



HHS Public Access

Author manuscript

Int J Med Microbiol. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Int J Med Microbiol. 2017 December ; 307(8): 533–541. doi:10.1016/j.ijmm.2017.09.002.

Metabolome-Wide Association Study of Peripheral Parasitemia in *Plasmodium vivax* Malaria

Luiz Gustavo Gardinassi^{1,2}, Regina Joice Cordy^{2,3}, Marcus V. G. Lacerda^{4,5,6}, Jorge L. Salinas⁷, Wuelton M. Monteiro^{4,5}, Gisely C. Melo^{4,5}, André M. Siqueira⁸, Fernando F. Val^{4,5}, ViLinh Tran^{1,2,9}, Dean P. Jones^{1,2,9}, Mary R. Galinski^{2,3,10}, and Shuzhao Li^{1,2,9}

¹Division of Pulmonary, Allergy and Critical Care Medicine, School of Medicine, Emory University, Atlanta, GA, USA

²Malaria Host–Pathogen Interaction Center, Atlanta, GA, USA

³International Center for Malaria Research, Education and Development, Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA

⁴Gerência de Malária, Fundação de Medicina Tropical Dr Heitor Vieira Dourado, Manaus, AM, Brazil

⁵Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, Manaus, AM, Brazil

⁶Instituto Leônidas & Maria Deane (FIOCRUZ), Manaus, AM, Brazil

⁷Rollins School of Public Health, Emory University, GA, USA

⁸Instituto Nacional de Infectologia Evandro Chagas (FIOCRUZ), Rio de Janeiro, Rio de Janeiro, Brazil

⁹Clinical Biomarkers Laboratory, Department of Medicine, Emory University, Atlanta, GA, USA

¹⁰Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, GA, USA

Abstract

Conflict of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The study was approved by the Ethics Institutional Review Board (IRB) from the Fundação de Medicina Tropical Dr Heitor Vieira Dourado and CONEP, Manaus, Brazil (IRB approval #: CAAE: 12516713.8.0000.0005); and all protocols and documentation were reviewed and sample shipments approved by the Emory IRB. Written informed consent was obtained from all of the participants or their parents or legal guardians.

Authors' contributions L.G.G. performed the data analysis and wrote the manuscript. J.L.S., F.F.V., M.V.G.L., W.M.M., G.C.M. and A.M. S. performed the clinical study, including patient assessment and sample collection. V.T. performed the metabolomics sample analysis. R.J.C and S.L. supervised the data analysis. M.V.G.L., W.M.M., D.P.J., M.R.G. and S.L. designed and supervised the study. All authors participated in manuscript preparation.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Background—*Plasmodium vivax* is one of the leading causes of malaria worldwide. Infections with this parasite cause diverse clinical manifestations, and recent studies revealed that infections with *P. vivax* can result in severe and fatal disease. Despite these facts, biological traits of the host response and parasite metabolism during *P. vivax* malaria are still largely underexplored. Parasitemia is clearly related to progression and severity of malaria caused by *P. falciparum*, however the effects of parasitemia during infections with *P. vivax* are not well understood.

Results—We conducted an exploratory study using a high-resolution metabolomics platform that uncovered significant associations between parasitemia levels and plasma metabolites from 150 patients with *P. vivax* malaria. Most plasma metabolites were inversely associated with higher levels of parasitemia. Top predicted metabolites are implicated into pathways of heme and lipid metabolism, which include biliverdin, bilirubin, palmitoylcarnitine, stearyl carnitine, phosphocholine, glycerophosphocholine, oleic acid and omega-carboxytrienor-leukotriene B4.

Conclusions—The abundance of several plasma metabolites varies according to the levels of parasitemia in patients with *P. vivax* malaria. Moreover, our data suggest that the host response and/or parasite survival might be affected by metabolites involved in the degradation of heme and metabolism of several lipids. Importantly, these data highlight metabolic pathways that may serve as targets for the development of new antimalarial compounds.

Keywords

Metabolomics; host-pathogen interactions; heme; glycerophospholipid

Background

Malaria is a life-threatening vector-borne disease caused by *Plasmodium* parasites that affects millions of individuals every year (WHO, 2016). Infections with *P. vivax* account for almost half of all cases of malaria outside Sub-Saharan Africa, leading to significant morbidity worldwide, besides causing severe and fatal disease (Lacerda et al., 2012; Mahgoub et al., 2012). While many efforts have been made to understand the burden and host response to infections with *P. falciparum* (Mueller et al., 2009), the biologic perturbations induced by infections with *P. vivax* are still largely unknown. *P. falciparum*'s blood-stages invade and replicate inside of red blood cells (RBCs) at any age, whereas *P. vivax* preferentially targets reticulocytes (immature erythrocytes that typically comprise about 1–2% of circulating RBCs) (Lim et al., 2016). A recent study demonstrated that reticulocytes exhibit a more complex metabolic phenotype than mature erythrocytes, suggesting that *Plasmodium* host cell tropism of distinct species may be caused by differences in the parasite's intrinsic metabolism (Srivastava et al., 2015). The *P. vivax* preference for reticulocytes contributes to lower parasitemias, which likely accounts for slower progression of the disease and reduced lethality rates of *P. vivax* malaria when compared with *P. falciparum* (Anstey et al., 2012; Howes et al., 2016; Mueller et al., 2009).

Hyperparasitemia, defined by a peripheral blood slide showing 4% infected RBCs, has been long considered as a criterion of severe falciparum malaria (Severe Malaria, 2014), however, the association between parasitemia and disease severity during *P. vivax* malaria is not clear. A recent study demonstrated that parasitemia is a poor predictor of three or more

severity criteria in *P. vivax* malaria patients (Siqueira et al., 2015). Nevertheless, high parasitemia was associated with fatal disease (Valecha et al., 2009), while another study demonstrated that peripheral *P. vivax* biomass has been underestimated and is associated with both systemic inflammation and disease severity (Barber et al., 2015). Indeed, increased parasitemia reflects the availability of an optimal nutritional environment together with the inability of the host to limit and control parasite growth. In this context, parasitemia correlates with levels of inflammatory and immunomodulatory mediators in the plasma of individuals with *P. vivax* malaria (Barber et al., 2015; Mendonça et al., 2013). Overall, these findings suggest that progression of *P. vivax* malaria can be affected by the density of parasites, and indicate that the metabolic response of the host and pathogen are also influenced by the parasite biomass during infections with *P. vivax*.

Metabolomics represents a powerful analytical approach to uncover the activity of physiological and pathological processes (Li et al., 2016) for the discovery of biomarkers of infectious diseases, including malaria (Park et al., 2015; Salinas et al., 2014). Nuclear magnetic resonance or gas/liquid chromatography coupled with mass spectrometry (GC/LC-MS) have been applied to understand the metabolic changes in models of host-*Plasmodium* interactions *in vitro* (Lakshmanan et al., 2012; MacRae et al., 2013; O'Hara et al., 2014; Olszewski et al., 2009; Park et al., 2015; Sana et al., 2013) and *in vivo* (Basant et al., 2010; Ghosh et al., 2012, 2013; Olszewski et al., 2009; Sengupta et al., 2013; Tritten et al., 2013). However, studies of human malaria are limited to evaluation of plasma from patients infected with *P. falciparum* (Lakshmanan et al., 2012; Surowiec et al., 2015; Sengupta et al., 2016) or urine from patients infected with *P. vivax* (Sengupta et al., 2011).

In view of the lack of understanding about host-pathogen interactions during infections with *P. vivax*, we used a high resolution metabolomics platform to investigate the associations between parasitemia and the plasma metabolome from patients with *P. vivax* malaria. The abundance of several metabolites varied according to the levels of parasitemia, whereby top predicted metabolites are involved in the metabolism of heme and lipids. This indicates that perturbations of these metabolic pathways are linked to the dynamics of parasite burden, and they may have an impact on the host response and parasite survival.

Methods

Study population and clinical evaluation

In this study, we used retrospective plasma samples obtained from patients with *P. vivax* malaria at the Fundação de Medicina Tropical Dr Heitor Vieira Dourado (FMT-HVD), as reported previously (Melo et al., 2014; Siqueira et al., 2015). Plasma samples were obtained under standard laboratorial procedures and stored at -80°C . Samples were shipped in dry ice to the Clinical Biomarkers Laboratory at Emory University, where they were also stored at -80°C . Briefly, all patients underwent an initial clinical characterization and physical examination followed by antimalarial treatment according to appropriate guidelines. At admission, laboratorial evaluation consisted of full blood count and biochemical analyses (hemoglobin, alanine aminotransferase, aspartate aminotransferase and creatinine), as reported (Melo et al., 2014; Siqueira et al., 2015). Diagnosis and quantification of parasitemia were performed with thick blood smear (TBS) microscopy. Each blood slide

was analyzed independently at least two times, whereby the number of asexual and sexual parasites were quantified by counting either 500 leukocytes or 500 parasites. Parasitemia was determined by the mean of two readings of parasites and the white blood cell counts from total blood. Mono-infections with *P. vivax* were confirmed by PCR (Melo et al., 2014; Siqueira et al., 2015). Patients were classified into three categories of parasitemia: low (10–999 parasites/ μ L), moderate (1,000–9,999 parasites/ μ L) and high (> 10,000 parasites/ μ L) according to previous studies (Ketema and Bacha, 2013; Kotepui et al., 2015).

Liquid chromatography and high-resolution mass spectrometry

Sample processing and high-resolution mass spectrometry analysis was performed as described previously (Park et al., 2012; Soltow et al., 2013; Go et al., 2015b; Jin et al., 2016; Walker et al., 2016; Cribbs et al., 2016; Li et al., 2017). Acetonitrile (2:1, v/v) was added to 65 μ L of plasma containing 14 stable isotope internal standards ([13C6]-D-glucose, [15N]-indole, [2-15N]-L-lysine dihydrochloride, [13C5]-L-glutamic acid, [13C7]-benzoic acid, [3,4-13C2] cholesterol, [15N]-L-tyrosine, [trimethyl-13C3]-caffeine, [15N2]-uracil, [3,3-13C2]-cystine, [1,2-13C2]-palmitic acid, [15N, 13C5]-L-methionine, [15N]-choline chloride, and 2'-deoxyguanosine-15N2,13C10-5'-monophosphate). Proteins were removed by centrifugation (13,200 x rpm at 4 °C) for 10 min. The resulting supernatant was transferred to an autosampler vial for LC-MS analysis, using a LTQ Velos Orbitrap mass spectrometer (Thermo Fisher). Injection volume was 10 μ l for each run. Reverse phase chromatography was accomplished using a 100x2.1mm, 5 μ M C18 column (Higgins Analytical) and, an acetonitrile gradient (where FA = 2% formic acid, W = water, ACN = acetonitrile). During the first 2 minutes, the gradient consisted of 5% FA, 60% W and 35% ACN, followed by an 8 min gradient of 5% FA, 0% W and 95% ACN. During the first 6 min, the flow rate was 0.35 ml/min and then changed to 0.5 ml/min for the remaining 4 min. Before a new injection, the column was subjected to solution consisting of 2% formic acid in acetonitrile and equilibrated to initial conditions for 2 min. Mass spectral data was acquired with positive electrospray ionization and the full scan of mass-to-charge ratio (m/z) ranged from 85 to 2000 at a resolution of 60,000. Operating conditions were used as follow: spray voltage of 4.5 kV, sheath gas flow 45 (arbitrary units), auxiliary gas flow of 5 (arbitrary units), capillary temperature of 275°C, maximum injection time of 500 milliseconds and AGC target of 5×10^5 . Xcalibur software (Thermo Fisher Scientific) was used to convert raw files to .cdf format and peak detection, noise filtering, m/z , and retention time alignment, and feature quantification were performed with apLCMS (Yu et al., 2009) and xMSanalyzer (Uppal et al., 2013). Quality control samples (pooled human plasma used to evaluate instrument stability, without clinical significance) was included in every batch of 20 samples, and each sample was run in triplicate. Each metabolite feature is defined by m/z and retention time, with intensity values associated with each replicate. Further quality control included the exclusion of technical replicates with overall Pearson correlation coefficient $r < 0.7$. Data were averaged among analytical replicates, log₂ transformed and normalized by the mean. Only features detected in more than 70% of all samples (3,670) were used in further analysis. Missing values were imputed using half mean of the feature across all samples.

Five different levels of metabolite identification are observed in the scientific literature (Schrimpe-Rutledge et al., 2016; Schymanski et al., 2014; Sumner et al., 2007). These include metabolite identification through comparison of 2 or more orthogonal properties between experimental data and authenticated chemical standards (level 1); putatively annotated compounds matching to both m/z and retention time of a previously characterized authenticated chemical standard (level 2); putatively annotated compounds matching to m/z from databases (level 3); putatively characterized compound classes (level 4), and unknown compounds (level 5). Therefore, putative annotation (level 3) of top significant metabolite features was determined by m/z matching to METLIN and KEEG databases (mass accuracy under 10 ppm, including multiple adducts). Over forty metabolites in this dataset matched to our in-house library constructed with LC-MS/MS of commercially available reference standards (annotation of level 2 confidence), including palmitoylcarnitine, stearyl carnitine, which were significantly associated with *P. vivax* parasitemia. The *mummichog* software (version 1.0.7) was used for pathway analysis (mass accuracy under 10 ppm) (Li et al., 2013). The metabolomics data used in this study are available at the public repository Metabolomics Workbench, under the accession number ST000578.

Statistical analyses

Statistical analyses were carried out using the R Language and Environment for Statistical Computing (R) 3.2.0 (Ihaka and Gentleman, 1996). Only data from patients with *P. vivax* malaria were used in statistical analyses. ANOVA or Kruskal-Wallis followed by Dunn's pairwise multiple comparisons procedure were used to identify significant differences between independent groups of parasitemia. Linear regression models adjusted by age and gender were used to determine the associations between plasma metabolites and parasitemia. False discovery rate (FDR) was computed using Benjamini-Hochberg method. Spearman's rank correlations were used to evaluate associations between parasitemia and clinical features. Euclidian distance method and ward linkage algorithm were used for hierarchical clustering.

Results and Discussion

Study subjects and clinical characteristics

All individuals in this study, which included 50 females and 100 males with mean age of 35.9 years and standard deviation of 15.3, presented symptoms of malaria and had confirmed mono-infection with *P. vivax*. Evaluation of clinical data between categorical groups of parasitemia identified significant differences in levels of hemoglobin ($P=0.0005$), RBC ($P<0.0001$), white blood cell (WBC) ($P=0.0055$), platelets ($P=0.0211$), ALT ($P=0.033$) and AST ($P=0.034$). Consistent with previous reports, we also identified significant correlations between parasitemia and levels of clinical parameters that include hemoglobin, RBCs, WBCs and platelets (Ketema and Bacha, 2013; Kotepui et al., 2015; Demissie and Ketema, 2016; Rodrigues-da-Silva et al., 2014). Demographics and clinical characteristics of the study population are described in Table 1.

Significant associations between plasma metabolites and *P. vivax* parasitemia

Our untargeted, high-resolution metabolomics platform measured over 20,000 metabolite features. After filtering by missing values, 3,670 features were used in the subsequent analysis. Unsupervised principal component analysis (PCA) of all samples revealed a clear pattern of two clusters among malaria patients and of replicates of quality control samples (Fig. S1), which demonstrates the robustness of the analytical method. Using the method of MWAS (Metabolome Wide Association Study (Bictash et al., 2010; Chadeau-Hyam et al., 2010; Nicholson et al., 2008), we investigated the metabolic phenotypes associated with distinct levels of parasitemia in *P. vivax* malaria. Categorical comparison of the plasma metabolome of patients with distinct levels of parasitemia using a univariate analysis of variance (ANOVA) identified the differential abundance of 286 metabolite features with FDR < 0.05 (Fig. 1A). One-way hierarchical clustering of highly significant metabolite features (50 features with FDR < 0.001) demonstrates that the intensity of plasma metabolites is mainly reduced with higher levels of parasitemia (Fig. 2A). To gather further insights into the associations between plasma metabolites with distinct levels of parasitemia, we fitted linear regression models adjusted by age and gender using parasitemia as a continuous variable. This identified 368 metabolite features associated with levels of parasitemia with FDR < 0.05 (Fig. 1B). Of note, linear regression models resulted in twice as many of highly significant metabolite features (103 features with FDR < 0.001), whereby hierarchical clustering analysis retrieved two major clusters, one small cluster of metabolites with intensities increasing with elevated levels of parasitemia (Fig. 2B); and one composed of several sub-clusters, confirming that the majority of plasma metabolites are reduced with increasing levels of parasitemia (Fig. 2B). Over 230 metabolite features were selected by both statistical methods (Fig S2). However, corrections for age and gender in linear regression analyses retrieved a larger number of metabolite features (Fig. 1B and Fig. S2). Biological factors such as age or gender influence the plasma metabolome of humans (Jové et al., 2015; Psychogios et al., 2011) and could impact the results from univariate ANOVA. Therefore, further analyses were focused on metabolite features selected by linear regression models, while those selected by ANOVA were retained only for comparison purposes.

By *m/z* matching to METLIN database (Smith et al., 2005), putative annotations (level 3) of top significant metabolites shown in Fig. 1 and Fig. 2 include phospholipids such as phosphocholine, glycerophosphocholine and several lysophosphatidylcholines (lysoPC). Of interest, the second most significant peak selected by linear regression (*m/z* - 526.3279, P-value = 1.37E-09, FDR = 2.52E-06) matched to a cyanobacterium toxin denominated antillatoxin A. The possibility of compounds similar to a cyanobacterial metabolite (not necessarily antillatoxin) being produced by *P. vivax* parasites and, secreted into the bloodstream during the course of the infection is intriguing, as *Plasmodium*'s apicoplast enzymes share high similarity to that of cyanobacteria (Okada, 2009).

To gather another perspective about the nature of top significant metabolite features (Fig. 2), *m/z* matching was performed with the KEEG database. This returned 106 or 231 matches to features depicted in Fig 2A or Fig 2B, respectively (Supplementary Table 1). Several *m/z* peaks matched to more than one compound and, a large number of features did not match to any chemical entity in any database. Considering the challenges of metabolomics analyses,

such as metabolite identification, a systematic and comprehensive methodological approach is required for appropriate interpretation and conclusions about these data (Li et al., 2013).

Pathways of heme and lipid metabolism are associated with *P. vivax* parasitemia

A recent development in the metabolomics field is the *mummichog* software, which uses pathway and network patterns to prioritize metabolite annotation (Li et al., 2013). We examined the biological context reflected by the metabolic profiles associated with *P. vivax* parasitemia using this computational tool. The most significant pathways overrepresented by metabolite features selected with both ANOVA and linear regression models include the porphyrin and heme metabolism, carnitine shuttle and glycerophospholipid metabolism (Table 2). Of note, metabolite features selected with univariate ANOVA identified an enrichment of the bile acid biosynthesis pathway that was not identified from the metabolites selected by linear regression. Accordingly, this result highlights that the synthesis of bile acids is indeed influenced by age (Einarsson et al., 1985), which indicates an advantage and higher accuracy of using linear regression models adjusted for confounding factors such as age and gender.

Some of the top predicted metabolites such as biliverdin, bilirubin, bilirubin-glucuronoside and bilirubin beta-diglucuronide are involved in porphyrin and heme metabolism (Table 2). The *m/z* peak matching to bilirubin was annotated with a confidence level 2, as this feature also matched to retention time and *m/z* of an authenticated chemical standard characterized previously in our laboratory (Go et al., 2015a). These metabolites exhibited higher abundance in the plasma of patients with increased levels of parasitemia (Fig. 3). Of interest, malaria has been characterized by hemolysis of both infected and uninfected erythrocytes (Fonseca et al., 2016; Joyner et al., 2016; Severe Malaria, 2014), leading to the release of cell-free hemoglobin and further heme prosthetic groups (Pamplona et al., 2007). The relatively low parasitemia observed in infections with *P. vivax*, and their preference to reticulocytes questions the relative contributions of parasite burden to hemolysis, especially of uninfected RBCs. However, several studies indicate that *P. vivax* exhibits a lower threshold of parasitemia associated with fever (Anstey et al., 2009; Karyana et al., 2008), and induces a greater inflammatory response when compared to *P. falciparum* (Karunaweera et al., 1992; Hemmer et al., 2006; Anstey et al., 2007). Moreover, the stability of RBCs are profoundly affected during active disease (Handayani et al., 2009; Lee et al., 2014). Noteworthy, patients with *P. vivax* malaria exhibit elevated levels of cell-free hemoglobin in plasma (Barber et al., 2016) and up-regulate levels of heme oxygenase-1 (HO-1) (Mendonça et al., 2013), which breaks down heme into biliverdin, carbon monoxide and iron. Levels of HO-1 and several other immunomodulatory and inflammatory mediators also correlate with *P. vivax* parasitemia during severe disease (Mendonça et al., 2013). Our data extend these findings, indicating that the abundance of metabolites generated by the metabolism of heme change according to levels of blood parasitemia. This association can be complex and affect the host in different ways. Heme oxygenase-1 is crucial for the survival of mouse models of malaria (Pamplona et al., 2007; Seixas et al., 2009), antagonizing the pathogenic effects of free heme, such as oxidative stress (Vinchi et al., 2013) and excessive inflammation (Dutra et al., 2014). However, another perspective is given by the direct immunomodulatory properties of biliverdin and bilirubin (Liu et al., 2008; Wegiel and Otterbein, 2012) and the

fact that *Plasmodium* parasites also metabolize heme into bilirubin (Okada, 2009). This has the potential to impact the function of leukocytes recruited to limit replication and facilitate parasite evasion.

Previous studies identified the differential abundance of serum lipids in malaria patients (Mesquita et al., 2016; Visser et al., 2013), however the mechanisms underlying this phenomenon are not clear. Our data provide insights into this process by demonstrating significant associations between parasitemia and pathways such as the carnitine shuttle and glycerophospholipid metabolism (Table 2). The abundance of metabolites putatively annotated as palmitoylcarnitine, stearoylcarnitine, heptadecanoyl carnitine and docosapentaenoyl carnitine decreased with higher levels of *P. vivax* parasitemia (Fig. 3). Both palmitoylcarnitine and stearoylcarnitine were also confirmed (annotation confidence level 2) by matched retention time and m/z to authenticated chemical standards characterized previously in our laboratory (Uppal et al., 2017). Of interest, those metabolites are involved in the carnitine shuttle pathway. The transfer of long-chain fatty acids across the inner mitochondrial membrane for β -oxidation is mediated by the carnitine shuttle pathway, whereas decreased abundance of related metabolites identified here might reflect increased uptake of host fatty acids by the parasite. Consistent with this hypothesis, we observed that levels of putatively annotated metabolites such as glycerophosphocholine and phosphocholine were also reduced with higher levels of parasitemia (Fig. 3). In contrast, we identified a significant increase in the abundance of the m/z peak 283.2627 (putatively annotated as oleic acid) with higher levels of parasitemia (Fig. 3), a fatty acid that is required for the intra-erythrocytic proliferation of *P. falciparum* (Mi-Ichi et al., 2007) and might also be essential for the growth of *P. vivax*. Indeed, malarial parasites scavenge, modify, and incorporate fatty acids and phospholipids from the host (Moll et al., 1988; Krishnegowda and Gowda, 2003), whereas elevated *P. vivax* parasitemia correlates with reduced levels of low and high-density lipoprotein in the serum of patients (Mesquita et al., 2016). Lipids are essential nutrients for parasite's proliferation, and for the conversion of heme into hemozoin (Ambele and Egan, 2012; Pisciotta et al., 2007), which is a detoxification strategy used by the parasite to survive inside the RBC while digesting hemoglobin. Our data highlight the importance of lipid metabolism during malaria episodes, while supporting the development of antimalarial compounds targeting pathways related to the uptake and metabolism of fatty acids and phospholipids (Ben Mamoun et al., 2010).

The metabolite tentatively annotated as omega-carboxy-trinor-leukotriene B4 (OCTLB4) exhibited decreased abundance with higher levels of parasitemia (Fig. 3). This eicosanoid has been associated with peroxisome deficiency disorders and chronic kidney disease (Mayatepek et al., 1993; Zhang et al., 2016); more recently it was detected in resting human platelets, but not in thrombin activated platelets (Slatter et al., 2016). While leukotriene B4 (LTB4) is an inflammatory molecule and chemoattractant that mediates the migration of neutrophils induced by heme (Monteiro et al., 2011), omega-oxidation is the main pathway used by human neutrophils to catabolize LTB4, regulating the inflammatory profiles of these cells (Shak and Goldstein, 1984). Taken together, those results suggest that the inflammatory stimulus induced by higher levels of parasitemia could impact the metabolism and signaling of activated leukocytes or platelets, leading to reduced abundance of OCTLB4.

Conclusions

In this study, we obtained high-resolution metabolomes of patients with *P. vivax* malaria, and successfully identified metabolites and pathways that are associated with parasitemia. Our data indicate that individuals with high parasitemia display increased activity heme degradation, and an overall reduction in the abundance of several lipids in the plasma. These results provide important insights into the host metabolic responses in *P. vivax* malaria. Despite of relatively low parasitemia, the inflammatory response induced by *P. vivax* might be responsible for the associations identified in this study. Future studies applying high-resolution metabolomics platforms will further enhance the understanding of the biology of infections with *P. vivax* and contribute to the identification of new drug targets and biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases; National Institutes of Health, Department of Health and Human Services [Contract No. HHSN272201200031C and NIH1S100D18006-01], and the National Center for Research Resources [ORIP/OD P51OD011132]. J.L.S. and F.F.V. were supported by the Fogarty International Center Global Health Fellowship [R25TW009337]. M.V.G.L. and W.M.M. were supported by CAPES, CNPq and FAPEAM.

We thank Karan Uppal for assistance with LC-MS data processing and discussion, and Esmeralda VS Meyer for administrative assistance. We also thank the members of the Malaria Host Pathogen Interaction Center for assistance with data management.

References

- Ambele MA, Egan TJ. Neutral lipids associated with haemozoin mediate efficient and rapid β -haematin formation at physiological pH, temperature and ionic composition. *Malar J.* 2012; 11:337.doi: 10.1186/1475-2875-11-337 [PubMed: 23043460]
- Anstey NM, Douglas NM, Poespoprodjo JR, Price RN. *Plasmodium vivax*: clinical spectrum, risk factors and pathogenesis. *Adv Parasitol.* 2012; 80:151–201. DOI: 10.1016/B978-0-12-397900-1.00003-7 [PubMed: 23199488]
- Anstey NM, Handojo T, Pain MCF, Kenangalem E, Tjitra E, Price RN, Maguire GP. Lung Injury in Vivax Malaria: Pathophysiological Evidence for Pulmonary Vascular Sequestration and Posttreatment Alveolar-Capillary Inflammation. *J Infect Dis.* 2007; 195:589–596. DOI: 10.1086/510756 [PubMed: 17230420]
- Anstey NM, Russell B, Yeo TW, Price RN. The pathophysiology of vivax malaria. *Trends Parasitol.* 2009; 25:220–227. DOI: 10.1016/j.pt.2009.02.003 [PubMed: 19349210]
- Barber BE, William T, Grigg MJ, Parameswaran U, Piera KA, Price RN, Yeo TW, Anstey NM. Parasite biomass-related inflammation, endothelial activation, microvascular dysfunction and disease severity in vivax malaria. *PLoS Pathog.* 2015; 11:e1004558.doi: 10.1371/journal.ppat.1004558 [PubMed: 25569250]
- Barber BE, William T, Grigg MJ, Piera KA, Chen Y, Wang H, Weinberg JB, Yeo TW, Anstey NM. Nitric Oxide-Dependent Endothelial Dysfunction and Reduced Arginine Bioavailability in *Plasmodium vivax* Malaria but No Greater Increase in Intravascular Hemolysis in Severe Disease. *J Infect Dis.* 2016; 214:1557–1564. DOI: 10.1093/infdis/jiw427 [PubMed: 27630198]

- Basant A, Rege M, Sharma S, Sonawat HM. Alterations in urine, serum and brain metabolomic profiles exhibit sexual dimorphism during malaria disease progression. *Malar J.* 2010; 9:110.doi: 10.1186/1475-2875-9-110 [PubMed: 20412601]
- Ben Mamoun C, Prigge ST, Vial H. Targeting the Lipid Metabolic Pathways for the Treatment of Malaria. *Drug Dev Res.* 2010; 71:44–55. DOI: 10.1002/ddr.20347 [PubMed: 20559451]
- Bictash M, Ebbels TM, Chan Q, Loo RL, Yap IKS, Brown IJ, de Iorio M, Daviglius ML, Holmes E, Stamler J, Nicholson JK, Elliott P. Opening up the “Black Box”: metabolic phenotyping and metabolome-wide association studies in epidemiology. *J Clin Epidemiol.* 2010; 63:970–979. DOI: 10.1016/j.jclinepi.2009.10.001 [PubMed: 20056386]
- Chadeau-Hyam M, Ebbels TMD, Brown IJ, Chan Q, Stamler J, Huang CC, Daviglius ML, Ueshima H, Zhao L, Holmes E, Nicholson JK, Elliott P, De Iorio M. Metabolic profiling and the metabolome-wide association study: significance level for biomarker identification. *J Proteome Res.* 2010; 9:4620–4627. DOI: 10.1021/pr1003449 [PubMed: 20701291]
- Cribbs SK, Uppal K, Li S, Jones DP, Huang L, Tipton L, Fitch A, Greenblatt RM, Kingsley L, Guidot DM, Ghedin E, Morris A. Correlation of the lung microbiota with metabolic profiles in bronchoalveolar lavage fluid in HIV infection. *Microbiome.* 2016; 4:3.doi: 10.1186/s40168-016-0147-4 [PubMed: 26792212]
- Demissie Y, Ketema T. Complicated malaria symptoms associated with *Plasmodium vivax* among patients visiting health facilities in Mendi town, Northwest Ethiopia. *BMC Infect Dis.* 2016; 16:436.doi: 10.1186/s12879-016-1780-z [PubMed: 27549864]
- Dutra FF, Alves LS, Rodrigues D, Fernandez PL, de Oliveira RB, Golenbock DT, Zamboni DS, Bozza MT. Hemolysis-induced lethality involves inflammasome activation by heme. *Proc Natl Acad Sci U S A.* 2014; 111:E4110–4118. DOI: 10.1073/pnas.1405023111 [PubMed: 25225402]
- Einarsson K, Nilsell K, Leijd B, Angelin B. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. *N Engl J Med.* 1985; 313:277–282. DOI: 10.1056/NEJM198508013130501 [PubMed: 4010739]
- Fonseca LL, Alezi HS, Moreno A, Barnwell JW, Galinski MR, Voit EO. Quantifying the removal of red blood cells in *Macaca mulatta* during a *Plasmodium coatneyi* infection. *Malar J.* 2016; 15:410.doi: 10.1186/s12936-016-1465-5 [PubMed: 27520455]
- Ghosh S, Sengupta A, Sharma S, Sonawat HM. Metabolic Perturbations of Kidney and Spleen in Murine Cerebral Malaria: 1H NMR-Based Metabolomic Study. *PLOS ONE.* 2013; 8:e73113.doi: 10.1371/journal.pone.0073113 [PubMed: 24039868]
- Ghosh S, Sengupta A, Sharma S, Sonawat HM. Metabolic fingerprints of serum, brain, and liver are distinct for mice with cerebral and noncerebral malaria: a ¹H NMR spectroscopy-based metabolomic study. *J Proteome Res.* 2012; 11:4992–5004. DOI: 10.1021/pr300562m [PubMed: 22838963]
- Go YM, Liang Y, Uppal K, Soltow QA, Promislow DEL, Wachtman LM, Jones DP. Metabolic Characterization of the Common Marmoset (*Callithrix jacchus*). *PLOS ONE.* 2015a; 10:e0142916.doi: 10.1371/journal.pone.0142916 [PubMed: 26581102]
- Go Y-M, Walker DI, Liang Y, Uppal K, Soltow QA, Tran V, Strobel F, Quyyumi AA, Ziegler TR, Pennell KD, Miller GW, Jones DP. Reference Standardization for Mass Spectrometry and High-resolution Metabolomics Applications to Exposome Research. *Toxicol Sci Off J Soc Toxicol.* 2015b; 148:531–543. DOI: 10.1093/toxsci/kfv198
- Handayani S, Chiu DT, Tjitra E, Kuo JS, Lampah D, Kenangalem E, Renia L, Snounou G, Price RN, Anstey NM, Russell B. High Deformability of *Plasmodium vivax* Infected Red Blood Cells under Microfluidic Conditions. *J Infect Dis.* 2009; 199:445–450. DOI: 10.1086/596048 [PubMed: 19090777]
- Hemmer CJ, Holst FGE, Kern P, Chiwakata CB, Dietrich M, Reisinger EC. Stronger host response per parasitized erythrocyte in *Plasmodium vivax* or *ovale* than in *Plasmodium falciparum* malaria. *Trop. Med. Int. Health TM IH.* 2006; 11:817–823. DOI: 10.1111/j.1365-3156.2006.01635.x [PubMed: 16772003]
- Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, Hay SI. Global Epidemiology of *Plasmodium vivax*. *Am J Trop Med Hyg.* 2016; :16–0141. DOI: 10.4269/ajtmh.16-0141 [PubMed: 28077739]

- Ihaka R, Gentleman R. R: A Language for Data Analysis and Graphics. *J Comput Graph Stat.* 1996; 5:299–314. DOI: 10.1080/10618600.1996.10474713
- Jin R, Banton S, Tran VT, Konomi JV, Li S, Jones DP, Vos MB. Amino Acid Metabolism is Altered in Adolescents with Nonalcoholic Fatty Liver Disease-An Untargeted, High Resolution Metabolomics Study. *J Pediatr.* 2016; 172:14–19.e5. DOI: 10.1016/j.jpeds.2016.01.026 [PubMed: 26858195]
- Jové M, Maté I, Naudí A, Mota-Martorell N, Portero-Otín M, Fuente M, Dla Pamplona R. Human Aging Is a Metabolome-related Matter of Gender. *J Gerontol A Biol Sci Med Sci.* 2015; :glv074.doi: 10.1093/gerona/glv074
- Joyner C, Moreno A, Meyer EVS, Cabrera-Mora M, Kissinger JC, Barnwell JW, Galinski MR. *Plasmodium cynomolgi* infections in rhesus macaques display clinical and parasitological features pertinent to modelling vivax malaria pathology and relapse infections. *Malar J.* 2016; 15:451.doi: 10.1186/s12936-016-1480-6 [PubMed: 27590312]
- Karunaweera ND, Grau GE, Gamage P, Carter R, Mendis KN. Dynamics of fever and serum levels of tumor necrosis factor are closely associated during clinical paroxysms in *Plasmodium vivax* malaria. *Proc Natl Acad Sci U S A.* 1992; 89:3200–3203. [PubMed: 1565611]
- Karyana M, Burdarm L, Yeung S, Kenangalem E, Wariker N, Maristela R, Umana KG, Vemuri R, Okoseray MJ, Penttinen PM, Ebsworth P, Sugiarto P, Anstey NM, Tjitra E, Price RN. Malaria morbidity in Papua Indonesia, an area with multidrug resistant *Plasmodium vivax* and *Plasmodium falciparum*. *Malar J.* 2008; 7:148.doi: 10.1186/1475-2875-7-148 [PubMed: 18673572]
- Ketema T, Bacha K. MaHPIC Consortium. *Plasmodium vivax* associated severe malaria complications among children in some malaria endemic areas of Ethiopia. *BMC Public Health.* 2013; 13:637.doi: 10.1186/1471-2458-13-637 [PubMed: 23834734]
- Kotepui M, Piwkhram D, PhunPhuech B, Phiwklam N, Chupeerach C, Duangmano S. Effects of malaria parasite density on blood cell parameters. *PloS One.* 2015; 10:e0121057.doi: 10.1371/journal.pone.0121057 [PubMed: 25807235]
- Krishnegowda G, Gowda DC. Intraerythrocytic *Plasmodium falciparum* incorporates extraneous fatty acids to its lipids without any structural modification. *Mol Biochem Parasitol.* 2003; 132:55–58. [PubMed: 14563537]
- Lacerda MVG, Fragoso SCP, Alecrim MGC, Alexandre MAA, Magalhães BML, Siqueira AM, Ferreira LCL, Araújo JR, Mourão MPG, Ferrer M, Castillo P, Martin-Jaular L, Fernandez-Becerra C, del Portillo H, Ordi J, Alonso PL, Bassat Q. Postmortem characterization of patients with clinical diagnosis of *Plasmodium vivax* malaria: to what extent does this parasite kill? *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2012; 55:e67–74. DOI: 10.1093/cid/cis615
- Lakshmanan V, Rhee KY, Wang W, Yu Y, Khafizov K, Fiser A, Wu P, Ndir O, Mboup S, Ndiaye D, Daily JP. Metabolomic analysis of patient plasma yields evidence of plant-like α -linolenic acid metabolism in *Plasmodium falciparum*. *J Infect Dis.* 2012; 206:238–248. DOI: 10.1093/infdis/jis339 [PubMed: 22566569]
- Lee WC, Malleret B, Lau YL, Mauduit M, Fong MY, Cho JS, Suwanarusk R, Zhang R, Albrecht L, Costa FTM, Preiser P, McGready R, Renia L, Nosten F, Russell B. Glycophorin C (CD236R) mediates vivax malaria parasite rosetting to normocytes. *Blood.* 2014; 123:e100–109. DOI: 10.1182/blood-2013-12-541698 [PubMed: 24652986]
- Li S, Park Y, Duraisingham S, Strobel FH, Khan N, Soltow QA, Jones DP, Pulendran B. Predicting Network Activity from High Throughput Metabolomics. *PLOS Comput Biol.* 2013; 9:e1003123.doi: 10.1371/journal.pcbi.1003123 [PubMed: 23861661]
- Li S, Sullivan NL, Roupheal N, Yu T, Banton S, Maddur MS, McCausland M, Chiu C, Canniff J, Dubey S, Liu K, Tran V, Hagan T, Duraisingham S, Wieland A, Mehta AK, Whitaker JA, Subramaniam S, Jones DP, Sette A, Vora K, Weinberg A, Mulligan MJ, Nakaya HI, Levin M, Ahmed R, Pulendran B. Metabolic Phenotypes of Response to Vaccination in Humans. *Cell.* 2017; 169:862–877. e17. DOI: 10.1016/j.cell.2017.04.026 [PubMed: 28502771]
- Li S, Todor A, Luo R. Blood transcriptomics and metabolomics for personalized medicine. *Comput Struct Biotechnol J.* 2016; 14:1–7. DOI: 10.1016/j.csbj.2015.10.005 [PubMed: 26702339]
- Lim C, Pereira L, Saliba KS, Mascarenhas A, Maki JN, Chery L, Gomes E, Rathod PK, Duraisingh MT. Reticulocyte Preference and Stage Development of *Plasmodium vivax* Isolates. *J Infect Dis.* 2016; 214:1081–1084. DOI: 10.1093/infdis/jiw303 [PubMed: 27432121]

- Liu Y, Li P, Lu J, Xiong W, Oger J, Tetzlaff W, Cynader M. Bilirubin Possesses Powerful Immunomodulatory Activity and Suppresses Experimental Autoimmune Encephalomyelitis. *J Immunol.* 2008; 181:1887–1897. DOI: 10.4049/jimmunol.181.3.1887 [PubMed: 18641326]
- MacRae JI, Dixon MW, Dearnley MK, Chua HH, Chambers JM, Kenny S, Bottova I, Tilley L, McConville MJ. Mitochondrial metabolism of sexual and asexual blood stages of the malaria parasite *Plasmodium falciparum*. *BMC Biol.* 2013; 11:67.doi: 10.1186/1741-7007-11-67 [PubMed: 23763941]
- Mahgoub H, Gasim GI, Musa IR, Adam I. Severe *Plasmodium vivax* malaria among sudanese children at New Halfa Hospital, Eastern Sudan. *Parasit. Vectors.* 2012; 5:154.doi: 10.1186/1756-3305-5-154
- Mayatepek E, Lehmann WD, Fauler J, Tsikas D, Frölich JC, Schutgens RB, Wanders RJ, Keppler D. Impaired degradation of leukotrienes in patients with peroxisome deficiency disorders. *J Clin Invest.* 1993; 91:881–888. DOI: 10.1172/JCI116309 [PubMed: 8450067]
- Melo GC, Monteiro WM, Siqueira AM, Silva SR, Magalhães BML, Alencar ACC, Kuehn A, del Portillo HA, Fernandez-Becerra C, Lacerda MVG. Expression Levels of pvcrt-o and pvmdr-1 Are Associated with Chloroquine Resistance and Severe *Plasmodium vivax* Malaria in Patients of the Brazilian Amazon. *PLOS ONE.* 2014; 9:e105922.doi: 10.1371/journal.pone.0105922 [PubMed: 25157811]
- Mendonça VR, Queiroz AT, Lopes FM, Andrade BB, Barral-Netto M. Networking the host immune response in *Plasmodium vivax* malaria. *Malar J.* 2013; 12:69.doi: 10.1186/1475-2875-12-69 [PubMed: 23433077]
- Mesquita TC, Martin TGO, Alves ER, Mello MBC, Nery AF, Gomes LT, Fontes CJF. Changes in serum lipid profile in the acute and convalescent *Plasmodium vivax* malaria: A cohort study. *Acta Trop.* 2016; 163:1–6. DOI: 10.1016/j.actatropica.2016.07.010 [PubMed: 27461878]
- Mi-Ichi F, Kano S, Mitamura T. Oleic acid is indispensable for intraerythrocytic proliferation of *Plasmodium falciparum*. *Parasitology.* 2007; 134:1671–1677. DOI: 10.1017/S0031182007003137 [PubMed: 17610764]
- Moll GN, Vial HJ, Ancelin ML, Op den Kamp JA, Roelofsen B, van Deenen LL. Phospholipid uptake by *Plasmodium knowlesi* infected erythrocytes. *FEBS Lett.* 1988; 232:341–346. [PubMed: 3378625]
- Monteiro APT, Pinheiro CS, Luna-Gomes T, Alves LR, Maya-Monteiro CM, Porto BN, Barja-Fidalgo C, Benjamim CF, Peters-Golden M, Bandeira-Melo C, Bozza MT, Canetti C. Leukotriene B4 mediates neutrophil migration induced by heme. *J Immunol Baltim Md.* 2011; 1950–186:6562–6567. DOI: 10.4049/jimmunol.1002400
- Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA. Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. *Lancet Infect Dis.* 2009; 9:555–566. DOI: 10.1016/S1473-3099(09)70177-X [PubMed: 19695492]
- Nicholson JK, Holmes E, Elliott P. The metabolome-wide association study: a new look at human disease risk factors. *J Proteome Res.* 2008; 7:3637–3638. DOI: 10.1021/pr8005099 [PubMed: 18707153]
- O’Hara JK, Kerwin LJ, Cobbold SA, Tai J, Bedell TA, Reider PJ, Llinas M. Targeting NAD⁺ Metabolism in the Human Malaria Parasite *Plasmodium falciparum*. *PLOS ONE.* 2014; 9:e94061.doi: 10.1371/journal.pone.0094061 [PubMed: 24747974]
- Okada K. The novel heme oxygenase-like protein from *Plasmodium falciparum* converts heme to bilirubin IX α in the apicoplast. *FEBS Lett.* 2009; 583:313–319. DOI: 10.1016/j.febslet.2008.12.015 [PubMed: 19073183]
- Olszewski KL, Morrisey JM, Wilinski D, Burns JM, Vaidya AB, Rabinowitz JD, Llinás M. Host-parasite interactions revealed by *Plasmodium falciparum* metabolomics. *Cell Host Microbe.* 2009; 5:191–199. DOI: 10.1016/j.chom.2009.01.004 [PubMed: 19218089]
- Pamplona A, Ferreira A, Balla J, Jeney V, Balla G, Epiphanyo S, Chora Â, Rodrigues CD, Gregoire IP, Cunha-Rodrigues M, Portugal S, Soares MP, Mota MM. Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. *Nat Med.* 2007; 13:703–710. DOI: 10.1038/nm1586 [PubMed: 17496899]

- Park YH, Lee K, Soltow QA, Strobel FH, Brigham KL, Parker RE, Wilson ME, Sutliff RL, Mansfield KG, Wachtman LM, Ziegler TR, Jones DP. High-performance metabolic profiling of plasma from seven mammalian species for simultaneous environmental chemical surveillance and bioeffect monitoring. *Toxicology*. 2012; 295:47–55. DOI: 10.1016/j.tox.2012.02.007 [PubMed: 22387982]
- Park YH, Shi YP, Liang B, Medriano CAD, Jeon YH, Torres E, Uppal K, Slutsker L, Jones DP. High-resolution metabolomics to discover potential parasite-specific biomarkers in a *Plasmodium falciparum* erythrocytic stage culture system. *Malar J*. 2015; 14:122.doi: 10.1186/s12936-015-0651-1 [PubMed: 25889340]
- Pisciotta JM, Coppens I, Tripathi AK, Scholl PF, Shuman J, Bajad S, Shulaev V, Sullivan DJ. The role of neutral lipid nanospheres in *Plasmodium falciparum* haem crystallization. *Biochem J*. 2007; 402:197–204. DOI: 10.1042/BJ20060986 [PubMed: 17044814]
- Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, Young N, Xia J, Knox C, Dong E, Huang P, Hollander Z, Pedersen TL, Smith SR, Bamforth F, Greiner R, McManus B, Newman JW, Goodfriend T, Wishart DS. The Human Serum Metabolome. *PLOS ONE*. 2011; 6:e16957.doi: 10.1371/journal.pone.0016957 [PubMed: 21359215]
- Rodrigues-da-Silva RN, da Lima JC Junior, de Fonseca BPF, Antas PRZ, Baldez A, Storer FL, Santos F, Banic DM, de Oliveira-Ferreira J. Alterations in cytokines and haematological parameters during the acute and convalescent phases of *Plasmodium falciparum* and *Plasmodium vivax* infections. *Mem. Inst. Oswaldo Cruz*. 2014; 109:154–162.
- Salinas JL, Kissinger JC, Jones DP, Galinski MR. Metabolomics in the fight against malaria. *Mem Inst Oswaldo Cruz*. 2014; 109:589–597. [PubMed: 25185001]
- Sana TR, Gordon DB, Fischer SM, Tichy SE, Kitagawa N, Lai C, Gosnell WL, Chang SP. Global Mass Spectrometry Based Metabolomics Profiling of Erythrocytes Infected with *Plasmodium falciparum*. *PLOS ONE*. 2013; 8:e60840.doi: 10.1371/journal.pone.0060840 [PubMed: 23593322]
- Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA. Untargeted Metabolomics Strategies—Challenges and Emerging Directions. *J Am Soc Mass Spectrom*. 2016; 27:1897–1905. DOI: 10.1007/s13361-016-1469-y [PubMed: 27624161]
- Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, Hollender J. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ Sci Technol*. 2014; 48:2097–2098. DOI: 10.1021/es5002105 [PubMed: 24476540]
- Seixas E, Gozzelino R, Chora A, Ferreira A, Silva G, Larsen R, Rebelo S, Penido C, Smith NR, Coutinho A, Soares MP. Heme oxygenase-1 affords protection against noncerebral forms of severe malaria. *Proc Natl Acad Sci U S A*. 2009; 106:15837–15842. DOI: 10.1073/pnas.0903419106 [PubMed: 19706490]
- Sengupta A, Ghosh S, Basant A, Malusare S, Johri P, Pathak S, Sharma S, Sonawat HM. Global host metabolic response to *Plasmodium vivax* infection: a ¹H NMR based urinary metabolomic study. *Malar J*. 2011; 10:384.doi: 10.1186/1475-2875-10-384 [PubMed: 22196439]
- Sengupta A, Ghosh S, Das BK, Panda A, Tripathy R, Pied S, Ravindran B, Pathak S, Sharma S, Sonawat HM. Host metabolic responses to *Plasmodium falciparum* infections evaluated by (¹H) NMR metabolomics. *Mol Biosyst*. 2016; 12:3324–3332. DOI: 10.1039/c6mb00362a [PubMed: 27546486]
- Sengupta A, Ghosh S, Sharma S, Sonawat HM. ¹H NMR Metabonomics Indicates Continued Metabolic Changes and Sexual Dimorphism Post-Parasite Clearance in Self-Limiting Murine Malaria Model. *PLOS ONE*. 2013; 8:e66954.doi: 10.1371/journal.pone.0066954 [PubMed: 23826178]
- Severe Malaria. *Trop Med Int Health*. 2014; 19:7–131. DOI: 10.1111/tmi.12313_2 [PubMed: 25214480]
- Shak S, Goldstein IM. Omega-oxidation is the major pathway for the catabolism of leukotriene B4 in human polymorphonuclear leukocytes. *J Biol Chem*. 1984; 259:10181–10187. [PubMed: 6088485]
- Siqueira AM, Lacerda MV, Magalhães BML, Mourão MP, Melo GC, Alexandre MA, Alecrim MG, Kochar D, Kochar S, Kochar A, Nayak K, del Portillo H, Guinovart C, Alonso P, Bassat Q. Characterization of *Plasmodium vivax*-associated admissions to reference hospitals in Brazil and India. *BMC Med*. 2015; 13:57.doi: 10.1186/s12916-015-0302-y [PubMed: 25889040]

- Slatter DA, Aldrovandi M, O'Connor A, Allen SM, Brasher CJ, Murphy RC, Mecklemann S, Ravi S, Darley-USmar V, O'Donnell VB. Mapping the Human Platelet Lipidome Reveals Cytosolic Phospholipase A2 as a Regulator of Mitochondrial Bioenergetics during Activation. *Cell Metab*. 2016; 23:930–944. DOI: 10.1016/j.cmet.2016.04.001 [PubMed: 27133131]
- Smith CA, O'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G. METLIN: a metabolite mass spectral database. *Ther Drug Monit*. 2005; 27:747–751. [PubMed: 16404815]
- Soltow QA, Strobel FH, Mansfield KG, Wachtman L, Park Y, Jones DP. High-performance metabolic profiling with dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) for study of the exposome. *Metabolomics Off J Metabolomic Soc*. 2013; 9:S132–S143. DOI: 10.1007/s11306-011-0332-1
- Srivastava A, Creek DJ, Evans KJ, Souza DD, Schofield L, Müller S, Barrett MP, McConville MJ, Waters AP. Host Reticulocytes Provide Metabolic Reservoirs That Can Be Exploited by Malaria Parasites. *PLOS Pathog*. 2015; 11:e1004882.doi: 10.1371/journal.ppat.1004882 [PubMed: 26042734]
- Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, Fan TW-M, Fiehn O, Goodacre R, Griffin JL, Hankemeier T, Hardy N, Harnly J, Higashi R, Kopka J, Lane AN, Lindon JC, Marriott P, Nicholls AW, Reily MD, Thaden JJ, Viant MR. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics Off J Metabolomic Soc*. 2007; 3:211–221. DOI: 10.1007/s11306-007-0082-2
- Surowiec I, Orikiiriza J, Karlsson E, Nelson M, Bonde M, Kyamanwa P, Karenzi B, Bergström S, Trygg J, Normark J. Metabolic Signature Profiling as a Diagnostic and Prognostic Tool in Pediatric *Plasmodium falciparum* Malaria. *Open Forum Infect Dis*. 2015; 2:ofv062.doi: 10.1093/ofid/ofv062 [PubMed: 26110164]
- Tritten L, Keiser J, Godejohann M, Utzinger J, Vargas M, Beckonert O, Holmes E, Saric J. Metabolic Profiling Framework for Discovery of Candidate Diagnostic Markers of Malaria. *Sci Rep*. 2013; 3:2769.doi: 10.1038/srep02769 [PubMed: 24067624]
- Uppal K, Soltow QA, Strobel FH, Pittard WS, Gernert KM, Yu T, Jones DP. xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data. *BMC Bioinformatics*. 2013; 14:15.doi: 10.1186/1471-2105-14-15 [PubMed: 23323971]
- Uppal K, Walker DI, Jones DP. xMSannotator: An R Package for Network-Based Annotation of High-Resolution Metabolomics Data. *Anal Chem*. 2017; 89:1063–1067. DOI: 10.1021/acs.analchem.6b01214 [PubMed: 27977166]
- Valecha N, Pinto RGW, Turner GDH, Kumar A, Rodrigues S, Dubhashi NG, Rodrigues E, Banaulikar SS, Singh R, Dash AP, Baird JK. Histopathology of fatal respiratory distress caused by *Plasmodium vivax* malaria. *Am J Trop Med Hyg*. 2009; 81:758–762. DOI: 10.4269/ajtmh.2009.09-0348 [PubMed: 19861606]
- Vinchi F, De Franceschi L, Ghigo A, Townes T, Cimino J, Silengo L, Hirsch E, Altruda F, Tolosano E. Hemopexin therapy improves cardiovascular function by preventing heme-induced endothelial toxicity in mouse models of hemolytic diseases. *Circulation*. 2013; 127:1317–1329. DOI: 10.1161/CIRCULATIONAHA.112.130179 [PubMed: 23446829]
- Visser BJ, Wieten RW, Nagel IM, Grobusch MP. Serum lipids and lipoproteins in malaria - a systematic review and meta-analysis. *Malar J*. 2013; 12:442.doi: 10.1186/1475-2875-12-442 [PubMed: 24314058]
- Walker DI, Uppal K, Zhang L, Vermeulen R, Smith M, Hu W, Purdue MP, Tang X, Reiss B, Kim S, Li L, Huang H, Pennell KD, Jones DP, Rothman N, Lan Q. High-resolution metabolomics of occupational exposure to trichloroethylene. *Int J Epidemiol*. 2016; 45:1517–1527. DOI: 10.1093/ije/dyw218 [PubMed: 27707868]
- Wegiel B, Otterbein LE. Go Green: The Anti-Inflammatory Effects of Biliverdin Reductase. *Front Pharmacol*. 2012; 3doi: 10.3389/fphar.2012.00047
- WHO. World Malaria Report 2016 [WWW Document]. WHO; n.d. URL <http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/> [accessed 12.15.16]

- Yu T, Park Y, Johnson JM, Jones DP. apLCMS—adaptive processing of high-resolution LC/MS data. *Bioinformatics*. 2009; 25:1930–1936. DOI: 10.1093/bioinformatics/btp291 [PubMed: 19414529]
- Zhang Z-H, Chen H, Vaziri ND, Mao J-R, Zhang L, Bai X, Zhao Y-Y. Metabolomic Signatures of Chronic Kidney Disease of Diverse Etiologies in the Rats and Humans. *J Proteome Res*. 2016; 15:3802–3812. DOI: 10.1021/acs.jproteome.6b00583 [PubMed: 27636000]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

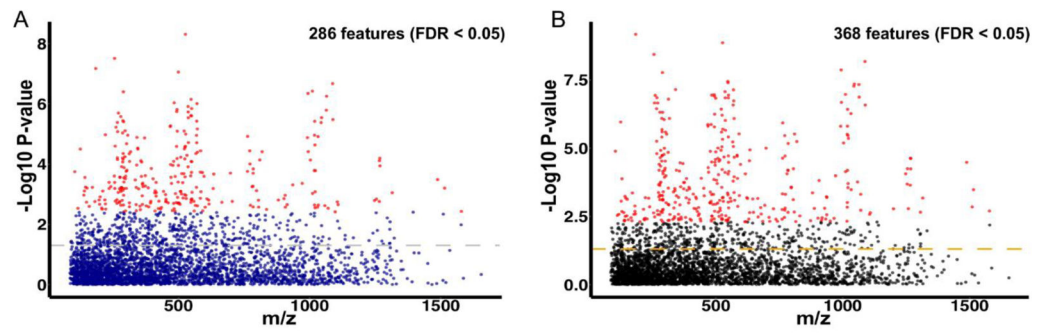


Figure 1. Significant associations between plasma metabolites and *P. vivax* parasitemia. (A) m/z features selected with univariate analysis of variance (ANOVA). (B) m/z features selected with linear regression adjusted by age and gender. Significant features were identified with a FDR < 0.05 and colored in red. Dashed lines represent a P-value of 0.05.

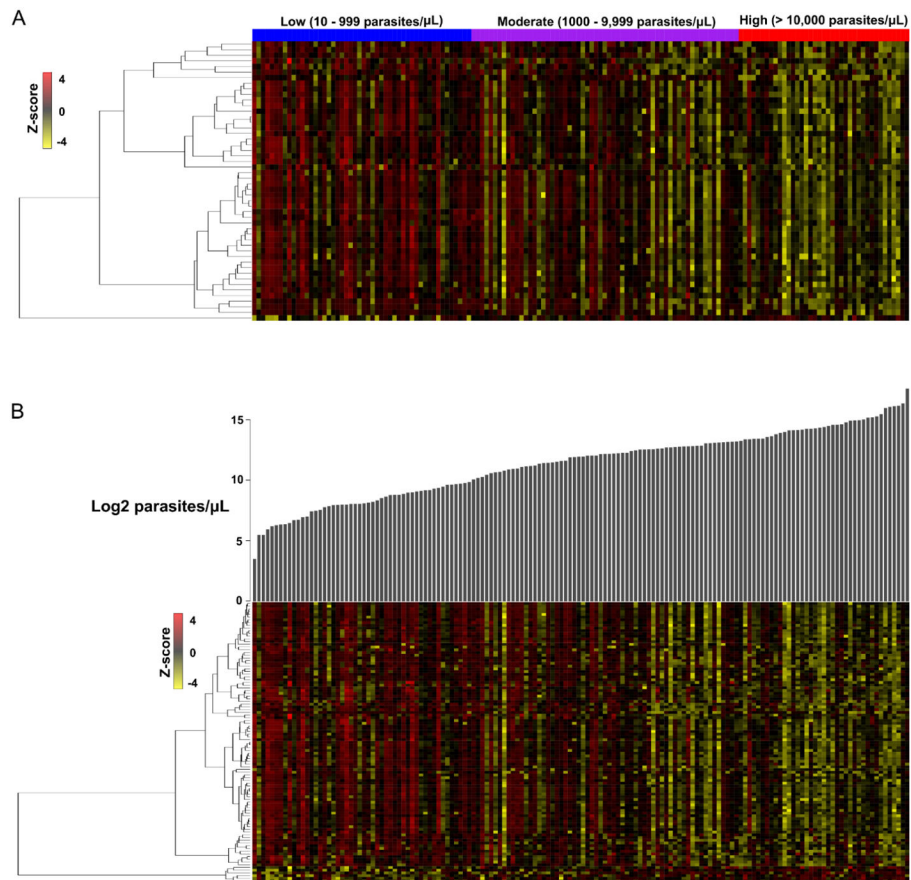


Figure 2. Dynamics of the abundance of metabolite features associated with *P. vivax* parasitemia. (A) One-way hierarchical clustering based on the intensity of highly significant metabolite features selected by ANOVA (FDR < 0.001, 50 *m/z* features). (B) One-way hierarchical clustering based on the intensity of highly significant metabolite features selected by linear regression models (FDR < 0.001, 103 *m/z* features). The yellow to red scale indicates lower to higher intensity levels based on a Z-score.

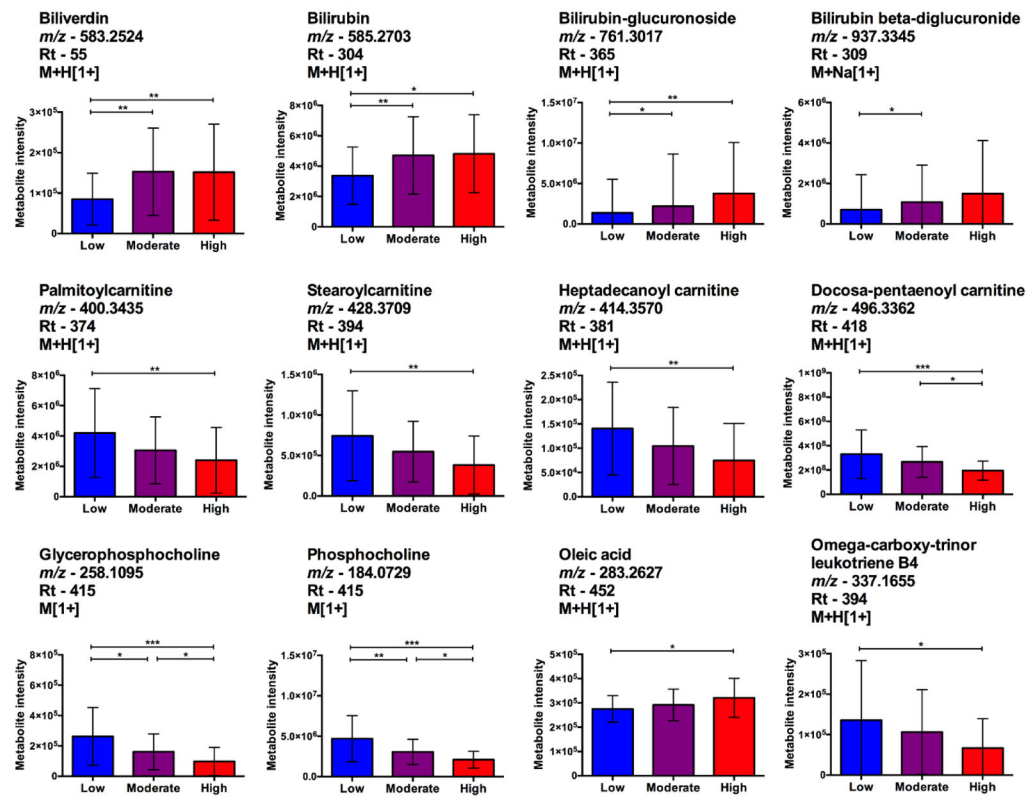


Figure 3.

Distribution of top predicted metabolites in patients with low, moderate and high levels of *P. vivax* parasitemia. Additional statistics were performed with Kruskal-Wallis followed by Dunn's pairwise multiple comparisons procedure; mean values, standard deviation (SD) and significance levels are shown (*, $P < 0.05$ and **, $P < 0.01$).

Table 1

Demographics and clinical characteristics of the study population

Parasitemia classification	Low (n = 50) (10–999 parasites/ µL)	Moderate (n = 61) (1,000–9,999 parasites/µL)	High (n = 39) (> 10,000 parasites/ µL)
Age, y	37.7 (13.4)	36.2 (16.0)	33.1 (16.2)
Male, n(%)	34 (68)	44 (72)	22 (56)
Female, n(%)	16 (32)	17 (28)	17 (44)
Hemoglobin (g/dL)	13.0 (2.2)	13.0 (2.2)	10.9 (3.0)
RBC (10 ⁶ /µL)	4.7 (0.9)	4.6 (0.8)	3.9 (1.0)
WBC (10 ³ /µL)	4.6 (1.8)	5.7 (2.8)	5.6 (2.0)
Platelets (10 ³ /µL)	117.5 (96.8)	94.7 (71.4)	94.6 (114.3)
ALT (U/L)	72.1 (58.3)	62.6 (75.8)	36.2 (31.2)
AST (U/L)	105.9 (84.3)	77.6 (78.8)	68.5 (62.7)
Creatinine (mg/dL)	0.8 (0.5)	0.8 (0.4)	0.8 (0.4)

Mean values and standard deviation are shown. RBC – red blood cells; WBC – white blood cells; ALT - alanine aminotransferase; AST - aspartate aminotransferase.

Table 2

Metabolic pathway analysis

Pathways	Features selected with ANOVA		Features selected with linear regression	
	Overlap size	<i>P</i> value	Overlap size	<i>P</i> value
Porphyryn and heme metabolism	3	0.00427	7	< 0.0001
Carnitine shuttle	4	0.0025	6	< 0.0001
Bile acid biosynthesis	5	0.0022		
Glycerophospholipid metabolism	4	0.0025	4	0.00056
Glycosphingolipid metabolism	3	0.00777	3	0.00181
Sialic acid metabolism	3	0.01356	3	0.00334
Fatty acid activation			3	0.00074
De novo fatty acid biosynthesis			3	0.00074
Squalene and cholesterol biosynthesis			3	0.00146
Xenobiotics metabolism			3	0.00484
Tyrosine metabolism			3	0.02098

Only pathways with three or more overlapping features are shown.