The polymorphism rs2234767 (-1377 G>A) in the *FAS* gene may be associated with *Plasmodium vivax* infection in the Brazilian Amazon

O polimorfismo rs2234767 (-1377 G>A) no gene FAS pode estar associado à infecção por *Plasmodium vivax* na Amazônia brasileira

DOI:10.34117/bjdv7n1-515

Recebimento dos originais: 10/12/2020
Aceitação para publicação: 20/01/2021

José Eduardo Gomes Arruda
PhD degree
Laboratory of Parasitology, Institute of Health Sciences, Federal University of Pará
Rua Augusto Corrêa s/n, Guamá, 66075-110, Belém, Pará, Brazil
E-mail: josearruda@ufpa.br

Felipe Bonfim Freitas
PhD degree
Laboratory of Retrovirus, Section of Virology, Evandro Chagas Institute
Rodovia BR-316 km 7 s/n, Levilândia, 67030-000, Ananindeua, Pará, Brazil
E-mail: felipebonfim@iec.gov.br

Gustavo Capatti Cassiano
PhD degree
Global Health and Tropical Medicine (GHTM), Universidade Nova de Lisboa
Rua da Junqueira, 100, 1349-008, Lisbon, Portugal
E-mail: gcapatti@hotmail.com

Ricardo Ishak
PhD degree
Laboratory of Virology, Institute of Biological Sciences, Federal University of Pará
Rua Augusto Corrêa s/n, Campus Básico, Guamá, 66075-110, Belém, Pará, Brazil.
E-mail: rishak@ufpa.br

Ricardo Luiz Dantas Machado
PhD degree
Center of Microorganisms Investigation, Fluminense Federal University
Avenida Visconde de Rio Branco s/n, Centro, 24020-141, Niterói, Rio de Janeiro, Brazil
E-mail: ricardomachado@id.uff.br

Marinete Marins Póvoa
PhD degree
Laboratory of Basic Research in Malaria - Entomology, Parasitology Section, Evandro Chagas Institute
Rodovia BR-316 km 7 s/n, Levilândia, 67030-000, Ananindeua, Pará, Brazil
E-mail: povoamm@gmail.com

Antonio Carlos Rosário Vallinoto
PhD degree
Laboratory of Virology, Institute of Biological Sciences, Federal University of Pará
Rua Augusto Corrêa s/n, Campus Básico, Guamá, 66075-110, Belém, Pará, Brazil
E-mail: vallinoto@ufpa.br

ABSTRACT
The present study investigated the association between fatty acid synthase (*FAS*) and *FAS* ligand (*FASL*) gene polymorphisms in 128 and 99 individuals with and without *Plasmodium vivax* infection, respectively, in an endemic area of the Brazilian Amazon. Polymorphism genotyping was performed by real-time PCR (qPCR). Analysis of allele and genotype frequency distributions in the *FAS*-670 (A>G), *FASL*-844 (C>T) and *FASL*-124 (A>G) positions did not indicate statistically significant differences between the populations studied here. However, differences in the genotype frequencies of *FAS*-1377 G>A between the group of individuals infected with *P. vivax* and the control group were statistically significant (p = 0.007), suggesting an association between this polymorphism and susceptibility to infection.

Keywords: Malaria, *Plasmodium vivax*, Polymorphism, Apoptosis.

RESUMO
O presente estudo investigou a associação entre polimorfismos do gene FAS e ligante FAS (FASL) em 128 e 99 indivíduos com e sem infecção por *Plasmodium vivax*, respectivamente, em uma área endêmica da Amazônia brasileira. A genotipagem do polimorfismo foi realizada por PCR em tempo real (qPCR). A análise das distribuições de frequência de alelo e genótipo nas posições FAS-670 (A> G), FASL-844 (C> T) e FASL-124 (A> G) não indicou diferenças estatisticamente significativas entre as populações aqui estudadas. No entanto, as diferenças nas frequências genotípicas de FAS-1377 G> A entre o grupo de indivíduos infectados por *P. vivax* e o grupo controle foram estatisticamente significativas (p = 0,007), sugerindo associação entre esse polimorfismo e a susceptibilidade à infecção.

Palavras- chave: Malaria, *Plasmodium vivax*, Polimorfismo, Apoptose

1 INTRODUCTION

Malaria is a public health problem in the Brazilian Amazon and, *Plasmodium vivax* accounts for 90% of infections malaria cases reported in Brazil [(1)]. Decreased peripheral blood lymphocytes are observed in patients with acute malaria. Sequestration in lymph nodes and the abnormal death of these cells by apoptosis are processes that may also be involved in the pathophysiology of the disease. Studies report that FAS (fatty acid synthase) membrane receptor-mediated apoptosis is an important cause of lymphopenia in malaria [(2,3)], although it was observed that the regulation of the immune response may be the result of apoptosis of mononuclear cells [(4)]. This regulation process, via the FAS

and FAS ligand (FASL) complex, is comparable to these processes in other infectious diseases [5,6].

It is believed that the *FAS*-670 A>G single nucleotide polymorphism (SNP) may play a role in gene regulation and expression [7]. In the *FAS*-1377 G>A SNP, the presence of adenine may be associated with functional alteration of the FAS receptor or a decrease in its expression [8]. In the *FASL* gene, the -844 C>T SNP seems to influence both the expression of the ligand and the signaling that it mediates [9]. It is not known whether the *FASL*-124 A>G SNP is associated with altered gene expression [7,10]. The present study investigated the association between different *FAS* and *FASL* gene polymorphisms and individuals with and without *P. vivax* infection in an endemic area of the Brazilian Amazon.

2 MATERIAL AND METHODS

Samples of 128 individuals (84 males and 44 females) with vivax malaria and 99 (50 males and 49 females) without malaria from the municipality of Goianésia do Pará, Pará, Brazil, were analyzed. Data on the ancestry of the samples were previously evaluated by Cassiano et al. [11]. The analysis showed that this population had 43.9% European, 31.6% African and 24.5% Amerindian ancestry. In this study we took into consideration the possible confounding factor of ethnicity by performing association analyses adjusted for admixture, as well as for age and gender. All control subjects were unrelated. Participants signed a consent form, and the project was approved by the Research Ethics Committee of the Evandro Chagas Institute (CAAE 0014.0.072.000-10).

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Alemanha), and the polymorphisms were genotyped by qPCR using the TaqMan® SNP method. For genotyping of the *FAS* gene polymorphisms, specific assays were used: C12123966_10 for *FAS*-1377 G>A and C95958811_10 for *FAS*-670 A>G (Life Technologies, Carlsbad, Califórnia, EUA). Protocols and primer and probe sequences are available upon request.

Statistical analyses were performed using R 3.0.0 software. Differences in proportions and means were tested by the Chi-square and Mann-Whitney tests, respectively. Genotypic deviations from Hardy-Weinberg Equilibrium (HWE) were evaluated using the exact HWE test described by Wigginton et al. [12]. Using the SNPassoc package [13], the associations between the SNPs evaluated were tested using logistic

regression models. The tests were evaluated using different genetic models. Linkage disequilibrium (D') between the loci pairs was determined using the genetics package [14]. Differences in haplotype frequencies were tested by logistic regression using the R haplo.stats package [15]. To adjust for multiple tests, Bonferroni correction was applied, and values of $p < 0.01$ were considered to be significant (0.05/4).

3 RESULTS

The observed allele, genotype and haplotype frequencies are presented in Tables 1 and 2. All SNPs were in HWE in the control group. There was a greater proportion of the *FAS*-1377A allele among the malaria patients than among the control subjects (25% vs 12%, $p = 0.0008$). After adjustment for possible confounders, homozygous individuals (AA) for the *FAS*-1377 G>A SNP were more frequent among malaria patients (OR = 6.82, 95% CI: 1.44-32.27, $p = 0.003$). Similarly, heterozygous individuals (TC) for the *FASL*- 844 C>T SNP were at increased risk of developing malaria (OR = 1.96; 95% CI: 1.08-3.57, $p = 0.03$). However, after Bonferroni correction, only the *FAS*-1377 G>A SNP remained significantly associated with malaria infection. There were no significant associations between the other SNPs (*FAS*-670 G>A and *FASL*-124 A>G) and malaria infection.

The D' was moderate among the SNP pairs in the *FAS* and *FASL* genes (D' = 0.51 and 0.60, respectively). For the *FAS* gene, four haplotypes were found (GG, GA, AG and AA) with frequencies of 41.5%, 39.3%, 15.1% and 4.1%, respectively. For the *FASL* gene, the identified haplotypes (CA, TA, TG and CG) had frequencies of 45.3%, 43.6%, 9.0% and 2%, respectively.

When analyzing only haplotypes with frequencies greater than 5%, no difference in haplotype frequencies was observed among the groups investigated (Table 3). One hypothesis for this lack of association may be that the patients involved in this study had no serious complications caused by *P. vivax*. In addition, the level of endemicity of the study area may have an effect on the epidemiology of the area and the frequency of some genotypes in the sample studied. These data need further investigation.

Table 1. Tests of genetic association between SNPs and malaria

Gene	rsID	Alternative name	Allele		MAF		Genotype comparison	OR (95% CI)	*p*-value	Adjusted OR (95% CI)	Adjusted *p*-value
			Major	Minor	Control	Case					
FAS											
	2234767	*FAS*-1377	G	A	0.12	0.25	AA vs GG + GA	6.93 (1.55-30.89)	0.0018	6.82 (1.44-32.27)	0.003
	1800682	*FAS*-670	G	A	0.43	0.44	GA vs GG + AA	1.40 (0.83-2.37)	0.21	1.25 (0.69-2.27)	0.46
FASL											
	763110	*FASL*-844	T	C	0.49	0.46	TC vs TT + CC	1.80 (1.06-3.06)	0.03	1.96 (1.08-3.57)	0.03
	5030772	*FASL*-124	A	G	0.09	0.12	AG vs AA + GG	2.03 (0.97-4.23)	0.05	2.18 (0.95-5.01)	0.06

MAF: minor allele frequency
OR: Odds ratio; 95% CI: 95% confidence interval
p-values adjusted for age and gender

Table 2. Genotype frequencies in malaria patients and healthy individuals

Gene	RsID	Alternative name	Genotype	Malaria infected n = 128	Uninfected n = 99	*p*-value
FAS	2234767	*FAS*-1377	GG	81 (0.63)	77 (0.78)	0.007
			GA	31 (0.24)	20 (0.20)	
			AA	16 (0.13)	2 (0.02)	
FAS	1800682	*FAS*-670				
			GG	35 (0.27)	32 (0.32)	0.46
			GA	74 (0.58)	49 (0.49)	
			AA	19 (0.15)	18 (0.18)	
FASL	763110	*FASL*-844				
			TT	33 (0.26)	29 (0.29)	0.06
			TC	73 (0.57)	42 (0.42)	
			CC	22 (0.17)	28 (0.28)	
FASL	5030772	*FASL*-124				
			AA	98 (0.76)	85 (0.85)	0.16
			AG	28 (0.22)	12 (0.12)	
			GG	2 (0.02)	2 (0.02)	

Table 3. Haplotype frequencies in the *FAS* and *FASL* genes in vivax malaria-infected and uninfected individuals

Haplotype	Malaria	Uninfected	OR (95% CI)	*p*-value
FAS$_{-1377/-670}$				
G/G	0.390	0.470	Reference	0.42
G/A	0.362	0.410	1.20 (0.74-1.95)	0.38
A/G	0.185	0.112	1.39 (0.68-2.83)	0.15
FASL$_{-844/+124}$				
C/A	0.432	0.461	Reference	0.53
T/A	0.440	0.446	0.99 (0.63-1.56)	0.96
T/G	0.114	0.060	2.02 (0.82-4.98)	0.13

OR: odds ratio; 95% CI: 95% confidence interval
p-values adjusted for age and gender

4 DISCUSSION

Most studies have sought to associate *FAS* and *FASL* gene polymorphisms with non-infectious parasitic diseases. A study developed in China [16] found an association between the *FAS*-1377 G>A polymorphism and the risk of lung cancer. However, Hashemi et al. [17] found no association between the *FAS*-1377 G>A polymorphism and breast cancer in samples from Iran. In addition, Zhong-Xing et al. [18] performed a meta-analysis with 17,858 cases and 24,311 controls using 169 studies collected from the PubMed, CNKI and Wanfang databases; their results suggested that the *G allele at position -1377 has a protective effect against the development of cancer. A study conducted in Turkey reported higher frequencies of the GG genotype in patients with rheumatoid arthritis. No association was found between the polymorphism studied and susceptibility and/or disease severity [19]. Similar results were observed in a study carried out in the Brazilian Amazon region on patients with hepatitis B virus (HBV) [20], which did not find an association between polymorphisms and infection.

Analysis of allele and genotype frequency distributions in the *FAS*-670 (A>G), *FASL*-844 (C>T) and *FASL*-124 (A>G) positions did not indicate that there were statistically significant differences between the populations studied here, suggesting that these SNPs are not associated with susceptibility to *P. vivax* infection. However, there was a statistically significant difference (p = 0.007) in the genotype frequencies of *FAS*-1377 (G>A) between the group of *P. vivax*-infected individuals and the control group, suggesting an association of the SNP with susceptibility to infection. To avoid a false positive result, we also measured the association among Fas and FasL and infection by controlling the effect of admixture. Because we ascertained that African, European and Native American admixture do not differ among cases and controls. We hypothesize that

this allele could be a prospective genetic marker for vivax malaria in endemic areas. In addition, as previously shown in a co-stimulatory molecules of the immune system gene [11], some polymorphisms differ across ethnic groups in the population. These differences may influence the pathophysiology of malaria. Herein, we reiterate the hypothesis that studies of polymorphisms should consider the ethnic composition of the population.

5 CONCLUSIONS

The innate immune response plays an important role in the survival of the parasite that causes malaria. However, future investigations aiming the analyses of FAS and FASL protein expression are still needed to corroborate to the development of vaccines and establishment of control measures for malaria in the human population.

ACKNOWLEDGEMENTS

We thanks all the subjects enrolled in the present study. This study was funded by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES), the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CPNq) and the Amazon Foundation of Support for Studies and Research (Fundação Amazônia de Amparo para Estudos e Pesquisas - FAPESPA).

REFERENCES

1- Sivep. Malária. Sistema de Informação de Vigilância Epidemiológica [updated 2017 Jan 20]. Available from: www.saude.gov.br/sivep_malaria.
2- Kern P, Dietrich M, Hemmer C, Wellinghausen N. Increased levels of soluble Fas ligand in serum in Plasmodium falciparum malaria. Infect. Immun. 2000; 68(5), 3061–63.
3- Matsumoto J, Kawai S, Terao K, Kirinoki M, Yasutomi Y, Aikawa M, et al. Malaria infection induces rapid elevation of the soluble Fas ligand level in serum and subsequent T lymphocytopenia: possible factors responsible for the differences in susceptibility of two species of Macaca monkeys to Plasmodium coatneyi infection. Infect. Immun. 2000; 68(3), 1183-88.
4- Riccio EKP, Júnior IN, Riccio LRP, das Graças Alecrim M, Corte-Real S, Morgado M, et al. Malaria associated apoptosis is not significantly correlated with either parasitemia or the number of previous malaria attacks. Parasitol. Res. 2003; 90(1), 9–18.
5- Badley AD, Pilon AA, Landay A, Lynch, DH. Mechanisms of HIV-associated lymphocyte apoptosis. Blood. 2000; 96(9): 2951-64.
6- Cossarizza A. Apoptosis and HIV infection: about molecules and genes. Curr. Pharm. Des. 2008; 14(3): 237-44.
7- Zhang Z, Wang LE, Sturgis EM, El-Naggar AK, Hong WK, Amos CI, et al. Polymorphisms of FAS and FAS ligand genes involved in the death pathway and risk and progression of squamous cell carcinoma of the head and neck. Clin. Cancer Res. 2006; 12(18): 5596–602.
8- Sibley K, Rollinson S, Allan JM, Smith AG, Law GR, Roddam PL, et al. Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia. Cancer Res. 2003; 63(15); 4327-30.
9- Wu J, Metz C, Xu X, Abe R, Gibson AW, Edberg JC, et al. A novel polymorphic CAAT/enhancerbinding protein beta element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. J. Immunol. 2003; 170(1): 132-38.
10- Pinti M, Troiano L, Nasi M, Moretti L, Monterastelli E, Mazzacani A, et al. Genetic polymorphisms of Fas (CD95) and FasL (CD178) in human longevity: studies on centenarians. Cell. Death Differ. 2002; 9(4): 431–38.
11- Cassiano GC, Santos EJ, Maia MH, Furini Ada C, Storti-Melo LM, Tomaz FM, et al. Impact of population admixture on the distribution of immune response co-stimulatory genes polymorphisms in a Brazilian population. Hum. Immunol. 2015; 76(11): 836-42.
12- Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of hardy-weinberg equilibrium. Am. J. Hum. Genet. 2005; 76(5): 887-93.
13- Gonzalez JR, Armengol L, Sole X, Guino E, Mercader JM, Estivill X, et al. SNPassoc: an R package to perform whole genome association studies. Bioinformatics. 2007; 23(5): 644–45.
14- Warnes G, Gorjanc F, Leisch M. Genetics: population genetics. [updated 2017 Jan 20] Available from: http://CRAN.R-project.org/package=genetics.
15- Sinnwell JP, Schaid DJ. Schaid, haplo.stats: Statistical analysis of haplotypes with traits and covariates when linkage phase is ambiguous. http://CRAN.Rproject.org/package=haplo.stats (accessed 20.01.17).
16- Zhang X, Miao X, Sun T, Tan W, Qu S, Xiong P, et al. Functional polymorphisms in cell death pathway genes FAS and FASL contribute to risk of lung cancer. J. Med. Genet. 2005; 42(6), 479–84.

17- Hashemi M, Fazaeli A, Ghavami S, Eskandari-Nasab E, Arbabi F, Mashhadi MA, et al. Functional polymorphisms of FAS and FASL gene and risk of breast cancer - pilot study of 134 cases. PLoS One. 2013; 8(1): e53075.
18- Zhong-Xing Z, Yuan-Yuan M, Hai Zhen M, Jian-Gang Z, Li-Feng Z. FAS-1377 G/A (rs2234767) polymorphism and cancer susceptibility: a meta-analysis of 17,858 cases and 24,311 controls. PLoS One. 2013; 8(8): e73700.
19- Yildir S, Sezgin M, Barlas IO, Turkoz G, Ankarali HC, Sahin G, et al. Relation of the Fas and FasL gene polymorphisms with susceptibility to and severity of rheumatoid arthritis. Rheumatol. Int. 2013; 33(10): 2637–45.
20- Santana BB, Viegas ML, Conde SR, Ishak MO, Ishak R, Vallinoto AC. FAS and FASL gene polymorphisms are not associated with hepatitis B virus infection based on a case-control study in a Brazilian population. Dis. Markers. 2013; 35(6): 741-46.