Duffy Blood System and G6PD Genetic Variants in *P. Vivax* Malaria Patients From Manaus, Amazonas, Brazil

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Abstract

Over a third of the world's population lives at risk of potentially severe Plasmodium vivax induced malaria. Unique aspects of the parasite's biology and interactions with the human host make it harder to control and eliminate the disease. Glucose-6-phosphate dehydrogenase (G6PD) deficiency and Duffy-negative blood groups are two red blood cell variations that confer protection against malaria. Molecular genotyping was performed in 225 patients with severe and non-severe malaria, which revealed 29 (12.94%) carriers of the G6PD 202$^{AG/GG}$ and 43 (19.19%) with G6PD 376$^{GA/AA}$. For the Duffy genotype, 70 (31.11%) were phenotyped as Fy(a + b-), 98 (43.55%) Fy(a + b+), 56 (24.9%) Fy(a-b+) and 1 (0.44%) Fy(a-b-). The *FY*01/FY*02* genotype was more frequent in both non-severe and severe malaria. However, the frequency increased when SNP c.376A > G was also present. In women, the *FY*01/FY*02* allele occurred concomitantly with c.376A > G more frequently in non-severe malaria, whereas in men, this synergism was more frequent in severe malaria. In addition, G202A/A376G genotypes were more frequent in severe malaria, with c.202G > A (RR = 5.57 − p < 0.001) and c.376A > G (RR: 4.49 − p < 0.001) strongly associated with the trials malaria (p < 0.001). Parasite count and density were not observed to specifically associate with variants in G6PD or Duffy. However, Duffy phenotype Fy(a-b+) (p = 0.003) and genotype FY*02/ FY*02 (p = 0.007) presented the highest values parasitemia density of the vivax malaria. Research on G6PD and Duffy antigen deficiencies has been valuable, particularly when focused on densely populated areas. We concluded that c.202G > A and c.376A > G SNPs were risk factors for the development of severe vivax malaria and that molecular diagnosis before treatment may be necessary in the Amazonian population and uncomplicated malaria showed a greater frequency of variation for GATA and G6PD variants than severe malaria.

Introduction

Malaria is a disease transmitted by the female Anopheles mosquito, and its etiological agent is a protozoan of the genus Plasmodium. In Brazil, the Plasmodium species *P. vivax, P. falciparum*, and *P. malariae* are found; other species known to cause human malaria are *P. ovale* and *P. knowlesi* (1−4).

Malaria is one of the most serious public health problems worldwide, and in tropical and subtropical regions, this parasitic disease is the leading cause of social and economic problems (6−7). In Brazilian epidemiology, approximately 90% of the cases result from P. vivax infection, and in 2018, over 33,000 cases were reported in a single region i.e. the state of Amazonas (8).

Susceptibility to malarial infection includes individuals who have contracted the disease several times. However, the severity depends on the relationship between the host, its immunological vulnerability, the Plasmodium ssp. responsible for the infection, and parasitic density. The presentation of individuals with malaria may include severe anemia, coagulation disorders, prominent thrombocytopenia, and numerical or functional changes in leukocytes with spleen involvement (9−11). In endemic regions of P. vivax infection, there have been cases of clinical complications and mortality, which have led to the characterization of P. vivax malaria as a serious or even fatal disease; however, these findings are uncommon characteristics of previous infections (12−13).

Although little is known of the pathophysiology, the progression and aggravation of P. vivax malaria is mainly associated with anemia, occasionally due to severe hemolysis (14−16). Other pathophysiological events, such as oxidative stress, may influence the development of clinical conditions (17, 18).

Some studies report that the Duffy glycoprotein acts as a possible facilitator in the process of erythrocyte invasion by *P. vivax*. Blood system antigens act as receptors for P. vivax merozoite ligands that contain DBL domains across alleles. These Duffy blood group system antigens (Fy^a, Fy^b, Fy3, Fy5, and Fy6) are encoded by two co-dominant allelic forms *FY*01* and *FY*02* that differ by the SNP in position c.125A > G of exon (19, 20). The most frequent SNP found in the

population is at position c.-67T > C in the GATA promoter region, characterized by the allele *FY*02N.01*, silencer of Fy^b expression only in erythroid cells, and the Fy(a-b-) phenotype when homozygous is denoted *FY*02N.01 / FY*02N.01* which is known to be a protective factor against vivax malaria infection.

Another phenotype associated with weak Fy^b antigen expression is determined by SNP c.256C > T and c.298G > A. These polymorphisms occur within the first intracellular loop of the Duffy protein resulting in reduced expression of the Fy^b antigen. The frequency of $Fy^{b\ weak}$ (*FY*02W.01*) is approximately 2% in Caucasians. Some polymorphisms resulting in $Fy^{a\ weak}$ (*FY*01W.01*) has not been observed. (21, 22).

Glucose 6-phosphate dehydrogenase (G6PD) is an enzyme involved in the pentose monophosphate pathway. Deficiency of this enzyme leads to free radical-mediated oxidative damage to erythrocytes, causing hemolysis. G6PD deficiency frequency is linked to the inheritance of X chromosome(s) with disease causing variant(s), which are highly prevalent in people of African, Asian, and Mediterranean descent (23, 24). In females, there are selective advantages with G6PD A-, which is characterized by the combination of variants A376G (c.376A > G) with variants: G202A (c.202G > A), A542T, G680T or T968C, which in the heterozygous state have selective advantage against severe malaria (25–27). Through natural selection, this G6PD deficiency is prevalent (8%) in populations where malaria is endemic (28).

Considering that the clinical severity between endemic areas can be complex with multifactorial influence, among these participations of genetic factors, inherited hemoglobinopathies and alterations in G6PD become relevant in the investigation of these comorbidities of *P. vivax* malaria cases in endemic areas because they may also be factors affecting phenotypic heterogeneity (29).

This is mainly because malaria caused by *P. vivax* challenges control strategies and elimination (30). The unique parasite biology, which involves the formation and subsequent reactivation of latent forms in the liver, and the ability to infect or vector before symptoms occur, favors the perpetuation of the parasitic life cycle (31). In this regard, there is added difficulty in tracking infected individuals owing to subpatent infections (32). This study aimed to determine the frequencies of Duffy alleles and G6PD c.202G > A and c.376A > G variants in malaria patients, to relate susceptibility and resistance to acquired P. vivax infections.

Methodology

This study was based on a cross-sectional model. Inclusion criteria were patients of both sexes with a diagnosis of severe (hospitalized) and uncomplicated (outpatient) malaria, from the Tropical Medicine Foundation Dr. Heitor Vieira Dourado (FMT-HDV), treated at the Clinical Research Ward (PES CLIN) hospital in the city of Manaus, state of Amazonas, Brazil. Data from medical records from March 2013 to April 2016 were included.

Research was initiated after formal release and acceptance by the institution in question, FMTAM, and was approved by the Research Ethics Committee (CEP). To ensure patient welfare, all individuals subjected to research signed the Free and Clarified Commitment Term (FICF), in compliance with CNS Resolutions 196/96.

The samples were used in two projects of this study: A larger study entitled "Clinical characterization complicated malaria for Plasmodium vivax", approved by the National Research Ethics Commission (CONEP), in June 2009, opinion no. 343/2009, protocol No.25.000.011.792/2009-15, and the second, smaller study, was approved by the Research Ethics Committee of (FMT-HVD) (study number 343/2009), entitled "Study of DUFFY Polymorphisms in patients infected with Plasmodium vivax" with opinion no. CAAE-0004.0.112.000−11 on 12/29/2011.

Approximately 0.5 mL peripheral blood samples were collected in the tube with an anticoagulant such as EDTA (ethylenediaminetetraacetic acid disodium salt) at a concentration of 1.5 mg / mL for blood counts, and aliquots in

1.5 mL tubes were used for extraction of nuclear DNA. An addition 0.6 mL was collected in a tube without anticoagulants for biochemical analyses.

Immediately after blood collection, hematological determinations were performed on the automated counter - ABX Pentra 80 (Horiba Diagnostics, Montpellier, FR) and biochemistry was performed on a Beckman Coulter (Inc, CA, US).

Dna Analysis

DNA was extracted from 200 µL of whole blood according to the QIAamp DNA Mini Kit (Qiagen, Hilden, DE) manufacturer's protocol (Cat No./ID 51304). After extraction, the DNA was quantified with a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Massachusetts, EUA), and stored at -20ºC.

Duffy Genotyping

The DNA amplification step was divided into two: a conventional PCR for amplification of the sequence of interest, followed by restriction enzyme digestion by PCR-RFLP, one for Duffy blood group (Duffy PCR) and one for the GATA Box variant (GATA PCR).

Genotyping for Duffy blood system groups was performed with synthetic oligonucleotides FYAB1 (5 'TCC CCC TCA ACT GAG AAC TC 3') and FAB2 (5 'AAG GCT GAG CCA TAC CAG AC 3'). Amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide. After PCR amplification was confirmed, the products were processed with BanI restriction enzyme digestion and incubated for at least 4 hours at 37ºC. The enzyme digestion product was observed on a 1.5% agarose gel stained with ethidium bromide.

A gene phenotypes and genotypes Duffy followed according to names for FY (ISBT 008) Blood Group Alleles (23).

For verification of c.-67T > C SNP in the GATA Box promoter, *FY*01/FY*02* and *FY*02/FY*02* genotype samples were used. Synthetic oligonucleotides FYN1 (5 'CAA GGC TGA CCC TA 3') and FYN2 (5 'CAT GGC ACC GTT TGG TTC AG 3') were used for GATA PCR.

The GATA PCR product was treated with a *StyI* restriction enzyme and incubated for at least 4 hours at 37 °C. The enzyme digestion product was observed on a 2.5% agarose gel. GATA normal genotypes were shown with 108 and 81 bp bands, while GATA mutated showed an additional 61 bp band.

Samples with genotype *FY*01/FY*02* and *FY*01/FY*02* were used to verify the SNPs c.265C > T and c.298G > A in the *FY*01W.02* coding region and for Fy^X.

The PCR product was treated with the restriction enzyme *MspAI* to verify the SNP c.265C > T and incubated for 4 hours at 60 °C; the *MwoI* restriction enzyme for the SNP c.298G > A and incubated for 4 hours at 37 °C, and visualized on an 8% polyacrylamide gel. The mutated genotypes for the SNP c.265C > T had an additional band of 161 bp and c.298G > A had an additional band of 343 bp.

G6pd Genotyping

For characterization of the variants, Real Time PCR (qPCR) was performed with the QuantStudio™ 3 Real-Time PCR System(Applied Biosystems, Thermo Fisher Scientific®) using TaqMan® probes specific for each polymorphism. The amplification reaction was performed for a final volume of 12uL/reaction, which contained 5uL of 2x TaqMan Universal Master Mix, 0.3uL of 20x SNP Genotyping Assay, 4.8uL of sterile water and 2.0uL of DNA (~ 100 ng) of the sample. The

G6PD variants analyzed in this project were chosen based on globally observed frequencies and their clinical importance according to WHO classifications.

STATISTICAL ANALYSIS

Data were entered into a database using Graphpad-Prism 5.0 software (Graphpad Software, San Diego, CA-US) and IBM SPSS Statistics, version 19 (IBM Corp., Armonk, NY, US), organized by variable type. The analysis of qualitative or categorical variables of three or more groups was performed by the non-parametric Chi-square test ($x2$), duly corrected by the Mantel-Haenszel and Yates tests. The analysis of groups with only two categorical variables were performed with the Fisher's exact test. Confidence intervals of 95% and prevalence ratios were calculated for these variables.

Results And Discussion

Patients positive for P. vivax infection treated at the Tropical Medicine Foundation of Amazonas hospital in Manaus, Brazil with a confirmed diagnosis of a monoinfection were enrolled in this study of Duffy/G6PD genotypes and their implications in disease pathogenesis. Patients with comorbidities, hemoglobinopathies, mixed Plasmodium infections, and viral infections were excluded. The World Health Organization (WHO) criteria: uncomplicated and severe were used to classify malaria patients, as described previously (33). There were 225 patients diagnosed with P. vivax malaria included in the study, 52.4% were male.

Genotyping of c.202G > A resulted in 29 (12.9%) positive patients, with 4.4% heterozygous and 0.44% homozygous females, while 8% of males were hemizygous. The c.376A > G was identified in 43 (19.1%) patients, with 18 (8%) heterozygous and 2 (0.88%) homozygous females, while 23 (10.22%) males were hemizygous.

Analysis of the Duffy system in the malaria patients showed a phenotypic distribution of 70 (31.1% - Fy(a + b-), 98 (43.55%) - Fy(a + b+), 56 (24.9%) - Fy(a-b+) and 1 (0.44%) - Fy(a-b-). Table 1 shows the genotypic distribution for the Duffy system, with predominance of the genotype *FY*01/FY*02* in the population. These results indicate that the Manaus-Amazonas region has individuals who express three Duffy phenotypes Fy(a + b+), Fy(a + b-) and Fy(a-b+) more frequently with expression of *FY*01* and *FY*02* antigens.

Table 1
Allele frequency of the Duffy blood group from *vivax* malaria patients.

Phenotypes	Genotypes	Percentage (%)
Fy(a + b+)	*FY * 01 / FY * 02*	42,66
Fy(a + b-)	*FY * 01 / FY * 02N.01*	16,00
	*FY * 01 / FY * 01*	15,11
Fy(a-b+)	*FY * 02 / FY * 02*	16,00
	*FY * 02 / FY * 02N.01*	8,88
Fy(a + ʷ)	*FY*01 / FY * 01W.02*	0,88
Fy(a-b-)	*FY * 02N.01 / FY * 02N.01*	0,44

The *FY*01/FY*02* genotype was present at higher frequencies in uncomplicated and severe malaria, 45.3% and 39.2%, respectively (Fig. 1). The presentation was more expressive and had a highly severe clinic course when the c.376A > G variant was present. In females, the *FY*01/FY*02* allele in combination with the c.376A > G variant was present at a higher frequency in cases of uncomplicated malaria, and in males this combination of variants occurred at a higher

frequency in severe malaria cases. Furthermore, when the clinical severity of malaria was compared with G6PD genotypes c.202/c.376A > G which demonstrated a higher frequency of severe malaria when compared with c.202G > A (RR = 5.57, 95% CI: 2.25–17.79, p < 0.001); c.376A > G (RR: 4.49, 95% CI: 1.99–8.43, p < .001) strongly associated with previous malaria episodes (p < 0.001) (Table 2).

Table 2
Genotypic frequency of G6PD variants among patients with uncomplicated and severe *vivax* malaria.

G6PD Genotypes	MALARIA N (%)		RR (CI)	p-value	PREVIOUS MALARIA EPISODES N (%)		RR (CI)	p-value
	Severe	Uncomplicated			Yes	No		
$202^{GA/AA}$	22 (26.2)	07 (6.0)	5.57 (2.25–17.79)	< .001*	17 (25.8)	08 (6.3)	5.11 (2.02–12.63)	< .001*
202^{GG}	62 (73.8)	110 (94,0)			49 (74.2)	118 (93.7)		
$376^{AG/GG}$	30 (32.6)	13 (10,6)	4.09 (1.99–8.43)	< .001	25 (33.8)	17 (12.6)	3.54 (1.75–7.13)	< .001*
376^{AA}	62 (67.4)	110 (89.4)			49 (66.2)	118 (87.4)		
$202^{GA/AA}$ / $376^{AG/GG}$	17 (21,5)	03 (2,7)	10.54 (2.83–35.66)	< .001**	13 (21.0)	07 (5.6)	4.47 (1.68–11.88)	< .001*
202^{GG} / 376^{AA}	62 (78,5)	110 (97,3)			49 (79.0)	118 (94.4)		
RR: Relative Risk (CI): Interval of Confidence * χ2 test (Yates's corrected) * * Fisher's exact test N: cases								

For c.202G > A, the highest frequency among females was the Fy(a + b+) and Fy(a + b-) phenotype in severe malaria cases and it was not found at the same frequency in the Fy(a-b+) phenotype, which was frequent among cases of uncomplicated malaria. In males, all Duffy phenotypes were more frequent in severe malaria, with Fy(a + b-) representing the largest number of cases. For c.376A > G, we observed a higher number of cases with the polymorphism for the Fy(a + b) phenotype in patients with severe malaria, with 47.36% of cases reported in males (data not showed).

The only allele *FY*02N.01/FY*02N.01* responsible for the Fy(a-b-) phenotype, was found in a single female patient with uncomplicated malaria, without the c.202G > A and c.376A > G variants. For uncomplicated malaria, the frequency of c.202G > A and c.376A > G variants decreased, particularly when these polymorphisms were concomitant. Severe malaria showed no variations in frequency for the c.202G > A variant, however, in the presence of A376G there was a slight decrease in frequency. When combined with the GATA variant, the c.202G > A and c.376A > G variants were observed at a lower frequency in uncomplicated malaria, but this finding did not reach statistical significance, despite a slight decrease in frequency. For severe malaria, the presence of the combined polymorphisms showed a moderate variation in frequency (Fig. 2).

Results showed no statistically significant association for women with *P. vivax* infection, nor with the frequency of mutated Duffy alleles. The authors do not speculate about the race of the participants due to the strong regional ancestral mix found in the Amazonian Caboclos, which originate with the arrival of Caucasians and Blacks to indigenous lands (Data not showed).

There was no significant association of parasite count and density with the Duffy antigen genotypes and phenotypes. However, However, Duffy phenotype Fy(a-b+) (p = 0.003) and genotype FY*02/FY*02 (p = 0.007) presented the highest values parasitemia density of the vivax malaria (Figs. 3, 4).

In this study, the highest frequency of the *FY*01/FY*02* genotype and the Fy(a + b+) and Fy(a + b-) phenotypes was found in malaria cases. These findings corroborate studies reported by Cavasini et al. (2007), which correlated the high frequency of the *FY*01* and *FY*02* alleles among *P. vivax* malaria patients. They concluded that the *FY*01/FY*02* and *FY*01/FY*01* genotypes were associated with a high frequency of Plasmodium vivax infection, and suggested that these individuals have a higher risk of disease (34).

The *FY*01/FY*02* and *FY*01/FY*01* genotypes are associated with increased frequency of *P. vivax* infection, while *FY*01/ FY*02W.01* and *FY*02/ FY*02W.01* were associated with low parasitic density levels (21).

The presence of a single case with null Duffy variants in this study supports a possible advantageous selection, driven by defense mechanisms against *P. vivax.* in endemic areas, which is corroborated by literature that has reported the Duffy negative blood group as a protective factor against *P. vivax* malaria infection (35).

In a study conducted in São Paulo, the phenotypic frequencies found in blood donors for Duffy blood system antigens were 19.8% for the phenotype Fy(a + b-) in Caucasians and 14.0% in Blacks, Fy(a + b+) in 41.4% of Caucasians and 1.6% of Blacks, Fy(a-b+) in 37.8% of Caucasians and 17.5% of Blacks and Fy(a-b-) in 1.1% of Caucasians and 66.9% of Blacks (36).

The results and clinical presentation of patients in this study support the hypothesis that G6PD deficiency does not confer a decreased risk of severe malaria infection (Table 3). Many controversies remain as hemizygous males and heterozygous females failed to have changes in frequency of uncomplicated malaria. However, the results showed that both male or female sex is independent and provides no protection against severe malaria.

Table 3
Genotypic frequency distribution of G6PD variants among uncomplicated and severe *vivax* malaria patients by the presence of Duffy GATA normal or mutated variants.

Malaria	Duffy GATA	c.202 G > A N (%)		p-value	c.376 A > G N (%)		p-value	c.202 G > A - c.376 A > G N (%)		p-value
		GG	GA/AA		AA	AG/GG		GG/AA	GA/AA - AG/GG	
Uncomplicated	Normal	86 (94.5)	05 (5.5)	.487**	86 (88.7)	11 (11.3)	.452**	86 (97.7)	02 (2.3)	.531**
	Mutated	24 (92.3)	02 (7.7)		24 (92.3)	02 (7.7)		24 (96.0)	01 (4.0)	
Severe	Normal	45 (77.6)	13 (22.4)	.181*	45 (70.3)	19 (29.7)	.252*	45 (83.3)	09 (16.7)	.107*
	Mutated	17 (65.4)	09 (34.6)		17 (60.7)	11 (39.3)		17 (68.0)	08 (32.0)	
Total	Normal	131 (87.9)	18 (12.1)	.087*	131 (81.4)	30 (18.6)	.249*	131 (92.3)	11 (7.7)	.043*
	Mutated	41 (78.8)	11 (21.2)		41 (75.9)	13 (24.1)		41 (82.0)	09 (18.0)	

* χ2 test (Yates's corrected) * * Fisher's exact test N: cases

For the process of parasite invasion into red blood cells, Duffy protein is functionally important, and it has been shown that in regions where the malaria transmission rates are average, as in the inhabitants of the Amazon, this protein is naturally immunogenic (37).

In a study with *P. vivax* malaria patients the *FY*01* and *FY*02* alleles were found to have low, medium, and high parasitic density, but in the presence of the GATA variant, genotypes with alleles *FY*02N.01* and *FY*02W.01*, were found only in patients with low parasitic density and low symptomatology (21).

A study performed in the state of Pará with a population of African descent demonstrated the presence of the c.202G > A variant was 0.060, the Duffy blood group included 24.3% Duffy negative and 41.3% individuals heterozygous for *FY*02^W*. The frequency of the *FY*02^W* allele was 41.0%. These findings support the monitoring of individuals with G6PD deficiency for use of primaquine during the routine care of Afro-descendant communities of the Trombetas, Erepecuru, and Cumná rivers, to assess risks of hemolytic crisis in recurrent cases of malaria in the region (38).

In recent decades, studies have revealed the complexity of Duffy system phenotypic and genotypic variation and variants of G6PD (A-) have a significant impact on the distribution of human populations in areas where malaria is endemic. The Duffy system and G6PD are, therefore, polymorphic systems that offer great challenges to researchers not only due to their academic importance, but also given their potential applications in the treatment of vivax malaria (39).

That is, where natural selection occurs within a population endemic for malaria, natural adaptations may result from genetic variation that provides a partial defense mechanism against P. vivax infections (40).

Conclusion

This study reports the frequency of G202A and A376G variants and Duffy alleles in patients with severe and uncomplicated malaria in Manaus, Amazonas, Brazil.

The presence of the G202A and A376G variants is a risk factor for the development of severe P. vivax malaria. Before treatment, molecular diagnosis for the G202A and A376G variants in patients diagnosed with malaria may be necessary in the Amazonian population.

The FY*01/FY*02 Duffy genotypes was more frequent than the null expression genotype in the vivax malaria patients and the FY*01/FY*02 genotype demonstrated greater association with severe malaria cases.

We found one only of the uncomplicated vivax malaria patient with Duffy phenotype Fy(a-b-).

Genotypes with heterozygous GATA variants were not protective against malaria infection.

Duffy phenotype Fy(a-b+) and genotype FY*02/ FY*02 presented the highest values parasitemia density of the vivax malaria.

Uncomplicated malaria showed a greater frequency of variation for GATA and G6PD variants than severe malaria.

Declarations

AUTHORS' CONTRIBUTIONS

- Natália Santos Ferreira and Jéssica Lorena dos Santos Mathias performed the collection of samples and performed the practical and laboratorial parts of the entire project.
- Natalia Santos Ferreira, Jéssica Lorena dos Santos Mathias and Anne Cristine Gomes Almeida performed the molecular tests for the genotyping of G6PD and Duffy genes polymorphisms. It should be noted that this project was the dissertation for his Master's Degree of Natalia Santos Ferreira and Jéssica Lorena dos Santos Mathias.
- Ana Carla Dantas and Fernanda Cozendey Anselmo assisted in the collection of samples and digitization of results in data analysis programs.
- Sérgio Roberto Lopes Albuquerque, Marcus Vinicius Guimarães Lacerda and Emerson Silva Lima assisted wrote the manuscript with support from Jose Pereira de Moura Neto.
- Marilda de Souza Gonçalves, Paulo Afonso Nogueira, Sérgio Roberto Lopes Albuquerque and Marcus Vinicius Guimarães Lacerda assisted in the development of the project and contributed to the final version of the manuscript.
- José Pereira de Moura Neto conceived the study, was the idealizer of the project and advised Natalia Santos Ferreira and Jéssica Lorena dos Santos Mathias. He helped supervise the project and assisted in sample collection and performed the analytical calculations and results simulations.
- The authors declare no conflict of interest and have no have financial relations with the industry. All donors were volunteers and provided (or a legal guardian provided) informed, written consent.

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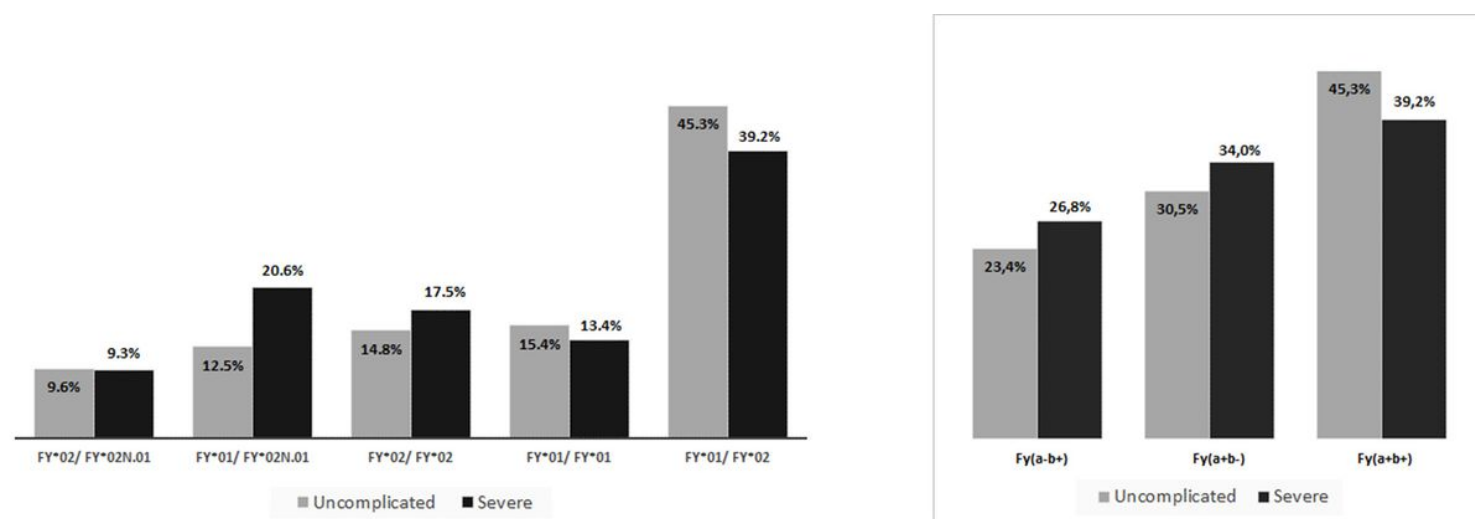
Figures

Figure 1

Genotypic and phenotypic distribution of Duffy antigens among uncomplicated and severe vivax malaria patients. Figure 1. The genotypes with null and weak expression of Duffy System were found in patients with uncomplicated malaria and the FY*01/FY*02 genotype demonstrated greater association with severe malaria cases.
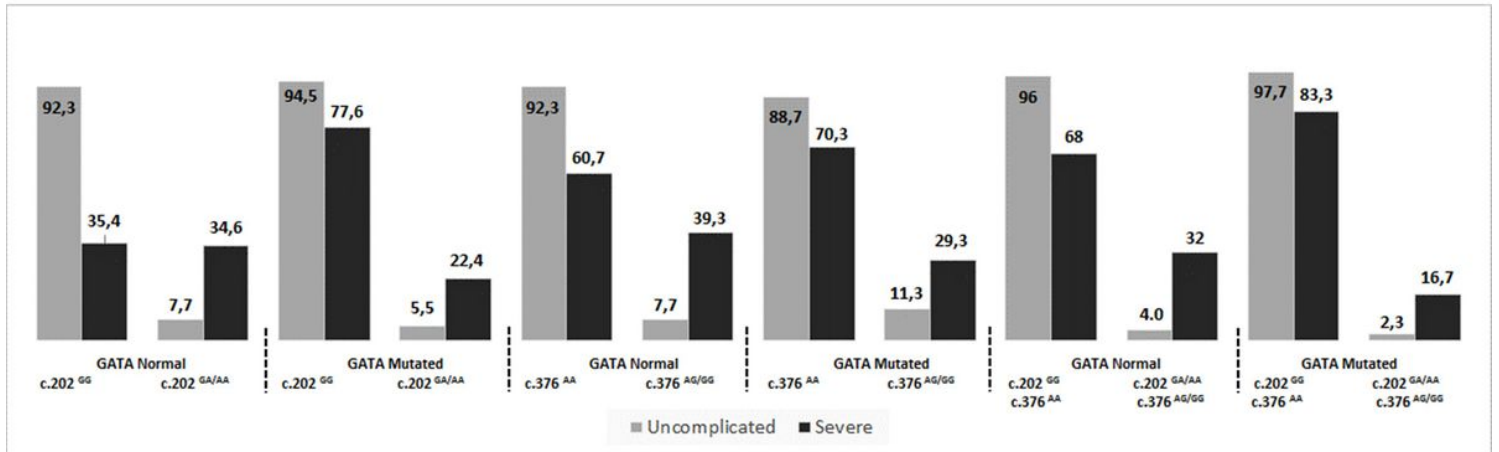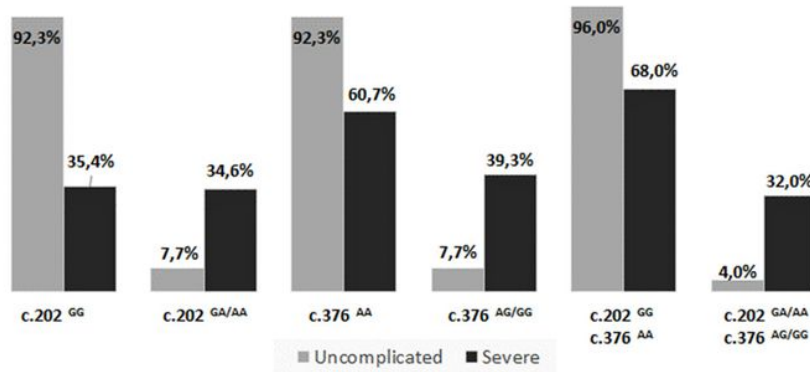
Figure 2

Distribution of c.202G>A and c.376A>G variants in the presence and absence of the Duffy variant among vivax malaria patients. Figure 2. The top panel shows that the presence of G202A and A376G variants were more frequent in cases of severe vivax malaria, with the lowest frequencies in uncomplicated malaria cases concomitant with the presence of other variants. On the lower panel, the GATA variant has been associated with virtually all records of GATA / G6PD variants in severe vivax malaria cases.
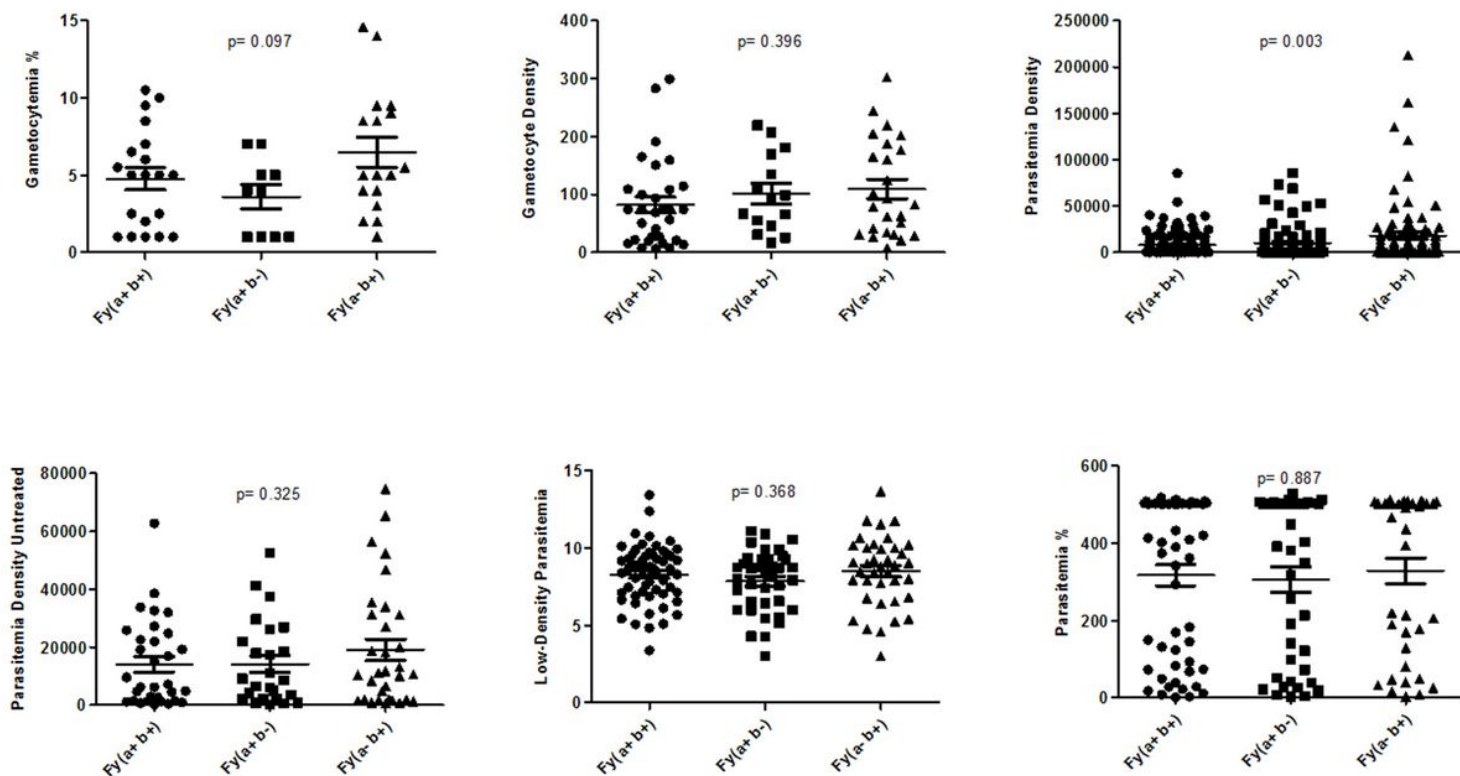
Figure 3

Level of parasite and densities counted by Duffy genotypes found in vivax malaria patient. Figure 3. There was statistical significance (p = 0.003) for parasitic density, mainly in the phenotype Fy(a-b+).
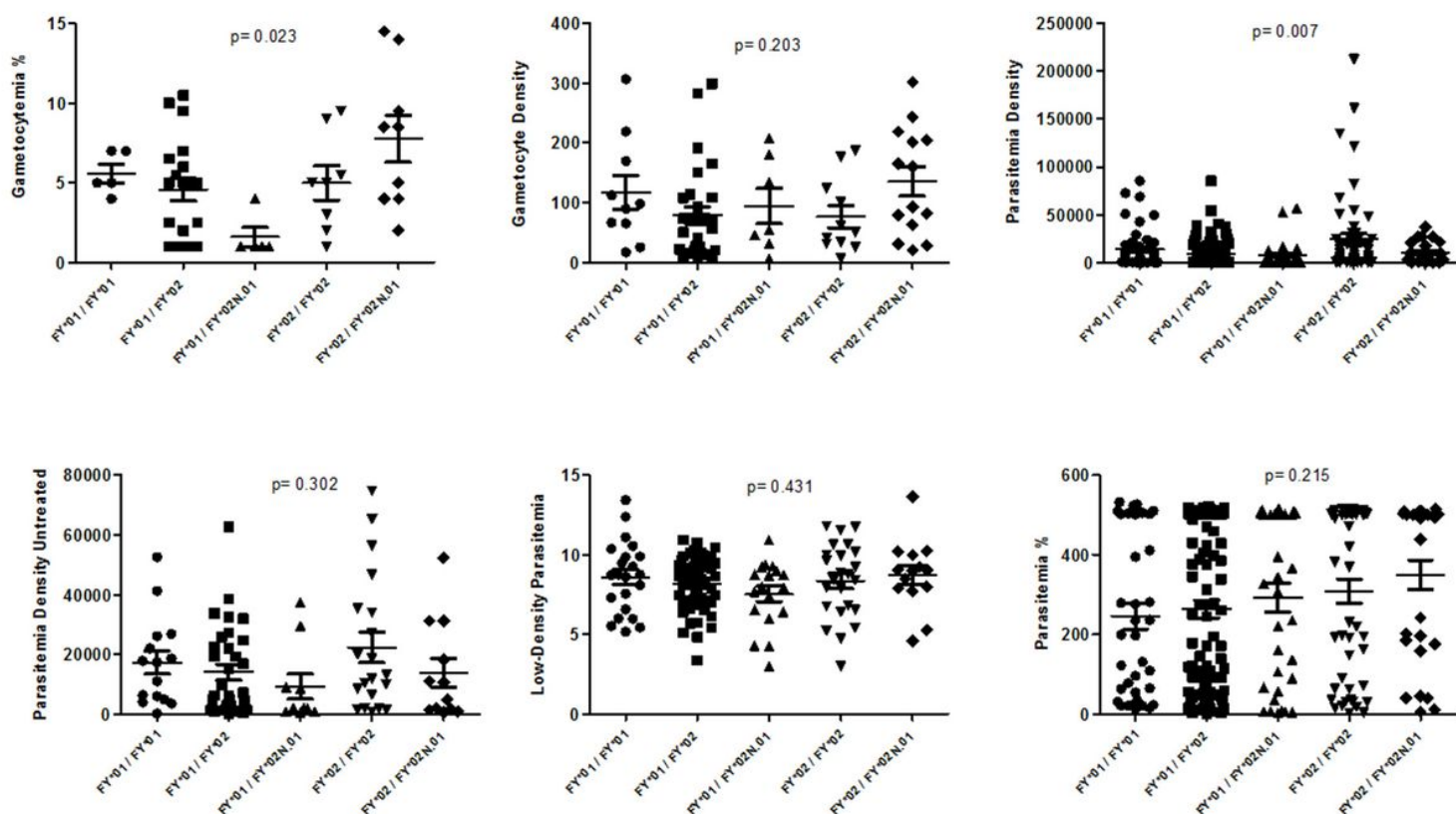

Figure 4

Level of parasite and densities counted by Duffy genotypes found in vivax malaria patients. Figure 4. The FY*02/ FY*02 genotype had the highest parasitic values, and when an FY*02 allele was present and expressed, these values were higher. The parasitic density was lower in the presence of the GATA variant.