areas, or people with reduced acquired immunity like primigravidae women. In malaria endemic regions, asymptomatic infections with low parasite densities are prevalent.

The clinical consequences of malaria take place during the asexual blood cycle of parasite life cycle. The clinical manifestations of malaria are classified as uncomplicated malaria (UM); severe malaria (SM) including severe malaria anemia (SMA) and cerebral malaria (CM). Malaria infection in pregnant women is considered as complicated malaria and known as pregnancy associated malaria (PAM).

During my PhD thesis i have been interested in P. falciparum genetic factors implicated in the pathogenesis of falciparum malaria. For that, i used isolates from Cameroun and Benin collected by our team from children less than six years of age. These children presented distinct clinical manifestation such as CM and UM. A group of children with asymptomatic infections had been collected and used as well.

Uncomplicated malaria (UM)

The most frequent features of uncomplicated malaria infection are chills, fever, sweat, headache, asthenia, weariness, myalgia, nausea and vomiting due to the liberation of hemozoin and the toxic bilirubin followed IE rupture. In case of delayed or subcurative treatment, the high temperature and the high parasite density would worsen the clinical case.

Treatment of uncomplicated malaria aims to eliminate the parasites from the body as rapidly as possible in order to prevent the progression of disease to severe form. An adequate treatment of the infection can attenuate its transmission to others and reduce the emergence of resistance to antimalarial agents.

The first line treatment for infants and young children with UM is artemisinin-based combination therapy (ACTs). A combination of two or more antimalarial medicines with different mechanisms of action and contrasting pharmacokinetics such as artemether / lumefantrine, artesunate / amodiaquine, artesunate / mefloquine, artesunate / sulfadoxine-pyrimethamine, and dihydroartemisinin / piperaquine is the treatment of choice for
uncomplicated falciparum malaria. Alternative therapy such as artemesunate / tetracycline or doxycycline or clindamycin, quinine plus tetracycline or doxycycline or clindamycin should be administered for seven days (WHO, 2010).

Severe malaria (SM)
In tropical endemic areas, severe malaria occurs primarily in children under 5 years of age whereas in industrialized countries, non-immune adults returning back from endemic zones are susceptible to develop severe malaria (Hatz et al. 2001; Leder et al. 2004). Several predisposing factors participate in this type of malaria and may lead to death, involving age more than 65 years, naïve non immune cases, female sex (especially pregnant), lack of prophylaxis, delay in medical treatment, degree of disease severity at admission and coexisting other health problems (Bruneel et al. 2003; Schwartz et al. 2001).

Severe P. falciparum infections cover a broad spectrum of complications, defined by the WHO as coma; generalized convulsions; cardio-vascular collapse; hemorrhagic syndrome; severe anemia (Hb<6 g/dl); pulmonary edema; hypoglycemia (<2.2 mmol/l); hypourexis (<400 ml/day); hemoglobinuria; and acidosis (PH <7.25). Of these, severe anemia, respiratory distress and cerebral malaria are the major grave outcomes of severe malaria (WHO, 2000 S1). In the absence of appropriate treatment, any of these complications could evolve rapidly and become fatal within hours or days.

Severe malaria anemia (SMA)

It is the most common clinical complication of severe P. falciparum malaria and the main cause of hospitalization of children in endemic areas. Severe anemia is characterized by arthralgia, paleness and low hemoglobin level <5 g/dl with parasite density > 10.000 p/µl (WHO, 2000 S1). This is probably a consequence of multiple causes such as erythrocyte hemolysis, suppressed synthesis of erythrocytes (Phillips et al. 1986) and increased spleen clearance for both uninfected and infected erythrocytes (Dondorp et al. 1999).

Respiratory distress (RD)

This outcome is possibly due to fluid retention and it is described by unusual or frequent deep breathing as a result of metabolic acidosis. This latter is enhanced by the low level of oxygen as a consequence of anemia and decreased microvascular blood flow arising from parasite sequestration and rosetting (Kaul et al.1991; Marsh et al. 1996).

Cerebral malaria (CM)

Patient with this form is characterized by last long unrousable coma accompanied with multiple seizures; as a result of blood brain barrier deterioration (Adams et al. 2002) and fever that may surpass 41°C. In the case of cerebral malaria, care should be taken to exclude other causes of coma, notably meningitis. This form is responsible for 15-20% of infant
mortality and is considered as the principle cause of long-term neurocognitive impairments in African children (Idro et al. 2010). In order to evaluate the state of consciousness of infected patient, the World Health Organization has adopted Glasgw coma scale (score between 6-7) for adult infected patients and Blantyre coma scale for children< 5 years of age. Blantyre score ranges from 0-5 and children with score ≤ 2 are diagnosed as cerebral malaria cases (Table 1, WHO 2000).

Table 1. A coma scale for children, Blantyre coma scale

<table>
<thead>
<tr>
<th>Type of stimulus</th>
<th>Observation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye movements</td>
<td>- Directed (e.g., follows mother’s face)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>- Not directed</td>
<td>0</td>
</tr>
<tr>
<td>Verbal response</td>
<td>- Appropriate cry</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>- response Moan or inappropriate cry</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>- None</td>
<td>0</td>
</tr>
<tr>
<td>Best motor response</td>
<td>- Localizes painful stimulus</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>- Withdraws limb from pain</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>- Non- specific or absent response</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>0 - 5</strong></td>
</tr>
</tbody>
</table>

The main goal of the treatment of severe malaria is to prevent death. In cerebral malaria cases, it aims to prevent neurological deficit.

In the case of cerebral malaria, parenteral full doses of any effective antimalarial drugs should be given as rapidly as possible. For adults and children artesunate IV or IM is indicated for a minimum of 24 hours. If it is not available, artemether or quinine is acceptable. After that, treatment should be completed with one of the following agents: ACT; artesunate plus clindamycin or doxycycline; or quinine plus clindamycin or doxycycline. A pre-referral treatment such as rectal artesunate, quinine IM or artesunate IM could be given, and then patient should be referred immediately to an appropriate health care for further treatment (WHO, 2010)
Pregnancy associated malaria (PAM)

In spite of the acquired immunity before pregnancy; pregnant women in malaria endemic zones are highly susceptible to malaria infections particularly during the first pregnancy where immunity decreases temporarily. During this period the placenta of pregnant woman offers a convenient environment for parasites with high affinity to placental receptors. *P. falciparum* parasites via VAR2CSA, the predominant parasite surface-expressed PfEMP1 protein in PAM, have an elevated capacity to adhere to chondroitin sulfate A (CSA) in the syncytiotrophoblasts of the placenta (Achur et al. 2008), provoking blood flow obstruction and disturbing the key source of nutrition for the fetus (Dorman et al. 2002). This phenomenon results in maternal anemia which itself has undesirable consequences as maternal death, premature delivery, spontaneous abortion, still birth and low birth weight (<2.500g) (Steketee et al. 2001).

The principal objective of treatment in pregnancy associated malaria case, is to save the life of the mother. During the first trimester, administration of quinine plus clindamycin for seven days has been indicated by the World Health Organization. In case of treatment failure, a combination of artesunate plus clindamycin for 7 days is indicated. During the second and third trimesters, sulfadoxine/pyrimethamine combination is recommended as a profylaxis. However, a treatment arteseminin combination therapy (ACT) is indicated.

The virulence of *P. falciparum* is partially due the capacity of IEs to cytoadher to endothelial cells of microvasculature of deep organs leading to obstruction of these vessels and organs disfunctions.

1.5. Cytoadherence property of *P. falciparum*

In order to pursue its erythrocyte life cycle, *P. falciparum* parasite shows an amazing ability to trick the host immune system and evade spleen-dependent clearance. One such phenomenon is called “cytoadherence” (Udeinya et al. 1981). It occurs once the erythrocytic parasites reach the mature stages (i.e. late-trphzoite and schizont), where they disappear from the peripheral blood and adhere to different venular endothelial cells of various organs including the brain (MacPherson et al. 1985; Pongponratn et al. 1991).

This sequestration of parasitized erythrocytes has sequels. It enhances the release of soluble mediators and activates the caspase pathways which in turn lead to vascular, neuronal and tissue apoptosis and blood brain barrier damage (BBB) in some cases of malaria (Pino et al. 2003; Wilson et al. 2008). In addition, the accumulation of infected erythrocytes results in microvascular clogging, ischemia, hypoxia, lactic acidosis and activation of inflammatory cytokines which increase endothelial cell adherence molecules (eCAM) and accelerate the adherence of additional populations of infected erythrocytes (van der Heyde et al. 2006).
However, the impact of sequestration on the host depends on host immunity, the affected tissues and parasite density.

During pregnancy-associated malaria the target of parasite sequestration is the placenta. Histological analysis of parasitized placenta showed inflammatory lesions with fibrinoid deposition leading to chronic intervillositis. This correlates with negative consequences such as spontaneous abortion, decreased intrauterine growth and increased fetal death (Rota et al. 2006).

In cerebral malaria the preferred niche of IEs is the brain microvasculature. There is mounting evidence that cytoadherence of IEs to brain microvascular endothelia is linked to cerebral malaria, which was confirmed by postmortem studies (Molyneux 1990). Analysis of brain tissues from 24 Thai patients who died of malaria revealed that the post capillary venules of the brain were packed with IEs compared to the other parts of the body including the lungs and the heart (Pongponratn et al. 1991). Similarly, another post mortem study of 21 Vietnamese individuals reported that accumulation of IEs within the microvascular endothelium of the brain was correlated with microvascular congestion and that was associated with premortem coma and shorter time to death (Ponsford et al. 2012).

The most important mediators of cytoadherence are variant surface antigens (VSA), mainly PfEMP-1 members of VAR family. These proteins are exposed at the surface of IEs allowing them to cling to various post capillary endothelial cells (Baruch et al. 1995).

The *P. falciparum* parasite has different ways to cytoadhere within the host. For that new terms have been coined to describe these mechanisms. The first one is known as “Rosetting”, where the infected erythrocytes bind spontaneously to uninfected erythrocytes and form rosettes resembling aggregates (Udomsangpetch et al. 1989). Rosettes in blood group A or B infected blood are larger and stronger that those in group O blood (Rowe et al. 2007; Udomsangpetch, 1993). Several lines of evidence have indicated that rosetting phenomenon is associated with severe malaria in sub-saharan Africa (Carlson et al. 1990; Treutiger et al. 1992), but not in South East Asia (Ho et al. 1991).

A comparative study of 79 Malian isolates from children with different clinical complications of severe malaria, has reported a contribution of rosetting in severe malaria pathogenesis compared to 91 patients with uncomplicated malaria (Doumbo et al. 2009). Similarly, another study of 111 isolates of Kenyan children with severe malaria and mild malaria found an increased capacity to form rosettes and giant rosettes among isolates from children with severe malaria compared to mild cases (Heddini et al. 2001). Horata et al identified a semi-conserved feature represented by a position of limited variability named (cyc/PolIV) that is located in DBLα of PfEMP-1 and associated with high rate of rosettes formation in both severe malaria and mild malaria groups (Horata et al. 2009).
The second term is “Clumping” where infected erythrocytes bind to platelets and form clumps (Pain et al. 2001). In vivo, this phenomenon could promote blood vessels occlusion and it is found to be associated with severe malaria (Mayor et al. 2011), particularly cerebral malaria since platelets express CD36 at their surfaces and induce the parasites binding even in absence of venular endothelial CD36 in the brain, thus they act as a bridge between brain endothelium and IEs. This mechanism of sequestration probably reorients the cytoadherence property of parasites and takes part in cerebral malaria pathogenesis (Wassmer et al. 2004). It has been suggested that thrombocytopenia could confer a protective mechanism for the host during cerebral malaria by decreasing platelet-mediated clumping of IEs (Wassmer et al. 2008).

Additional terms to express the distinct mechanisms of cytoadherence ability of infected erythrocyte comprise “binding to cells of immune system” such as plasma B cells, IgM, lymphocytes, monocytes, and dendritic cells (Donati et al. 2004; Ockenhouse et al. 1989; Urban et al. 1999)
1.5.1. Receptors contribute to cytoadherence of infected erythrocyte

A diverse array of endothelial receptors that are likely to participate in the adherence of IE to microvasculature endothelia has been identified. Several investigations attempted to describe a specific binding phenotype of isolates from severe malaria patients that is less frequent in uncomplicated malaria parasites. So far, no definitive candidate has been identified apart from chondroitin sulfate proteoglycan (CSPG) also known as CSA. It is believed that CSA is the main receptor of the VAR2CSA protein that is expressed at the surface of IE and can adhere to the syncytiotrophoblasts leading to placental sequestration of IEs during malaria in pregnant women (Fried and Duffy, 1996; Salanti et al. 2003).
Other potential receptors are: Thrombospondin (Roberts et al. 1985); Cluster Differentiation 36 (CD36) (Barnwell et al. 1989; Oquendo et al. 1989); Inter Cellular Adhesion Molecule-1 (ICAM-1) (Berendt et al. 1989); Vascular Cell Adhesion Molecule-1 (VCAM-1) (Ockenhouse et al. 1992); Complement Receptor-1 (CR-1) (Rowe et al. 1997); Platelet endothelial Cell Adhesion Molecule-1 (PECAM-1) (Treutiger et al. 1997); Integrin αvβ3 (Siano et al. 1998); P-selectin (Ho et al. 1998); Heparan sulfate (Vogt et al. 2003); Fractalkine (Hatabu et al. 2003); Fibronectin (Eda and Sherman. 2004); Neural Cell Adhesion Molecule (NCAM) (Pouvelle et al. 2007); gC1qR-HABP1-P32 (Biswas et al. 2007) and Endothelial Protein C Receptor (EPCR) (Turner L. et al. 2013). Most of these receptors have not been intensively explored and the role of *P. falciparum* ligands with each receptor in severe malaria is not well established.

Among these candidates, CD36 and ICAM-1 have been extensively investigated and were shown to be the most commonly used by parasite isolates (Udomsangpetch et al. 1996). However, our knowledge about the implication of these receptors in cytoadherence of IE comes from the human genetic polymorphisms associated with malaria severity as well as from cytoadherence assays under static or flow conditions by employing recombinant proteins coated on plastic dishes, cell line, receptor-bound beads or fluorescently labeled proteins.

### 1.5.2. Cluster Differentiation 36 (CD36)

This transmembrane glycoprotein (88-KD) is encoded by *cd36* gene on chromosome 7 and expressed on various cell types including phagocytes, microvascular endothelia; platelets; hepatocytes; retinal pigment epithelium; adipocytes and specialized epithelia of breast, kidney and gut. Thus, a broad spectrum of biological processes would be expected. It acts as anti-angiogenic factor; cellular receptor for thrombospondin, collagen, modified low-density lipoproteins and long-chain fatty. It is also involved in sensory perception (Silverstein and Febbraio 2009). The binding site in CD36 for *P. falciparum* ligands is located within 97-110 and 139-184 amino acids regions, as suggested by monoclonal antibodies and peptides against this protein (Baruch et al. 1999; Daviet et al. 1997). The parasite ligands for this receptor are PfEMP-1 variants (Baruch et al. 1996) encoded by group B and C of var genes through their N-terminal CIDR1α domain (Miller et al. 2002; Robinson et al. 2003).

CD36 is considered as the major host receptor for *P. falciparum* field isolates (Chaiyaroj et al. 1996; Newbold et al. 1997), though its pivotal role in malaria pathogenesis is still unclear. Whereas several laboratory assays did not note differences in CD36-binding patterns between field isolates from severe and mild malaria cases living in Africa (Heddini et al. 2001; Mayor et al. 2011; Rogerson et al. 1999), others have supported a correlation between uncomplicated malaria and binding to CD36 (Newbold et al. 1997; Ochola et al. 2011). Conversely, researchers in Asian endemic regions have shown that CD36 may play a part in malaria severity (Ho et al. 1991; Udomsangpetch et al. 1996) as it facilitates the binding of
high proportion of parasites throughout the body, promoting systemic infection that contributes to the severity of the disease.

The role of CD36 in the interaction between IEs and host immune system is elusive. While, adherence of IEs to macrophages via CD36 induces phagocytosis and enhances IEs clearance, (McGilvray et al. 2000), binding to dendritic cell (DC) via CD36 yields to DC dysfunction decreasing the adaptive immune response, thus exacerbating the infection (Urban et al. 2001). However, other finding suggests that IEs-binding to CD36 is not necessary to inhibit DC maturation; instead high parasite density can induce immunosuppression (Elliott et al. 2007).

It has been shown that CD36-binding is not involved in the rosetting process (Rowe et al. 2000); however, it is essential but not sufficient for the clumping phenomenon (Arman et al. 2013).

1.5.2.1. Role of cd36 gene polymorphism in malaria

Several studies suggested an important role of human gene polymorphism in the malaria severity but these investigations have reported contradictory conclusions.

In Thailand, a correlation between cd36 polymorphisms and protection against severe malaria has been detected. The frequencies of 14T to C allele in the upstream promoter region and 53G to T in the downstream promoter region of exon 8, were significantly higher in mild malaria than those of cerebral malaria cases, suggesting that such cd36 polymorphism may protect against cerebral malaria. Similarly, the frequency of repeat 12TG in intron 3 [in3 (TG) 12], which is linked with CD36 deficiency, was higher in uncomplicated malaria than in cerebral malaria cases. This polymorphism could alter the binding affinity of CD36 isoform to IEs, leading to decrease the risk of cerebral malaria (Omi et al. 2003).

In Africa however, researches led to confounding outcomes. Non-sense substitution mutation of T to G in cd36 gene at nucleic acid 188 of exon 10 leading to CD36 deficiency was found to bestow protection against severe malarial anemia (SMA), probably through decreased binding of IEs to microvascular endothelia (Chilongola et al. 2009; Pain et al. 2001). However, this finding contradicted with another study of African and Middle Eastern populations in which no correlation between CD36 deficiency and protection from severe malaria was observed (Fry et al. 2009).

These results concluded that the existence of distinct cd36 mutations in Africans and Asians populations might be related to alternative or additional selection pressures on cd36 and co-evolution between human host and parasite.

1.5.3. Inter Cellular Adhesion Molecule-1 (ICAM-1 CD54)

It is a transmembrane glycoprotein (90-115-KD) and a member of the immunoglobulin like superfamily. It is constituted of five immunoglobulin (Ig)-like domains. This protein is found as a dimer at the surface of cells and its N-terminal Ig-like domain displays binding sites for
fibrinogen, the Lymphocyte Function-associated Antigen-1 (LFA-1), cell-entrance site of human rhinovirus and *P. falciparum* PfEMP-1. This latter is situated on the BED side in the core of ICAM-1 dimer (Fig. 6) (Jun et al. 2001; Ockenhouse et al. 1992). Thus, ICAM-1 takes part in recognition and adherence between cells. The parasite ligands for this receptor are PfEMP-1s via DBLβ-C2 of group B, C and DBLβ3 of group A (Bengtsson et al. 2013; Howell et al. 2008; Oleinikov et al. 2009). ICAM-1 is expressed at a restricted level on several types of tissues such as leucocytes, vascular endothelial cells, mucous epithelia of the tonsils, hepatic sinusoids lining cells, spleen, thymus epitheliums, and some fibroblasts (Dustin et al. 2011). Upon pro-inflammatory cytokine stimulation e.g. TNF-α, IL-6 and INF-γ, ICAM-1 is up-regulated in various cell lineage including vascular endothelial cells, macrophages and leucocytes. The activated leucocytes bind to ICAM-1/LFA-1 and transmigrate in to the sites of inflammation (Caldenhoven et al. 1994).

Postmortem brain histopathology examination has reported high levels of ICAM-1 and co-localization with IEs in cerebral blood vessels (Turner G.D. et al. 1994). Furthermore, during malaria infection, ICAM-1 was found to be up-regulated on endothelial cells (Turner G.D. et al. 1998). However, *in vitro* cytoadherence assays led to contradictory outcomes about the implication of this receptor in malaria pathology. While several outcomes did not note any difference in binding pattern of ICAM-1 between parasites form severe and mild cases (Heddini et al. 2001; Rogerson et al. 1999), others pointed to an association between disease severity and binding to ICAM-1 (Newbold et al. 1997; Ochola et al. 2011).

However, it has been shown that clinical malaria isolates including from cerebral malaria, bound to CD36 10-fold higher than to ICAM-1; and 80% of ICAM-1-selected IEs bind to CD36 suggesting a crucial role of CD36 in IEs sequestration (Udomsangpetch et al. 1996). There is rising evidence that CD36 and ICAM-1 act in synergy to strengthen IEs sequestration in post capillaries endothelial cells (Heddini et al. 2001; McCormick et al. 1997; Yipp et al. 2000). ICAM-1 promotes rolling and static binding of infected erythrocytes whereas CD36 provides stronger stationary adherence, mentioning to possible complementary role between these receptors (Cooke et al. 1994).

### 1.5.3.1. Role of *icam-1* gene polymorphism in malaria

Polymorphism analyses of *icam-1* gene, found on chromosome 19, and its relation with malaria are inconclusive and contradictory. Fernandez-Reyes et al have identified a mutation in the N-terminal domain of ICAM-1 (known as ICAM-1*Kilifi*) with a gene frequency >30% associated with an increased risk of developing cerebral malaria in Kenya (Fernandez-Reyes et al. 1997). This polymorphism involves A to T substitution at position 179 resulting in a lysine to methionine replacement at position 29 in the BC-loop that interacts with rhinoviruses, lymphocytes, and infected erythrocytes. Similarly, Amodu et al found that the presence of the G allele at ICAM-1 exon 6 increase the risk of severe malaria in Nigerian children by 3.6 fold (Amodu et al. 2005). Conversely, three studies did not agree with those...
findings since no correlation between ICAM-1<sup>Kilifi</sup> polymorphism frequency and severe malaria has been detected (Bellamy et al. 1998; Fry et al. 2008; Ohashi et al. 2001). Surprisingly, a study in Gabon has shown that 55% of children with mild malaria were carriers to ICAM-1<sup>Kilifi</sup> whereas only 39% of those with severe malaria were carriers to this mutation and 52% of healthy children were ICAM-1<sup>Kilifi</sup> carriers, suggesting a protective role for this polymorphism (Kun et al. 1999).

Finally, mutagenesis analysis of ICAM-1 binding site for <i>P. falciparum</i> targeting alanine residues in the binding region resulted in 25 mutant proteins, e.g ICAM-1<sup>S22/A</sup> where alanine residue was replaced by serine at position 22 (Tse et al. 2004). In this study three different parasite lines (ItG, JDP8, A4) with different binding ability to wild type ICAM-1, (ICAM-1<sup>ref</sup>) were used to evaluate their adherence-capacities to a panel of mutant ICAM-1 proteins under flow and static conditions. The outcomes revealed that these parasite lines shared a similar binding region BED of ICAM-1, but they used different residues of ICAM-1 and showed variable binding phenotypes. This might be due to the variant PfEMP-1 expressed on the surface of each parasite line.

![The crystal structure of N-Terminal Ig-like domain of human ICAM-1 shown with fibrinogen, LFA-1 and <i>P. falciparum</i> binding sites. The binding site of Rhinovirus includes residues dispersed throughout Ig-like domain. The strands of the β-barrel are named from A to G. Some residues have been numbered for wild type ICAM-1. Source: (Tse et al. 2004).](image)

**Fig. 6:** The crystal structure of N-Terminal Ig-like domain of human ICAM-1 shown with fibrinogen, LFA-1 and <i>P. falciparum</i> binding sites. The binding site of Rhinovirus includes residues dispersed throughout Ig-like domain. The strands of the β-barrel are named from A to G. Some residues have been numbered for wild type ICAM-1. Source: (Tse et al. 2004).
1.5.4. Platelet endothelial Cell Adhesion Molecule-1 (PECAM-1 CD31)

Human PECAM-1 gene maps to chromosome 17, and the 130-kD protein is extensively expressed on monocytes, platelets and endothelial cells where it participates in endothelial cell-cell junction stabilization and mediates the transmigration of leucocytes into sites of inflammation (Gumina et al. 1996). It has been demonstrated that 54% of Kenyan isolates of cerebral malaria patients bound to PECAM-1 (Heddini et al. 2001), but in another study no association of this binding with severe malaria had been found (Newbold et al. 1997). The parasite ligand is PfEMP-1 through its CIDRα and DBL2δ domains (Chen et al. 2000), which bind to the N-terminal segment of Ig-like domains (the first four domains) (Treutiger et al. 1997).

Among the four existing polymorphisms at loci 125 and 563 [125 valine (V)-563 asparagine (N); 125V-563 serine (S); 125 leucine (L)-563N; and 125L-563S], The frequency Valine to asparagine (125 V/V-563 N/N) in PECAM-1 was found to raise the risk of cerebral malaria in Thailand (Kikuchi et al. 2001), and Leucine to Valine polymorphism at codon 125 was not found to confer protection against severe malaria in Kenya or Papua New Guinea (Casals-Pascual et al. 2001).

1.5.5. Complement Receptor-1 (CR1)

Complement regulatory protein-1 (160-250kD) is expressed on infected erythrocytes, leucocytes and dendritic cells (Khera and Das 2009). In vivo, CR1 binds to complement components such as C3b and C4b resulting in several essential functions such as phagocytosis and elimination of immune complexes from the blood circulation (Ahearn and Fearon 1989). CR1 has been defined as rosetting-mediating receptor because in the absence of CR1, Infected erythrocytes were unable to form rosettes (Rowe et al. 1997). Moreover, monoclonal antibodies against this receptor inhibited rosettes formation (Rowe et al. 2000). The parasite ligand is PfEMP-1 through its DBLα and the binding site is C3b of CR1 (Rowe et al. 2000). CR1 gene is harbored on chromosome 1 and it has been shown that Thai individuals with T-T genotype in promoter region are protected from cerebral malaria by enhancing the expression of erythrocyte-CR1 which in turn increases the clearance of immune complexes (Teeranaipong et al. 2008). By contrast, CR1 deficiency of human erythrocyte decreased the rosetting in Papua New Guinea and protected against severe malaria (Cockburn et al. 2004).

1.5.6. Endothelial Protein C Receptor (EPCR)

EPCR has recently been reported as a new mediator of infected erythrocytes adherence to microvascular endothelial cells via CDRα1 of domain cassette 8 and DC13 of PfEMP-1. It is encoded by proc gene on chromosome 20 and expressed primarily on arteries and veins endothelia and at low level on brain capillaries endothelia (Simmonds and Lane 1999). This
receptor (46-kD) provides anti-inflammatory and cytoprotective effects to endothelial cells by the assistance of activated protein C (APC)-EPCR signaling pathway. Further, antithrombotic activity and conservation of vascular integrity are also promoted by the same pathway supported by this protein (Mosnier et al. 2007). However, disruption of EPCR expression in the brain has been observed in cerebral malaria cases which gave rise to localized microvascular thrombosis and inflammation at site of sequestration (Moxon et al. 2013). Interestingly, it has been shown that PfEMP1 binds to EPCR near or at the same region of activated protein C leading to abortion of cytoprotective effects mediated by APC-EPCR on endothelial cells (Turner L. 2013). These findings are shedding the light on a novel therapeutic strategy.

1.6. *Plasmodium falciparum* genome

1.6.1. Composition and organization of genome

The nuclear genome of *P. falciparum* is haploid with 23 mega bases (Mb) dispersed throughout 14 chromosomes that vary in size from 0.643-3.29 Mb. Up to now, 5542 protein-coding genes have been identified. The average length of intonless genes is 2.3 Kb, with introns of an average size of 950 bp found in 54% of the genes (Gardner et al. 2002). This genome is distinct from the other eukaryotes as it rich in adenine (A) and thymine (T) bases that represent 80.6% of parasite genome. This confers instability to the genome and makes DNA arduous to handle. The genome holds 43 transfer RNAs (tRNA) and a few genes of ribosomal RNA (rRNA) that are classified according to the expression stage of parasite life cycle. Type A rRNAs are mainly expressed in human host whereas type S rRNAs are preferentially expressed in the *Anopheles* host (Waters 1994).

Apart from its nuclear genome, *P. falciparum* parasite has a small mitochondrial (6-kb) and an apicoplast (35-kb) genome. The latter is indispensable for parasite existence, since this organelle contributes to fatty acids, isoprenoids and haeme metabolism. In addition, about ten percent of proteins encoded by nuclear genes are targeted to the apicoplast (Gardner et al. 2002).

1.6.2. Chromosome structure

One of the properties of *P. falciparum* genome is the complexity of sub-telomeric regions that are composed of 5 blocks (SB 1-5), the localization and the composition of these blocks promotes mitotic recombination between telomers (Gardner et al. 2002). The parasite chromosomes carry conserved and polymorphic domains. The conserved domains are located within the centromeric regions where the house-keeping genes are dominant. The polymorphic domains are situated in subtelomeric blocks, rich in adenine and thymine and so likely to play a role in recombination, gene conversion between telomere proximal genes and chromosome pairing (de Bruin et al. 1994).
At least three variant gene families (rif, stevor, var) are implicated in the antigenic variation phenomenon. They are preferentially found in the sub-telomeric regions and separated from the telomers by many conserved tandem repeats from 1-6 namely telomere-associated repetitive elements (TARE) each with 21 base pair (Repetitives- 20) (Oquendo et al. 1986) (Fig. 9).

Sixty percent of P. falciparum genes code for hypothetical proteins with unknown function. A considerable proportion of products with specific function are proteins dedicated for antigenic variation followed by restricted repertoire of membrane transporters and metabolic enzymes (Gardner et al. 2002).

1.7. Variant surface antigens (VSA)

The spectacular ability of P. falciparum parasite to fool the host immune system, thus to ensure a chronic infection is associated with a set of variant surface antigens. VSA is a term used to describe variable surface antigens that are encoded by multigenic families and expressed at the surface of infected erythrocyte. These VSA are implicated in antigenic variation as well as in cytoadherence of IEs to host cells that lead to severe malaria under certain circumstances.

However, in malaria endemic regions, immunity to non-cerebral severe malaria is acquired after few malarial episodes (Gupta et al. 1999) and it appears to be directed against P. falciparum VSA. Indeed, parasites associated with severe malaria in non-immune young children, expressed limited and conserved subset of VSA which are recognized by IgG from adults more than the VSA expressed by parasites causing mild or asymptomatic infections (Nielsen et al. 2002).

So far, five families of VSA have been identified var (~60), rif (~150), stevor (~30), Pfmc2TM (~11) and surfin (~10) (Baruch et al. 1995; Blythe et al. 2009; Kyes et al. 1999; Lavazec et al. 2006; Winter et al. 2005) each with variable members of genes, predominantly localized in subtelomeric sites, the chromosomal regions subjected to gene recombination (Hernandez-Rivas et al. 1996; Freitas-Junior et al. 2000). Furthermore, var genes family is the best-characterized VSA member and is found to play a crucial role in malaria pathogenesis.

1.7.1. STEVOR and RIFIN

Although these two hyper variable families share sequence homology, stevor genes seem to be more conserved than rifin ones (Gardner et al. 1998). The 3D7 genome holds approximately 150 rif genes; most of which are organized head to head with var genes, and about 30 stevor genes. The majority of stevor genes are localized at the chromosome ends; contrary to var and rif which can be found in the center of parasite chromosomes (Gardner et al. 2002).

The proteins encoded by these genes, RIFIN and STEVOR, are small polypeptides ranging from 25 to 45 kD and characterized by putative signal sequence, followed by a semi-
conserved domain, an hyper variable region (HVR) and a conserved C-terminal domain (Cheng et al. 1998). These proteins are exported to the surface of infected erythrocytes (Blythe et al. 2008; Kyes et al. 2001) and display highly polymorphic amino acid sequences in *P. falciparum* isolates (Albrecht et al. 2006; Blythe et al. 2008), suggesting a potential implication in antigenic variation and hence in evading the defense mechanism (Fernandez et al. 1999; Niang et al. 2009).

The RIFIN family members have been classified into two sub-groups, type-A and type-B which further could be divided into B1, B2, B3 clusters and can be transcribed simultaneously with type-A (Joannin et al. 2008; Petter et al. 2007). Type-A RIFINs are larger than those of type-B due to the presence of 25 amino acids motif within the semi-conserved region (C1) of type-A. Indeed, this motif is situated at approximately 66-amino acids downstream of *Plasmodium* export element motif (PEXEL), but it is not found in B-type RIFINs (Fig. 7). Moreover, while type-A group contains 10 conserved cysteine residues, only 6 of them are located in type-B RIFINs (Joannin et al. 2008). Furthermore, solely type-A RIFINs are trafficked to the surface of parasitized erythrocytes via Maurer’s clefts whereas type-B RIFINs are retained within the parasites (Petter et al. 2007).

A comparison analysis of transcript abundance of *rif* and *var* using *P. falciparum* 3D7 and NF54 clones showed a distinct *rif* transcript pattern during gametocytes and sporozoites stages comparing to *rif* and *var* expression profiles during the asexual stages. In addition, a single variant of B1-type RIFIN dominated the sexual stages of *P. falciparum* 3D7 and NF54 suggesting an important role of this gene in the adherence during this stage and shedding the light on this protein as a potential transmission blocking vaccine candidate (Wang et al. 2010).

As RIFIN proteins, STEVOR antigens display two transmembrane domains; one of which is hypothetical (Fig. 7). Antibodies directed against STEVORS found that these proteins are expressed at the surface of the IE at schizogony, resulting in antigenic variability during this stage (Niang et al. 2009). These antigens are found in the rhoptries and at the surface of merozoites as well as among released component in the invading merozoites, suggesting a potential role during invasion and probably in immune evasion in the erythrocyte invasion process (Khattab et al. 2008).

Transcription profiles of *rif*-A, *rif*-B and *stevor* at different stages of parasite life cycle in the clinical isolates cultivated *in vitro* compared to 3D7 clone had been investigated by real time PCR (Bachmann et al. 2012. The results revealed two peaks of transcription of *rif*-A and *stevor* during the ring and trophozoite stages. In addition, clinical isolates showed tremendous transcript abundance of these variant surface antigens comparing with 3D7. Immunoflorescence analysis of these isolates detected the localization of RIFIN and STEVOR at young trophozoites, shizonts and merozoites. The findings led to propose a complex model of the expression of these polymorphic families throughout the asexual life cycle of
parasite which might be in relation with clinical outcomes of infection (Bachmann et al. 2012).

Fig. 7: Schematic organization of STEVOR, type-A and type-B RIFIN proteins. (Indel): insertion/deletion of 25 aminoacid consensus located in typ-A RIFIN, but not in type-B; (SP?): Potential signal; (TM): transmembrane domain; (V, V1, V2) are variable regions and (C1 and C2) are conserved regions. Source: (Joannin et al. 2011).

1.7.2. Var genes and PfEMP-1

The proteins encoded by the hyper variable var genes family are Plasmodium falciparum erythrocyte membrane proteins 1 (PfEMP-1) that range in size from 200-400 kDa (Baruch et al. 1995; Smith et al. 1995; Su et al. 1995). These antigens are trafficked to the surface of infected erythrocyte where they are anchored into electron dense protrusions, namely knobs (Igarashi et al. 1987) with the assistance of other proteins, mainly Knob Associated Histidine Rich Proteins (KAHRP) (Pologe et al. 1987). These antigens play a role of mediator between IEs and endothelial receptors, leading to sequestration of IEs throughout the body organs and bringing about severe complications. PfEMP-1 proteins are major targets of host defense. Pre-exposure to anti PfEMP-1 antibodies promotes selection and expression of specific antigens during a novel infection (Bull et al. 1998). Each individual parasite harbors ~ 60 copies of var genes dispersed throughout 14 chromosomes with a majority localized in the subtelomeric regions and the rest in the center of chromosomes. All these genes consist of two exons separated by a conserved intron. The larger one, exon I (3.5-9 kb) encodes the polymorphic extracellular region implicated in cytoadherence phenomenon which includes N-terminal segment (NTS); multiple domains of duffy binding like domain (DBL), one or two cysteine rich inter domain regions (CIDR) and a transmembrane domain (TM), while exon II (1-1.3 kb) codes for a semi-conserved cytoplasmic tail with an acidic terminal segment (ATS) (Fig. 8) (Gardner et al. 2002; Su et al. 1995).
Fig. 8: General characteristics of var genes. All members of var gene family consist of two exons separated by conserved intron. The variable extra cellular domain encoded by exon 1 and involves: N-terminal segment (NTS); Duffy binding like domains (DBL); cysteine rich inter domain regions (CIDR) and transmembrane domain (TM). The intracellular domain (exon 2) encodes a semi conserved amino acid terminal segment (ATS) Source: Modified from (Sherf, 2008).

Based on sequence homology of coding and non coding regions, chromosomal location, direction of transcription and domain structure of encoded proteins, var genes have been categorized into three major groups A, B and C, three conserved groups var1, var2 and var3, and intermediate groups B/A & B/C (Kraemer and Smith 2003; Lavstsen et al. 2003). All group A var genes (10 genes) possess ups A flanking sequence, situated in the sub telomeric sites and transcribed toward the telomere in the opposite direction of group B var genes (22 genes) which have a ups B sequence and are found near the telomere and their transcription is headed to the centromere. Group C var genes (13 genes) are flanked by ups C, located near the centromere and oriented toward the telomere. The intermediate groups B/A (4 genes) and B/C (10 genes) have upstream B sequence followed by the coding regions of group A or C respectively. Var1 ups D and var2csa ups E are highly conserved groups each with a unique sub telomeric gene; var3 is a conserved subfamily of group A with three small genes (Fig. 9) (Lavstsen et al. 2003).
Fig. 9: Genomic organization and nuclear position of *P. falciparum* var genes.

Global organization of subtelomeric regions of the 14 linear chromosomes shows that they all share non-coding repeats of various sizes called *TARE* 1–6 (telomere associated repeat elements) situated beside telomere repeats. Subtelomeric site is followed by a member of the group B *var* genes, usually accompanied by another group A *var* genes transcribed in the opposite direction. Group C *var* genes are clustering in the chromosome center and *rif* genes are often interspersed with *var* genes.

Source: Modified from (Scherf et al. 2008).

The extra cellular parts of PfEMP-1 proteins are composed of multiple Duffy binding-like domains (DBL) and one or two cysteine rich inter domain regions (CIDR), each of 300–650 amino acids. Each copy of *var* gene has its own length according to the number and types of domains including in its sequence (3.9–13 kb). However, four proteins, VAR2CSA and VAR3 PfEMP-1 members, do not belong CIDR domains (Rask et al. 2010; Smith et al. 2000) (Fig. 10).

Based on sequence similarity, DBL domains are categorized into DBL α, β, γ, ε, δ,ζ and five smaller distinct classes (four DBL domains of VAR2CSA and a DBL α/ζ of VAR3) (Rask et al. 2010; Smith et al. 2000). CIDR domains are classified into five distinct domains α, β, γ, δ, and pam (Rask et al. 2010; Smith et al. 2000). These subclasses can be further sub categorized into 147 subtypes (e.g. DBLα 1.1). In general, the subtypes of these domains are located in the N-terminal part of PfEMP-1 (e.g. DBLαCIDRαDBLβ/DBLY) and they are associated with ups of *var* groups, which is not the case of domains associated with C-terminal part (e.g. DBLδCIDRβ/YDBLεDBLζ) (Rask et al. 2010).
It has previously been shown that group A and B of var genes are differentially transcribed in severe malaria isolates, including cerebral malaria, as compared to parasites from uncomplicated malaria (UM) or asymptomatic infections (Jensen et al. 2004; Kyriacou et al. 2006; Rottmann et al. 2006). Moreover, group A var genes code for large and complex multi domains PfEMP-1, in agreement with studies that found that parasite isolated from patients with cerebral malaria express high molecular weight PfEMP-1 as compared to those from
non severe malaria isolates (Bian et al. 1999). Recently, it has been shown that the high level of group A var genes is correlated with severe malaria and rosetting phenotype in African children (Warimwe et al. 2012). This latter was found to be associated with respiratory distress while non-rosetting group A PfEMP-1 isolates was associated with impaired consciousness, thus the clinical outcomes of malaria infection are affected by specific group A var gene expressed by infecting parasites.

A remarkable up-regulation of var2csa by P. falciparum parasite isolates selected in vitro for adherence to CSA receptor was shown using real time PCR (Salanti et al. 2003). This unique gene codes for VAR2CSA protein, expressed at the surface of parasitized erythrocyte infected placenta (Magistrado et al. 2008). Currently, VAR2CSA is considered as the major ligand for parasite adherence to placenta intervillous space of pregnant women, and high plasma level of antibodies directed against VAR2CSA in pregnant women found to decrease the risk of low birth weight at delivery (Salanti et al. 2004).

A recent study employed alignment and distance tree analysis of 399 known domains of PfEMP-1 from seven genomes has identified 628 conserved minimal homology blocks describing most of PfEMP-1 sequences. In addition, 23 conserved domains, namely domain cassettes have been categorized (Rask et al. 2010). Some of these domains have been described previously such as DC1 for var1 (Salanti et al. 2002; Salanti et al. 2003), DC2 for var2csa (Salanti et al. 2003), and DC3 for var3 (Trimnell et al. 2006). A domain cassette is identified as a tandem of 2 to 4 domains of DBL and CIDR semi-conserved involved in many var genes and are located exclusively in N or C-terminal parts of PfEMP-1 (Rask et al. 2010). Var genes encoding domain cassette 8 (DC8) and domain cassette 13 (DC13) are of clinical significance. DC8 involves genes with upstream B followed by four domains NTSβDBLa2CIDRa1.1DBLβ12DBLγ4/6, whereas DC13 found in group A var genes and it is characterized by the tandem domains of DBLa1.7CIDRa1.4 (Fig. 11). The transcript abundance of these domain cassettes found to be higher for all severe malaria forms including cerebral malaria than mild malaria (Bertin et al. 2013; Lavstsen et al. 2012). Moreover, Tanzanian children revealed serological recognition for recombinant PfEMP-1 domains belonging to DC8 similar to group A domains and higher than group B and C domains (Lavstsen et al. 2012).
It has been demonstrated that *P. falciparum* parasites selected for binding to human brain microvascular endothelial cells (HBMEC) showed significant expression levels of PfEMP-1 DC8 genes (Claessens et al. 2012). In addition, plasma from convalescent Kenyan children exhibited higher antibody recognition profiles to HBMEC-selected parasites than those of unselected ones (Avril et al. 2012), suggesting the importance of these PfEMP-1 motifs in building protecting immunity to malaria.

PfEMP-1 proteins are seen as the major determinants in cytoadherence affinity of IEs to different receptors and that the binding sites of some receptors have been mapped to specific DBL and CIDR domains of PfEMP-1 (Fig. 12) (Baruch et al. 1997; Chen et al. 2000; Joergensen et al. 2010).

The head structure of CIDR1α domain of group B and C PfEMP-1 demonstrates a great ability to bind multiple receptors including CD36; PECAM-1 (along with DBL2δ) (Chen et al. 2000; Janes, 2011; Robinson et al. 2003).

Likewise, DBL domains show wide spectrum of binding capacity to different receptors such as heparin sulfate (HS) and blood group A antigen. They also mediate the adherence of infected erythrocytes to CR1 of uninfected erythrocytes through NTS-DBLα-CIDRY of varO (group A PfEMP-1) resulting in rosettes formation (Rowe et al. 1997; Vigan-Womas et al. 2012).

The infected erythrocytes bind to ICAM-1 through DBLβ-C2 domains of group B and C PfEMP-1 and the DBLβ3 of group A PfEMP-1 (Bengtsson et al. 2013; Howell et al. 2008; Oleinikov et al. 2009).

The tight regulation of PfEMP-1s expression endows the parasite a high degree of fitness. PfEMP-1s are employed in several biological processes allowing the parasite to modulate the
host immune response and to set a long lasting infection to ensure its transmission and survival. For instance, PfEMP-1 binds to dendritic cells, and inhibits their maturation resulting in decreased ability to induce T cell response, thus, decreasing the immune defense (Urban et al. 2001).

PfEMP-1 via its CIDR1α domain can also bind and induce B-lymphocytes of naïve donors. This interaction elicits the multiplication, augments the size of B lymphocytes and stimulates the secretion of non-immune IgM and cytokines (TNFa & IL-6). In addition, CIDR1α binds to Fab and FC fragments of human Ig (Donati et al. 2004).

Recently, a new mechanism used by parasites from pregnant women to by-pass the recognition of host’s antibodies has been described. The parasites employ non-functional epitopes of VAR2CSA to bind non-immune IgM which would mask PfEMP-1-specific IgG epitopes and confer a protection to IE from phagocytosis without affecting the binding of VAR2CSA to CSA in placenta (Barfod et al. 2011).

**Fig. 12:** Different binding phenotypes of var gene groups are potentially correlated with specific clinical outcomes. Source: (Kraemer and Smith 2006).

It has previously been shown that of the hyper variable repertoires of var genes, trophozoite parasite chooses single PfEMP-1 to be transcribed and expressed on the surface of infected erythrocytes at each life cycle (Dzikowski et al. 2006; Voss et al. 2006). However, these
observations have been contradicted with recent results which reported an ability of *P. falciparum* parasite to co-express two different PfEMP-1 antigens at the surface of IE (Joergensen et al. 2010).

### 1.8. Mutually exclusive expression and switching properties

The mutually exclusive transcription of *P. falciparum var* genes and the ability to switch between members are essential properties for the parasite life in order to avoid the host immune defense and to ensure chronic infection. The underlying strategies contributing to these mechanisms have not been clearly illustrated. However, several studies aimed to test the implication of epigenetic factors or specific genetic elements that may control monoallelic expression.

#### 1.8.1. Mutually exclusive expression property

Mutually exclusive expression of single *var* gene and keeping the rest of family members in a transcriptionally silent state is thought to be epigenetically controlled and it is linked to histone modification (Chookajorn et al. 2007; Lopez-Rubio et al. 2007). During the ring stage of intraerythrocyte life cycle, the 5' upstream of the actively transcribed *var* gene is extensively supplemented with di and trimethylation histone 3 lysine4 (H3K4me2 and H3K4me3) (Lopez-Rubio et al. 2007) and with acetylated trimethylation histone3 lysine 9 (H3K9me3ac) (Chookajorn et al. 2007). At the late stage of parasite life this gene loses its richness in H3K4me3 and H3K9me3ac and becomes temporarily silent, however it keeps H3K4me2 probably to transmit memory of the active gene to the following asexual life cycles. Conversely, the 5' flanking region of stably silent *var* genes are enriching with trimethylation histone3 lysine 9 (H3K9me3), which is highly associated with gene silencing by heterochromatin in the other eukaryotes. As yet, the shift of a silenced *var* gene from heterochromatin to a euchromatin nuclear region has not been clarified. However, these results suggest a competition between the two processes methylation and acetylation of H3K9 at the 5' upstream which affects the transcription activity and shed some light on the important role of 5' flanking region (Chookajorn et al. 2007; Lopez-Rubio et al. 2007). Another gene activation marker of the euchromatin is the alternative histone PfH2A.E, which is dynamically enriched in active *var* genes promoters during the ring stage of asexual life cycle, but it is not found in silent *var* genes promoters referring to its implication in the transcriptional activity of *var* genes (Petter et al. 2011).

Sterile transcript mediated by the conserved *var* gene intron between exon 1 and exon 2 is presumably involved in the regulatory mechanism of transcription. It has been shown that *var* gene silencing requires an interaction between *var* intron, which has promoter activity and another promoter within the upstream of coding region (Calderwood et al. 2003). Furthermore, each intron is able to repress only a single promoter, thus only one *var* gene is silenced without influencing the transcriptional mechanism of adjacent *var* promoters. This finding suggests that one-to-one pairing is indispensable for silencing and that mechanism of
var gene silencing is controlled for each individual gene instead of whole silent chromatin over a chromosomal region (Frank et al. 2006). It has been reported that during the late stage of intraerythrocyte life cycle, the non coding RNA (ncRNA) transcripts which initiated from a bi-directional promoter inside the conserved intron of var gene are physically associated with chromatin at var loci and they may act as transcription silencer (Epp et al. 2009).

1.8.2. Switching property of *Plasmodium falciparum*

The dynamic of antigenic variation known as switching rate, is one of unanswered questions about *P. falciparum* life and properties. Whereas in vitro investigation in the lack of immune pressure found that the parasites have the capacity to switch the expression of var genes immediately at a rate of 2% (Roberts et al. 1992), switching in human malaria parasites from infected volunteers showed that only one var gene type transcribed predominantly in different isolates with high switching rate estimated around 16% per generation (Peters et al. 2002). The data suggested that var gene switching property is due to a “reset” pattern i.e the same var gene is re-expressed as merozoites left the liver. In addition, switching from the first var gene to another might take place quickly after leaving hepatocytes, but the subsequent change-overs are carried out at slower rate.

Another switching model based on in vitro cultures where clones of parasites with selected specific cytoadherence pattern were used to measure “on rate” (switching to active state) and “off rate” (switching to inactive state). Results indicated that each var gene has its own on and off switching rate “ordered switch model” contrarily to “random switch model” which supposes that all var genes have the same switch rate. The outcomes showed that switching is not different between subtelomeric and central var genes (Horrocks et al. 2004).

1.9. Potential vaccine targets

*P. falciparum* undergoes a fascinating and complicated life cycle. The parasites spend most of their life inside human host cells and they are extracellular solely for a short period of time as sporozoites, merozoites. For each stage of development, only a small proportion of parasites are required to persist and to complete the parasite life cycle. In addition, at every step of life, *P. falciparum* is able to express a set of antigens that are principle contributors to host-immune escape. Up to date, a *P. falciparum* vaccine remains elusive. However, through the last decade; several trials have been attempted to interrupt the life cycle of the parasite by targeting multiple antigens.

The ultimate aim of our study is to find out *P. falciparum* genetic factors implicated in the pathogenesis of severe malaria, especially cerebral malaria. The proteins encoded by these genes could be interesting vaccine candidates. A suitable vaccine should be cost-effective and reduce the risk of antimalarial drug resistance developing. This vaccine candidate should
confer protection or at least prevent the progression towards the severe form of malaria; consequently the rate of mortality among African children due to severe malaria would be reduced.

The known vaccine candidates could be divided into three groups.

1) Pre erythrocytic candidates

- CircumSporozoite protein (CS): is considered as the principal antigen expressed at the sporozoite stage and it is necessary for parasite development. Region II plus in this protein plays a key role in sporozoite motility and hepatocyte invasion where it binds to heparin sulfate proteoglycan (Agnandji et al. 2011; Aly et al. 2009; Cerami et al. 1992). Recently, this protein has been targeted by the RTS,S/AS01 vaccine. Preliminary outcomes of phase III trial in African children found that the vaccinees showed approximately 50% less clinical malaria episodes for the first year. Conclusive results are expected later in 2014 (Agnandji et al. 2011).

- Thrombospondin Related Anonymous Protein (TRAP) of P. falciparum is located within the micronemes and on the surface of sporozoites. It holds an amino acid sequence that provides sporozoites with the ability to recognize and invade host hepatocytes (Muller et al. 1993).

- Liver Stage Antigen1 (LSA1) is a P. falciparum protein specifically expressed at liver stage. It is indispensable for exoerythrocytic schizogony. Indeed, without this antigen, schizontes lose their ability to differentiate into merozoites and to express the apical membrane antigen1 (AMA1) which is a crucial protein involved in erythrocyte invasion (Mikolajczak et al. 2011). The first Phase I/II trial for LSA-NRC recombinant protein with aluminum hydroxide adjuvant, reported good safety profile, robust antibody-antigen specificity and CD4+ T-cell responses. However, it could not confer protective immunity against experimental malaria challenge in the tested naive volunteers (Cummings et al. 2010).

2) Blood stage targets

- The Glutamate-Rich Protein (GLURP) of P. falciparum is expressed in both liver and erythrocytic stages. Naive participants have been inoculated with long synthetic peptide LSP-GLURP85-213 formulated with aluminum hydroxide or montandine ISA720 for Phase I vaccine trial. The peptide was able to induce a specific antibody response and cytophilic IgG1 antibodies against GLURP 85-213 which could inhibit parasite growth in vitro (Hermsen et al. 2007).

- The Merozoite Surface Protein1 (MSP1) is one of blood stage parasite proteins that are required for erythrocyte invasion. A vaccine based on the 42kDa C-terminal fragment of MSP1 (known as falciparum malaria protein1, FMP1/AS02) was administered to healthy Kenyan children for Phase IIb clinical trial. Although it was well-tolerated and showed a
favorable safety profile in those infants, FMP1 was not an efficient vaccine because it did not provide a protection against first falciparum malaria episode and was not able to decrease the parasitemia (Ogutu et al. 2009).

- Merozoite Surface Protein2 (MSP2): A combination B vaccine containing part of the 3D7 MSP1, MSP2 and the ring infected erythrocyte surface antigen (RESA) combined with montandine ISA720, has been evaluated for Phase I-Ilb clinical trial. The product decreased the parasitemia by 62% in children living in Papua New Guinea, but it was not efficient to reduce the frequency of malaria episodes (Genton et al. 2002).

- Merozoite Surface Protein3 (MSP3): A long synthetic peptide containing the conserved region of MSP3 (MSP3-LSP) adjuvanted with montandine ISA720 or aluminum hydroxide, had been produced to immunize healthy volunteers for Phase I trial. MSP3-LSP was well tolerated and without severe side effects. It was also capable to trigger a strong and a specific immune response including T-cells, INF-γ and cytophilic IgG1 (Audran et al. 2005; Druilhe et al. 2005).

- GMZ: is a fusion protein of MSP3-GLURP with aluminum hydroxide was produced for Phase Ia clinical trial. It demonstrated a capacity to elicit a specific antibody-antigen reaction and to induce memory B cells which were recognized during the 12 months follow-up of the study (Esen et al. 2009).

- The Apical membrane protein1 (AMA1) is located at P. falciparum surface and expressed during the asexual blood stage of parasite life. Phase II study was conducted in healthy Malian children to investigate the immunogenicity and the safety of AMA1-Combination 1 (C1) comprising two recombinant allelic proteins (AMA-1FVO and AMA-13D7) with Alhydrogel® adjuvant. Results showed that in spite of the desirable safety profile of this combination, it was moderately immunogenic and had no influence on the level of parasitemia (Sagara et al. 2009).

- The Erythrocyte binding antigen 175 (EBA175) is a P. falciparum conserved protein that mediates the parasites binding to the host’s erythrocytes. A recombinant EBA175 vaccine combined with aluminum phosphate was inoculated into malaria naïve adults from the United States. This vaccine was safe and well tolerated. It was capable to elicit antibody response and to inhibit parasite growth in vitro (El Sahly et al. 2010).

- The Reticulocyte binding protein homologue 5 (Rh5) is a special member of the reticulocyte binding homologue family (PfRh) which is involved in erythrocyte invasion and adherence to infected erythrocyte surface. This antigen is distinct from the other members as it is smaller and has no C-terminal transmembrane domain. It is located in the rhoptries of merozoites and associated to the tight junction of the parasite, suggesting a crucial role in the penetration of merozoites into the infected erythrocyte (Baum et al. 2009).
*P. falciparum* merozoite has multiple alternative pathways to invade the infected erythrocyte, which hampers the researches to define and develop an adequate invasion-blocking vaccine targets (Baum et al. 2005).

- Serine repeat antigen 5 (SERA5) is an asexual intra erythrocytic stage protein expressed in the mature parasites i.e. late trophozoites and schizonts and released into the lumen of parasitophorous vacule. Phase la clinical trial evaluated the capacity of SE36/AHG, which is a recombinant molecule of SERA5 with aluminum hydroxide gel, in healthy adults. It showed a favorable safety profile and highly sero conversion against SE36 with increasing age, but it did not induce an antibody response during the primary malaria infections (Horii et al. 2010).

- Other set of asexual intra erythrocytic stage targets:

  Members of variant surface antigen (VSA) families which are highly polymorphic and include: VAR (PfEMP-1), STEVOR, RIFIN, SURFIN and PFMC2TM. Of these protein families VAR, RIFIN and STEVOR families have been investigated. The majority of these proteins are expressed and transported to the surface of infected erythrocytes. Members of VSA were found to mediate the cytoadherence of IEs to endothelial host receptors throughout the body. Therefore these antigens are potential vaccine candidates against malaria pathogenesis.

  The most well studied member of the PfEMP-1 as a vaccine candidate is VAR2CSA. This protein is considered as a major antigen in parasites infecting pregnant women, conferring the parasites a high tropism towards syncytiotrophoblasts of the placenta. The sequestration of IEs in the placenta is at the origin of severe clinical effects on mothers and children (Salanti et al. 2004). It has been shown that multigravidae women acquire adherence-blocking-antibodies against VAR2CSA which prevent from harmful consequences of Pregnancy Associated Malaria (PAM) supporting the idea of a vaccine targeting VAR2CSA protein (Salanti et al. 2004). Several domains combinations of VAR2CSA with suitable adjuvants have been used to immunize animals and to test the binding inhibitory efficacy of elicited IgG. These domains include DBL4ε-ID4, NTS-DBL1X, NTS-DBL1X-Id1, Id1, Id1-DBL2X and DBL2X which were able to trigger high antibodies responses with similar efficacy as pooled plasma from immune multigravidae African women (Bordbar et al. 2012; Magistrado et al. 2011; Pinto et al. 2012). These antigens will be tested for their safety and efficacy in vaccine trials against PAM in the next two years.

  The vaccine that we seek should be able to induce a specific antibody response against specific PfEMP-1 types exported at the surface of IE that would prevent the cytoadherence of IEs to microvascular endothelial receptors in the deep organs such as the brain. As a consequence, the non-adhered IEs would circulate in the peripheral blood where they would be filtered out by the spleen. Thus, this vaccine could be very efficient to hinder the progression to complicated forms of malaria and reduce the risk of developing cerebral malaria.
3) Sexual stages targets or Transmission Blocking Vaccine (TBV)

- **Pfs25**: is a 25 kDa protein located at the surface of zygotes and ookinetes. Because Pfs25 is expressed solely during the parasites life in the mosquito midgut, no immunity against this protein can be acquired naturally (Kaslow et al. 1989). However, Pfs25 with montadin ISA51 (Pfs25/ISA51) was a vaccine candidate for Phase I clinical trial in American naïve volunteers. Although the results found an outstanding antibody response to this protein and that specific antibodies could inhibit the transmission of parasites to mosquitoes in *ex vivo* assays, this vaccine was astonishingly reactogenic and was not well-tolerated by participants (Wu et al. 2008).

- **Pfs230**: is a large protein, expressed in early gametocytes for few hours after leaving the human infected erythrocyte to the mosquito midgut. Recombinant protein spanning part of this antigen was able to elicit antibodies responses that recognized the gametocytes surfaces and reduced the capacity of parasites to infect mosquito by 71.2-89.8% (Williamson et al. 1995).

- **Pfs48/45** is specifically expressed by gametocytes inside the human host and located at the surface of macrogamets *P. falciparum* parasites. Without this antigen, parasites fertilization could not take place (Outchkourova et al. 2008).

Thus, the complexity of *P. falciparum* parasites hinders the efficacy of these vaccine candidates and incites investigators to explore the parasite proteins implicated in pathogenesis to identify candidates that could be efficient against clinical malaria.
2. Aims of study

*Plasmodium falciparum* malaria causes diverse clinical manifestations ranging from asymptomatic infection to uncomplicated and severe malaria including cerebral malaria. The heterogeneity of these clinical outcomes is due to both host and parasite factors. Though, the biological mechanisms implicated in the progression from mild malaria towards severe clinical state are poorly known, the variation in the genetic and epigenetic background of parasite populations may be involved the parasites virulence thus, leading to several clinical manifestations of malaria. Therefore, the relationships between parasite genetic characteristics and malaria-related pathology need to be further investigated.

The cytoadherence of infected erythrocytes to microvascular endothelial cells in deep organs is believed to be an important virulence factor. This process results from the interaction between parasite proteins named variant surface antigens (VSA) expressed on the surface of infected erythrocytes (IEs) and human receptors expressed at sites of cytoadherence. Yet, the cytoadherence of IEs to a given receptor has not been attributed to a distinct clinical manifestation of malaria.

Several endothelial receptors that may participate in the cytoadherence of IEs to microvascular endothelia have been identified, and few studies attempted to correlate a particular binding phenotype of IEs with a given clinical presentation of malaria. Of these receptors, CD36 and Inter Cellular Adhesion Molecule-1 (ICAM-1) were shown to be used by the majority of patient-derived parasites.

We hypothesized that differential in gene expression could be at the origin of phenotypic variation of parasites resulting in specific interaction with the host which may lead to a particular manifestation of malaria.

The overall aim of this PhD thesis was to gain increased knowledge about the parasite genetic factors implicated in cerebral malaria.

The specific objectives of this work were 1) to analyze the transcriptomes of isolates obtained from asymptomatic carriers and patients with uncomplicated or cerebral malaria. We also investigated the transcriptomes of the 3D7 clone and the 3D7-Lib line that expresses severe malaria associated-variant surface antigen. 2) To examine the potential role of a set of up-regulated genes revealed by arrays analysis in IEs cytoadherence phenotype to a particular receptor.

Firstly, we applied DNA microarrays hybridization analysis to compare gene expression profiles of isolates from Cameroonian children with cerebral malaria; uncomplicated malaria and asymptomatic carriers at ring and mature stages. We identified and confirmed by RT-qPCR deferentially transcribed genes in CM and 3D7-Lib line comparing to AM and 3D7 clone, (Manuscript 1)

Secondly, among the genes in up-regulated CM-associated parasites as compared to AM-associated parasites, UPS A1, A3, B and domain cassettes DC8 and DC13 var genes groups were found to be highly induced. Hence, we aimed to elucidate their potential implication in the pathogenesis of cerebral malaria by promoting increased cytoadherence to specific
receptors CD36, ICAM-1 and CSPG (CSA). Therefore, we studied the adhesive phenotype of clinical fresh isolates, collected during two field missions (June-September 2012 and May-July 2013), from Beninese children with cerebral malaria and uncomplicated malaria by static binding assay. We then performed RT-qPCR to assess the transcription level of var groups A, B, var2, var3, domain cassettes DC8 and DC13 of these isolates to identify a relationship between the cytoadherence phenotype to a specific receptor with the transcription pattern of particular Pfemp-1 variant, (Manuscript 2).
Chapter 2

Gene transcriptomic pattern of *Plasmodium falciparum* in children with cerebral malaria and asymptomatic carriers

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Abstract

The mechanisms underlying the heterogeneity of clinical malaria remain largely unknown. We hypothesized that differential gene expression contributes to phenotypic variation of parasites which results in a specific interaction with the host, leading to different clinical features of malaria. In this study, we analyzed the transcriptomes of isolates obtained from asymptomatic carriers, and from patients with uncomplicated or cerebral malaria. We also investigated the transcriptomes of the 3D7 clone and that of the 3D7-Lib line that expresses severe malaria associated-variant surface antigens.

Our findings revealed a specific up-regulation of genes involved in pathogenesis, adhesion to host cell and erythrocyte aggregation in parasites from patients with cerebral malaria and 3D7-Lib line. We did not find any significant difference between the transcriptome of parasites from cerebral and uncomplicated malaria. The difference between isolates from asymptomatic children and cerebral malaria concerned genes coding for surface antigens, Maurer’s cleft proteins, transcriptional factor proteins, and proteins implicated in protein transport. We also found an important variation in the expression of variant surface multigenic families like var, rif and phist genes. The most outstanding difference was the up-regulation of UPS A1, A3 and UPS B1 of var genes containing architectural domains of DC4, DC5, DC8, DC13 and their neighboring rif genes in isolates from cerebral malaria and 3D7-lib. The predominance of type A var gene transcripts containing DC4, DC5, DC8, and DC13 in parasites from children with cerebral malaria strengthens the association of these var genes with cerebral malaria pathogenesis. Therefore, deciphering the mechanisms by which proteins encoded by these genes are implicated in malaria pathology may provide new opportunities to control malaria disease. In addition, the concomitant regulation of these genes with other genes in their chromosomal neighborhood suggests a common mechanism of gene regulation in *P. falciparum*.

Keywords:
*Plasmodium falciparum*, transcriptome, cerebral malaria, asymptomatic parasitemia, microarrays- hybridization, variant surface antigens
Background

Of the five malaria species that infect humans, *Plasmodium falciparum* is the most common in Africa, and is the species that causes the most severe disease. According to the World Health Organization (WHO), the majority (86%) of the estimated 660,000 annual deaths related to malaria are due to *P. falciparum* infections that occur in children under the age of five years old living in Africa (Greenwood et al. 1991; Murray et al. 2012; Organization 2012; Snow et al. 2005). Despite an adequate antimalarial treatment, 10 to 30% of patients with severe malaria die, and 25% of survivors of cerebral malaria develop chronic neuro-cognitive impairment (Newton et al. 1998). Indeed, the dramatic loss of lives due to malaria and, the negative economic impact associated with the disease lead to the loss of approximately 412 billion US dollars in African countries yearly, further retarding their economic development (Sachs et al. 2002). At present, the only means to effectively control malaria in human hosts remains the use of antimalarial drugs. However, malaria parasites are capable of developing resistance to all currently available drugs, and only a few promising novel drug candidates are under development. Furthermore, despite several candidates undergoing early phases of clinical development, a highly effective vaccine against malaria parasites is still unavailable. The identification of new therapeutic targets or vaccine candidates is hampered by the lack of knowledge on various molecular mechanisms used by malaria parasites to survive within the human host.

*P. falciparum* infection causes a large spectrum of disease manifestations, ranging from asymptomatic infection (AM) to uncomplicated malaria (UM) and severe and complicated malaria. Only a small proportion of individuals (Organization 2011) develop severe disease, including cerebral malaria (CM), severe malaria-associated anemia (SMA), and other life-threatening clinical syndromes, such as acute renal failure, acute respiratory distress, pulmonary edema, hemoglobinuria, disseminated intravascular coagulation, and circulatory collapse (Pongponratn et al. 1991). In areas of low transmission, severe malaria also occurs in older children (i.e. > 5 years old) and adults. After repeated malaria episodes, individuals develop natural protective immunity and considerably decrease their risk to develop severe and complicated malaria, suggesting acquired immunity and protection against severe disease (Marsh et al. 1989; Bull et al. 1998; Dodoo et al. 2001). The heterogeneity of clinical manifestations is due to both human and parasite factors (Mackintosh et al. 2004; Ranjit et al. 2005; Kwiatkowski et al. 2005).

Although the biological processes involved in the progression from acute uncomplicated malaria towards severe clinical state are mostly unknown, we hypothesized that the variation of clinical manifestations depends on parasite gene expression changes.

The relationship between parasite genetic profiles and malaria-related pathologies needs to be established to further understand the pathogenesis of severe and complicated malaria. Genomic research on *P. falciparum* has advanced considerably since the entire genome sequence of the *P. falciparum* reference clone 3D7 was determined. The *P. falciparum* 23-Mb genome is AT-rich and comprises 14 nuclear chromosomes, a 6-kb mitochondrial genome, and a 35-kb plastid-like apicoplast genome (Gardner et al. 2002). The genome sequencing project revealed a large proportion of predicted genes which are likely to be involved in the parasite evasion mechanisms from the human immune system, and host-parasite interactions. The majority of these
latter genes encode amplified gene families, such as the hypervariable variant surface antigen (VSA) genes \textit{(var, rif, stevor, and pfmc2TM)} that mediate evasion from the host immune system, and genes involved in host cell invasion pathways. Gene transcription profiling of the intraerythrocytic developmental cell cycle, proteomic studies, as well as \textit{in silico} sequence computational analysis have led to a substantial progress in defining gene functions and genetic regulatory networks (Le Roch et al. 2003; Bozdech et al. 2003; Kuss et al. 2012; Patra et al. 2008). However, the absence of data from a large number of \textit{P. falciparum} field isolates associated with different clinical manifestations, ranging from asymptomatic carriage to uncomplicated malaria and severe disease, tends to limit our knowledge on the biological processes involved in parasite virulence.

It has been shown that parasites isolated from children with severe malaria (SM) express a limited and conserved set of VSA antigens (VSA-SM) that are more strongly and more commonly recognized by IgG from malaria-exposed individuals than VSA expressed by parasites infecting children with uncomplicated malaria (VSA-UM) (Staalsoe et al. 2003). Therefore, the identification of VSA-SM encoding genes may reveal new therapeutic and vaccine targets for malaria control.

The aim of this study was to analyze and compare the transcriptomes of \textit{P. falciparum} parasites isolates from asymptomatic carriers and patients with clinical malaria, including UM and CM, by DNA microarray hybridization, in order to investigate potential virulence factors associated with severe clinical manifestations. As controls for our experiments, we analyzed and compared the transcriptome of the 3D7 reference clone to that of the 3D7 Lib-line that expresses VSA-SM related to severe pathogenesis (Staalsoe et al. 2003).

**Patients, materials and methods**

**Patients**

After obtaining verbal informed consent from children parents or guardians, Cameroonian schoolchildren aged <12 years old were mass-screened in November 2009 to identify asymptomatic \textit{P. falciparum} malaria parasite carriers (AM) in Ekoundouma, a village located about 5 km west of Yaoundé (Latitude:3.8644652°, Longitude:11.5138617°). Thick smears prepared from fingerpricked capillary blood samples were stained with 10% Giemsa, and the parasite density of \textit{P. falciparum} was determined by microscopy. Children with positive thick blood smear who had not taken antimalarial treatment within the previous two weeks and who were afebrile at the time of mass screening and during the previous one week were enrolled after obtention of verbal informed consent from their parents or their caretakers. Children with gametocytemia, mixed infections with \textit{P. ovale} and/or \textit{P. malariae}, or fever (axillary temperature > 37.5°C) were excluded. Schoolchildren with >1,000 asexual parasites/μL of blood and symptoms associated with malaria were treated with artesunate-amodiaquine combination and paracetamol, as recommended by the Cameroonian Ministry of Public Health.

Symptomatic patients with acute uncomplicated malaria (UM) were enrolled among patients consulting spontaneously at the Nlongkak Catholic missionary dispensary in Yaoundé from January to December 2009. The inclusion criteria were as follows: age < 12 years old, \textit{P. falciparum} parasitemia ≥ 0.1% (or > 5,000 asexual parasites/μL of blood), absence of other \textit{Plasmodium} species, fever
(axillary temperature > 37.5°C) at the time of consultation or history of fever within 24 h preceding consultation, absence of signs and symptoms of severe and complicated malaria, as defined by the WHO, and denial of recent self-medication with an antimalarial drug. The enrolled patients were treated with artesunate-amodiaquine and paracetamol.

Children presenting cerebral malaria (CM) were enrolled at Olembe hospital in Yaoundé, Cameroon, between April 2008 and December 2009, and at the Centre National Hospitalier Universitaire Hubert Koutoucou Mega (CNHU-HKM) in Cotonou, Benin, in 2012 and 2013 between June and August. CM was defined as a Blantyre coma score of ≤ 2 persisting for 30 min and/or at least two seizures within 24 h preceding consultation, and no other obvious cause of coma. The patients were given the appropriate treatment and necessary medical care at the hospital, according to the current guidelines for the treatment of severe and complicated malaria recommended by the Cameroonian and the Beninese Ministries of Health.

Clinical history, physical and neurological examinations, as well as laboratory examinations, including *P. falciparum* parasitemia, creatinine, glycemia, and C-reactive protein were registered on an ad-hoc data form.

After written informed consent was obtained from symptomatic children’s parents or legal guardian, *P. falciparum*-infected blood was collected by venipuncture. Approximately 3–5 mL of blood was collected in ethylene diamine tetraacetic acid (EDTA)-coated tubes. The study protocol was reviewed and approved by the Cameroonian National Ethics Committee and the Cameroonian Ministry of Public (Health authorization N° 028/CNE/DNM/07) as well as by the ethics committee of the Research Institute of Applied Biomedical Sciences, Cotonou, Benin (N°006/CER/ISBA/12 and N°21/CER/ISBA/13).

We obtained verbal informed consent from all parents or gardians of schoolchildren who participated in the current study, including those for which venous blood was not collected. Verbal informed consent was in agreement with the national ethic committee rules and recommendations at the time the study was performed.

For all symptomatic children enrolled in the study, written informed consent was obtained from their parents or legal guardians. The consents were recorded on paper sheets.

The written informed consent and the way of its recording were approved by the two ethic committees.

**Cultivation of *P. falciparum* parasites**

Two laboratory-adapted parasite lines were used. Cryopreserved stocks of 3D7 reference clone and 3D7-Lib line derived from 3D7 (Wang et al. 2009) were cultivated for 10 days using the standard method developed by Trager and Jensen (Trager et al. 1976). Parasites were synchronized, and ring and late trophozoite stages were isolated using plasmagel (Jensen et al. 1978). The 3D7-Lib line was obtained by several rounds of incubation with Liberian hyper-immune plasma using anti-IgG coupled with streptavidine-conjugated Dynabeads, as previously described (Staalsoe et al. 2003). The 3D7-Lib line was shown to express group A *var* and neighboring *rif* genes, suggesting that it mainly expresses variable surface antigens (VSAs) associated with severe disease (Kirchgatter et al. 2002; Bull et al. 2005; Kyriacou et al. 2006).
Plasmodium falciparum isolates maturation

Venous samples containing late trophozoites were excluded, and only samples with 100% ring stage parasites were used in the present study. Samples were washed in cold phosphate-buffered saline (PBS) by centrifugation and divided into two fractions of 200 µL cell pellets. One fraction was immediately stored at -80°C in Trizol (Life Technologies) for RNA extraction. The second aliquot was cultivated for 24 to 38 hours to obtain late trophozoites. Isolates that developed into at least 85% of late trophozoite stages were harvested and stored at -80°C in Trizol.

Microarray design and hybridization

For each clinical group, three samples were constituted by pooling equivalent amounts of parasite RNA from six isolates to perform array hybridization. To ensure that the same quantity of RNA from each isolate was analyzed, we used isolates of approximately the same parasitemia. For the controls (3D7 clone and 3D7-Lib line), three samples (at either ring or late trophozoite stage) were obtained from three independent parasite cultures.

Microarrays were manufactured by Agilent Technologies using SurePrint in situ synthesis technology (Agilent Technologies France SAS, Les Ulis, France). Fifteen slides of eight arrays each containing 14,882 oligonucleotides covering 5,534 Open Reading Frame (ORF) sequences derived from the P. falciparum database were used. The oligonucleotides were designed using oligoarray2 software (Rouillard et al. 2003) by setting the general parameters to 55-60 bases for oligonucleotides length with a melting temperature between 79 and 81°C, and with a 3’ bias of 1,500 nucleotides since RNases preferentially act from the 5’ end. The melting temperature range was relaxed for genes for which no oligonucleotide could be found with the default parameters. The array contained two sets of oligonucleotides. The first set was designed on the basis of the 3D7 sequence genome. These oligonucleotides were chosen within the coding sequences (CDS) of protein-coding genes in the nuclear genome. A minimum of three oligonucleotides per gene were designed when possible. To avoid cross-hybridization with human genes, cross-hybridization was computed using both 3D7 and human genome libraries. The second set of oligonucleotides was designed to hybridize to genes exhibiting large sequence variations, including var, rif, stevor and Pfmc-2TM multigenic families. For these genes, all sequences available in the GenBank and those kindly provided by our collaborators (T. Lavstsen, Centre for Medical Parasitology, University of Copenhagen, Denmark) were retrieved and added to the array. Sequence alignment using Clustal W and Clustal X version 2.0 (Larkin et al. 2007) was performed for each family or sub-family, and specific oligonucleotides were designed. For the other genes with high sequence variations (i.e., genes with high numbers of single nucleotide polymorphisms [SNPs]), sequences were retrieved from PlasmoDB, and specific oligonucleotides were designed.

Total RNA from 200 µl of P. falciparum pellet and laboratory-adapted parasites (3D7 and 3D7-lib) was extracted using chloroform-isopropanol method as recommended by the manufacturer. The integrity of the total RNA from each sample was checked using Agilent 2100 Bioanalyser (Agilent Technologies, France). For reverse transcriptase (RT)-real time PCR (rtPCR), RNA was treated with DNAs at 37°C for 15 min. The absence of contaminating DNA was assessed by 40 cycles of rtPCR using primers targeting seryl tRNA transferase and fructose biphosphate aldolase genes, which are
highly conserved P. falciparum housekeeping genes. Total RNA (6–10 μg) was transcribed using the SuperScript First-Strand Synthesis System® (Life Technologies) and random hexamer oligonucleotides mixture. The newly synthesized cDNA was labeled using SuperScript® indirect cDNA labeling system (Invitrogen, Carlsbad, CA) to generate green fluorescent Cyanin-3 labeled cDNA according to the manufacturer’s instructions. The quantity of labeled cDNA was measured using Nanodrop® (Thermo Fisher Scientific, Villebon-sur-Yvette, France) to ensure that the same quantity of labeled cDNA was deposited for each array hybridization. After quantification of the fluorescent specific activity of Cy3-labeled cDNA, the quantities of targets were normalized at 100 pmol of Cy3, and hybridized for 24 h at 65°C. The slide was washed with water and dried at room temperature. Slides were scanned using GenePix 4000 A scanner (Axon), and the images were analysed using (GenePix5 software, Axon). Dusted and poorly fluorescent spots (below the background level) were excluded from analysis. After array gridding, data were exported as gpr files for statistical analysis.

Quantitative real-time rtPCR

Quantitative rtPCR was performed to confirm array results. For each target gene, a pair of primers was designed to obtain specific DNA sequence fragments that span less than 350 bp. Primer sequences are presented in Table 1 supplemented Data S1. Individual real-time rtPCR amplifications were carried out in a final volume of 20 μl in a Frame Star 384-well plate (4titude® France, Bagneux, France) containing 1x final concentration of SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma-Aldrich, Saint-Quentin Fallavier, France), 100 nM of gene-specific primers and 1 μl cDNA. rtPCR was performed on using 7900 HT (Applied Biosystems®, Saint Aubin, France). The thermal cycle program was as follows: 10 minutes of pre-incubation at 95°C followed by 95°C x 15s and one minute at 60°C x 1 min for 40 cycles. All samples were run in triplicate. Threshold cycles (Ct) and melting curves were analyzed using Applied Biosystems ABI SDS software 2.3. The level of amplification referred to the number of cycles at which the PCR product was detectable and confirmed by the melting curve for each gene. Normalized data were used to quantify the relative levels of a given mRNA between samples using the ΔΔCt analysis (Hooper et al. 2001). Before proceeding to the relative quantification using this method, the similarity in PCR amplification efficiency of the target gene and a house keeping gene (argenyl tRNA synthetase, PF3D7_1218600 / PFL0900c) used as internal controls was checked.

To validate the array results by rt-qPCR, a set of 14 genes were chosen randomly from genes that were differentially expressed between the clinical groups and between 3D7 and 3D7-Lib laboratory-adapted parasites. These genes were grouped into three categories, depending on their fold change on the arrays: down-regulated genes defined as Fold change [FC] value < 2 units of expression), moderately up-regulated genes (FC value, 2–7 units of expression), and highly up-regulated genes (FC value > 7 units of expression). To further confirm our findings, another rt-qPCR was performed for a set of var gene family found to be up-regulated by arrays analysis using a new batch of parasite RNAs isolated from children with CM or UM. The relative expression between the two conditions was calculated by the 2^ΔΔCt method (Hooper, 2001 #6151) and the transcript abundance of var genes was expressed as Transcript units (Tu), defined as Tu= 2^[ Tu] (Lavstsen et al. 2012).
Statistical analysis

Data generated from the arrays were analyzed using the R software 2.14.1 R Core Team (2012) http://www.R-project.org/ (Gentleman et al. 2004) and the Bioconductor limma package (Smyth et al. 2005). Since hybridizations included only one condition per array, the resulting one-color hybridization signal was first loaded in a limma-like RG structure with the red foreground signal set to 1. Background signals were set to 0 for both colors, and the RG data structure was transformed into MA one using the RG.MA function, with logarithmic ratios in log 2 (green signal/red signal). A quantile normalization between arrays was applied to the full data set (Smyth et al. 2003). After exclusion of flagged (invalid) and control spots, differentially expressed genes were extracted using linear models and the empirical Bayes method implemented in limma (Smyth et al. 2004), followed by a Benjamini and Yekutieli (BY) P-value adjustment with error value set to 0.05 (Benjamini et al. 2001).

Mean fold changes of differential expression between pairs of conditions were computed and a cut-off value of 2 was applied. Transcripts were considered as differentially expressed when all their probes had a fold change higher than 5 and a p-value lower than 0.05. The rank-based non-parametric Spearman’s test (Rho) was used to correlate qPCR and array results, and the Kendall test was used to test the correlation between our results and the published arrays data. The means of continuous variables were compared using the one-way analysis of variance, followed by Bonferroni multiple comparison post-test. Statistical tests were performed using SigmaStat 3.5 (Systat Software, Inc. Point Richmond, CA). The significance level was set at $P < 0.05$.

Bioinformatics analysis

Gene Ontology term analysis of differentially genes set:
Functional analysis of the differentially expressed genes was performed using the GOstats package in Bioconductor with default settings (Falcon et al. 2007) and Sanger GeneDB P. falciparum gene annotations. The supplementary file lists the top eight most overrepresented GO terms in each set (table 2).

Clustering analysis: Differentially expressed genes were clustered according to their fold change using the neighbor joining algorithm with Lance-Williams dissimilarity updates (hclust function from R 2.15.1) starting from euclidian distance on fold changes.

Results

Characteristics of patients

A total of 54 patients were enrolled in the study: 18 children with cerebral malaria (CM), 18 children with acute uncomplicated malaria (UM), and 18 children with asymptomatic malaria (AM). The clinical and biological characteristics of these patients are summarized in Table 1. The mean age differences between patients of the three clinical groups were statistically significant ($P < 0.05$). At
enrolment, mean body temperatures were comparable between CM and UM groups \((P > 0.05)\). As expected, the asymptomatic carriers were all afebrile, and their mean body temperature was significantly lower \((P < 0.05)\) than that of the other two clinical groups. The CM group had significantly lower hemoglobin values than the UM group \((P < 0.05)\). The geometric mean parasite densities of CM and UM groups were not significantly different \((P > 0.05)\). Asymptomatic carriers had a significantly lower parasite density compared with that of other patient groups \((P < 0.05)\). These differences in parasite densities were corrected before performing the arrays. To avoid bias in the expression pattern of abundant transcripts due to high parasitemia, RNAs from highly parasitized CM and UM isolates were diluted to obtain similar concentrations of RNA as in AM isolates.

**Gene expression pattern of field isolates**

For each clinical group, three pools of six isolates were built and hybridized on arrays at both ring and late trophozoite stages. These isolates were collected from children with CM, UM, or AM. The transcriptome of isolates at the same asexual developmental stage was compared to that of the 3D7 reference clone. Furthermore, the transcriptome of clinical isolates from children with CM was also compared to that from children with AM. We also compared the transcriptome of the parent clone 3D7 to that of the selected 3D7-Lib. Figure 1 presents the MA plot of differentially expressed genes between these two conditions. Our transcriptomic data from 3D7 reference clone was also compared to those of the published database (Bartfai et al. 2010), which yielded a high correlation between the two transcriptomic data.

The transcriptome of the rings was obtained by hybridization of cDNA from a mixture of 80% rings, 5% late trophozoites, and 15% schizonts for laboratory-adapted parasite lines. All field isolates consisted of 100% ring stages. In parallel, the transcriptome of late trophozoites of both laboratory-adapted parasites and clinical isolates was analyzed by cDNA hybridization using a mixture of 85% late trophozoites, 10% schizonts, and 5% rings.

Using one-color labeling method, the mean green fluorescence intensity after removing the background signal reflects the presence or absence of cDNA corresponding to each gene among the 5,534 analyzed ORFs. The analysis of transcriptomic data of 3D7 and 3D7-Lib ring stage parasites provided 168 transcripts with a mean fluorescence level below the background, which were therefore considered to be untranscribed. Likewise, in late trophozoite stages, 61 of these 168 transcripts were also not transcribed. However, the transcriptome data of field isolates at late trophozoites stage showed only 38 transcripts with fluorescence intensities below the background level. Transcriptomic data from 3D7 reference clone and field isolates at the same developmental stages were highly correlated \((r = 0.79)\) in most \((4,837\) to \(5,344)\) transcribed ORFs. These results suggest that we detected the majority of isolate transcripts at both ring and late trophozoite stages. The lists of non-transcribed genes in 3D7, 3D7-Lib, CM-associated isolates, and AM-associated isolates are provided in supplementary information Tables 2, 3, 4, 5I, respectively.

**Differentially expressed genes in the 3D7-Lib and 3D7**

3D7-Lib expresses VSAs that are potentially implicated in severe and complicated malaria, including cerebral malaria. The comparison of this selected 3D7-Lib to the parent 3D7 reference clone
showed some differences in the expression profile. A total of 395 transcripts were up-regulated in 3D7-Lib late trophozoites, as compared to the corresponding developmental stage of 3D7. Of these 395 genes, 52 displayed 3- to 9-fold higher expression in 3D7-Lib. Some of these genes encode ring exported proteins REX1, REX2, REX3, erythrocyte membrane protein PfEMP1 members, membrane-associated histidine-rich protein (MAHRP1), histidine-rich protein II (HRPII), *Plasmodium* exported proteins (PHISTa, PHISTb, PHISTc), DnaJ protein, putative serine/threonine protein kinase family (FIKK4.2) and hypothetical proteins. A total of 296 genes were slightly down-regulated, exhibiting 2- to 3-fold change in the expression level. The comparison of ring-stage transcriptomes revealed 30 up-regulated genes, ranging from 4- to 30-fold increased expression, in 3D7-Lib, and five down-regulated genes. The majority of the overexpressed genes encoded proteins belonging to PfEMP1 and RIFIN families, exported proteins, knob-associated histidine-rich protein (KAHRP), proteins with DNAJ domain, and some hypothetical proteins. The group A var genes [PF3D7_1100200 / PF11_0008, PF3D7_1150400 / PF11_0521, PF3D7_1300300 / PF13_0003], and PF3D7_0425800 /PFD1235w, and neighboring rif genes [PF3D7_1100300 / PF11_0009, PF3D7_1150300 / PF11_0520, PF3D7_1300400 / PF13_0004, and rif PF3D7_0425700 / PFD1230c] were up-regulated in 3D7-Lib, as compared to 3D7, with an expression level ranging from 2.5 to 26-fold (Table 5, SI). These genes were found to be up-regulated in 3D7-Lib in a previous study (Wang et al. 2009).

**Identification of differentially expressed genes in CM compared with AM.**

Only the comparison between late trophozoites from CM and AM patients showed statistically significant differentially expressed genes, leading to 99 up-regulated transcripts and 135 down-regulated transcripts (Table 5, SI). However, the comparison between parasites from CM and UM patients, or from AM and UM patients showed no significant differences in the transcriptomic pattern.

Up-regulated genes in CM parasites compared to AM parasites exhibited 3 to 14 higher expression rates, while down-regulated genes showed 3- to 22-fold change. The 15 most up-regulated genes encode *Plasmodium* exported proteins, glycophorin-binding proteins (GBP), hypothetical proteins, proteins encoding VSA, such as PfEMP1, RIFIN, other surface proteins like tryptophan- and threonine-rich antigen, and ring-infected erythrocyte surface antigen (RESA). Of the 99 up-regulated genes in CM isolates, 39% were also up-regulated in 3D7-Lib, and include members of rif A1 and var A group, some of them being topologically located in the neighborhood. In parallel, 135 transcripts were down-regulated in CM parasites, as compared to AM parasites. Among these 135 transcripts, 36 showed a 5- to 22-fold decrease in transcription and correspond to genes encoding rhoptry-associated proteins (RAP1, RAP2, and RAP3), merozoite surface proteins (MSP3, MSP7, and MSP9), Maurer’s cleft 2 transmembrane domain protein, and hypothetical proteins.

We then compared CM and AM-associated isolates transcriptomes to the laboratory-adapted 3D7 one. Significant differences in gene expression between field maturated trophozoites from individuals with CM and those with AM, compared to the 3D7 were observed. In CM-associated isolates, 182 transcripts were up-regulated and 85 were down-regulated, while in AM-associated parasites 40 were up-regulated and 41 were down-regulated. The top list of up-regulated transcripts in CM
parasites presented 2 to 9-fold changes and the most down-regulated genes presented fold changes ranging from 2 to 16 for the majority of transcripts with only one transcript that was not transcribed. 50 to 67% of up-regulated genes in both CM- and AM-associated parasites encode exported proteins that are located on the RBC surface: PHIST, RIFIN, STEVOR, PfMc-2TM and PFEMP1 members. In addition to the up-regulation of VSA-encoding genes, like var and rif, we observed the up-regulation of genes encoding Skeleton Binding Protein1 (SBP1), HRPIII, glycoporphin binding protein (GBPH2), as well as genes encoding *Plasmodium* conserved proteins for which no function has been assigned yet. Down-regulated genes in CM-associated parasite isolates include other members of *Plasmodium* exported proteins such as Pfmsp11 and genes encoding variant surface proteins belonging to RIFIN, PfMc-2TM, STEVOR, and PFEMP1 as well as other genes [PF11_0442, PF13_0090, PFC0185w, PF11_0381] encoding transcription factors with AP2 domain, ADP-ribosylation factor, membrane skeletal protein IMC1, respectively. PF10_0343 coding for S antigen was the most repressed transcript in CM-and AM-associated parasites, respectively. This gene might not be transcribed in these isolates.

The non-significant differences in the transcription pattern between CM and UM parasites may result from a similar transcription pattern between these two parasite populations or may be due to the inability of arrays to detect slight variations. Therefore, in order to confirm the specific regulation pattern of these genes in CM-associated parasites, we examined the transcription pattern of UPS A1, A3, and B1 var groups found to be highly up-regulated in another set of RNAs collected from children presenting CM or UM. We found that these genes were significantly up-regulated in CM-associated parasites, as compared to UM-associated parasites (Fig 3) and that our results were in agreement with our arrays outcomes.

**Confirmation of array results using quantitative real-time rt-PCR**

The differential transcription level obtained by DNA hybridization arrays was further confirmed by quantitative PCR using cDNA from the samples used for array hybridization (Fig. 2) and cDNA from a second set of samples (Fig. 3). A highly significant correlation (rho = 0.775; P < 0.0011, Spearman’s rank correlation) between array and qPCR results was found, indicating that both methodological approaches yielded similar results and identified the genes that were either down- or up-regulated with accuracy (Fig. 2; S1).

**Bioinformatics analysis of differentially expressed genes**

Gene Ontology term analysis of up-regulated genes in CM-associated parasites compared to AM-associated parasites, as well as in 3D7-Lib at ring and late trophozoite stages compared to 3D7 revealed GO terms of pathogenesis as the most overrepresented term (Table 2). Other over represented GO terms included cytoadherence to microvasculature mediated by symbiont protein, antigenic variation, or interaction with host.

Clustering analysis performed for up-regulated genes according to their fold change exhibited a network of two clusters in up-regulated gene sets for the three compared conditions, CM versus AM, 3D7-Lib T versus 3D7 T, and 3D7-lib R versus 3D7 R. For CM up-regulated genes, the first cluster contained three genes with PF3D7_0201700 / PFD0085c; encoding acylcoA gene separated from
PF3D7_1301200 / PF13_0010 gene encoding GBPH2 and PF3D7_0701900 / PF07_0004 encoding *Plasmodium* exported protein. Similarly, comparison of the first cluster of 3D7-Lib T versus 3D7 contained PF10_0038; gene encoding 40S ribosomal protein (S20e) separated from PF3D7_0935900 / PFI1735c REX1, PF3D7_1301700 / MAL13P1.61 *Plasmodium* exported protein, (GEXP07), PF3D7_1001500 / PF10_0019 early transcribed membrane protein 10.1 (ETRAMP10), as well. The first cluster of 3D7-Lib R up-regulated genes also contained four genes with KAHRP-encoding gene with the highest fold change followed by three PfEMP1 encoding genes; *PF13_0003*, *PF11_0521* and *PFD1235w*.

In the three conditions, the second cluster contained the remaining up-regulated genes within two sub-networks in which some of the connected genes were grouped according to their chromosomal location or sequence and functional similarities, as PF3D7_0201600 / PFB0080c, encoding PHISTb, PF3D7_0201800/PFB0090c, encoding RESA- like protein with PHIST and DNAJ domains.

**Discussion**

Microarray-based transcriptomic studies are powerful approaches that have been successfully applied to identify many virulence genes in bacterial and cancer cell systems (*Mahan* *et al.* 1993; *Alizadeh* *et al.* 2000). Due to the limited amount of RNA that can be obtained from clinical samples of *P. falciparum*-infected individuals, only a few studies have investigated whole- transcriptomes of clinical isolates (Daily *et al.* 2004; Daily *et al.* 2005; Daily *et al.* 2007; Tuikue Ndam *et al.* 2008; Vignali *et al.* 2011). These studies have demonstrated substantial differences in gene expression patterns between clinical isolates and laboratory-adapted *P. falciparum* strains. This difference in gene transcription between *in vitro* and *in vivo* conditions is probably related to the biological processes and adaptation that the parasites undergo to survive within the human host or in artificial *in vitro* environment. The host-parasite interactions that require gene activation include parasite sequestration and immune system evasion, sexual differentiation for parasite transmission, as well as various metabolic processes that are essential for *in vivo* parasite survival.

The present study aimed to compare and analyze the whole transcriptome of *P. falciparum* parasites isolated from patients with distinct clinical features, including asymptomatic carriage, acute uncomplicated malaria (UM), and cerebral malaria (CM), in order to gain new insights into regulation of gene transcription underlying malaria pathology. Our results indicate noticeable variation in gene transcription pattern of parasite isolates compared to 3D7, as well as between the parent 3D7 and selected 3D7-Lib. Interestingly, we observed a consistent variation in the gene transcription pattern between parasites isolated from children with CM and parasites isolated from asymptomatic children. Although the array hybridization failed to detect differentially expressed genes between CM and UM, and between UM and AM parasites, our rt-PCR results indicate that the up-regulation of specific *var* gene groups in CM-associated parasites, which was not observed in AM- and UM-associated parasites, might be of biological significance. It is likely that genes differentially expressed in parasites associated with CM, compared to those from asymptomatic carriers, are implicated in the biological mechanisms involved in CM pathogenesis, such as cytoadherence of parasitized erythrocytes to brain endothelial cells, leading to an enhanced inflammation, both locally at the parasite-host cell binding
site and elsewhere far from the binding site via the action of cytokines. These assumptions are reinforced by our test for enrichment on gene ontology categories of up-regulated genes in 3D7-Lib and CM-associated parasites, which yielded the overrepresentation of pathogenesis, regulation of cell adhesion, cytoadherence to microvasculature, erythrocytes aggregation, and antigenic variation GO terms.

We then focused our attention on genes induced both in isolates from children with CM and in isolates from children with AM. These genes were also up-regulated in 3D7-Lib, compared to 3D7. This finding allowed us to develop an in vitro model for further extensive studies on these gene candidates.

We also compared our transcriptomic results to published data and found that the majority of transcripts that were not transcribed in our study in ring and trophozoite stages of 3D7 and 3D7-Lib were also absent in previous array data (Le Roch et al. 2003; Bozdech et al. 2003), suggesting untranscribed ORFs in in vitro cultivated parasites. However, many transcripts absent from the 3D7 transcriptome were present in our transcriptomic data of field isolates and in the transcriptome of other clinical isolates (Vignali et al. 2011), suggesting that these transcripts are not essential for asexual in vitro survival within the RBC. Among the undetected transcripts in ring and trophozoite stages in 3D7 clone, most were amplified gene families that encode (PFEMP1, RIFIN, STEVOR, and PFMC2TM) and unknown hypothetical proteins. The difference in the expression of amplified gene families may be due to an inherent variable expression between isolates or to the high number of distinct parasite populations in field isolates. In our previous study in the same study site, 60% of infections were multiclonal and harbored up to seven distinct parasite populations (Basco et al. 2004). It is also likely that these represent variants that do not exist in 3D7.

We also compared our transcriptomic data from ring stage and late trophozoite stage of 3D7, as well as from parasite isolates, to those of the corresponding stages of 3D7 obtained by RNA sequencing (Bartfai et al. 2010) and found a highly significant correlation (p = 2.2e-16, Kendall test) between these data. These results demonstrate the robustness of the array hybridization method to provide consistent data that are comparable to RNA sequencing and reflect the conserved nature of basic biological processes, including general metabolism and cell cycle, in both in vivo and in vitro conditions. This high correlation is consistent with the predominance of ring stage parasites in the peripheral blood of infected individuals and the changes in the transcription pattern leading to the progression and development towards the late trophozoite stage.

Differentially transcribed genes in parasites from CM patients compared to those of AM-associated parasites are expected to provide insights into parasite-associated virulence factors. We investigated the function of 99 up-regulated genes in parasites from children with CM compared to the parasites from children with AM, and found that 28% of up-regulated genes encode exported proteins, with the majority (67%) of them being VSA-encoding genes, such as var, rif, phist, and surfin. These genes are implicated in parasite sequestration and immune evasion. Interestingly, var group A/Ups A1, A3, and/or UpsB1, [PF3D7_1150400 / PF11_0521, PF3D7_1300300 / PF13_0003, PF3D7_0937600 / PF1820w, PF3D7_0632800 / PFF1595c, PF3D7_0425800 / PFD1235w] were up-regulated in CM-associated isolates and in the 3D7-Lib line, while [PF3D7_0200300 / PFB0020c PF3D7_0400400 / PFD0020c] were up-regulated only in CM-associated parasites. 3D7 PF3D7_1150400 / PF11_0521 displays DBLα-1.7 and CIDR-α1.4, while PF3D7_0400400 / PFD0020c displays CIDRα-1.1, DBLβ12, DBLγ6. These architectural domains characterize Domain Cassettes
DC13 and DC8, respectively, which were shown to be transcribed at higher levels in parasites from children with severe malaria in Tanzania (Lavtsen et al. 2012) and Benin (Bertin et al. 2013), as compared to the parasites from children with uncomplicated malaria. Similarly, *PF3D7_1300300 / PF13_0003, PF3D7_0425800 / PFD1235w* contain DC5 which also seems to be associated with severe malaria (Lavtsen et al. 2012) and parasite binding to the human receptor PECAM1 (Berger et al. 2013). Our findings are consistent with these earlier results. Furthermore, the transcription of *PF3D7_0400400 / PFD0020c* and that of its orthologs IT4var19, HB3var3, and IT4var7 were also shown to be highly induced in parasite lines selected for binding to human brain endothelial cells (HBEC) (Claessens et al. 2012; Avril et al. 2012). These genes also encode DC8 and DC13-containing PfEMP1s and antibodies raised against the NTS- DBLα1 and the NTS-DBLα1-CIDRα1 domains of HB3var3 inhibit the binding of infected erythrocytes on HBEC, providing further support of their implication in cytoadherence (Claessens et al. 2012). In addition, recently the endothelial protein C receptor (EPCR) was identified as receptor for DC8 and DC13-containing PfEMP1s, suggesting a crucial role of EPCR binding in the pathology of malaria (Turner L. 2013). Interestingly, anti-PfEMP1 IgG antibodies targeting the recombinant DBLβ9 of PF13_0003, DBLβ3, DBLγ13, CIDRα1.6 of PFD1235w, CIDRα1.4 of PF11_0521 were also found in children exposed to malaria (Lavtsen et al. 2012), suggesting a selective expression of these PfEMP1 variants by parasites infecting children that lead to an activation of the immune system and response by antibodies synthesis.

Similarly, 3D7 *PF13_0003*, which is characterized by DBL1α1 and CIDR1γ that are shared with other genes, such as PaloAlto89F5 VarO, IT4/I29, and IT4var60, is implicated in rosetting (Vigan-Womas et al. 2008; Albrecht et al. 2011). This gene was up-regulated in parasites from children with CM and was highly induced in 3D7-Lib. The N-terminal region of DBL1α1 plays a role in the formation of pseudo-rosettes on the surface of a fibroblast-like cell line COS and baculovirus-infected cells expressing individual PfEMP1 domains (Vigan-Womas et al. 2008; Rowe et al. 1997). In the present study, the up-regulation of exclusively UPS A1 and UPS A3 var groups in isolates from children with CM is in strong agreement with the results obtained in other studies (Kirchgatter et al. 2002; Kyriacou et al. 2006; Jensen et al. 2004; Warimwe et al. 2012) reinforcing the relationship between specific PfEMP1 protein expression and the development of CM.

Var genes are known to be ubiquitously transcribed at the early ring stage with restriction to only one transcript during mature trophozoites stage (Scherf et al. 1998; Chen et al. 1998). Our finding on the differential up-regulation of var genes in late trophozoites may be inherent to the high proportion of ring stages in our mRNA preparation from cultivated isolates as well as in laboratory-adapted parasites. *Rif* genes *PF3D7_0631800 / PFF1545w, PF3D7_1041000 / PF10_0403, PF3D7_1041000 / PFC1100w, PF3D7_1100300 / PF11_00520, PF3D7_0425700 / PFD1230c, PF3D7_1100300 / PF11_0009, phist a, b, c PF3D7_1478000 / PF14_0752, PF3D7_1253300 / PFL2565w, PF3D7_0702100 / MAL7P1.7, PF3D7_0936900 / PF1175w, PF3D7_0836000 / MAL8P1.4, PF3D7_0219700 / PF900900c, and PF3D7_1300300 / PF13_0003 pfemp1 were also up-regulated in 3D7- Lib and/or in both 3D7-Lib and parasites from CM patients. The *phist a PF3D7_1478000 / PF14_0752* was previously reported to be up-regulated in parasite lines selected for binding to HBEC (Claessens et al. 2012). The *rif* genes are composed of a large family of about 150 clonally variant genes grouped into two subfamilies, A- and B-type RifINs, according to their subcellular localization and 25-amino acid
sequence, which occurs only in A-type RIFINs (Joinnin et al. 2008). The A-type RIFINs are thought to be trafficked to the surface of the infected erythrocyte (Khattab et al. 2008), while some B-type RIFINs have been associated with the extracellular stages, merozoites and gametocytes (Mwakalinga et al. 2012). The majority of the gene family members are located near the var genes within the sub-telomeric regions of the chromosomes. These regions are subjected to recombination, increasing their diversity. In 3D7-Lib, the rif PF3D7_1150300 / PF11_00520, PF3D7_1100300 / PF11_0009, PF3D7_0425700 / PFD1230c, PF3D7_1300400 / PF13_0004, were concomitantly up-regulated with their neighboring var genes PF3D7_1150400 / PF11_0521), PF3D7_1100200 / PF11_0008, PF3D7_0425800 / PFD1235w, PF3D7_1300300 / PF13_0003. These genes may be under the control of the same promoter region.

Other genes, such as PF3D7_1301200/PF13_0010, PF3D7_1401000/PF14_0010, and PF3D7_0501300/PFE0065w, were also found to be highly up-regulated in parasites from children with CM. PF3D7_1401000/PF14_0010 and PF3D7_1301200/PF13_0010, encode GBPH and GBPH2, respectively. These proteins belong to Maurer’s cleft proteins (Lanzer et al. 2006), either transiently during their export to the infected erythrocyte membrane, like PfEMP1, RIFIN (Petter et al. 2007), SURFIN (Winter et al. 2005), STEVOR (Kaviratne et al. 2002), PfMC-2TM (Sam-Yellowe et al. 2004), or are constitutive proteins of the Maurer’s cleft itself, as Plasmodium skeleton-binding protein 1 (PfsBP1) which is encoded by PF3D7_0501300/PFE0065w (Sam-Yellowe et al. 2004). PfsBP1 was demonstrated to be implicated in PfEMP1 trafficking to the surface of erythrocytes (Spycher et al. 2006). PF3D7_1478000 / Pf 14_0752 encodes MAHRP1 (Phist a), a Maurer’s cleft resident protein also essential for PfEMP1 trafficking to the surface of infected red blood cells (Spycher et al. 2006; Spycher et al. 2006). These two genes were transcribed at higher levels in field isolates, compared to 3D7. MAHRP1 was among the 15 most up-regulated genes in parasite lines selected to adhere on HBEC (Claessens et al. 2012).

Among the regulated genes, some code for antigenic proteins, such as P. falciparum sporozoite surface threonine- and asparagine-rich protein (STARp) (PF3D7_07023005 / PF07_0006, P. falciparum ring-infected erythrocyte surface antigen (RESA) PF3D7_1149200 / PF11_0509. These genes were found to be up-regulated in clinical isolates, as compared to laboratory-adapted strains (Mackinnon et al. 2009). The genes implicated in the transcription process, including PF3D7_0318200 / PFC0805w DNA-directed RNA polymerase II, PF3D7_1433500 / PF14_0316 putative DNA topoisomerase II, PF3D7_1220100 / PFL0970w PF3D7_1231600 / PFL1525c pre-mRNA splicing factors were transcribed at higher levels in CM-associated parasites, compared to AM-associated parasites and 3D7. PF3D7_1220100 / PFL0970w was highly expressed in parasites from children and pregnant women (Vignali et al. 2011). However, the transcription factors with ApiAp2 domains PF3D7_1439500 / PF14_0374, PF3D7_1143100 / PF11_0442 were slightly down-regulated in CM-and AM-associated parasites, compared to 3D7 which is consistent with the findings of Vignali et al (Vignali et al. 2011).

Several differentially expressed genes in the present study were similarly regulated in other studies (Mackinnon et al. 2009), supporting the variable nature of expression pattern of these genes between field isolates and 3D7 clone and the robustness of array hybridization method to produce reproducible results despite technical differences between laboratories. For instance, (REX1) ring-exported protein 1, PF3D7_0935900 / PFI1735c, (REX2) ring-exported protein 2, PF3D7_0936000 / PFI1740; (GIG) gametocytogenesis-implicated protein PF3D7_0935600 / PFI1720w, (STARp) sporozoite threonine- and asparagine-rich protein PF3D7_0702300 / PF07_0006; lysophospholipase, putative
PF3D7_0702200 / PF07_0005, conserved *Plasmodium* protein with unknown function PF3D7_0702400 /PF07_0007, (Pfg27) gamete antigen 27/25 PF3D7_1302100 / PF13_0011 were all up-regulated in AM-and CM-associated isolates analyzed in our study and were also among the top 20 up-regulated genes in clinical isolates in a previous study (Mackinnon et al. 2009). REX1, REX2 and GIg genes are located in close proximity in chromosome 9, while STAR and putative lysophospholipase as well as a conserved *Plasmodium* gene are located within a close distance in chromosome 7, suggesting co-regulation of gene transcription due to their proximity. Similarly, when examining the topological location of all up-regulated genes in parasites from CM patients compared to those from AM patients, three to 12 genes were located on the same chromosome at a distance ranging from 5 to 100 kb, a distance at which the variation of expression can be controlled by a single promoter or the same epigenetic mechanism.

In conclusion, we identified a set of specifically up-regulated genes in *parasites collected from* CM patients, which are likely to be implicated in the pathogenesis of cerebral malaria. Furthermore, our findings on type A *var* genes containing DC5, DC8 and DC13 in isolates from children with CM and in the 3D7-Lib are in agreement with other published studies (Lavstsen et al. 2012; Claessens et al. 2012; Avril et al. 2012; Warimwe et al. 2012; Normark et al. 2007), supporting the potential implication of these genes in CM.

These results call for further investigations on PfEMP1 members related with severe malaria for a better understanding of malaria-associated pathologies. In addition to PfEMP1 group A1, A3 and B1 subset, other genes encoding exported proteins as well as non-exported proteins, were up-regulated in CM-associated parasites. The potential role of these genes in CM deserves to be further investigated and may allow the identification of new targets for malaria control.

**Authors contribution**

RT, PD, NTN, AA, CR, MAD, and JYC conceived the study design. TA carried out RNA extraction, and RT performed qPCR experiments and participated in the analysis of results. GN and MAD carried out statistical analysis. EB and NTN designed oligonucleotides used in the arrays. ME carried out gene ontology term analysis. CWW performed selection of parasite strains. GNA participated in patient recruitment. RT performed sample collection, in vitro cultivation of isolates, arrays hybridization, analysis of results. TA, RT, LKB, CWW, EB, and PD wrote the manuscript. All authors have read, corrected, and approved the final manuscript.

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Table 1. Clinical and biological characteristics of *P. falciparum*-infected patients and asymptomatic carriers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cerebral malaria(^1)</th>
<th>Uncomplicated malaria</th>
<th>Asymptomatic carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of enrolled patients</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Mean age ± SD (range) (months)</td>
<td>15.3 ± 6.6 (6–25)</td>
<td>28.9 ± 15.9 (7–60)</td>
<td>65.3 ± 7.4 (48–72)</td>
</tr>
<tr>
<td>Sex ratio F/M (n)</td>
<td>0.8 (8/10)</td>
<td>1.0 (9/9)</td>
<td>1.25 (10/8)</td>
</tr>
<tr>
<td>Geometric mean parasitemia (95% confidence interval; range) (asexual parasites/μl of blood)</td>
<td>30,000 (9,550–94,000)</td>
<td>50,100 (38,200–65,600)</td>
<td>3,460 (2,400–5,000); 1,120–9,630</td>
</tr>
<tr>
<td>Mean Body Temperature (°C)</td>
<td>39.0 ± 1.1</td>
<td>38.9 ± 0.8</td>
<td>36.8 ± 0.4</td>
</tr>
<tr>
<td>Mean hematocrit ± SD(%)</td>
<td>24.6 ± 3.7</td>
<td>29.9 ± 5.7</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^1\)mean Blantyre score, 2/5; mean blood glucose level ± SD, 1.17 ± 0.29 g/L; mean creatinine ± SD, 12.7 ± 33.7 mg/dL. In two cases, symptoms of cerebral malaria occurred concomitantly with acute respiratory distress or hemoglobinuria. ND, not determined.
Table 2: Top eight significant GO terms for Gene Ontology term analysis of differentially expressed genes set in cerebral malaria isolates and 3D7-Lib compared to asymptomatic malaria isolates and 3D7 clone, respectively.

<table>
<thead>
<tr>
<th>Best GOs</th>
<th>P-value</th>
<th>Gene Ontology term description.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0009405</td>
<td>5 x 10^{-4}</td>
<td>Pathogenesis</td>
</tr>
<tr>
<td>GO:0020013</td>
<td>9 x 10^{-4}</td>
<td>modulation by symbiont of host erythrocyte aggregation</td>
</tr>
<tr>
<td>GO:0022407</td>
<td>9 x 10^{-4}</td>
<td>regulation of cell-cell adhesion</td>
</tr>
<tr>
<td>GO:0030155</td>
<td>9 x 10^{-4}</td>
<td>regulation of cell adhesion</td>
</tr>
<tr>
<td>GO:0034109</td>
<td>9 x 10^{-4}</td>
<td>homotypic cell-cell adhesion</td>
</tr>
<tr>
<td>GO:0034110</td>
<td>9 x 10^{-4}</td>
<td>regulation of homotypic cell-cell adhesion</td>
</tr>
<tr>
<td>GO:0034117</td>
<td>9 x 10^{-4}</td>
<td>erythrocyte aggregation</td>
</tr>
<tr>
<td>GO:0034118</td>
<td>9 x 10^{-4}</td>
<td>regulation of erythrocyte aggregation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Best GOs</th>
<th>P-value</th>
<th>Gene Ontology term description.</th>
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<tbody>
<tr>
<td>GO:0009405</td>
<td>4 x 10^{-17}</td>
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</tr>
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<td>GO:0044406</td>
<td>6 x 10^{-12}</td>
<td>adhesion to host</td>
</tr>
<tr>
<td>GO:0022407</td>
<td>9 x 10^{-12}</td>
<td>regulation of cell-cell adhesion</td>
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<td>GO:0034109</td>
<td>9 x 10^{-12}</td>
<td>homotypic cell-cell adhesion</td>
</tr>
<tr>
<td>GO:0034117</td>
<td>9 x 10^{-12}</td>
<td>erythrocyte aggregation</td>
</tr>
<tr>
<td>GO:0051701</td>
<td>3 x 10^{-09}</td>
<td>interaction with host</td>
</tr>
<tr>
<td>GO:0051805</td>
<td>9 x 10^{-05}</td>
<td>evasion or tolerance of immune response of other organism involved in symbiotic interaction</td>
</tr>
<tr>
<td>GO:0020033</td>
<td>2 x 10^{-04}</td>
<td>antigenic variation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Best GOs</th>
<th>P-value</th>
<th>Gene Ontology term description.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0009405</td>
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<td>GO:0030155</td>
<td>9 x 10^{-17}</td>
<td>regulation of cell adhesion</td>
</tr>
<tr>
<td>GO:0034109</td>
<td>9 x 10^{-17}</td>
<td>homotypic cell-cell adhesion</td>
</tr>
<tr>
<td>GO:0034117</td>
<td>9 x 10^{-17}</td>
<td>erythrocyte aggregation</td>
</tr>
<tr>
<td>GO:0020035</td>
<td>2 x 10^{-16}</td>
<td>cytoadherence to microvasculature, mediated by symbiont protein</td>
</tr>
<tr>
<td>GO:0051701</td>
<td>2 x 10^{-13}</td>
<td>interaction with host</td>
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</tr>
<tr>
<td>GO:0006950</td>
<td>1 x 10^{-09}</td>
<td>response to stress</td>
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The reported p-values are based on the “OddsRatio score”, a measure of gene-enrichment (Falcon et al.2007).
**S1 appendix:** List of up-regulated and down-regulated genes in 3D7 versus 3D7-Lib and in isolates from children with cerebral malaria versus isolates from asymptomatic children.
Lists of untranscribed genes in 3D7, 3D7-Lib, CM ans AM.
The increase or decrease (fold change) and the Bonferroni \( P \)-value correction are presented.

**List of up-regulated genes in 3D7-LibR vs 3D7R:**

<table>
<thead>
<tr>
<th>[New Gene ID]</th>
<th>[Previous ID]</th>
<th>[Product Description]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF3D7_0100300</td>
<td>PFA0015c</td>
<td>var-like erythrocyte membrane protein 1</td>
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<td>PF3D7_0115700</td>
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<td>PF3D7_0201600</td>
<td>PFB0080c</td>
<td>Plasmodium exported protein (PHISTb), unknown function</td>
</tr>
<tr>
<td>PF3D7_0201800</td>
<td>PFB0090c</td>
<td>RESA-like protein with PHIST and DnaJ domains</td>
</tr>
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DEAD/DEAH box helicase, putative
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cytoadherence linked asexual protein 3.2 (CLAG3.2)
cytoadherence linked asexual protein 3.1 (CLAG3.1)
membrane skeletal protein IMC1-related (ALV2)
conserved protein, unknown function
serine/threonine protein kinase, putative (ARK2)
TPR domain containing protein
conserved Plasmodium protein, unknown function
conserved Plasmodium protein, unknown function
circumsporozoite- and TRAP-related protein (CTRP)
mitochondrial ribosomal protein L29/L47 precursor, putative
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cdc2-related protein kinase 4 (CRK4)
kinesin-like protein, putative
P-type ATPase, putative (ATPase7)
ATP-dependent RNA helicase, putative (DOZI)
conserved Plasmodium protein, unknown function
normocyt binding protein 1, reticulocyte binding protein homologue 1
conserved Plasmodium protein, unknown function
conserved Plasmodium protein, unknown function
flap endonuclease 1 (FEN1)
GTPase, putative
DNA polymerase alpha
conserved Plasmodium protein, unknown function
RING zinc finger protein, putative
RNA binding protein, putative
regulator of chromosome
condensation, putative transcription factor with AP2 domain(s) (ApiAP2)

**List of up-regulated genes in CMT vs AMT:**

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PF3D7_0114100  PFA0680c  Pfmc-2TM Maurer’s cleft two transmembrane protein (MC-2TM)
PF3D7_0206200  PFB0275w  metabolite/drug transporter, putative
PF3D7_0206800  PFB0300c  merozoite surface protein 2 (MSP2)
PF3D7_0207600  PFB0340c  serine repeat antigen 5 (SERAS)
PF3D7_0210000  PFB0450w  secretory complex protein 61 gamma subunit (Sec61-gamma)
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PF3D7_0302200  PFC0110w  cytoadherence linked asexual protein 3.2 (CLAG3.2)
PF3D7_0302500  PFC0120w  cytoadherence linked asexual protein 3.1 (CLAG3.1)
PF3D7_0316600  PFC0725c  formate-nitrite transporter, putative
PF3D7_0320900  PFC0920w  histone H2A variant, putative (H2A.Z)
PF3D7_0321400  PFC0945w  protein kinase, putative
PF3D7_0414900  PFD0720w  conserved ARM repeats protein, unknown function
PF3D7_0423500  PFD1110w  glideosome associated protein with multiple membrane spans 2 (GAPM2)
PF3D7_0423700  PFD1120c  early transcribed membrane protein 4 (ETRAMP4)
PF3D7_0501500  PFE0075c  rhoptry-associated protein 3 (RAP3)
PF3D7_0501600  PFE0080c  rhoptry-associated protein 2 (RAP2)
PF3D7_0502900  PFE0140c  mitochondrial inner membrane TIM10 associated protein, putative
PF3D7_0503400  PFE0165w  actin-depolymerizing factor 1 (ADF1)
PF3D7_0505800  PFE0285c  small ubiquitin-related modifier, putative (SUMO)
PF3D7_0508000  PFE0395c  6-cysteine protein (P38)
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PF3D7_0518300  PFE0915c  proteasome subunit beta type 1, putative
PF3D7_0522600  PFE1130w  conserved protein, unknown function
PF3D7_0525800  PFE1285w  membrane skeletal protein IMC1-related
PF3D7_0609800  PFF0485c  zinc finger protein, putative
List of up-regulated genes in CMT vs 3D7T:

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Plasmodium exported protein, unknown function
erythrocyte membrane protein 1 (PFEMP1), pseudogene
inner membrane complex protein 1a, putative (IMC1a)
membrane skeletal protein IMC1-related (ALV2)
conserved Plasmodium protein, unknown function
hypothetical protein
circumsporozoite- and TRAP-related protein (CTRP)
conserved Plasmodium protein, unknown function
ABC transporter, putative
conserved Plasmodium protein, unknown function
riffin (riffin (3D7-rifT3-7))
Plasmodium exported protein, unknown function, pseudogene
normocyte binding protein 1, reticulocyte binding protein homologue 1
dipeptidyl peptidase 3 (DPAP3)
conserved Plasmodium protein, unknown function
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erthrocyte membrane-associated antigen
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PF3D7_0937000  PFI1790w  Plasmodium exported protein (PHISTb), unknown function
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PF3D7_1373400  MAL13P1.535 rifin (RIF)
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PF3D7_0615100  PFF0730c  enoyl-acyl carrier reductase (ENR)
PF3D7_0617500  PFF0847w  rifin, pseudogene
PF3D7_0617600  PFF0850c  stevor (Stevor)
PF3D7_0618400  PFF0890c  conserved Plasmodium membrane protein, unknown function
PF3D7_0624800  PFF1195c  conserved Plasmodium protein, unknown function
PF3D7_0625100  PFF1215w  sphingomyelin synthase, putative
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PF3D7_0670060  MAL8P1.215 Plasmodium exported protein, unknown function, pseudogene
PF3D7_0701000  MAL8P1.211 erythrocyte membrane protein 1 (PfEMP1), pseudogene
PF3D7_0712500  MAL7P1.52  rifin, pseudogene
PF3D7_0712600  PF07_0051  erythrocyte membrane protein 1, PfEMP1 (VAR)
PF3D7_0716700  PF07_0069  conserved Plasmodium protein, unknown function
PF3D7_0718800  PF07_0076  conserved Plasmodium protein, unknown function
PF3D7_0718900  MAL7P1.97  conserved Plasmodium protein, unknown function
PF3D7_0723200  MAL7P1.124 conserved Plasmodium protein, unknown function
PF3D7_0805900  MAL8P1.136 conserved Plasmodium protein, unknown function
PF3D7_0808800  PF08_0105  rifin (RIF)
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PF3D7_1335500 MAL13P1.177 conserved Plasmodium protein, unknown function
PF3D7_1348700 MAL13P1.245 conserved Plasmodium protein, unknown function
PF3D7_1348800 MAL13P1.246 E1-E2 ATPase, putative
PF3D7_1357300 PF13_0306 dynein light chain, putative
PF3D7_1361300 MAL13P1.305 conserved Plasmodium protein, unknown function
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**Genes untranscribed in CM and AM isolates:**

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List of qPCR primers:

**Highly up regulated genes**

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PF3D7_1300300 / PF13_0003  CACAGGTATGGGAAGCAATG CCATACAGCCGTGACTGTTC

**Moderately regulated genes**

PF3D7_0425800 / PF1235w  AAACACGTTGAATGGCGATA GACGCCGAGGAGGTAAATAG
PF3D7_1144000 / PF11_0454  CAGGAAGTGCTCAGCTACAT CTGACATGACCAGAGATAGC
PF3D7_1150400 /PF11_0521 TGCTGAAGACCAAATTGAGC TGCTGAAGACCAAATTGAGC
PF3D7_0401500 /PF0065w CCCGTGGATATGTTCTTGC GGTTGCTATACTGCCAGTAA
PF3D7_0100300 /PFA0015c CGTAAAACATGGTGGGATGA GGCCCATTCAAGTAAACCAC

down regulated genes
PF3D7_1200600 /PFL0030c TGGTGATGGTACTGCTGGAT TTTATTTTCGGCAGCATTTG
PF3D7_0532700 /PFE1620c GATAGTGGTCGTAAGAGGT CGTTTGAGCATCTCATCATG

Figure. 1. Mean MA plot associated with the comparison of cerebral malaria with asymptomatic malaria (mean log ratio of expression versus mean log intensity)
Figure. 1 SI: Histogram of raw p-values obtained from limma for the comparison of cerebral malaria with asymptomatic malaria.
Figure 2: The expression level measured by quantitative RT-qPCR for arrays data confirmation. 

**PFB0106c** and **PF13_0003** transcripts were highly up-regulated (fold change>6) by arrays in 3D7-Lib-Ring (R) versus 3D7-R and 3D7-Lib-Late Trophozoite (LT) versus 3D7-LT, respectively. **PFD1235W**, **PF11_0521**, **PFA0015c**, **PFD0065w**, and **PF11_0454** transcripts were moderately up-regulated (fold change range [2.5-5]) in 3D7-LT versus 3D7Lib-LT, CM-LT versus AM-LT, CM-LT versus 3D7- LT and 3D7-Lib-R versus 3D7-R, respectively. **PFE1620C** and **PFL0030C** transcripts were down-regulated (Fold change range [4 to 0]) in CM-LT versus 3D7-LT.
Figure 2 SI: Spearman’s rank correlation ($r = 0.775; P < 0.001$) between arrays hybridization and qPCR methods for gene expression analysis.

Figure (3). Transcription pattern of UPS A1, A3, and B1, var genes in parasites from CM and UM. The differential in transcription was compared between groups by the Mann-Whitney U test, significant $P$-values are shown in the figure.
Chapter 3

Cytoadherence phenotype of *P. falciparum* infected erythrocytes is associated with specific *Pfemp-1* expression in parasites from children with cerebral malaria

Talleh Almelli\textsuperscript{1,2}, Nicaise Tuikue Ndam\textsuperscript{1,2}, Sem Ezimégnon\textsuperscript{3}, Maroufou J. Alao\textsuperscript{4}, Charles Ahouansou\textsuperscript{3}, Gratien Sagbo\textsuperscript{5}, Annick Amoussou\textsuperscript{6}, Philippe Deloron\textsuperscript{1,2}, Rachida Tahar\textsuperscript{1,2}

1 Institut de Recherche pour le Développement (IRD), UMR 216 Mère et Enfant Face aux Infections Tropicales, Paris, France

2 PRES Sorbonne Paris Cité, Université Paris Descartes, Faculté de Pharmacie, Paris, France

3 Centre d’Etude et de recherche sur le Paludisme Associé à la Grossesse et l’Enfance (CERPAGE), Cotonou, Bénin

4 Département de pédiatrie, Hôpital Mère-enfant de la lagune (HOMEL), Cotonou, Bénin

5 Service de pédiatrie, Centre National Hospitalo-Universitaire (CNHU), Cotonou, Bénin

6 Service de pédiatrie, Hôpital de zone de Suru-Léré, Cotonou, Bénin

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Footnote

None of the results reported in this manuscript have been presented in a meeting.
Abstract.

**Background:** Cytoadherence of *P. falciparum*-infected erythrocytes (IEs) in deep microvasculature endothelia plays a major role in the pathogenesis of cerebral malaria (CM). This biological process is thought to be mediated by *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) and human receptors such as CD36 and ICAM-1. As yet, the relationship between the expression of PfEMP-1 variants and cytoadherence phenotype during CM is not well established.

**Methods:** We examined the cytoadherence phenotypes of IEs to CD36, ICAM-1, CSPG (CSA) and assessed the transcription patterns of A, B, var2, var3, var genes groups and Domain Cassettes DC8 and DC13 in parasites from children with CM and uncomplicated malaria (UM). We investigated whether the cytoadherence phenotype was related to a specific transcription profile of Pfemp-1 variants.

**Results:** We found that parasites from CM patients bind more to CD36 than those from UM patients, however no difference was observed in binding ability to ICAM-1 and CSPG. Parasites from CM isolates highly transcribed groups A, B, var2, var3, DC8 and DC13 as compared to those from UM isolates. The high transcription levels of var genes belonging to group B positively correlated with increased binding level to CD36.

**Conclusion:** Our data show that CM isolates bind significantly to CD36, and that this was correlated with high transcription level of group B var genes, supporting their implication in malaria pathogenesis.

**Keywords:**
*Plasmodium falciparum*, cerebral malaria, uncomplicated malaria, var genes, domain cassette, transcript abundance, cytoadherence, CD36, ICAM-1, CSPG.
Introduction

Cerebral malaria (CM) is the most serious manifestation of *falciparum* malaria. It is responsible for 15-20% of infant mortality (Idro et al. 2010). There is strong evidence that sequestration of IEs within the brain microvascular endothelia plays a key role in CM pathogenesis (MacPherson et al. 1985, Pongponratn et al. 1991). Such phenomenon protects the mature parasites from spleen-dependent clearance (van der Heyde et al. 2006). The adhesive properties of IE are attributed to *P. falciparum* Erythrocyte Membrane Protein-1 (PfEMP-1) expressed at the surface of IE (Su et al. 1995). Encoded by ~60 members of *var* genes family, Pfemp-1s are divided into three main groups (A, B, C) and three relatively conserved groups *var1*, *var2*, and *var3* (Baruch et al. 1995, Kraemer and Smith 2006, Su et al. 1995).

It has been shown that groups A and B *var* genes are differentially transcribed in severe malaria isolates compared with parasites from uncomplicated malaria (UM) or asymptomatic infections (Jensen et al. 2004, Kyriacou et al. 2006, Rottmann et al. 2006). Recent studies have shown that PfEMP-1s are characterized by 23 conserved architectural motifs named domain cassettes (DC), and that DC8 and DC13 are of clinical significance (Rask et al. 2010). The transcription levels of these DCs are higher in parasites from severe malaria, including CM, than in those from UM (Bertin et al. 2013, Lavstsen et al. 2012). Moreover, *P. falciparum* parasites selected for binding to human brain microvascular endothelial cells (HBMEC) express high levels PfEMP-1 DC8 genes (Claessens et al. 2012), and plasma from malaria convalescent children exhibited higher antibody recognition profiles to HBMEC-selected parasites than unselected ones, suggesting the importance of PfEMP-1 motifs in building protective immunity to clinical malaria (Avril et al. 2012, Claessens et al. 2012).

In vitro experiments identified a few endothelial receptors that participate in the adherence of IEs to the microvasculature. CD36 and Inter Cellular Adhesion Molecule-1 (ICAM-1) are the most commonly used by patient parasites (Chaiyaroj et al. 1996). As yet, no definitive receptor implicated in the pathogenesis of malaria has been named, apart from chondroitin sulphate proteoglycan (CSPG) in the case of parasites isolated from pregnant women (Salanti et al. 2003). CD36 has been presented as a major receptor involved in the cytoadherence for *P. falciparum* isolates (Ho et al. 1991, Newbold et al. 1997). Few studies have found no difference in CD36-binding patterns between parasites from severe malaria and those from UM patients (Heddini et al. 2001, Mayor et al. 2011, Rogerson et al. 1999), others have reported an association between UM and high binding level to CD36 (Newbold et al. 1997, Ochola et al. 2011). Postmortem brain examination has reported high levels of ICAM-1, which co-localized with IEs in cerebral blood vessels (Turner G. D. et al. 1994). However, other studies did not detect any difference in binding to ICAM-1 in parasite isolates from severe or mild cases (Heddini et al. 2001, Rogerson et al. 1999), others suggested an association between disease severity and ICAM-1-binding (Newbold et al. 1997, Ochola et al. 2011).
The implication of CD36 and ICAM-1 in the pathogenesis of CM, and their roles in combination with PfEMP-1 variants expression needs to be clarified. We combined binding phenotype examination by static binding assay and transcript abundance analysis of parasites freshly collected from Beninese children with CM or UM to investigate the relationship between parasite PfEMP-1 variants expression and binding phenotypes to CD36, ICAM-1 and CSPG.

Materials and methods

Ethics statement

This study was approved by the ethical committee of the research Institute of applied biomedical sciences, Cotonou, Benin (N°006/CER/ISBA/12 and N°21/CER/ISBA/13).

Study design, malaria patients

This study was conducted in Cotonou in southern Benin, during the 2012 and 2013 malaria transmission periods (June-September and May-July, respectively). This area is characterized by two rainy seasons during which malaria infection is mainly caused by *P. falciparum*, with approximately 33 infected bites per individual annually (Akogbeto 1995).

Children under five years of age presenting to Hôpital Mère-enfant de la lagune (HOMEL), Centre National Hospitalier Universitaire Hubert Koutoucou Mega (CNHU-HKM), or to Hôpital Suru-Léré were screened on arrival by the malaria rapid diagnostic test (DiaQuick Malaria *P. falciparum* Cassette, Dialab®) and were recruited in the study if they presented CM or UM. CM was defined as a microscopically confirmed *P. falciparum* infection and a Blantyre coma score ≤ 2, with the exclusion of any other cause of coma. UM was defined as a *P. falciparum* parasitemia accompanied by fever, headache or myalgia without signs of severity and/or evidence of vital organ dysfunction, as defined by the WHO. After obtaining informed and written consent from parents or guardians, 2 to 4 ml of venous blood samples were collected into tubes containing Citrate Phosphate Dextrose Adenine (CPDA). *P. falciparum* infections were confirmed by Giemsa-stained thick blood smears and parasitemia was recorded as the number of parasites/µL of blood. After diagnosis, all participants were treated according to the national malaria program policy.

Sample preparation and parasite culture

Fresh parasitized blood samples were treated as follows: plasma and buffy coat were removed from the blood, and erythrocytes pellet was washed twice in RPMI-gentamicin. One hundred µl were conserved in TRIzol® reagent (Life Technologies), and stored at -80°C for RNA extraction. Another 100 µl were spotted on Whatman 3MM filter paper, and stored at room temperature for DNA extraction and *msp* genotyping. Five hundred µl were cultured in RPMI1640 medium (LONZA). Parasites were cultured for less than 48 hours until they...
reached mature stage, then they were purified using magnetic columns (MACS, Miltenyi Biotec) for binding assay.

**Binding assay**

Static binding assays were carried out for 27 UM and 38 CM fresh isolates at 20 % parasitemia in 100 x 15-mm Petri dishes as described (Bigey et al. 2011). Twenty µl of each recombinant protein diluted in PBS were spotted on Petri dish and incubated overnight at 4°C in a sealed humid container. The following recombinant proteins were used: CSPG at 5 µg/ml (Decorin, Sigma-Aldrich, France); CD36 and ICAM-I at 10 µg/ml (R&D, Lille, France). Spots were then blocked with 3 % bovine serum albumin (BSA) in PBS for 30 minutes at 37°C in a humid chamber. Purified parasites were washed with PBS, centrifuged for 10 min at 2500 rpm, resuspended in 3 % BSA-RPMI, and 20 µl of parasite suspension were added to each spot, and incubated for 12 minutes at room temperature. Unbound erythrocytes were washed off gently with PBS using an automated washing system. Bound cells were fixed with 1.5 % gluteraldehyde in PBS for 10 minutes, Giemsa-stained, and counted microscopically. The number of IEs bound to each receptor was determined by counting 10 fields using a 40x objective, and expressed as number of IEs bound per mm². For each sample, the binding assay was done in duplicate to all proteins in the same plate. All results shown are averages of the binding level of duplicate spots per sample. As a negative control, 1 % BSA was used to assess nonspecific binding.

**Parasite RNA extraction and cDNA synthesis**

cDNAs were synthesized from 36 UM and 51 CM thawed samples preserved in TRIzol® reagent, following the manufacturer’s instructions. RNAs were then treated with DNase I (BioLabs), and absence of genomic DNA was assessed by 40 cycles of RT-PCR with fructose-bisphosphate aldolase primers (Salanti et al. 2003). Reverse transcription of RNA was performed by Thermoscript® (Life Technologies) with random hexamers and oligo dt primers, following manufacturer’s recommendations.

**Transcript abundance of var genes**

Individual RT-PCR reactions were carried out on a Rotorgene® thermal cycler (Corbett Research) using 1 X SYBR Green® (Bioline), and 1.25 µM of specific primer pairs for group A (A1, A2, A3), group B (B1), var2csa and var3 of var genes. A set of primers to target DC8 and DC13 was also used (Lavstsen et al. 2012). The endogenous controls used were fructose-bisphosphate aldolase and seryl-tRNA synthetase (Salanti et al. 2003). Cycling conditions were 95°C for 1 minute, followed by 40 cycles of 95°C for 30 seconds, 54°C for 40 seconds, and 68°C for 50 seconds. Data were analyzed by the Rotorgene software for which the threshold cycle was set at 0.025. The specificity of each PCR product was verified by a specific melting curve for each pair of primers. The transcript abundance was calculated by relative quantification using two housekeeping genes \((\Delta C_T)_{\text{var_primer}} = C_T \text{ var_primer} - C_T \text{ DC8, DC13})
average_control primers). Transcript units were then determined as \( Tu = 2^{(\Delta Ct)} \), as described by (Lavstsen et al. 2012).

**Genomic DNA extraction and Pfmsp1 & Pfmsp2 genotyping**

Genomic DNA was extracted from 36 UM and 51 CM filter papers using the Chelex 100 resin method, as described (Plowe et al. 1995). Specific primers were used to amplify block 2 Pfmsp1 and block 3 Pfmsp2 by PCR (Snounou et al. 1999). Multiplicity of infection (MOI) was determined as the highest Pfmsp1 or Pfmsp2 allele number found in each sample.

**Statistical analysis**

Binding data were analyzed qualitatively from the number of IEs bound to a given receptor minus the number of IEs bound to BSA. Then, for each receptor, isolates were divided into two groups of low binders and high binders, according to the median binding level of CM and UM samples together. The distribution of low and high binders to a given receptor in both CM and UM groups was compared by Chi-square test. The Mann-Whitney U test was used to compare the transcript abundance of var group genes and DC8 and DC13 between CM and UM groups. Correlation between binding profile and transcript level of dominant var gene which was transcribed in more than 50% of samples was achieved by the Spearman’s rank test. Data were plotted and statistical tests were performed by GraphPad, V5 (Prism software). Differences of each comparison were statistically significant when \( P \)-value <0.05.

**Results**

A total of 88 children were enrolled, of those 52 children presented with CM and 36 with UM. Of 52 children with CM, 21 presented CM alone, while the others had overlapping clinical syndromes: 25 children also presented with severe anemia (SA), and 16 with hyperparasitemia (HP) (10 children with CM presented both SA and HP). The clinical and biological features of these patients are summarized in Table 1. There was no difference in sex ratio, age, and mean body temperature between the two groups (all \( P > 0.05 \)). The hemoglobin level was lower (\( P < 0.0001 \)) while parasite density was higher in the CM as compared to the UM children (\( P < 0.01 \)). All children with UM survived, while 19 patients (36.5%) with CM died. Multiple infections were recorded in both groups with a mean range of multiplicity of infection of 3.4 clones in CM and 2.2 clones in UM infections (\( P < 0.0001 \)).

**Binding profiles**

Due to the low parasitemia of UM isolates and probably to pre-collection anti malarial treatment for CM children, some parasites cultures failed to reach mature stages. Therefore, we could only investigate the binding phenotypes for 27 of the 36 UM and 38 of the 52 CM isolates. We found that all isolates, except two (63 of 65), bound to both CD36 and ICAM-1, and 48 isolates bound weakly to CSPG (Table 2).
The distribution of low and high binders isolates to a given receptor in both CM and UM groups shows that the prevalence of CM-high binders to CD36 was significantly higher than UM-high binders ($P = 0.007$), while no significant difference in the binding to ICAM-1 ($P = 0.4$) or CSPG ($P = 0.4$) was noted between the parasites from these two clinical groups (Figure 1).

**Transcript levels of var genes groups and DC8 & DC13**

Transcription level (Tu) of A, B, var2, var3, DC8 and DC13 var genes were analyzed by RT-qPCR in 36 UM and 51 CM isolates. Group A, B, var2, var3, DC8 and DC13 were significantly transcribed to a higher level in CM than in UM isolates (Table 3, Figures 2 and 3). However, no significant difference in the transcription levels of the A2, DBLy4/6 and DBLaCIDRα genes was observed between CM and UM isolates.

**Correlation between binding phenotype and transcription level of var genes**

We examined the relationship between binding profiles and transcription levels of var genes groups and DCs for each isolate considering only highly transcribed genes determined as “dominants transcripts”.

Group B var genes were the predominant transcripts in 51% of samples. Tu of group B var genes was positively correlated with the binding level to CD36 ($r = 0.46$, $P = 0.005$), but not to ICAM-1 ($r = 0.05$, $P = 0.7$), nor to CSPG ($r = 0.1$, $P = 0.2$). Group A var genes were the predominant transcripts in 49% of samples, and no significant correlation was detected between the Tu and the binding levels to CD36 ($r = 0.2$, $P = 0.2$), ICAM-1 ($r = 0.1$, $P = 0.4$) and CSPG ($r = -0.02$, $P = 0.1$).

Likewise, DC8 and DC13 genes were predominant in 78.5% and in 21.5% of samples respectively. Tu of DC8 and DC13 were not correlated with binding levels to CD36 ($r = -0.005$, $P = 0.9$, and $r = 0.09$, $P = 0.7$, respectively), ICAM-1 ($r = 0.1$, $P = 0.4$, and $r = -0.06$, $P = 0.8$, respectively) and CSPG ($r = 0.05$, $P = 0.7$, and $r = -0.1$, $P = 0.5$, respectively).

**Discussion**

Cytoadherence of IEs to cerebral microvasculature cell lining is considered to be a major factor in CM pathogenesis (MacPherson et al. 1985, Raventos-Suarez et al. 1985). This mechanism is probably mediated by PFEMP-1 variants expressed at the surface of IEs (Baruch et al. 1996). However, the ligand-receptor interactions leading to IE-binding to brain microvascular endothelium are not well understood.

In order to investigate the potential role of PfEMP-1 variants in cytoadherence to host receptor, we used clinical isolates from individuals with cerebral or uncomplicated malaria to
examine the cytoadherence phenotypes to ICAM-1, CD36 and CSPG, and analyzed the transcription profiles of the var genes.

We show that CD36 is the main binding receptor used by clinical isolates irrespective of the degree of disease severity, in agreement with earlier findings considering a strong implication of CD36 in the cytoadherence phenomenon (Chaiyaroj et al. 1996, Ho et al. 1991, Newbold et al. 1997). By contrast to previous studies, CD36-binding level was higher in CM than in UM isolates, suggesting an important role of CD36 in the parasite sequestration during CM.

Conversely, in agreement with previous studies using static binding assay (Heddini et al. 2001, Rogerson et al. 1999), binding phenotypes to ICAM-1 or CSPG were similar in CM and UM isolates. A higher binding level to ICAM-1 in CM than in UM isolates was seen under flow conditions, as probably flow assay better simulates physiological wall shear stress, under which ICAM-1 promotes IEs rolling and binding (Ochola et al. 2011). Moreover, it has been shown that *P. falciparum* isolates, including those from CM, bind to CD36 10-fold higher than to ICAM-1, and that 80% of ICAM-1-selected IEs bind to CD36, suggesting an association of CD36 with disease pathogenesis as it facilitates the binding of a high proportion of parasites throughout the body (Udomsangpetch et al. 1996). In addition, under flow conditions, CD36 offers greater and more efficient adherence property than ICAM-1 (Cooke et al. 1994). These results are in agreement with ours as a higher binding level of IEs to CD36 than ICAM-1 has been observed.

Despite the fact that CD36 is poorly expressed in brain vascular endothelia, Turner et al. reported low but significant association between CD36 expression and IEs sequestration in brain vessels (Turner G. D. et al. 1994). Moreover, IEs binding to cerebral endothelial cells may take place indirectly through platelets accumulated in the brain vasculature of CM patients (Wassmer et al. 2004). Platelets express a high level of CD36; this may promote IEs binding and sequestration in the brain, despite the low CD36 expression on brain microvascular endothelium. These cells act as a bridge between brain endothelium and IEs via CD36-binding. This mechanism of sequestration possibly reorients the binding properties of parasites, contributing to CM pathogenesis (Wassmer et al. 2004).

PfEMP-1 variants expressed on the surface of IEs determine the cytoadherence properties of parasite populations (Baruch et al. 1996). These proteins are well-described ligands, and studies have shown that they recognize and interact with more than one receptor independently of IEs subpopulations. One PfEMP-1 variant may allow the cytoadherence to more than one host receptor through its semi-conserved N-terminal head structure DBL1α and CIDR1α (Chen et al. 2000). Likewise, DBLβC2 and DBLβ3 bind ICAM-1 (Bengtsson et al. 2013, Oleinikov et al. 2009), the tandem NTS-DBLα-CIDRα of varO is essential for IEs binding and rosette formation (Vigan-Womas et al. 2012). CIDRα1 of DC8 and DC13 var genes is a potential ligand of endothelial protein C receptor (Turner L. et al. 2013).
In our study, the transcription of *Pfemp-1* variants including groups A, B, *var3*, DC8, and DC13 was clearly up-regulated in CM parasites compared to UM ones, in agreement with previous studies (Bertin et al. 2013, Doritchamou et al. 2012, Kyriacou et al. 2006, Lavstsen et al. 2012, Rottmann et al. 2006), and supports the implication of these genes in CM pathogenesis. *Var2csa*, the gene encoding VAR2CSA, is transcribed to a much higher level in isolates from pregnant than from non-pregnant women (Salanti et al. 2003, Tuikue Ndam et al. 2005). Some CM isolates expressed *var2csa* at low but higher level than UM isolates, but binding to CSPG was consistently very weak and similar in both groups, indicating that VAR2CSA does not take part in children’s malaria pathogenesis. The higher *var2csa* expression level in selected CM isolates may due to the higher parasitemia and higher MOI of children presenting with CM compared to those with UM.

The transcription level of group B genes positively correlated with the binding level of IEs to CD36, confirming the earlier findings that parasite ligands for CD36 are PfEMP-1 variants encoded by groups B and C *var* genes through their CIDR1α domain (Robinson et al. 2003; Smith et al. 1998). Functional analysis using mutagenesis modifications in the M2 region of CIDR1α linked CD36 binding ability to specific CIDR1α residues (Gamain et al. 2001). Recombinant protein of CIDRa1.1 of DC8 also inhibits the binding of IEs to brain endothelium cells, suggesting a role of this domain in binding phenotypes involved in cerebral malaria (Avril et al. 2012).

The transcription levels of DC8 and DC13 were higher in CM than in UM isolates, and were not correlated with the binding levels to CD36, ICAM-1, nor CSPG. DC8, DC13 are part of few genes from groups B and A, respectively. It is likely that our study did not involve enough of isolates expressing DC8 and DC13, and lacked of power to detect such correlation between the binding phenotypes and the transcription patterns.

In conclusion, CM isolates bind more to CD36 than UM isolates, and no difference was observed in binding levels to ICAM-1 and CSPG. CM isolates transcribed groups A, B, *var2*, *var3*, DC8 and DC13 at higher levels than UM isolates, and the high transcription level of group B *var* genes correlated with increased binding level to CD36. These results support the role of these PfEMP1 variants in CM. For its survival, the parasite develops sophisticated mechanism to bind host receptors and establish chronic infections. Hence, identifying parasites ligands involved in IEs sequestration during cerebral malaria could be targeted to prevent the disease.

**Notes**

**Acknowledgments.** We would like to thank all children and their parents for participating in this study. We are grateful to the pediatricians and the nurses in HOMEI, CNHU-HKM and Suru Léré for their help in sample collection. We thank Prof Ayvivi and Dr Layla for facilitating fieldwork. UMR216 is part of the Laboratory of Excellence GR-Ex, reference ANR-

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Potential conflicts of interests. All authors declare that they have no competing interests.
Table 1. Parasitological and Clinical Characteristics of Children Enrolled in Cotonou, 2012-2013. Comparisons between *P. falciparum* isolates from children presenting with cerebral (CM) and uncomplicated malaria (UM) were achieved by the Mann-Whitney U test

<table>
<thead>
<tr>
<th></th>
<th>Cerebral Malaria (N = 52)</th>
<th>Uncomplicated Malaria (N = 36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio (female/male)</td>
<td>23/29</td>
<td>18/18</td>
<td>0.2</td>
</tr>
<tr>
<td>Age (months), median [range]</td>
<td>42 [5-72]</td>
<td>33 [8-58]</td>
<td>0.09</td>
</tr>
<tr>
<td>Parasitemia (P/µl), median</td>
<td>75,806 [889-3,600,000]</td>
<td>49,453.5 [1,164-246,857]</td>
<td>0.01</td>
</tr>
<tr>
<td>Hemoglobin (g/dl), median [range]</td>
<td>5.15 [0.8-12.6]</td>
<td>7.45 [5.2-12.5]</td>
<td>0.0001</td>
</tr>
<tr>
<td>Blantyre score, median [range]</td>
<td>2 [0-2]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Temperature, median [range]</td>
<td>38°C [35.5°C-40°C]</td>
<td>38.5°C [36.5°C-40°C]</td>
<td>0.15</td>
</tr>
<tr>
<td>Multiplicity of infection, median [range]</td>
<td>3.4 [1-6]</td>
<td>2.2 [1-6]</td>
<td>0.0001</td>
</tr>
<tr>
<td>Number of deaths</td>
<td>19</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
**Table 2. Receptor Binding Data.** Values are the mean number of infected erythrocytes bound (range) /mm² to a given receptor minus the number of infected erythrocytes bound to BSA.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Cerebral Malaria (n = 38)</th>
<th>Uncomplicated Malaria (n = 27)</th>
<th>All Samples (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36</td>
<td>350.5 (0-2552)</td>
<td>85.1 (0-379)</td>
<td>244.0 (0-2552)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>48.3 (0.6-215)</td>
<td>39.3 (0-294)</td>
<td>45.2 (0-294)</td>
</tr>
<tr>
<td>CSPG</td>
<td>11.3 (0-62)</td>
<td>9.6 (0-72)</td>
<td>10.7 (0-72)</td>
</tr>
</tbody>
</table>

**Table 3. Transcript Abundance of var Genes from Groups A, B, var2, var3, DC8 and DC13.**

Comparisons between *P. falciparum* isolates from children presenting with cerebral (CM) and uncomplicated malaria (UM) were achieved by the Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Primers ID</th>
<th>T⁺ CM median [IQR]</th>
<th>T⁺ UM median [IQR]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1.4 [1-6.8]</td>
<td>1 [0-1.7]</td>
<td>0.01</td>
</tr>
<tr>
<td>A2</td>
<td>1.2 [1-5.3]</td>
<td>1 [1-6.3]</td>
<td>0.8</td>
</tr>
<tr>
<td>A3</td>
<td>19.6 [4.3-33]</td>
<td>6.6 [1-14.3]</td>
<td>0.02</td>
</tr>
<tr>
<td>B1</td>
<td>9.3 [1-64]</td>
<td>1 [1-2.6]</td>
<td>0.001</td>
</tr>
<tr>
<td>var2</td>
<td>1 [1-4.4]</td>
<td>1 [0-1]</td>
<td>0.001</td>
</tr>
<tr>
<td>var3</td>
<td>17.1 [1-34]</td>
<td>1.4 [1-10.4]</td>
<td>0.008</td>
</tr>
<tr>
<td>CIDRα1.1 (DC8)</td>
<td>2.11 [1-11.8]</td>
<td>1 [1-2.3]</td>
<td>0.02</td>
</tr>
<tr>
<td>DBLβ12 (DC8)</td>
<td>2.8 [1-13.9]</td>
<td>1 [1-4.2]</td>
<td>0.03</td>
</tr>
<tr>
<td>DBLγ4/6 (DC8)</td>
<td>1 [0-1]</td>
<td>1 [0-1]</td>
<td>0.4</td>
</tr>
<tr>
<td>DBLaCIDRα (DC8)</td>
<td>0 [0-1]</td>
<td>0 [0-1 ]</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Figure 1. Prevalence of high-binder isolates of children presenting cerebral (CM, blue bars) and uncomplicated malaria (UM, yellow bars) on CSPG, ICAM-1 and CD36. The difference in prevalence to a given receptor was tested by the Chi-square test; significant $P$-value is shown in the figure.
Figure 2. Transcript levels of var genes from group A, B, var2 and var3 in *P. falciparum* isolates from children presenting with either cerebral (CM) or uncomplicated malaria (UM). Transcript patterns of specific var genes were compared between groups by the Mann-Whitney U test, significant *P*-values are shown in the figure.

Figure 3. Transcript abundance of DC8 and DC13 targeted by CIDRα1.1, DBLB12, DBLα1.7, and CIDRα1.4 primers in *P. falciparum* isolates from children presenting with either cerebral (CM) or uncomplicated malaria (UM). Transcript patterns were compared between groups by the Mann-Whitney U test, significant *P*-values are shown in the figure.
Chapter 4

Discussion and Perspectives

Comparative transcriptomic analysis using microarrays is a robust technique that has improved our knowledge of the relationship between gene expression patterns and their potential roles in biological processes (Le Roch et al. 2003; Bozdech et al. 2008). Furthermore, differences in gene expression profiles between *P. falciparum* isolates and laboratory-adapted lines have been revealed (Daily et al. 2004; Ndam, et al. 2008). These variations of genes transcriptions between in vitro and in vivo conditions may associate with the biological mechanisms and the adaptation of parasites within the host or with the in vitro conditions. The host-parasite interactions that require gene activation involve different metabolic pathways essential for in vivo parasite survival, sexual differentiation for parasite transmission, parasite cytoadherence and immune evasion.

The overall aim of my PhD thesis was to gain more insights of the parasite genetic factors implicated in cerebral malaria and the potential relation of gene expression profiles of a specific subset of *var* genes with cytoadherence phenotype to a particular receptor.

In order to attain our goals we first compared the whole transcriptome of *P. falciparum* parasites isolated from children with distinct clinical manifestations, including uncomplicated malaria (UM) and cerebral malaria (CM) and from asymptomatic carriers (AM) at ring and late trophozoite stages to investigate gene transcription patterns underlying malaria pathology. As controls, gene expression profiles of 3D7-Lib line were compared with 3D7 clone. In addition, transcriptomic patterns of isolates were compared to 3D7 clone as well.

Our results revealed consistent differences in the transcription profiles of parasite isolates compared to 3D7, and between the selected 3D7-Lib compared to 3D7. Moreover, we detected important differences in gene transcription patterns between parasites from children with CM compared to those from asymptomatic children.

The up-regulated genes that could be implicated in CM pathogenesis are those that code for proteins involved in cytoadherence of IEs to brain endothelial receptors and antigenic variation that would allow the parasites to avoid antibodies responses. This result was supported by gene ontology test of up-regulated genes in CM and 3D7-Lib which showed over representation of pathogenesis, regulation of cell adhesion, cytoadherence to microvasculature, erythrocytes aggregation and antigenic variation.

The majority of 3D7 and 3D7-Lib transcripts that were not detected in our arrays were also found also absent in other studies (Le Roch et al. 2003; Bozdech et al. 2003), suggesting un-transcribed ORFs in cultured parasites. However, many of those transcripts were found in our transcriptome data of field isolates and in the transcriptome of other clinical isolates (Vignali et al. 2011), suggesting that these genes are not useful for in vitro-survival of asexual-stage parasites. Of the undetected transcripts in ring and trophozoite stages of 3D7 clone variant surface antigen (VSA) families (PfEMP-1, RIFIN, STEVOR, and
PFMC2TM) and unknown hypothetical proteins. The variable expression profiles of VSA families in parasite isolates is probably due to different gene transcriptions between isolates, to the high number of multiclonal infections in field isolates (1-7) as well as to the fact that six isolates were pooled to obtain sufficient quantity of RNA for hybridization. It is also likely that these represent variants that do not exist in 3D7.

Furthermore, we compared our transcriptomic outcomes of parasite isolates and 3D7 at ring and late trophozoite stages to transcriptomic data obtained by RNA sequencing of 3D7 at corresponding stages (Bartfai et al. 2010) and observed significant correlation (p = 2.2e-16, Kendall test). These outcomes support the efficiency of the array hybridization assay in providing coherent data that are comparable to RNA sequencing method and demonstrate the conserved nature of fundamental biological processes such as metabolic pathways and cell cycle, within in vivo and in vitro conditions. This higher correlation is concordant with the predominance of ring stage parasites in the peripheral blood circulation of infected individuals, and with the changes in gene transcription profiles during the progression to the late trophozoite stage.

Moreover, differentially transcribed genes in CM isolates compared to AM parasites suggests an association with parasite-virulence factors. Our arrays detected 99 up-regulated genes in CM compared to AM parasites in late trophozoite stage. Twenty eight genes code for exported proteins with a majority of VSA encoding genes such as var, rif, phist, and surfin. These genes are potentially implicated in parasite cytoadherence and immune avoidance. Interestingly, group A and group B var genes such as Pf11_0521, Pf13_0003, Pfi1820w, PfD1235w, PfF1595c were over expressed in CM isolates and in 3D7-Lib line, whereas PfB0020c and PfD0020c were up-regulated only in CM isolates. Pf11_0521 displays DBLα-1.7 and CIDRα1.4, while PfD0020c displays CIDRα-1.1, DBLβ12 and DBL-γ6. These architectural domains characterize Domain Cassettes DC13 and DC8, respectively, and were found to be transcribed at tremendous levels in cerebral malaria isolates from Tanzanian (Lavstsen et al. 2012) and Beninese children (Gwladys et al. 2012) compared to mild cases isolates.

Likewise, DC5-containing genes such as Pf13_0003 and PfD1235w were also up-regulated in CM and 3D7-Lib. It has been shown that DC5 is associated with severe malaria (Lavstsen et al. 2012), and it is implicated in parasites adherence to the human receptor PECAM1 (Berger et al. 2013).

The transcription level of PfD0020c and that of its orthologs IT4var19, HB3var3, and IT4var7 were remarkably elevated in parasite lines selected for binding to human brain endothelial cells (HBEC) (Claessens et al. 2012, Avril et al. 2012). These genes code for DC8 and DC13-containing PfEMP-1s and directed antibodies against the NTS-DBLα1 and the NTS-DBLα1-CIDRα1 domains of HB3var3 inhibit the binding of infected erythrocytes to HBEC, supporting their role in cytoadherence (Avril et al. 2012). Recently, endothelial protein C receptor (EPCR) has been identified as a new receptor candidate for PfEMP-1s through CIDRα1.1 of DC8, suggesting the implication of EPCR-binding in malaria pathogenesis (Turner
Interestingly, anti-PfEMP-1 IgG antibodies targeting recombinant proteins representing DBLy6 and CIDRα1.1 of PfD0020c, DBLβ9 of Pf13_0003, DBLβ3, DBLy13, CIDRα1.6 of PfD1235w and CIDRα1.4 of Pf11_0521, with regard to serological recognition, showed that children in malaria endemic regions are exposed to parasites expressing these domains at an early age (Lavstsen et al. 2012). This exposure promotes the induction of the immune system and the synthesis of suitable antibodies against these variants. Moreover, DBLα1 of 3D7 Pf13_0003, which is shared with other genes, such as Pal Alto89F5 VarO, IT4/I29, and IT4var60, found to be implicated in rosetting (Albrecht et al. 2011; Vigan-Womas et al. 2008). This gene was over expressed in CM isolates and in the 3D7-Lib. The N-terminal region of DBLα1 plays a role in pseudo-rosettes formation on the surface of a fibroblast-like cell line COS and baculovirus-infected cells expressing individual PfEMP-1 domains (Rowe et al. 1997; Vigan-Womas et al. 2008).

In the present study, the up-regulation of UPS A and UPS B var groups in parasites from children with CM is consistent with findings of the other studies (Jensen et al. 2004; Kirchgatter et al. 2002; Kyriacou et al. 2006, Rottmann et al. 2006, Warimwe et al. 2012), strengthening the relationship between specific PfEMP-1 protein expression and the development of CM.

Other VSA antigens found to be up-regulated in 3D7- Lib line and/or in parasites from CM children are rif genes PfF1545w, Pf10_0403, PFC1100w, Pf11_00520, PfD1230c, Pf11_0009, phist a, b, c genes Pf14_0752, PfL2565w, MAL7P1.7, PfL1785w, MAL8P1.4, Pb0900c. The phist a Pf14_0752 was previously shown to be over expressed in parasite lines selected for binding to HBEC (Claessens et al. 2012). The rif family consists of ~150 clonally variant genes classified into two groups A- and B-type rifins, basing on their sub-cellular localization and the presence of a 25-amino acid motif within the semi-conserved region (C1) of type-A rifin (Joannin et al. 2008). The A-type RIFINs are transported to the surface of the infected erythrocyte (Khattab et al. 2008), whereas specific B-type RIFINs have been associated with the extracellular stages; merozoites and gametes (Mwakalinga et al. 2012). Most of rifin family members are situated close to var genes within the sub-telomeric regions of the chromosomes. These regions are subjected to recombination, which increases the diversity of these genes. Our results showed that in 3D7-Lib, rif Pf11_00520, Pf11_0009, PfD1230c, Pf13_0004, were concomitantly up-regulated with their neighboring var genes Pf11_0521, Pf11_0008, PfD1235w, Pf13_0003 suggesting a unique promoter controls the expression of these genes.

Furthermore, non-VSA genes such as Pf13_0010, Pf14_0010, and PfE0065w, were also found to be highly up-regulated in CM isolates. Pf13_0010 and Pf14_0010 code for GBPH2 and GBPH, respectively. These antigens are members of Maurer’s cleft proteins (Lanzer et al. 2006), either temporarily during their trafficking to the infected erythrocyte surface with PfEMP-1, RIFIN (Petter et al. 2007), SURFIN (Winter et al. 2005), STEVOR (Kaviratne et al. 2002), PfMC-2TM (Sam-Yellowe et al. 2004), or as constitutive proteins of the Maurer’s
cleft itself, as *Plasmodium falciparum* skeleton-binding protein 1 (PfSBP1) which is encoded by *PfE0065w* (Blisnick et al. 2000). This protein is shown to be involved in PFEMP-1 transporting to the erythrocyte surface (Cook, BM et al. 2006). *Pf14_0752* encodes MAHRP1 (Phist a), a Maurer's cleft resident protein that is also necessary for PFEMP-1 trafficking to the surface of infected red blood cells (Spycher et al. 2006, Spycher et al. 2008). Our findings demonstrated that the last two genes were transcribed at higher levels in the field isolates comparing to 3D7. In addition, MAHRP1 was among the 15 non-VSA up-regulated genes in parasite lines selected to adhere on HBEC (Claessens et al. 2012).

Other differentially transcribed genes in CM isolates are those which code for antigenic proteins, such as *P. falciparum* sporozoite surface threonine- and asparagine-rich protein (STARP) *Pf07_0006*, *P. falciparum* ring-infected erythrocyte surface antigen (RESA) *Pf11_0509*. These genes were found to be up-regulated in clinical isolates comparing with laboratory-adapted strains (Mackinnon et al. 2009). Some genes implicated in the transcription process, including *Pfc0805w* DNA-directed RNA polymerase II, *PfL1525c* pre-mRNA splicing factors were highly transcribed in CM isolates as compared to AM and 3D7 parasites.

Our study presented some regulated genes that were also differentially transcribed in other study (Mackinnon et al. 2009). These outcomes suggest a variable expression pattern of these genes between field isolates and 3D7 clone. In addition, these findings support the powerful of transcriptomic analysis by microarray hybridization to obtain reproducible data although different techniques had been used between laboratories. Of these genes, ring-exported protein 1 (REX1) *Pf1735c*; ring-exported protein 2 (REX2) *Pf1740;* gametocytogenesis-implicated protein (GIG) *Pf1720w;* sporozoite threonine- and asparagine-rich protein (STARP) *Pf07_0006;* lysophospholipase, putative *Pf07_0005,* conserved *Plasmodium* protein with unknown function *Pf07_0007, (Pfg27) gamete antigen 27/25 *Pf13_0011* were all up-regulated in AM and CM isolates used in our study, and were also among the top 20 over expressed genes in clinical isolates of another study (Mackinnon et al. 2009). REX1, REX2 and GIG genes are found in close distance in chromosome 9 whereas STARP, putative lysophospholipase and a conserved *Plasmodium* gene are localized within close proximity in chromosome 7, proposing a co-regulation of gene transcription due to their proximity. Likewise, three to 12 up-regulated genes in parasites from CM patients compared to AM parasites were harbored on the same chromosome at a distance ranging from 5 to 100 kb where the variability of gene transcription is likely dominated by a single promoter or the same epigenetic mechanism.

On the other hand, cytoadherence of IEs to cerebral microvasculature beds is considered as a major factor in the pathogenesis (MacPherson et al. 1985, Raventos-Suarez et al. 1985). In vitro experiments have identified several endothelial receptors that may participate in the adherence of IEs to microvasculature endothelia, and few studies attempted to identify a specific binding phenotype of parasites causing severe malaria which is unlikely or rarely
shown in uncomplicated malaria isolates. As yet, no definitive candidate has been incriminated, apart from chondroitin sulfate proteoglycan the main receptor of VAR2CSA involved in placental sequestration during malaria in pregnant women (Salanti et al. 2003). Of these receptors, CD36 and ICAM-1 are the most well investigated receptors and the most commonly used by patient parasites (Chaiyaroj et al. 1996). Our knowledge of the role of these receptors in cytoadherence of IE comes mainly from binding assays under static or flow conditions.

However, cytoadherence studies require specific experimental platform as well as particular biological material like “P. falciparum clinical isolates, parasite strains, and mammalian cells expressing the desired receptor or receptors as recombinant proteins” which make the assays difficult to perform resulting in scarce data particularly those obtained from patients with distinct clinical malaria features.

In the second part of my PhD thesis, we aimed to elucidate the potential implication of var genes variants which were found to be up-regulated in CM isolates in our microarrays analysis and may be involved in the pathogenesis of cerebral malaria by promoting increased cytoadherence to specific receptors host such as CD36, ICAM-1. To investigate whether the expression of these PfEMP-1 variants is related to the binding phenotypes to CD36, ICAM-1 or CSPG of clinical parasites isolates, we examined the binding patterns of fresh parasite isolates from Beninese children with cerebral or uncomplicated malaria to ICAM-1, CD36 and CSPG using static binding assay and analyzed the transcripts abundance of groups A, B, var2, var3, DC8 and DC13 for these isolates.

Our results show that CD36 is the major receptor used by clinical isolates regardless the degree of disease severity, consistent with earlier findings demonstrated a strong implication of CD36 in the cytoadherence of IEs (Chaiyaroj et al. 1996; Ho et al. 1991; Newbold et al. 1997). In contrary to previous studies, we report for the first time that CD36-binding levels were higher for parasites from CM that for those from UM isolates, suggesting a potential implication of CD36 in CM pathogenesis.

Furthermore, we did not observe any difference in binding level to ICAM-1 or CSPG between CM and UM parasites isolates. This result is in agreement with previous investigations where static binding assays were used (Heddini et al. 2001; Rogerson et al. 1999). However, the difference in binding levels to ICAM-1 between CM and UM isolates was only detected under flow conditions, which may simulate physiological wall shear stress, under which ICAM-1 promotes IEs rolling, reducing blood flow and providing cytoadherence (Ochola et al. 2011). Further studies are needed to strengthen this observation.

Moreover, It has been found that malaria parasites, including those from CM, bind to CD36 10 times higher than to ICAM-1, and that 80 % of IEs selected for ICAM-1-binding, adhered also to CD36, proposing an association of CD36-binding with disease pathogenesis as it assists the binding of a high proportion of parasites throughout the body, promoting
systemic infection that contributes to the severity of the disease (Udomsangpetch et al. 1996). In addition, it has been shown that under flow conditions, CD36 provides higher and more efficient adherence property than ICAM-1 (Cooke et al. 1994). These results are in agreement with ours as a high binding level of IEs to CD36 than ICAM-1 has been observed.

Despite the fact that CD36 is expressed at restricted level in vascular endothelia of the brain, a low but significant association between CD36 expression and IEs sequestration in brain vessels has been observed (Turner G.D. et al. 1994). Further, IEs binding to cerebral endothelial cells probably takes place indirectly by the mean of platelets accumulated in brain vasculature of CM patients (Wassmer et al. 2003). The platelets are known to express high level of CD36 at their surfaces, thus they would promote parasite binding even in the absence of CD36 expression on brain microvascular endothelium. Therefore, these cells would act as a bridge between brain endothelium and IEs through CD36-binding. This mechanism of cytoadherence possibly reorients the adherence properties of parasites and contributes to CM pathogenesis (Wassmer et al. 2004).

PfEMP-1 variants trafficked to the surface of IEs determine the cytoadherence patterns of parasite populations. These proteins are well-investigated ligands, and it has been demonstrated that these antigens have the ability to recognize and bind more than one receptor independently of IEs subpopulations (McCormick et al. 1997). One PfEMP-1 variant promotes the cytoadherence to more than one host receptor through its semi-conserved N-terminal head structure DBL1α and C1DR1α (Chen et al. 2000). Simirarily, DBLβ-C2 of groups B, C and DBLβ3 of group A bind ICAM-1 (Bengtsson et al., 2013; Howell et al. 2008; Oleinikov et al. 2009), the tandem NTS-DBLα-CIDRY of varO is essential for IE binding and rosettes formation (Vigan-Womas et al. 2012), and CIDRα1 of DC8 and DC13 var genes is a potential ligand of endothelial protein C receptor (Turner L et al. 2013).

In this study, we found a noticeable up-regulation in the transcription of Pfemp-1 variants including groups A, B, var3, DC8, and DC13 in CM parasites compared to UM ones which is in agreement with previous studies (Bertin et al. 2013; Doritchamou et al. 2012; Kyriacou et al. 2006; Lavstsen et al. 2012; Rottmann et al. 2006;) and supports their implications in CM pathogenesis.

It is believed that the transcription level of var2csa the coding gene of VAR2CSA is higher in isolates from pregnant than non-pregnant women (Salanti et al. 2003, Tuikue Ndam et al. 2005). Surprisingly, we found that some CM parasites transcribed var2csa at higher levels than UM parasites which might be due to the higher parasite density and higher multiplicity of infection of CM parasites comparing to UM isolates. However, the binding level to CSPG was low and no significant difference was detected between the two groups pointing out the non-important role of VAR2CSA in children malaria pathogenesis.

More interestingly, we show a correlation between the transcription level of group B and the binding level of IEs to CD36, confirming the earlier findings reporting that parasite ligands for
CD36 are PfEMP-1 variants encoded by group, B and C var genes through CIDR1α domain (Robinson et al. 2003; Smith et al. 1998). Furthermore, functional analysis using mutagenesis modifications in the M2 region of CIDR1α have linked the binding activity to CD36 to specific residues within CIDR1α (Gamain et al. 2001). CIDRα1.1 of DC8 was also found to inhibit the binding of IEs to brain endothelium cells suggesting a role of this domain in binding phenotypes involved in cerebral malaria (Avril et al. 2012).

The transcription levels of DC8 and DC13 were higher in CM than UM isolates in agreement with several findings (Bertin et al. 2013; Cleassens et al. 2012; Lavstsen et al. 2012). However, the transcription levels of these DC were not correlated with the binding levels to CD36, ICAM-1 or CSPG. DC8, DC13 are part of few genes of group B and A respectively, probably more isolates are needed to detect such correlation between the binding phenotypes and the transcription patterns.

In conclusion, the transcriptomic analysis using DNA microarrays that we achieved is a powerful technique. It allowed us to explore the majority of genetic variations between isolates from cerebral malaria, uncomplicated malaria and asymptomatic carriers at the same time. Thus a distinct gene expression profile has been identified in cerebral malaria isolates comparing to asymptomatic carriers. Of these genes, groups A and B var genes containing DC4, DC5, DC8 and DC13 are of clinical interest. These genes are probably implicated in the pathogenesis of cerebral malaria. The implication of the proteins encoded by these genes in virulence, namely PfEMP-1, arises from their ability to mediate the cytoadherence of IEs to post capillary endothelial cells.

Therefore, we applied static cytoadherence test to study the role of var group genes, which were found to be up-regulated in cerebral malaria isolates by the microarrays. We show that parasites from CM isolates bind more to CD36 than those from UM isolates and that they transcribed groups A, B, var2, var3, DC8 and DC13 higher than those from UM isolates. The high transcription level of group B var genes correlated with increased binding level to CD36. These results support the role of these PfEMP1 variants in CM pathogenesis.

However, as for any research investigation, our study has certain scientific and technical limitations. Indeed, we did not find out any differences in the expression patterns between cerebral malaria and uncomplicated malaria isolates when DNA microarray has been used. This may due to the fact that fewer numbers of isolates from each group had been used (only 18 isolates from each group, CM and UM), which might be insufficient to detect the distinct genetic variation between these two groups. Moreover, although DNA microarray approach is high throughput and cost effective, it still suffers lack of perfect processing methods and acceptable sensitivity (Tu et al. 2004).

On the other hand, studying the cytoadhesion phenotypes of P. falciparum isolates from CM and UM children using static binding assay did not show any significant difference in binding levels to ICAM-1 between these two groups. This might be due to several reasons: first, it is
possible that there is a degree of uncontrolled variation in clinical definition of malaria, resulting in over or under estimation of the binding properties of parasites to their cognate receptor, if any exists. This is due to the fact that we classified the clinical groups based on signs and symptoms presented by children at the moment of admission to hospital. Thus, cerebral malaria isolates drawn from peripheral blood at the time of enrollment, may not represent those sequestering and causing damages of host tissues. Likewise, uncomplicated malaria in children may have evolved towards severe malaria. In both cases, this may interfere with the relationship between isolate characteristics and disease classification.

Moreover, the multiplicity of infection (MOI), where each parasite population may exhibit a specific binding phenotype, is an additional confounding factor. In our cytoadherence study, the MOI for CM isolates was 3.4 clones while it was 2.2 clones for UM. If one clone displays a specific adhesion property, and sequesters in brain microvasculature endothelia leading to CM, while another clone circulates in the peripheral blood representing different binding phenotype at the moment of sampling, then the observed phenotype would not match the real clone causing cerebral malaria.

Finally, static binding system employed in our study does not necessarily reflect the intensity of ligand-receptor that occurs in vivo. This model may not confer the appropriate receptor conformation, accessibility or mobility thereby the number of IEs bound in vitro may not represent the real proportion of parasites that sequester in vivo. The development of new binding assays closely mimicking physiological ligand-receptor interaction will provide relevant knowledge on receptor affinity to IEs.

As perspectives, it would be interesting to pursue the exploring of the role of VSA and non-VSA genes, found to be upregulated in CM isolates by our transcriptomic analysis, in the pathogenesis of cerebral malaria. This could be achieved by the production of recombinant proteins and their corresponding antibodies, which would allow as to:

- Evaluate the capacity of these proteins to induce a specific antibody response (ELISA).
- Conduct binding assays (static and flow) to analyse the cytoadherence phenotypes of these proteins to endothelial receptors such as CD36 and ICAM-1.
- Carry out inhibition binding assays to test the ability of specific antibodies to abolish the binding of these proteins to the tested receptors.

Inhibition the binding of parasite ligand to its preferred/specific receptor by specific antibodies or suitable agonist molecules could be an efficient strategy to prevent severe complication of P. falciparum infections, such as cerebral malaria.
Reference


Calderwood MS, Gannoun-Zaki L, Wellem TE, Deitsch KW. 2003. Plasmodium falciparum var genes are regulated by two regions with separate promoters, one upstream of the coding region and a second within the intron. J Biol Chem 278: 34125-34132.


Cummings JF, Spring MD, Schwenk RJ, Ockenhouse CF, Kester KE, et al. 2010. Recombinant Liver Stage Antigen-1 (LSA-1) formulated with AS01 or AS02 is safe, elicits high titer antibody and induces IFN-gamma/IL-2 CD4+ T cells but does not protect against experimental Plasmodium falciparum infection. Vaccine 28: 5135-5144.


Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, et al. 2004. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in


Le paludisme, présent particulièrement en Afrique, peut être responsable d’une maladie très grave, le neuropaludisme qui se caractérise par une phase de coma. Les jeunes enfants sont particulièrement vulnérables aux accès palustres car ils ne disposent pas d’une immunité naturelle suffisante. Celle-ci se constitue au fil des années et des infections palustres successives.

But du projet : Pour aider au développement d’un vaccin contre le paludisme grave, notre équipe se propose d’étudier les accès graves du paludisme au niveau des parasites.

Conduite de l’étude:

- Si vous acceptez que votre enfant participe à notre étude, nous lui prélèverons une petite quantité de sang veineux dans un tube alors qu’il est malade.

- Le sang servira à caractériser les parasites du paludisme au laboratoire du CERPAGE. Aucune autre étude ne sera réalisée sur le sang de votre enfant.

- Votre enfant sera normalement pris en charge par le service de pédiatrie du CNHU. Le projet n’intervient pas dans le traitement.

- Pour vous remercier de votre aide dans ce projet, une partie du traitement sera pris en charge sous forme de médicaments à récupérer gratuitement à la pharmacie du CNHU, ainsi que l’examen de goutte épaisse.
Participation volontaire/Abandon : Votre participation est totalement volontaire et ne vous coûtera rien. Vous pouvez choisir de ne pas participer ou d’arrêter à tout moment. Ceci n’aura aucun effet sur votre prise en charge par l’hôpital.

Confidentialité : Toutes les informations recueillies resteront confidentielles. Les observations médicales seront collectées uniquement par votre médecin ou ses assistants. Votre nom ne sera pas utilisé pour identifier vos échantillons. Votre nom, ainsi que toute autre information confidentielle, ne seront pas utilisés dans un rapport écrit.

Questions : Si vous avez une question au sujet de l’étude, vous pouvez contacter le médecin de votre enfant ou le principal responsable de cette étude.

Médecin : Pr Ayivi, service de pédiatrie, CNHU, Cotonou, Bénin
Responsable de l’étude au Bénin : Nicaise T. NDAM, IRD, 08 BP 841, Cotonou ; Email : Nicaise.Ndam@ird.fr
Responsable de l’étude en France : Rachida Tahar UMR 216 Université Paris Descartes 4, Avenue de l’observatoire Paris 75006 Tel 00331 53 73 99 33 email Rachida.Tahar@ird.fr
Informed Consents

Projet: FPPG
Etude des facteurs parasitaires impliqués dans le paludisme grave
UMR 216- CERPAGE- ISBA

CONSENTEMENT ECLAIRE

J’ai pris connaissance de l’information ci-dessus, que l’on m’a expliquée.
Je l’ai comprise.
J’ai eu l’occasion de poser des questions.
Une réponse satisfaisante a été apportée à toutes mes questions.
J’accepte délibérément que mon enfant participe à l’étude.
J’accepte que le prélèvement de sang de mon enfant soit conservé à l’Institut de Recherche pour le Développement pour des études complémentaires.
J’ai reçu une copie de la note d’information sur l’étude.
Je comprends que si j’ai des questions au sujet de cette étude, ou si je décide d’abandonner l’étude, je peux contacter mon médecin ou le principal responsable.

Date :
Le formulaire de consentement a-t-il été lu au participant ? Oui / Non
Nom de l’enfant : Nom du parent :
Signature du parent :

(Empreinte de l’index si la personne ne peut pas signer)

Nom de la personne qui a recueilli le consentement :
Signature de la personne qui a recueilli le consentement :
Projet: FPPG
Étude des facteurs parasitaires impliqués dans le paludisme grave
UMR 216- CERPAGE- ISBA

Fiche d’inclusion (Accès Simple) – à remplir par le médecin de l’hôpital

Date : _____________________ Lieu : _____________________
Nom de la personne remplissant le questionnaire : ______________________________

IDENTIFICATION

NOM et PRENOM de l’enfant : _________________________________________________
NOM et PRENOM des parents : _________________________________________________
Age : ________________________ (ans + mois) Sexe : F M
Ethnie : ___________________
Quartier d’habitation: ___________________

HISTORIQUE DU PALUDISME DE L’ENFANT :
L’enfant a-t-il déjà été hospitalisé pour un accès palustre grave ? OUI NON
Si oui : combien de fois ? ______________________________
date de la dernière hospitalisation : ________________________

L’enfant a-t-il pris des antipaludiques au cours des dernières semaines ? OUI NON
Si oui, quel(s) médicament(s) ? ______________________________

L’enfant prend-il une prophylaxie antipalustre ? OUI NON
Si oui : quel médicament ? ______________________________
à quelle posologie ? ______________________________

L’enfant dort-il sous moustiquaire ? OUI NON

PALUDISME ACTUEL :

Depuis quand l’enfant a-t-il de la fièvre ? ______________________________
L’enfant a-t-il eu des convulsions ? ____________________________

L’enfant a-t-il reçu un traitement avant de venir à l'hôpital ? OUI NON

Si oui, lequel ? ______________________________________________

A quelle posologie ? __________________________________________

ETAT CLINIQUE (À REMPLIR PAR LE SOIGNANT) :

T° axillaire ou anale (à préciser): _________________________

Décrivez en quelques lignes l’état clinique de l’enfant à son arrivée à l’hôpital :

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Diagnostic : ............................................................................................................................
Projet : FPPG

Etude des facteurs parasitaires impliqués dans le paludisme grave

UMR 216- CERPAGE- ISBA

Fiche d’inclusion (Paludisme cérébral) – à remplir par le médecin de l’hôpital

Date : _____________________  Lieu : _____________________

Nom de la personne remplissant le questionnaire : _______________________________

IDENTIFICATION

NOM et PRENOM de l’enfant : _________________________________________________

NOM et PRENOM des parents : _______________________________________________

Age : ______________________ (ans + mois)  Sexe : F  M

Ethnie : _____________________

Quartier d’habitation: ______________________

HISTORIQUE DU PALUDISME DE L’ENFANT :

L’enfant a-t-il déjà été hospitalisé pour un accès palustre grave ? OUI  NON

Si oui : combien de fois ? ________________________________

date de la dernière hospitalisation : _______________________

L’enfant a-t-il pris des antipaludiques au cours des dernières semaines ? OUI  NON

Si oui, quel(s) médicament(s) ? ________________________________

L’enfant prend-il une prophylaxie antipalustre ? OUI  NON

Si oui : quel médicament ? ________________________________

à quelle posologie ? ________________________________

L’enfant dort-il sous moustiquaire ? OUI  NON

PALUDISME ACTUEL :

Depuis quand l’enfant a-t-il de la fièvre ? ________________________________
L’enfant a-t-il eu des convulsions ? ____________________________

L’enfant a-t-il reçu un traitement avant de venir à l’hôpital ? OUI NON

Si oui, lequel ? ________________________________________________

A quelle posologie ? __________________________________________

**ÉTAT CLINIQUE (À REMPLIR PAR LE SOIGNANT) :**

T° axillaire ou anale (à préciser): ____________________________

L’enfant est-il comateux ? _______________ A-t-il des convulsions ? _______________

Calcul du score de Blantyre :

<table>
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<th>Mouvement des yeux</th>
<th>Cotation</th>
<th>Résultat</th>
</tr>
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<td>Dirigés (suit le visage de la mère)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Non dirigés</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Réponse verbale</th>
<th>Cotation</th>
<th>Résultat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appropriée</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Inappropriée ou gémissements</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aucune</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meilleure réponse motrice</th>
<th>Cotation</th>
<th>Résultat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localisation du stimulus douloureux</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Retrait du membre à la douleur</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Réponse non spécifique ou absente</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| **Total** | **0 - 5** |
**PARACLINIQUE**

| FS DP  
| GLYCEMIE |
|---|---|

| Ponction Lombaire | GB  
| Protéino-rachie  
| Glyco-rachie |

| NFS | Taux d’Hb  
| VGM  
| TCMH  
| Leucocytes |

Autre bilan paraclinique:............................................................................................................
TRAITEMENT
- Quinine ...........................................................................................................................................
   Nombre de jour IV ………
   Nombre de jour PER OS ……
- Autre antipaludiques ..........................................................................................................................
- SG 10% [ ]
- SG 30% [ ]
- Transfusion [ ]
- Anticonvulsants [ ] préciser ...........................................................................................................
- Autres traitements [ ] ...........................................................................................................................

EVOLUTION
- Evaluation du Score de Blantyre après quininie : H2 H4 H6 H12
  J1 J2 J3 J7

Marquer le délai retour à la conscience ...........
- AUTRE PROBLEME (Hémolyse, insuffisance rénale …)

- Période et durée d’hospitalisation ..................................................................................................

Exécut [ ]
Sortie contre avis médical ou évasion [ ]

Décès [ ]

Décrivez en quelques lignes l’état clinique de l’enfant à son arrivée à l’hôpital :
.................................................................................................................................................................
.................................................................................................................................................................
.................................................................................................................................................................
Diagnostic: