



## Research Article

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# The Interleukin-1 $\beta$ (-511T/C) is Associated with Ulcerative Colitis

Mayara Tavares<sup>1\*</sup>, Camilla De Lima<sup>2</sup>, Valéria Martinelli<sup>3</sup>, Maurílio De Lucena<sup>4</sup>, Francisco Lima<sup>5</sup>, Adriana Telles<sup>6</sup>, Lucas Brandão<sup>6</sup> and Mário De Melo Júnior<sup>6</sup>

<sup>1</sup>Department of Cell Biology and Genetics, Federal University of Rio Grande do Norte, Brazil

<sup>2</sup>Laboratory of Immunopathology Keizo Asami, Federal University of Pernambuco, Brazil

<sup>3</sup>Department of Gastroenterology, University Hospital, Federal University of Pernambuco, Brazil

<sup>4</sup>Department of Proctology, Barão de Lucena Hospital, Brazil

<sup>5</sup>Department of Surgery, University Hospital, Federal University of Pernambuco, Brazil

<sup>6</sup>Departament of Pathology, Federal University of Pernambuco, Brazil

**\*Corresponding author:** Mayara Costa Mansur Tavares, Department of Cell Biology and Genetics, Federal University of Rio Grande do Norte, CEP: 59078-970, Brazil.

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## Abstract

**Purpose:** Inflammatory bowel disease (IBD) is a group of illnesses whose primary manifestation is inflammation. The most common typical phenotypes are ulcerative colitis (UC) and Crohn's disease (CD). Although the precise etiology remains obscure, several reports have indicated that dysfunction of the mucosal immune system play an important role in its pathogenesis. This study aimed to analyzing the genes polymorphisms of immune response in Brazilian patients with IBD.

**Methods and results:** 95 patients were analyzed for the caspase activation and recruitment domains 15/ NOD like receptor 2 (CARD15/NOD2) (rs2066844 and rs2066845), NOD like receptor – (NLRP1) (rs12150220), NLRP3 (rs35829419) and interleukin (IL)-1 (rs16944) genes polymorphisms. The anatomic-clinical form of CD predominant was both, fistulizing and inflammatory each (35.18%), followed by structuring (27.77%) and 1.85% structuring and fistulizing in the same patients. 91 healthy subjects composed the control group. The statistical analysis was performed using R program. NOD like receptor pyrin domain containing 1 and 3 and caspase activation and recruitment domains 15/ NOD like receptor 2 genes R702W and G908R variants were not associated to inflammatory bowel disease susceptibility. We found that AG genotype of interleukin-1beta was associated with the development of UC.

**Conclusion:** Our findings suggest that the IL-1 single nucleotide polymorphism is involved with UC and may be contributing to pathogenesis in Brazilian population.

**Keywords:** Inflammasome; Interleukin; Inflammatory bowel disease; Single nucleotide Polymorphism

## Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract. Crohn's disease (CD) and Ulcerative Colitis (UC) are the two main spectrum of IBD, representing a heterogeneous and multifactorial condition. While CD presents discontinuous regions of transmural inflammation, affecting from the mouth to the anus, the inflammatory process in UC is limited to the mucosa and submucosa affecting all or part of the colon or rectum extension without alternating injury [1].

The inflammatory response is the main phenotype of IBD, being expressed as the result of the continuous exposition of the gastrointestinal tract to a variety of antigens (enteric bacteria and foods) in genetically susceptible individuals [2].

Recently, progress has been achieved in the molecular understanding of the immune response in the onset of disease [3]. Several receptors of the extracellular interleukin (IL) and toll-like receptors families (TLR) have been implicated in IBD [4].

Single nucleotide polymorphism (SNPs) at IL-1 and NLRP3 genes were linked to the pathogenesis of intestinal inflammation and CD susceptibility [5-8].

In a recent meta-analysis of genome-wide association studies for CD and UC, 163 IBD-associated loci were identified. Many of them are associated with both UC and CD. These regions contain candidate genes for a variety of functions like autophagy, microbe recognition, lymphocyte signaling and others [9].

This study aimed to investigating polymorphisms associated to inflammasomes in patients with IBD. We analyzed 5 SNPs involved in inflammasome activation: 2 variants in the CARD15/NOD2 (R702W and G908R), 1 in NLRP1, 1 in NLRP3 and 1 in IL-1 genes in Brazilian individuals.

## Materials and Methods

A cross-sectional study was performed in patients affected by IBD. The patients were recruited at Gastroenterology Clinic of the Hospital Barão de Lucena/SES/PE and Clínicas Hospital of Pernambuco, Federal University of Pernambuco (Recife, Brazil) between June 2013 and January 2014. The diagnosis of IBD was based on clinical, laboratory, imaging, endoscopic and histological features according to the criteria of the Lennard-Jones (1989). The information of age of diagnostic, gender, location and CD of disease behavior were organized according to the Vienna Classification [10]. Blood samples were collected in tube containing anti-coagulated with ethylenediaminetetraacetic acid (EDTA). The genetic analyses were conducted at the Laboratory of Immunopathology Keizo Asami of the Federal University of Pernambuco (Recife, Brazil).

We enrolled ninety-five patients between 14 and 81 years old (medium: 42.07/ median: 41 years old/  $\sigma = 13,92740738$ ). Clinical data collected from includes the age at diagnosis and disease behavior (structuring, inflammatory or fistulizing). The predominant gender was similar between male (47/95; 49.47%) and female (48/95; 50.53%). The age at diagnosis of the disease prevailed in subjects under 40 years (55.79%).

Of the 95 cases, 41 (43.16%) had UC and 54 (56.84%) had CD. Of all the 54 CD diagnoses in our study, 19 patients (35.18%) were diagnosed as having non-structuring/non-fistulizing CD, 15 patients (27.77%) with structuring CD, 19 (35.18%) with fistulizing CD and 1 (1.85%) patient with both, structuring and fistulizing CD. All data are included in Table 1.

The control group comprised 91 subjects who underwent endoscopy and who had no clinical or laboratory evidence of IBD. Ninety-one volunteers were analyzed to NLRP1, 85 other individuals of the same group were genotyped for NLRP3, 83 for CARD15 R702W variant, 80 for CARD15 G908R variant and 84 volunteers for IL-1.

## Ethical issues

This study was previously approved by the Ethics Committee of the Health Sciences Center of Federal University of Pernambuco (CEP/CCS/UFPE) (protocol 222/2010), and all patients agreed to participate by signing the Free and Informed Consent Form.

## Genomic DNA extraction

Genomic DNA was extracted from 250  $\mu$ L of periferic blood using Rapid protocol Mini Salting Out method and digestion with proteinase K, presented in Tecnical Handbook of Twelfth International Histocompatibility Workshop and Conference (1996), with modifications to amounts used.

## SNPs selection and Genotyping

We analyzed 2 variants in the CARD15/NOD2 gene (R702W and G908R, corresponding, respectively to rs2066844 and rs2066845), 1 in NLRP1 gene (rs12150220), 1 in NLRP3 gene (rs35829419) and 1 SNP in IL-1 gene (rs16944). Genotyping was performed using commercially available fluorogenic allele specific probes (Taqman Probes, Applied Biosystems, Foster City, CA, USA) with the ABI7500 Real Time PCR platform (Applied Biosystems, Foster City, CA, USA). Allelic discrimination followed as recommended by the manufacturer and analyzed using the SDS software 2.3 (Applied Biosystems, Foster City, CA, USA).

## Statistical analysis

Statistical analysis was realized using the R program. The influence of each polymorphism on the risk of developing IBD was estimated by odds ratio (OR) using a confidence interval (CI) of 95% for the parameters. Hardy-Weinberg equilibrium was tested. The prevalence of different genotypes in patients and controls was analyzed by the Fisher.

## Results

Statistical analysis univariate has indicated that women have a later age of diagnosis than men (p-value = 0.003463) Table 1.

The anatomical and clinical forms showed no association with polymorphisms studied by univariate analysis Table 1.

**Table 1:** Characterization of patients.

	UC n=41 (43.16%)	DC n=54 (56.84%)
Gender		
Male	21 (51.22%)	26 (48.15%)
Female	20 (48.78%)	28 (51.85%)
Age of diagnosis		
< 40 years	17 (41.46%)	25 (46.3%)
≥40 years	24 (58.54%)	29 (53.7%)
Family History	6 (14.63%)	5 (9.26%)
No Family History	35 (85.37%)	49 (90.74%)
Eating habits		
Diet	29 (70.73%)	21 (38.89%)
No diet	12 (29.27%)	33 (61.11%)
Clinical Characteristics CD		
Anatomo-clinical form		
Non-structuring/Non-fistulizing		19 (35.18%)
Structuring		15 (27.77%)
Fistulizing		19 (35.18%)
More than one form		1 (1.85%)

The genotype distributions of CARD15/NOD2, NLRP1, NLRP3 and IL-1 are in agreement with Hardy-Weinberg equilibrium, except the healthy group to NLRP1 ( $p=0.0082$ ) and patients' group to IL-1 (IBD and UC; both  $p=0.0000$ ).

The presence C allele in NLRP3 gene was shown to be very frequent, nevertheless there was no statistical difference was seen between the groups analyzed Table 2.

**Table 2:** The genotype frequencies of NLRP1, NLRP3, CARD15/NOD2 Var F702W and CARD15/NOD2 Var G908R gene polymorphisms and their association with risk of IBD (CD and UC).

SNP	HC		IBD	IBD CD	IBD UC	HCXIBD		HCXCD		HCXUC	
	Alleles/ Genotypes	n (%)	n (%)	n (%)	n (%)	OR (CI 95%)	p-value	OR (CI 95%)	p-value	OR (CI 95%)	p-value
NLRP1	A	118 (65)	118 (68)	60 (65)	58 (71)	1	0.6294	1	0.9431	1	0.4241
	T	64 (35)	56 (32)	32 (35)	24 (29)	0.88 (0.56- 1.36)		0.98 (0.58- 1.66)		0.76 (0.43- 1.34)	
	AA	44 (48)	39 (45)	20 (43)	19 (46)	1		1		1	
	AT	30 (33)	40 (46)	20 (43)	20 (49)	1.5 (0.79- 2.85)	0.2758	1.47 (0.68- 3.18)	0.4391	1.54 (0.71- 3.37)	0.3715
	TT	17 (19)	8 (9)	6(13)	2 (5)	0.53 (0.21- 1.37)	0.2735	0.78 (0.27- 2.26)	0.8427	0.27 (0.06-1.3)	0.156
NLRP3	C	168 (99)	138 (99)	61 (98)	77 (99)	1	0,7566	1	0,6919	1	0,5790
	A	2 (1)	2 (1)	1 (2)	1 (1)	1,22 (0,17- 8,75)		1,38 (0,12- 15,46)		1,09 (0,1- 12,21)	
	CC	83 (98)	68 (97)	30 (97)	38 (97)	1		1		1	
	CA	2 (2)	2 (3)	1 (3)	1 (3)	1,22 (0,12- 15,82)	0,7551	1,38 (0,12- 15,82)	0,6900	1,09 (0,1- 12,42)	0,5766
	AA	0 (0)	0 (0)	0 (0)	0 (0)	ND	ND	ND	ND	ND	ND
NOD2/ R702W	C	151 (91)	128 (93)	57 (92)	71 (93)	1	0,7219	1	0,9742	1	0,6944
	T	15 (9)	10 (7)	5 (8)	5 (7)	0,79 (0,34- 1,81)		0,88 (0,31- 2,54)		0,71 (0,25- 2,03)	
	CC	70 (84)	60 (87)	27 (87)	33 (87)	1		1		1	
	CT	11 (13)	8 (12)	3 (10)	5 (13)	0,85 (0,18- 2,73)	0,9327	0,71 (0,18- 2,73)	0,8550	0,96 (0,31-3)	0,8218
	TT	2 (2)	1 (1)	1 (3)	0 (0)	0,58 (0,05- 6,59)	0,8844	1,30 (0,11- 14,89)	0,6571		0,8433
NOD2/ G908R	G	159 (99)	123 (99)	59 (98)	64 (1)	1	0,5933	1	0,9422	1	0,6345
	C	1 (1)	1 (1)	1 (2)	0 (0)	1,29 (0,08- 20,88)		2,69 (0,17- 43,79)			
	GG	79 (99)	61 (98)	29 (97)	32 (1)	1		1		1	
	GC	1 (1)	1 (2)	1 (3)	0 (0)	1,30 (0,16- 44,99)	0,5920	2,72 (0,16- 44,99)	0,9419	ND	0,6337
	CC	0 (0)	0 (0)	0 (0)	0 (0)	ND	ND	ND	ND	ND	ND
IL-1	G	90 (54)	88 (47)	49 (47)	39 (48)	1	0,2847	1	0,3627	1	0,4484
	A	78 (46)	98 (53)	55 (53)	43 (52)	1,28 (0,85- 1,95)		1,30 (0,79- 2,11)		1,27 (0,75- 2,16)	
	GG	25 (30)	9 (1)	9 (17)	0 (0)	1		1		1	
	GA	40 (48)	70 (75)	31 (60)	39 (95)	4,86 (0,88- 5,27)	0,0003	2,15 (0,88- 5,27)	0,1382	ND	0,0000
	AA	19 (23)	14 (15)	12 (23)	2 (5)	2,05 (0,73- 5,72)	0,2637	1,75 (0,61- 5,01)	0,4305	ND	0,3942

The same could be seen in the presence to C allele in CARD15/NOD2 gene R702W variant and also the presence to G allele in CARD15/NOD2 gene G908R variant Table 2.

We observed no significant relationship between the CARD15/NOD2 gene R702W and G908R variants in both controls and IBD patients Table 2.

NLRP1 gene polymorphism have shown no significant association between genotypic and allelic frequencies in the groups studied (HC x IBD). We can say there is no significant difference in the co-dominant model when compared the IBD UC to control groups ( $p=0.059$ ) Table 2.

Through the univariate and multivariate analysis, it was found that AA and AG genotype of IL-1 was associated with the development of CD and UC, respectively. The IL-1 SNP is involved with IBD (GAXAA,  $p$ -value=0.0003) Table 2.

## Discussion

Several inflammasome complexes could be activated and release IL-1 $\beta$  have been described [11]. Genetics polymorphisms in inflammasome has been linked to susceptibility common diseases.

The gene polymorphism in IL-1 -511T in Brazilian patients with periodontitis was associated with the disease in whites and mulattos' patients [12]. rs1143634 in IL-1 was also significantly associated to the HIV-1 infection in Brazilian population [13]. In other studies, it was verified this association in UC.

While the NLRP3 inflammasome regulates a variety of inflammatory and autoimmune diseases, IL-1 $\beta$  production contributes to intestinal inflammation. Recently, NLRP3-induced production of IL-1 $\beta$  have been associated to protection against colitis [14].

Our findings suggest that the genes polymorphisms in the NLRP3 and CARD15/NOD2 G908R and R702W variants were not associated to IBD susceptibility.

Various studies report the influence of polymorphism NLRP3 gene in several diseases, but results have been contradictory. No evidence of association of NLRP3 SNP rs35829419 with abdominal aortic aneurysms patients was from observed New Zealand [15]. A study performed in North of China, minor allele of rs35829419 was absent in Alzheimer's disease patients and only present in controls, suggesting that the A allele is the protective allele against the risk of developing late-onset Alzheimer's disease [16]. The same can be seen in the Pontillo study (2012b). The results of rs10754558 suggest a protective effect against HIV-1 infection but the rs35829419 not to be associated with HIV infection. The conflicting results can be attributed to genetic variations between different populations or variant phenotypes of patients in the study [17].

However, a study conducted in Japan in patients with allergic diseases evaluated two polymorphisms associated with NLRP3 gene (rs4612666 and rs10754558) and found these polymorphisms was associated with anaphylactic shock induced by food. In our study, we obtained no results statistically significant. This polymorphism can increase the IL-1 beta production and to develop IBD.

A research conducted in Netherlands, CD was associated with R702W ( $p=0.008$ ) for heterozygotes [18]. Other study carried out in south of Italy, CD patients showed NOD2/CARD15 R702W mutation significantly most frequent when compared with control group and UC patients ( $p=0.001$  and  $p=0.03$ , respectively) [19]. Our findings suggest no correlation to IBD, in Brazilian patients.

In literature, we found no association between of the gene NOD2/CARD15/G908R variant and diseases of different population groups studied [19,20]. In our study we did not observe statistically significant association.

NLRP1 polymorphism gene was not associated with HIV-1 infection according Pontillo et al. (2012b) study. However, in another study of Pontillo et al. (2012a) in patients with lupus erythematosus, there was an association of NLRP1 SNP rs2670660 with the predisposition to disease. In present study, NLRP1 gene not contributes to pathogenesis in Brazilian population.

Few studies of association between genes polymorphisms of immune response and inflammatory bowel disease have been conducted at the moment. The data about this disease in Brazilian population are scarce. Thus, it is important to evaluate different aspects of population genetic profile that might be interfering with the response of patients to the same treatment of IBD [21].

Our data suggesting an association between IL-1 gene polymorphism and the development of Ulcerative Colitis

This study showed current and important information of inflammasome gene polymorphisms in inflammatory bowel disease. It is necessary to develop other studies involving major quantity of individuals and to associate aspects socioeconomics, clinical and epidemiological.

## Competing Interests

The authors declare that there are no conflicts of interest (economic or otherwise).

## Author Contribution

MCMT designed the study, coordinated and carried out the experiments and drafted the manuscript; CADL participated in the experiments of DNA extraction and sequencing of the samples and performed the data analysis; VFM and MTL performed the sample collection; FEAL and AMST contributed to the clinical diagnosis; MRMJ and LACB conceived the study, performed the data analysis and reviewed the manuscript; LACB corrected the manuscript.

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