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A Slightly Decreased Humoral Response and Higher Proportion of Female Gametocytes in Dry Season Infection in Malian Children

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Abstract

Malaria transmission is highly seasonal in Bandiagara, Mali. During the high transmission season, children experience up to four clinical episodes of malaria. Malaria immunity increases seasonally due to repeated exposure to P. falciparum. The molecular mechanisms underlying the dynamics of host immunity and its influence on parasite gene expression between seasons is unclear. In this study we analyzed the gene expression profiles of hosts and parasites in children living in Bandiagara with symptomatic malaria. We simultaneously sequenced host and parasite mRNA using dual-RNA-sequencing of 20 blood samples collected from 10 children with symptomatic falciparum malaria during the wet and dry seasons. We generated 75-119 million reads and found similar gene expression profiles in hosts and in parasites between seasons. In addition, we observed an increased in the proportion of plasma cells in wet season. Moreover, expression of *EPHB1* also increased in this season. The *EPHB1* gene is expressed in dendritic cells that promote adaptive immunity. For the parasite, we found similar developmental stages between seasons except an increase in the proportion of female gametocytes in the dry season. Our data suggest that the host and parasite profiles are overall similar during the wet and dry seasons, with only slight differences possibly associated with adaptative immunity. This suggests a novel paradigm of gene expression of *P. falciparum* in the dry season.

Keywords: Plasma Cells, *EPHB1 Gene*, Immunoglobin Complex, Host-Parasite Interactions, *P. falciparum*, Dual-RNA-sequencing, Seasonality.

List of Abbreviations: BCR: B Cells Receptors; cTfh: circulating T follicular cells, EPHB1 : Ephrin type-B receptor 1, Tfh: T Follicular Cells; MBCs: Memory B Cells; PfEMP1: *Plasmodium falciparum* Erythrocyte Membrane Protein 1; IGLV3-10: Immunoglobulin Lambda Variable 3-10; IGLV1- 40: Immunoglobulin Lambda Variable 1-40; IGLV3-27: Immunoglobulin Lambda Variable 3-27

Introduction

Several programs and strategies have been implemented over the past decades to better control malaria in sub-Saharan Africa, thereby leading to a decrease in the burden of the disease. However, this achievement has stalled according to the last reports of the World Health Organization [1]. The complexity of host-parasite interactions [2] and environmental factors [4] that favor *Plasmodium* transmission represent barriers to further decreases as well as the potential eradication of the disease. Populations living in malaria endemic areas gradually develop immunity to *P. falciparum* over years of exposure. Initially, children develop immunity to severe malaria and later to the uncomplicated infection [4]. Indeed, children living in endemic areas have a low level of Memory B cell IgG [5] that expands in correlation with age and exposure [6]. Acquired immunity during infection wanes drastically in the absence of continued exposure [7]. Cellular and molecular mechanisms of this immunity are not very well elucidated, and this knowledge gap constitutes a major hurdle in our ability to prevent the occurrence of the disease and to develop an effective vaccine.

Clinical manifestations of malaria result from the invasion of erythrocytes by merozoites and their multiplication within these erythrocytes. Therefore, analysis of gene expression from blood samples can contribute to better understanding malaria pathogenesis and host immunity. Many studies have analyzed the human and *P. falciparum* transcriptomes separately and simultaneously to investigate immune cells and parasite behavior in malaria infections. Indeed, transcriptional profiling of children with acute malaria has shown that *P. falciparum*-specific atypical B cells positively regulate the pathways that are involved in plasma cell differentiation and genes that promote B- and T-cell interactions [8]. This includes a clonal link between *P. falciparum*-specific atypical B Cells, activated B cells, and classical Memory B Cells (MBCs) via *BCR gene* sequencing and reveals a similar degree of somatic hypermutation [8]. It has also been shown that TCR sequences are shared by blood circulating T follicular cells (cTfh) and Tfh residing within lymph nodes [9], allowing the study of the latter via the bloodstream. Moreover, our previous work revealed that human gene expression shows stronger innate response in Malian younger children whereas stronger adaptative responses in older children [10]. In these Malian children, neutrophils and T cells levels are proportional and inversely proportional to the parasitemia respectively [10]. These suggest that parasitemia induce neutrophil recruitment and T cell migration in secondary lymphoid organs. In the same cohort, we showed in the context of *P. falciparum* and *P. ovale* infections that the human transcriptome was not clustered according to the parasite species whereas parasites genes are regulated differently within species [11].

The sequencing of the *P. falciparum* genome identifies genes involved in host-parasite interactions and immune escape factors [12]. Sequencing efforts have shown the diversity of parasite antigens whose expression differs according to the life stages, representing a major challenge to host immunity. *P. falciparum* gene expression analysis in children with the sickle cell trait phenotype HbAS suggests that parasites downregulate transcripts involved in protein folding and export machinery, oxidative stress responses, and chaperone proteins that have a contribution to reduce *its* pathogenicity [13]. The expression of the variant gene *PfEMP1* has been suggested at the hepatocyte stage [14].

Although gene expression profiles and malaria clinical severity have been explored using this approach [10,15,16], few studies have employed sequencing methods to investigate both human and parasite gene expression profiles between wet and dry seasons. Indeed, parasites isolated in the dry season show a distinct transcriptional profile from those of the wet season in subjects with asymptomatic malaria [17]. In the dry season, *P. falciparum* may decrease expression of ligands that promote tissue-specific sequestration of infected erythrocytes and rosetting. This would allow an increase in splenic clearance and several months of submicroscopic parasitemia [17]. We previously investigated the transcriptome profiles of children with uncomplicated malaria living in an endemic area of Bandiagara in Mali between 2009-2014 using the dual RNA-sequencing approach, allowing for simultaneous sequencing of host and parasite transcriptomes during an episode of malaria (i) at the beginning and end of one transmission season and (ii) at the end and beginning of two consecutive transmission seasons. In this study children genes expression showed an adaptive immune signature later during the transmission season, while it remains relatively stable between transmission seasons. For *P. falciparum* the genes expression is relatively stable within and between the transmission seasons. Then, we hypothesized that dual RNA sequencing will show that the expression of immune genes in Bandiagara children will exhibit a more adaptive signature in the rainy season than in the dry season, while *P. falciparum* will show a decrease in the expression of genes associated with virulence in the dry season.

Material and Method

Ethics Statement

The institutional review boards of the University of Maryland, Baltimore (FWA00007145) and that of the University of Sciences, Techniques and Technologies of Bamako, Mali (FWA00001769) approved the protocol. We obtained informed consent from children's parents and assent from teenagers.

Study design

Whole blood samples were collected from a large incidence study that took place in Bandiagara, Mali from 2009 to 2014. The longitudinal study enrolled 400 children, aged 0-14years, that were followed throughout five wet and dry seasons [18]. Briefly, blood was collected in PAXgene tubes from children presenting with symptomatic malaria during scheduled and unscheduled visits and with *Plasmodium* infection as confirmed by thick smear.

We selected 10 children who had one uncomplicated malaria infection in the dry season and one uncomplicated malaria infection in the previous or subsequent wet season (Table 1). Any malaria episode occurring from November to May and from August to October was classified as a dry season and a rainy season infection respectively.as described previously [19].

Table I: Study participant characteristics

PID	Gender	Year of birth	Symptoms	Hb	Age at infection (in years)	Temperature	Season	Parasitemia
A1	M	2008	UM	AA	2	39.4	Dry	225
A2	M	2008	UM	AA	1	40.1	Wet	64650
B1	F	2007	UM	AS	3	38.9	Dry	325
B2	F	2007	UM	AS	5	38.9	Wet	4650
C1	M	2007	UM	AS	5	38	Dry	87600
C2	M	2007	UM	AS	2	38	Wet	3613
D1	M	2008	UM	AA	3	36.7	Dry	3875

D2	M	2008	UM	AA	3	39.7	Wet	62100
E1	F	2003	UM	AA	7	36.7	Dry	1400
E2	F	2003	UM	AA	7	39.5	Wet	36375
F1	F	2008	UM	AA	3	39.8	Dry	18300
F2	F	2008	UM	AA	2	38.8	Wet	24900
G1	M	2004	UM	AA	6	39.1	Dry	82950
G2	M	2004	UM	AA	7	36.8	Wet	49175
H1	F	2005	UM	AA	5	38.1	Dry	4575
H2	F	2005	UM	AA	5	37.4	Wet	9375
I1	F	2006	UM	AA	4	38.4	Dry	625
I2	F	2006	UM	AA	4	39	Wet	112800
J1	M	2000	UM	CC	11	36.9	Dry	26325
J2	M	2000	UM	CC	11	37.5	Wet	30475

A—J represent subject and 1 is dry season infection and 2 is wet season infection. M is male volunteers, F is female volunteers. UM: uncomplicated Malaria which can have as symptoms an axillary temperature greater than 37.5°C, headache, joint pain, vomiting, diarrhea, or abdominal pain and presence of malaria parasites

Inclusion/Non-inclusion criteria

We included children who were permanently residing in Bandiagara, provided written informed consent for genetic analysis and attended more than 40 scheduled visits (40 months) during the incidence study [18]. We included children with uncomplicated malaria (axillary temperature greater than 37.5°C, headache, joint pain, vomiting, diarrhea, or abdominal pain and parasitemia). We did not include children with severe malaria (cerebral malaria, severe anemia and respiratory distress) and who had received any antimalarial treatment before visit.

RNA-sequencing and reads processing

We extracted total RNA from each whole blood sample using MagMax blood RNA kits (Themo Fisher). We then depleted rRNA, selected polyA and prepared stranded libraries with the NEBNext Ultra II Directional RNA Library Prep Kit (NEB). An Illumina NovaSeq 6000 was used to sequence cDNA libraries and generated 75 to 119 million paired-end reads of 150bp per sample. The sequences generated from each sample were aligned twice with hisat2 to concatenated *P. falciparum* 3D7 and human hg38 genome references (downloaded, respectively, from plasmoDB v.55 and NCBI human genes). First, we aligned the sequences with hisat2 default parameters and then with max-intronlen 5000. We selected reads mapped to the human genome from the BAM files generated with the default parameters and the reads mapped to the *P. falciparum* genome from the BAM files generated with a maximum intron length of 5,000bp. Markduplicates PICARD was used to remove PCR duplicates from all files.Subread FeaturesCounts v1.6.4 and gene annotations downloaded from PlasmoDB (*Plasmodium* genes) and NCBI (human genes) were used for determining the numbers of reads mapped to each gene for each sample [20].

We have shown previously that in *P. falciparum* infections, host genes generally cluster by individual responses (21). To minimize potential confounding due to this individual variation in response, we used a paired design, comparing samples within individuals.

Gene expression analysis

We normalized gene expression into counts per million (CPM), separately for human and for parasite genes. To filter out lowly expressed genes, we retained for further analyses genes that met at least a 10 CPM threshold in 50% of samples. Normalized via trimmed mean of M-values TMM was used to normalize the reads counts. A quasi-likelihood negative binomial generalized model was used to statistically test for differential expression in bioconductor package EdgeR (v 3.32.1) i) with and without the leukocyte proportions as covariates for human genes and ii)) with and without the proportions of each *P. falciparum* lifecycle stages as covariates for parasite genes. All results were corrected for multiple testing using the False Discovery Rates [22].

Gene expression deconvolution

We estimated, for each sample the proportion of i) each blood leukocyte subset ii) each *Plasmodium* developmental stage using CIBERSORTx (23,24). For human gene deconvolution, we used the validated reference leukocyte gene signature matrix LM22 (25), consisting of 547 genes differentiating 22 immune subtypes (grouped to eight categories in our analysis). For *P. falciparum* deconvolution, we used as reference a custom signature matrix derived from *P. berghei* scRNA-sequencing data [23]. We analyzed variations in leukocytes and parasite stage proportions between seasons using paired T-test analysis.

Results

Characteristics of participants and transcriptional profiles of host and parasite revealed by dual RNA sequencing

A total of ten children aged from 1 to 14 years, matched according to gender (five girls and five boys) were enrolled in this study. Most participants had the hemoglobin phenotype HbAA, while two had HbAS and one had HbCC. Parasitemia levels ranged from 225 to 112,800 parasites/µL (Table 1).

After library preparation, RNA-sequencing reads from each blood sample were mapped to the *P. falciparum* (3D7) and human genomes (GRch38). 251,707–3,542,989 (0.41-9.61%) reads were mapped to the *P. falciparum* genome reference and 12,008,304–64,831,798 (90-99%) reads were mapped to human genome reference, allowing robust analysis of gene expression for 2,921 *P. falciparum* genes and 10,941 human genes.

We then conducted principal component analysis (PCA) to identify possible outliers and to visualize how the samples clustered based on the parasite and host gene expression. Overall, these analyses indicated that global variations in host or parasite gene expression were not noticeably affected by the season of the infection, nor distinctly clustered by individuals (Figure 1). To minimize potential confounding by individual differences in response to infection, we used a paired design, comparing gene expression of samples collected from each child in the dry and wet seasons. The analysis of differential gene expression between samples from both seasons allows for identification of potential genes influenced by seasonality.

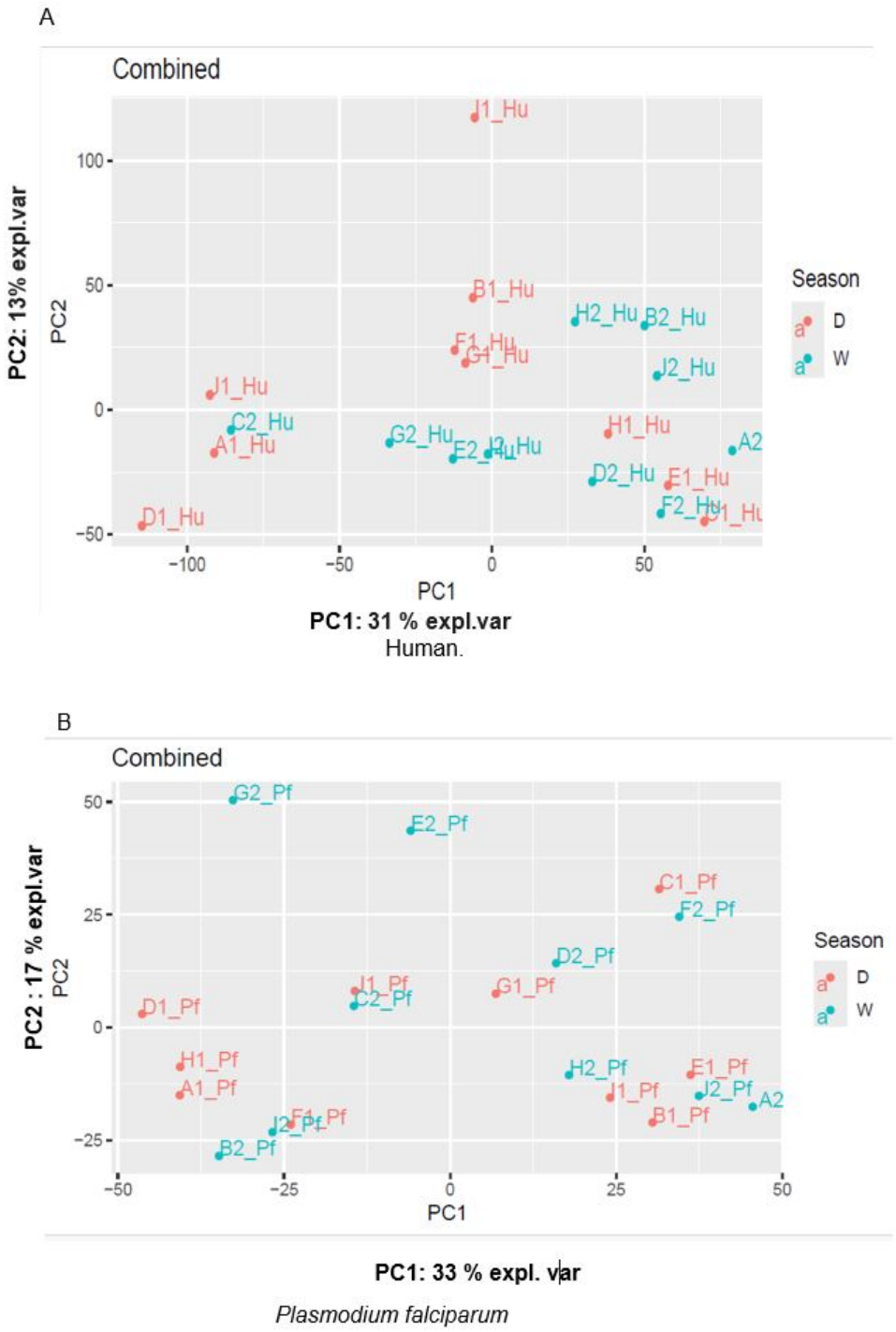

Figure 1: Principal Component Analysis of human (A) and *P. falciparum* (B) gene expression

Reads generated samples during the dry season are shown in red and those generated in wet season are shown in green. Each participant is represented twice: with one dry season sample and one wet season sample.

Host gene expression analysis suggested lower humoral response in dry season

We tested whether the expression of specific host or parasite genes differed between dry and wet season infections. For host gene expression, only three genes were differentially expressed between dry and wet season infections (false discovery rate (FDR) < 0.1) after adjusting for parasitemia. Three components of the immunoglobulin complex (*IGLV3-10, IGLV1-40, IGVL3-27*) were significantly more expressed in wet season infections (Figure 2b). These genes are expressed by plasma cells

and constitute the V region of the variable domain of immunoglobulin light chains, which are responsible for antigen recognition. These findings might reflect minor differences in the humoral response between wet- and dry-season infections.

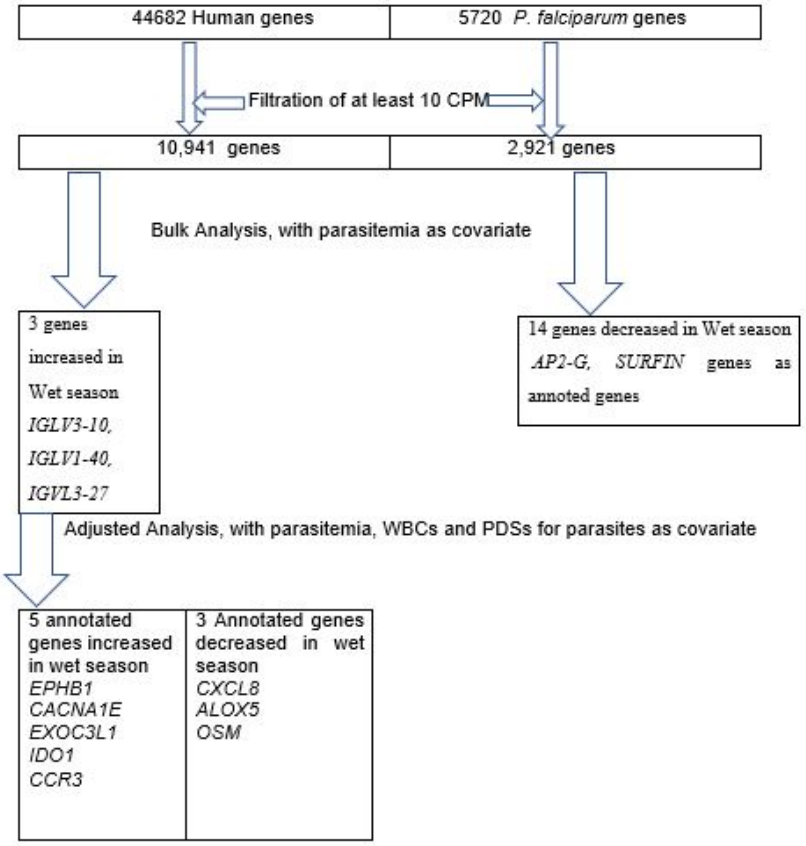

Figure 2a: Overview of the differential host and parasite gene expression analysis in the wet season compared to the dry season. WBCs: White blood Cells, PDSs: Parasites Developmental Stages, CPM: Counts Per Million.

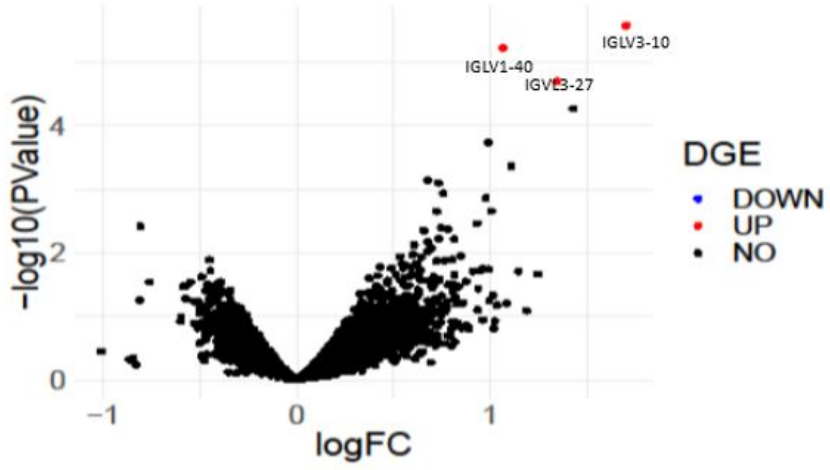

Figure 2b: Differential expression of host genes using parasitemia as a covariate in the wet season. Compared to dry season. Three genes of the immunoglobulin light chain lambda (in red) were upregulated in the wet season; black dots indicate genes whose expression do not variate between season.

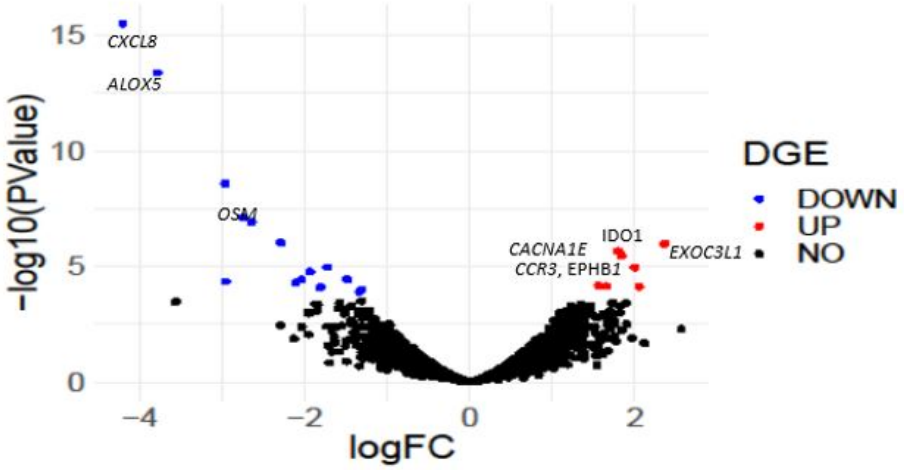

Figure 2c: Differential expression of host genes in wet season compared to dry season. Differential expression has been adjusted using levels of B cells, T cells, eosinophils, neutrophils, and natural killer cells and parasitemia as covariates. Upregulated genes are in red and downregulated genes are in blue and black dots indicate genes whose expression do not variate between season.

We then estimated the proportion of each white blood cell subset directly from the RNA-sequencing data using gene expression deconvolution (Figure 3). We tested whether the immune cell composition differed statistically between paired infections during the wet and dry seasons. We noted an increased level of plasma cells in the wet season (p= 0.004, figure 3b) which could suggest an increased humoral response in the wet season. However, two individuals had plasma cell proportions higher than the median, which could drive this significant statistical difference.

Of note, we did not discriminate between memory B cell subsets. Further, we did not detect any significant difference between dry and wet season infections in most white blood cell proportions (B cells p = 0.1; T cells, p= 0.7; Neutrophil, p= 0.9, Monocyte, p= 0.4; Figure 3b).

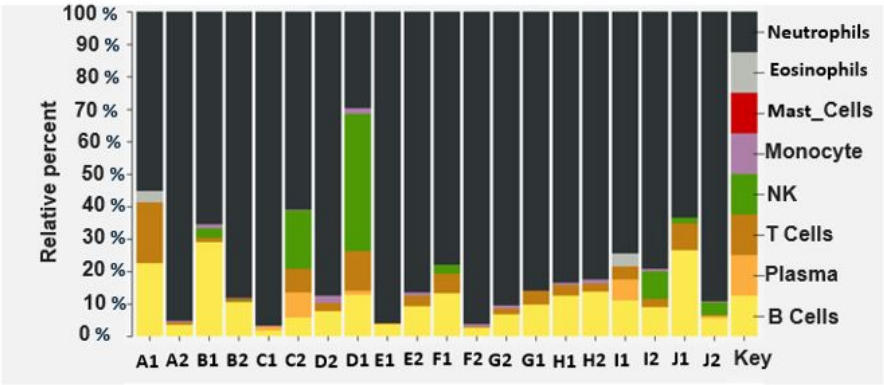

Figure 3a: The relative proportion of white blood cells generated from bulk dual_RNA_Seq. Black bars represent neutrophils, grey bars represent eosinophils, red bars represent mastocytes, purple bars represent monocytes, green bars represent natural killer cells, dark brown bars represent T cells, brown bars represent plasma cells and yellow bars represent B cells.

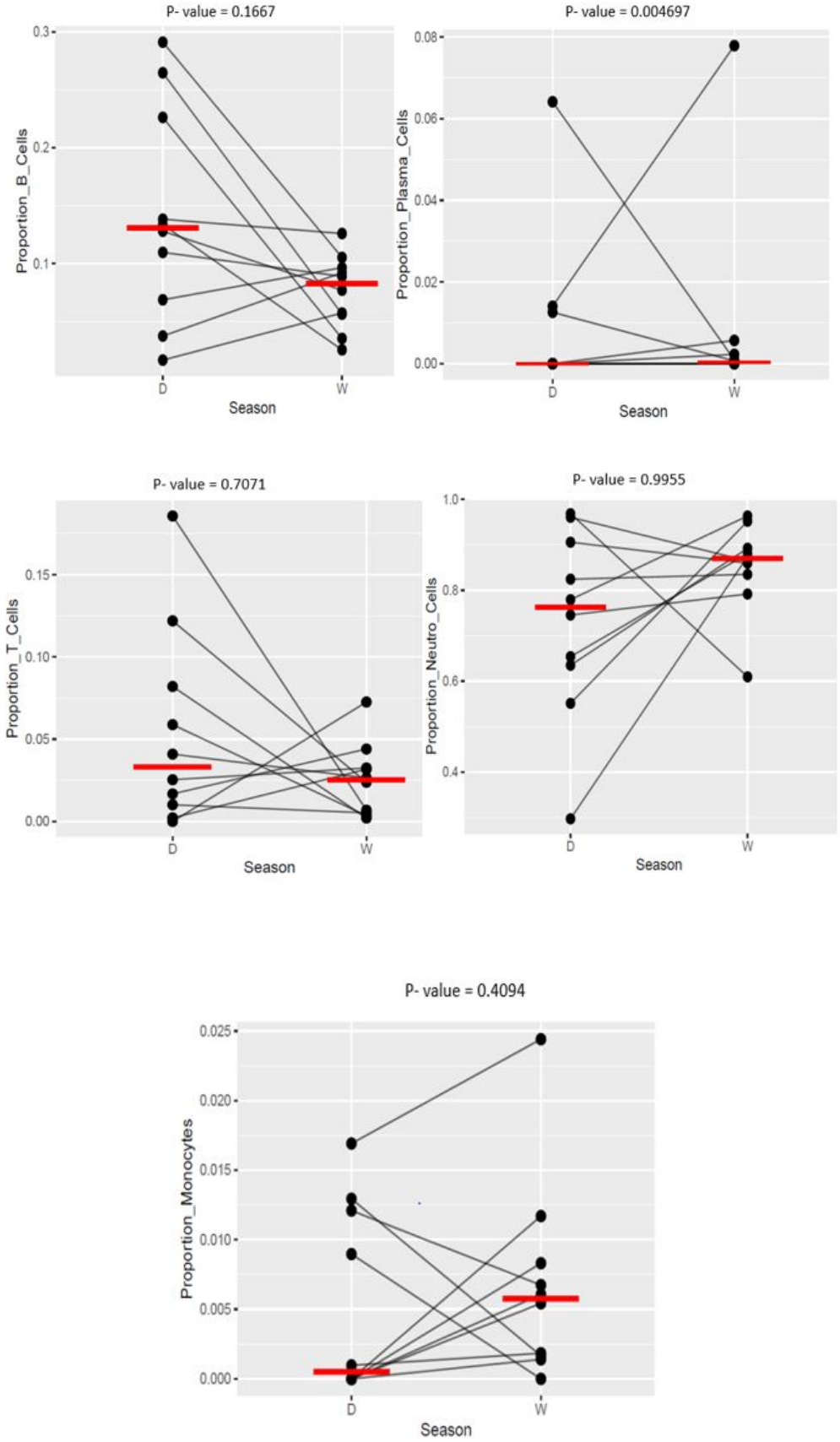

Figure 3b: Change in the relative proportion of white blood cells between infections occurring in the dry and wet seasons. The variation of the proportion of B and T cells and neutrophils between the dry and wet season, with the thin black lines joining es-

timates from the same individual. The proportion of T cells, monocytes, Neutro= neutrophils does not change between seasons. However, plasma cells showed a significant increase in wet season. All comparisons utilize Student's paired t-test with significance was defined as P ≤ 0.05. The red bar corresponds to the median for each group. Note the difference in y-axis scale due to differences in the proportion of each immune cell subtype. D= Dry Season and W= Wet Season.

Since differences in cell composition may influence gene expression analyses, we repeated this analysis after adjusting for the relative percentage of white blood cells in each sample and for parasitemia. After adjustment, 22 host genes were differentially expressed between wet and dry season infections (Figure 2c). Among these genes, we found interestingly, elevated expression of *EPHB1* gene, a receptor tyrosine kinase, in the wet season. This gene is expressed in plasmacytoid dendritic cells, monocyte-derived dendritic cells, and B cells [26]. This gene is involved in dendritic cell differentiation and maturation [27]. In addition, we noted a higher expression of three other annotated genes, each having a role in cell physiology and influencing immune cell functionality: *CACNA1E, EXOC3L1*, and *IDO1*. *CACNA1E* encodes a subunit of a voltage-gated calcium channel involved in calcium ion transport into cells and is involved in the activation of human neutrophils. [28]. EXOC3L1 is involved in cell exocytosis and highly correlated with immune infiltration [29] . IDO1 is an enzyme involved in tryptophan catabolism expressed by many immune cells such as dendritic cells, monocytes, macrophages, and T-cells. It has an immunoregulatory role in T cells proliferation [30]. In addition, chemokine receptor CCR3 was also highly expressed in the wet season.

Genes with lower expression in wet season infections included the pro-inflammatory cytokine such as CXCL8 and ALOX5 enzyme, that belongs to a lipoxygenase gene family. OSM, which encodes for leukemia inhibitory factor.

Overall, we observed a higher abundance of plasma cells during the wet season. Furthermore, after adjusting for variations in cell composition, we identified gene expression differences which are in favor of an increase of plasma cells.

Higher female gametocyte level in malaria symptomatic infections in the dry season.

Looking at *P. falciparum* genes expression, 10 genes were significantly less expressed in wet season than in dry season infections (FDR < 0.1) (Figure 4a). Interestingly, this included *AP2-G* (PF3D7_1222600), a gene encoding the transcription factor that is the master regulator of gametocyte commitment [31]. We noted also the lower expression of *SURFIN* genes PF3D7_1301800 (*SURFIN 13.1*), PF3D7_0113600, (*SURFIN 1.2*); and PF3D7_0424400, (*SURFIN 4.2*) (FDR< 0.1) in the wet season.

We evaluated variations in parasitemia between infections occurring during the dry and wet seasons and found no differences overall (p= 0.7, Figure 5a). This is somewhat surprising given the low level of parasitemia typically reported in *P. falciparum* infections during the dry season [17, 32]. When we repeated this analysis after adjusting for the relative percentage of parasite developmental stage difference, we did not see any differential expression between seasons (Figure 4b).

We then estimated the proportion of the different parasite developmental stages in each infection using gene expression deconvolution. For all samples, we found a large predominance of ring and trophozoite stages, consistent with the sequestration of mature parasites in *P. falciparum* infections and the overrepresentation of transcriptionally active trophozoites in gene expression deconvolution [11] (Figure 5b).

The parasite stage composition was similar in infections from the dry and wet seasons (trophozoites: p = 0.2; rings: p = 0.5; male gametocytes: p = 0.9, Figure 5c), suggesting that the parasite's development is not affected by the season. However, we found a significantly higher proportion of female gametocytes in the dry season (p <0.001, Figure 5c).

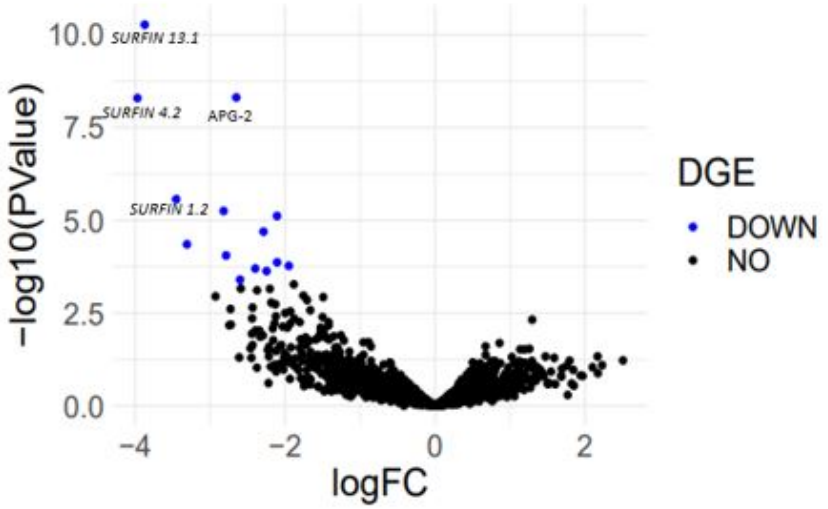

Figure 4a: Differential expression of Pf genes between the dry and wet seasons using parasitemia as a covariate. Genes downregulated in the wet season are shown in blue. No parasite genes were upregulated in the wet season compared to the dry season.

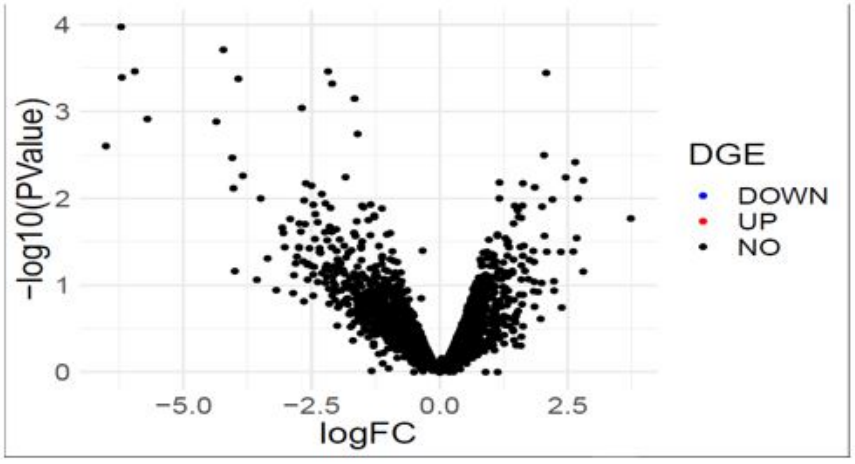

Figure 4b: Differential expression of *Pf* genes between the dry and wet seasons using proportion of trophozoites, ring, female gametocyte, parasitemia as covariates.

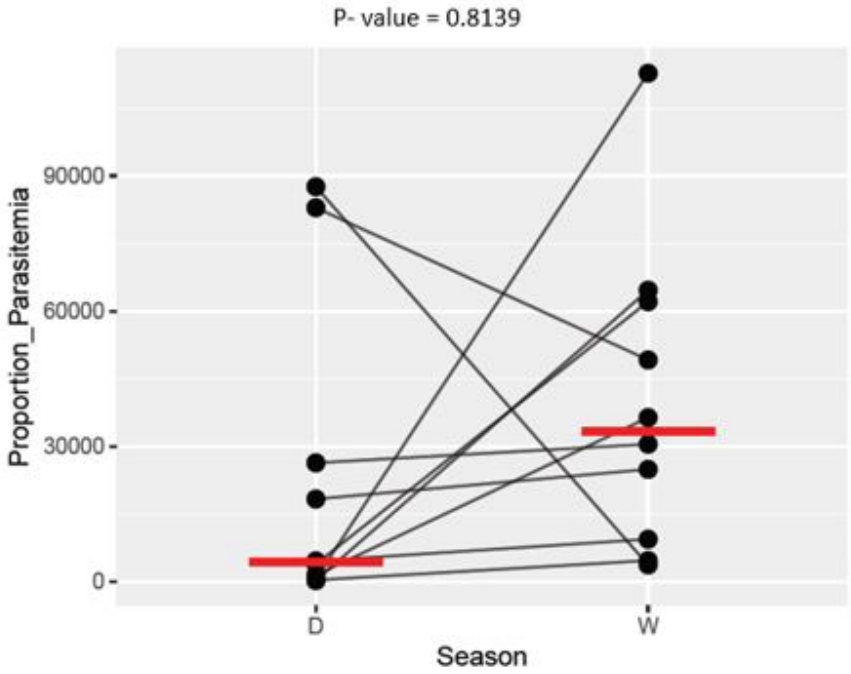

Figure 5a: Variation of the proportion of Parasitemia between dry and wet season, with the thin black lines joining estimates from the same individual. All comparisons utilize Student's paired t-test with significance was defined as $P \leq 0.05$. The red bar corresponds to the median for each group. Note the difference in y-axis scale due to differences in the proportion of parasitemia. D= Dry Season and W= Wet Season.

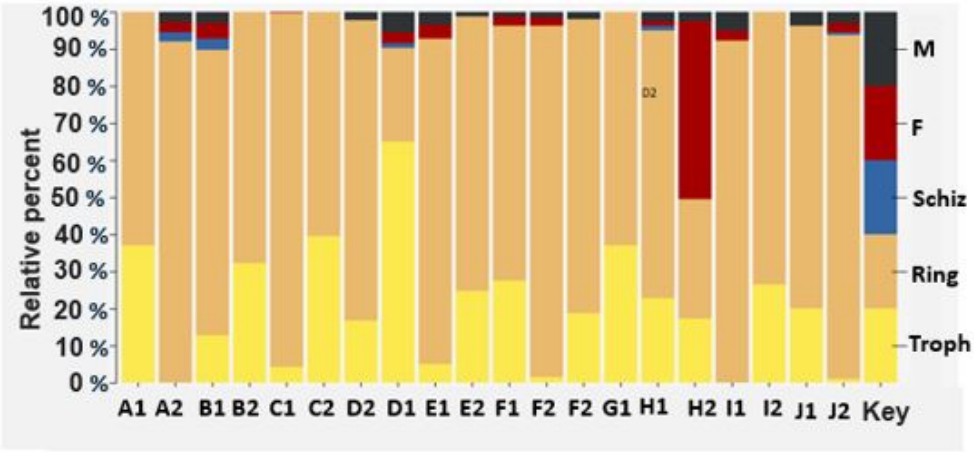

Figure 5b: The relative proportion of parasite developmental stages generated from the bulk dual_RNA_Sequencing. Black bars represent the male gametocytes, reds bar is the female gametocyte, blue bar is the schizont stage, light brown bar is the ring stage, yellow bar is the trophozoite stage.

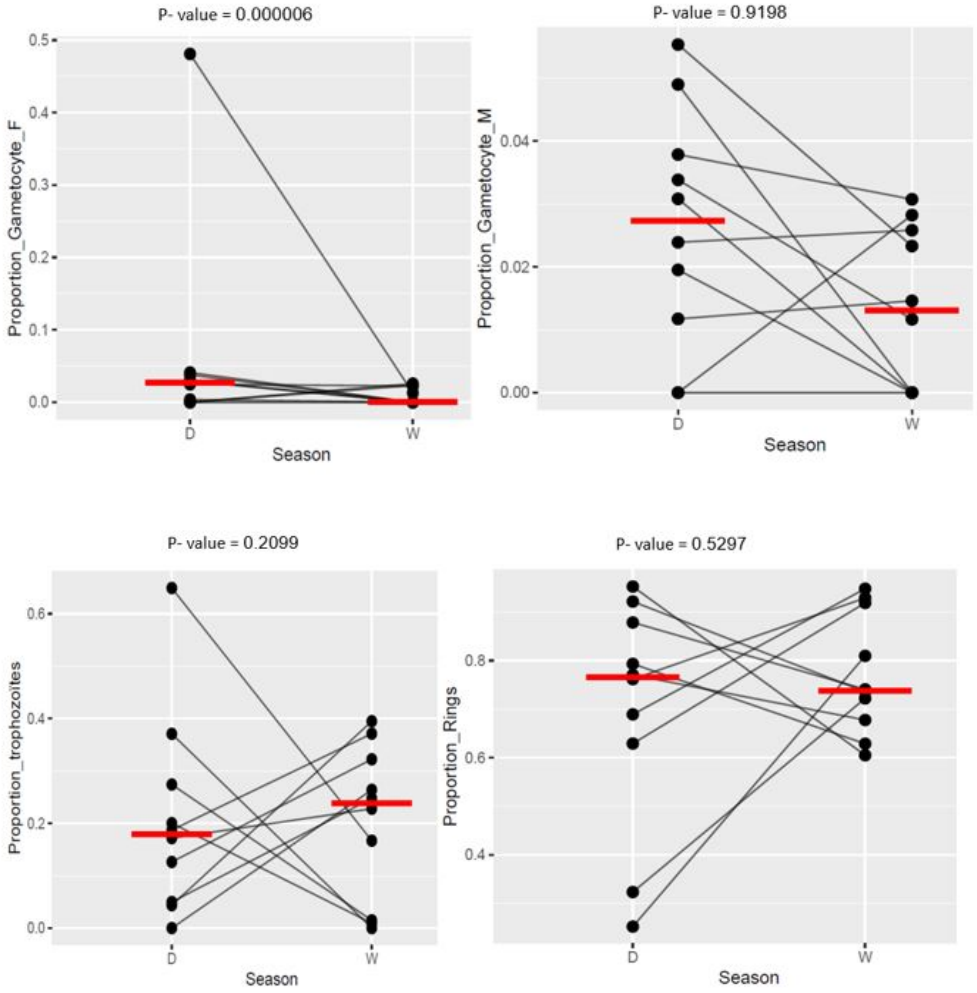

Figure 5c: Parasite developmental stage variation between seasons. Variation of the proportion of trophozoites, Rings, and Gametocytes Males and females between dry and wet season, with the thin black lines joining estimates from the same individual. Only Gametocytes females decreased from dry to wet season and the rest of parasite stages did not change. The red bar corresponds to the median for each group. All comparisons utilize Student's paired t-test with significance was defined as $P \leq 0.05$ Note the difference in y-axis scale due to differences in the proportion of parasite developmental stage. D= Dry Season and W= Wet Season.

Discussion

The complexity of host-parasite interactions and environmental factors that promote *Plasmodium* transmission hamper the potential eradication of the disease. Using the dual RNA-Sequencing approach, we investigated human and parasite gene expression in children infected with *P. falciparum* in the Bandiagara region. Our findings show that host and *P. falciparum* gene expression do not differ dramatically between uncomplicated malaria infections occurring in the dry and wet seasons. However, we noticed a higher plasma cell level in malaria transmission season infections with bulk RNA-sequencing suggesting a difference in antibody response between wet and dry seasons. Previous studies have described how humoral immunity is acquired in children during the transmission season and wanes during a long low transmission season or a decrease in exposure [33, 34]. However, It has also been shown that an anti-MSP-1 antibody was higher in dry rather than wet season infections and that this observation is positively correlated with age [35]. It would be interesting to measure the antibodies levels with microarray. The results of this study are globally consistent with our previous study showing that host immunity remains relatively stable be-

tween two consecutive wet seasons [36]. Since we did not examine the different subsets of memory B cell. It would be interesting to study the dynamics and contributions of these cells with respect to malaria seasonality.

We noted also an increased level of *EPHB1* gene in the wet season after adjusting for the relative percentage of white blood cells in each sample and parasitemia. This gene encodes for a receptor tyrosine kinase and is expressed by many immune cells such as plasmacytoid dendritic, monocyte-derived dendritic cells and B cells [26].This gene is also involved in the co-stimulation of T cell [37]. This has never been reported in malaria immunity but it has been involved in dendritic cell differentiation and maturation in non-small cell lung cancer [27]. It has also been shown that phosphorylation of EPHB1 through COX-2–dependent signaling pathways is crucial for DC maturation [27]. The role of this gene needs to be investigated in malaria context.

Its ligands EFNB1 (ephrin-B1) is also crucial for T cell development [38], and plays an essential role in T cell–T cell co-operation, enhancing T cell sensitivity to antigen stimulation [39]. This finding is interesting as it suggests a potential DC and T cells interaction mediated by ephrin-B1 and EPHB1 in malaria, which could promote B-cell activation. Moreover, EFNB1/EPHB1 signaling has been shown to enhance lymphocyte migration and the production of pro-inflammatory cytokines such as TNF-α and IL-6 during inflammation [40] supporting the idea that this pathway may contribute to immune cell communication and activation in malaria infection.

With regards to *P. falciparum,* our study showed minor gene expression differences between the dry and wet season that contrasts with previous findings [17]. Malaria infections occurring in the dry season in asymptomatic participants have often been found to have sub-microscopic parasitemia in different settings [41]. Indeed, those studies compared participants with different infection presentations (clinical malaria in the wet season vs asymptomatic infection in the dry season) [17] or with different malaria endemicities (high vs low transmission area) [42]. These findings may have been confounded by parasite gene expression differences associated with different disease outcomes [42]. Our previous study which compared two episodes of uncomplicated malaria occurring in the same individual in two successive transmission seasons did not show any difference in parasite gene expression. This design eliminates inter-individual variations and those due to clinical expression of malaria [36].

We noted a lower proportion of female gametocytes in the wet season compared to dry season. We do not know why this decrease of female gametocyte occurs. This has not yet been described. However, most studies have shown an increase in the number of gametocyte in general during the rainy season, which is consistent with transmission [43, 44]. Moreover, one study has shown that gametocyte prevalence was associated with age, but not with seasonality [45]. We also noted lower expression of PF3D7_1222600(*AP2-G*) and some *SURFIN* gene variants in the wet season. *SURFIN* expression has not been well-studied, particularly in comparison to proteins encoded by *var* genes. SURFIN 4.2 protein is involved in merozoite invasion of red blood cells (RBCs) [46] and is suspected to bind endothelial cells because of its link with iRBC and with PfEMP-1 [47]. Further studies are needed to elucidate the role of SURFIN.

It is important to note that the differential gene expression observed in the parasite is due the effect of the developmental stages. Indeed, when the developmental stages were taken in the analysis the differential gene expressions were not seen. This contrasts with the previous study that showed that parasites isolated in the dry season show a distinct transcriptional profile from those of the wet season in subjects with asymptomatic malaria [17].

We note some limitations in this study. First, the definition of dry and wet season could be improved. We defined as "wet season" the period between August and October corresponding to the rainy and high malaria transmission season. The dry season was defined as the period between November and May. In Bandiagara, the dry season is comprised of two sub-seasons**:** the cold dry season (November through February) and the hot dry season (March through May). In some years, transmission season can extend to the beginning of the cold dry season. As the majority of our dry season samples were collected in the cold dry season, it might explain the relative similarity of parasitemia between seasons [48]. The ideal dry season period in Bandiagara for

study collection would be March, April, and May. Second, there was a variability in age of participants. Our participants differed in age, varying from 1 – 14 years old. We previously showed that the age of the participant contributed significantly to differences in host gene expression in terms of malaria [10, 11]. The maturation of the immune system of children could be part of this difference [43]. The host factors including immunity background and the hemoglobinopathies that influence parasite gene expression [13,17] should also be considered in any follow-up studies of the influence of seasonality on gene expression of hosts and parasites.

We included symptomatically infected children and treated different levels of disease progression. As adaptative immunity takes several weeks to develop, the time between infection and diagnosis could influence the gene expression profile.

Conclusion

The gene expression profile of hosts and parasites change slightly between dry and wet season infections. Our work is a pilot study of host gene expression that shows decreased levels of plasma cells and *EPHB1* gene expression in dry season *P. falciparum* infections. While parasite gene expression showed an increased level of female gametocyte in the dry season. Our data suggests a novel paradigm of gene expression of *P. falciparum* in the dry season which needs further investigation.

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