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A polymorphism in the *IL1B* gene (rs16944 T/C) is associated with cutaneous leishmaniasis caused by *Leishmania guyanensis* and plasma cytokine interleukin receptor antagonist

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ABSTRACT

Nod-like Receptor Protein3 (NLRP3) inflammasome in macrophages infected with *Leishmania sp*. enhances the secretion of IL-1β. Excess IL-1β production is linked to disease severity in patients with cutaneous leishmaniasis (CL) caused by *L. mexicana*. Blockade of the NLRP3 inflammasome in cell cultures from skin biopsies of patients with CL caused by *L. braziliensis* inhibited the release of IL-1β. We hypothesized that common single nucleotide polymorphisms in the *IL1B* and in its receptor antagonist *IL1RN* genes may be predictive of CL caused by *L. guyanensis*. The SNPs –511T/C (rs16944) and + 3954C/T (rs1143634) of the *IL1B* and *IL1RN* VNTR (rs2234663) were assessed in 881 patients with CL and 837 healthy controls by PCR-RFLP and direct PCR respectively. Plasma cytokines levels were also assayed. The plasma levels of IL-1β were higher in patients compared to control subjects. In contrast, increased plasma levels of IL-1Ra were observed in controls. The rs16944 C/C genotype was more common among the patients (OR = 1.5 [95%CI 1.1–2.0]; P = 0.004) and the C allele suggests susceptibility to CL (OR = 1.2 [95%CI 1.1–1.4]; P = 0.003). The rs16944 C/C genotype shows a tendency to correlate with lower levels of the IL-1Ra cytokine. Low levels of IL-1Ra cytokine and rs16944 C/C genotype seem to confer susceptibility to *L. guyanensis*-infection in the Amazonas.

1. Introduction

Leishmaniasis, an infectious vector-borne disease caused by *Leishmania* species, affects more than 12 million people worldwide and remains a major health problem in the tropical and subtropical regions of Asia, the Middle East, sub-Saharan Africa and South America [1]. Over 20 different *Leishmania* species are identified to cause Leishmaniasis. The disease is transmitted by sand fly, the phlebotomines. *Leishmania*-infection displays a wide spectrum of clinical manifestations. It ranges from asymptomatic, spontaneously healing skin lesions, to more chronic cutaneous (CL) or mucocutaneous lesions (ML) and the life threatening visceral leishmaniasis (VL) that can be fatal in the absence of treatment or to 5–15% even with treatment.

CL is the most common with 0.7 to 1.2 million of new cases each year. In Brazil, the estimated annual incidence of CL cases is around 72,000 to 119,000 [2]. *L. braziliensis*, *L. guyanensis*, *L. lainsoni*, *L. naiffi* and *L. linbergi* are the major species causing CL in Brazil. *L. guyanensis* is the main etiological agent of CL in the Amazonas.

Extensive studies in *L. major*-infected murine model of CL demonstrated that the clinical course of the disease depends mainly on the balance of pro-inflammatory cytokines produced by Th1 cells and anti-inflammatory cytokines produced by Th2 cells and subsets of regulatory T cells [3].

Leishmania species can activate the NLRP3 inflammasome in infected macrophages and enhance the secretion of interleukin-1beta (IL-1β) [4,5]. IL-1β production due to NLRP3 confer resistance in C57BL/6 to *L. amazonensis*, *L. braziliensis* and *L. infantum chagasi* but not to *L. major* infection [5]. In contrast, knockout C57BL/6 mice for any

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component of the NLRP3 inflammasome such as apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), or Caspase1/11 are resistant to a *L. major* Seidman strain that can cause chronic lesions in C57BL/6 [6]. Knockout C57BL/6 mice for IL-1β or IL-1 receptor signaling are also resistant to *L. major* Seidman strain [6]. Similarly, BALB/c mice known to develop non-healing and disseminating lesions to *L. major* become resistant in the absence of the NLRP3 inflammasome components [4]. Caspase 1/11 and NLRP3 knockout mice infected with *L. braziliensis* manifest reduced pathology [7]. A genome-wide transcriptional study of skin biopsies specimens of ulcer lesions from patients with CL caused by *L. braziliensis* showed an upregulation of *IL1B* and genes involved in the NLRP3 inflammasome [8]. Blockade of the NLRP3 inflammasome in cell cultures from skin biopsies of patients with CL inhibited the release of IL-1β [7]. Excess of IL-1β production are linked to increased disease severity in patients with CL caused by *L. Mexicana* [9].

Taken together, these studies suggest that the genetic background of the host together with the *Leishmania* species play key role in the displaying of the clinical manifestations and IL-1β may be one of the key components involving in either susceptibility or resistance to *Leishmania*-infection. Indeed, IL-1 family members are involved in various autoinflammatory and immunopathologies disorders and in shaping a coordinated immune response against infectious pathogens [10].

The interleukin-1 gene cluster is located on chromosome 2q and spread within 430 Kb containing *IL1A*, *IL1B* and *IL1RN*. *IL1A* and *IL1B* encode the pro-inflammatory cytokines IL-1α and IL-1β respectively while *IL1RN* their endogenous receptor antagonist, IL-1Ra. Three single nucleotide polymorphisms (SNP) in *IL1B* at positions –511C/T (rs16944), –31C/T (rs1143627) and +3954C/T (rs1143634) base pairs (bp) from the transcriptional sites have been studied in various inflammatory and infectious diseases [11]. The functional effects of these polymorphisms on the expression of IL-1β are conflicting [12–15]. The *IL1RN* possesses an 86-bp variable number of tandem repeats (VNTR) (rs2234663) in intron 2. This VNTR has also been studied in several infectious diseases [16–20].

In attempting to determine whether the SNPs rs16944, rs1143634 and rs2234663 may confer either protection or susceptibility to CL caused by *L. guyanensis* in the Amazonas, we also investigate if these SNPs have any influence on the expression of the genes by assaying circulating plasma cytokines in healthy subjects with no history of leishmaniasis from the endemic areas of leishmaniasis.

2. Materials and methods

2.1. Study population

The study was conducted in the perirural areas of Manaus, the capital city of the Amazonas State where *L. guyanensis* is endemic. All participants were enrolled between January 2009 and March 2016. The patients with CL were followed at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD), the referral center for treatment of tropical diseases including leishmaniasis. The healthy controls with no history of CL and devoid of any scar suggestive of CL were recruited from the same endemic areas and shared similar environments as the patients with CL. Most of the participants are agricultural workers and has been living in the areas for more than five years. This study population is mainly an admixture of Native American Ancestry with European ancestry. This population is of origin of nearly 50 to 60% of Native American, 40 to 50% of European and around 10% of African origin [21].

Eight hundred and eighty-one (881) patients with CL caused by *L. guyanensis* and 837 healthy controls were included in the study. Among the patients with CL, 663 (75.2%) patients were male (mean age ± SD, 33.97 ± 13.85 years) and 218 (24.8%) were females (36.49 ± 15.77 years). In the controls group, 566 (67.6%) were male (41.63 ± 17.54 years) and 271(32.4%) were female (39.46 ± 17.78). Overall, the mean age among the patients with CL and controls is 34.58 ± 14.37 years and 40.92 ± 17.64 years respectively. The controls are slightly older ($p < 0.0001$).

2.2. Ethical statement

The Research Ethics Committee of the FMT-HVD approved this study (File Number CAAE: 09995212.0.0000.0005). All of the participants or their responsible party for individuals < 18 years of age provided written informed consent for the collection of samples and subsequent analysis.

2.3. Biological sample collection

Five mL of peripheral blood was collected by venipuncture into EDTA-containing Vacutainer (Becton Dickinson) for DNA extraction and Cytokines assay. From all the patients with CL, a biopsy of the lesion was performed for the identification of *Leishmania* species. The collection of plasma from patients and controls for cytokines analysis only started from January 2013 to March 2016.

2.4. Identification of Leishmania spp

The diagnosis of patients with CL was confirmed by direct microscope examination of Giemsa-stained specimens for the presence of *Leishmania* parasites from lesion scarifications. DNA was prepared from lesion biopsy specimens of all the patients with CL. *Leishmania vianna* specific PCR with discrimination between *L. braziliensis* and *L. guyanensis* was performed as described elsewhere [22,23]. Species identification also was through direct nucleotide sequencing of a fragment of the HSP70 and miniexon genes. The following pair of primers: HSP70F: 5′GGACGAGATCGAGCGCATGGT-3′ and HSP70R: 5′-TCCTTCGACGCT CCTGGTTG-3′ for HSP70 and Mini-ExonF: 5′-TATTGGTATGCGAAACT TCCG-3′ and Mini-ExonR: 5′-ACAGAAAACTGATACTTATATAGCG-3′ were used to amplify separately a fragment of 233 bp for HSP70 and 227 bp for Mini-Exon respectively. Nucleotide sequencing was performed with the kit BigDyes from Applied Biosystem (Thermofisher, MA USA) following the protocols suggested by the manufacturer.

2.5. DNA extraction and SNP genotyping

The DNA extraction was according to the proteinase K and salting-out method [24]. The SNPs *IL1B* –511T/C (rs16944) present in the promoter region and +3954 C/T (rs1143634) in Exon two were performed by a polymerase chain reaction-restriction fragment length polymorphism technique with the restriction enzymes *Ava I* and Taq I (New England Biolabs, Ipswich, MA, United States) respectively. The following pairs of primers flanking the SNP: Forward 5́-TGGCATTGAT CTGGTTCATC-3́ and Reverse 5́-GTTTAGGAATCTTCCCACTT-3́ for rs16944 were used to generate a fragment of 305 bp and for rs1143634 Forward 5́-GTTGTCATCAGACTTTGACC-3́ and Reverse 5́-TTCAGTTCA TATGGACCAGA-3́ to yield 250 bp fragment. For both SNPs, the PCR reactions were optimized in a final volume of 25 μL under the cycling conditions of an initial denaturation step of 94 °C for 5 min; 40 cycles of 94 °C for 30 secs; 53 °C for 30 secs and 72 °C for 30 secs; and a final step of 72 °C for 7 min. The PCR mix contains: 0.2 μM of each primer (Thermofisher, MA USA), 40 nM of dNTP (Thermofisher, MA USA), 2.0 mM of $MgCl_2$ (Thermofisher, MA USA), 1 U of Taq DNA polymerase (Thermofisher, MA USA) and 50 ng of DNA. A volume of 10 μL of the PCR products was digested with 2 units of the respective restriction enzyme and size separated in a 3% agarose (Ultrapure Agarose, Thermofisher, MA USA) gel electrophoresis. When the C allele is present for rs16944, the 305 bp yields two fragments of 190 and 115 bp and remains uncleaved in the presence of the T allele. Similarly, for the rs1143634, the 250 bp fragment is cleaved by *Taqa I* to yield two

fragments of 114 and 136 bp in the presence of the C allele and remains uncut for the T allele.

For the *IL1RN* VNTR rs2234663, the primers (Forward 5́-CTCAGC AAC ACTCCTAT-3́ and Reverse 5́-TCCTGGTCTGCAGGTAA-3́) were used. The PCR mix and cycling conditions were similar as above. Allelic discrimination was by fragment size separation in a 2% agarose gel electrophoresis.

2.6. Cytokines assay by Luminex

Plasma samples were kept frozen at –80 °C until the measurement of the levels of cytokines. The concentrations of circulating plasma IL-1β and IL-1Ra cytokines were determined using the multiplex cytokine commercial kit Bio-PlexPro™ Human Cytokine Grp I Panel 27-Plex (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions in the Bio-plex 200 Protein Array System (Luminex Corporation, Austin, TX, USA).

2.7. Statistical analysis

The Mann-Whitney test and χ^2 were used to compare age and sex between the patients and control subjects, respectively. The genotype and allele frequencies were determined by direct gene counting. The Hardy-Weinberg Expectation (HWE) was determined by comparing the observed genotypes with the expected under HWE for the estimated allele frequency. For comparison of genotypes and alleles frequencies, logistic regression analysis was applied with the help of the website http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. The Linkage disequilibrium and comparisons of haplotypes were determined with the Haploview software (v.4.2). For the comparison of the effects of SNPs on plasma cytokine levels, the Mann-Whitney and Kruskal-Wallis test were applied using the GraphPad Prism (v.6.1) software.

3. Results

3.1. Genotypes and alleles distributions of the SNPs of the IL1B and the VNTR of IL1RN

The *IL1B* SNP rs16944 was assessed in 872 patients with CL and 812 controls while rs1143634 in 881 patients with CL and 837 controls. The number of genotyped patients and controls among the SNPs are different due to unsuccessful PCR for some of the samples. The SNPs were chosen from the HAPMAP as they are tag SNPs covering a distance of 8399 bp of the gene. Notably, rs16944 is in complete linkage disequilibrium with another SNP rs1143627 in all populations while rs1143634 with SNP rs3136558. For this reason, both SNPs are the most studied in the literature. The *IL1RN* VNTR rs2234663 was analyzed in 796 patients with CL and 813 controls. All the SNPs studied were in HWE in both groups except the rs1143634 deviated among the controls (p = 0.01). The distributions of the genotypes and allele frequencies for the two SNPs and VNTR are shown in Table 1 and 2 respectively.

The distribution of the rs16944 genotypes differs significantly between the patients and controls (χ^2 = 8.8; p = 0.003). The rs16944 CC genotype was significantly higher in patients (OR = 1.5 [95%CI 1.1–2.0]; p = 0.005). In the dominant model, carriers of C allele were compared with the TT genotypes and the difference is significant suggesting the C allele as a risk factor (OR = 1.3 [95%CI 1.1–1.6] p = 0.009). The comparison of alleles also showed that C allele is associated with susceptibility (OR = 1.2 [95%CI 1.1–1.4] p = 0.003).

For the *IL1B* rs1143634 there was an excess of genotype TT (4.3%) in controls than in patients (2.0%). The genotype TT confer protection (OR = 0.5 [95%CI 0.3–0.8] p = 0.0094) while CC genotypes susceptibility (OR = 2.1[95%CI 1.2–3.8] p = 0.0094). In the dominant model, carriers of the C allele compared to TT homozygotes differed significantly showing the C allele as a risk factor (OR = 2.2; [95%CI 1.2–3.8] p = 0.007).

Table 1
Genotypes and alleles distributions of the polymorphisms rs16944 and rs1143634 between patients with Cutaneous Leishmaniasis and control subjects.

SNP	CL	Control	OR	CI (95%)	p Value
IL1B (rs16944)	*N* = 872	*N* = 812			
CC	172 (19.7)	128 (15.8)			
TC	441 (50.6)	394 (48.5)			
TT	259 (29.7)	290 (35.7)			
C	785 (45)	650 (40)			
T	959 (55)	974 (60)			
Comparisons					
	CC vs TT		1.5	1.1–2.0	0.0046
	CC vs TC		1.3	1.0–1.6	0.04
	CC + TC vs TT		1.3	1.1–1.6	0.0085
	C vs T		1.2	1.1–1.4	0.0034
IL1B (rs1143634)	*N* = 881	*N* = 837			
CC	641 (72.8)	607 (72.5)			
CT	222 (25.2)	194 (23.2)			
TT	18 (2)	36 (4.3)			
C	1504 (85.4)	1408 (84)			
T	258 (14.6)	266 (16)			
Comparisons					
	TT vs CC		0.5	0.3–0.8	0.0094
	TT vs CT		0.4	0.2–0.8	0.0056
	TT vs CC + CT		0.5	0.3–0.8	0.007
	T vs C		0.9	0.8–1.1	0.3092

Table 2
Genotypes and alleles distribution of IL1RN intron 2 variable number tandem repeat (VNTR) in patients with Cutaneous Leishmaniasis and controls subjects.

SNP	CL	Control	OR	CI (95%)	p Value
IL1RN	*N* = 796	*N* = 813			
1/1	344 (43.2)	320 (39.4)	1.2	1.0–1.4	0.1285
1/2	349 (43.8)	376 (46.2)	0.9	0.7–1.1	0.3581
1/3	6 (0.8)	13 (1.6)	0.5	0.2–1.2	0.1808
1/4	5 (0.63)	7 (0.86)	0.7	0.2–2.3	0.8002
2/2	79 (9.9)	86 (10.6)	0.9	0.7–1.3	0.7265
2/3	5 (0.63)	3 (0.37)	1.7	0.4–7.2	0.7007
2/4	7 (0.9)	6 (0.73)	1.2	0.4–3.6	0.9695
4/4	1 (0.12)	2 (0.24)	0.5	–	0.9854
1	1048 (65.8)	1029 (63.3)	1.1	1.0–1.3	0.1269
2	519 (32.6)	562 (34.6)	0.9	0.8–1.1	0.2645
3	6 (0.38)	16 (0.98)	0.4	0.1–1.0	0.0611
4	13 (0.82)	15 (0.92)	0.9	0.4–1.9	0.8964

Concerning the *IL1RN* VNTR, four alleles were observed in this population (Allele 1: 410 bp; 2: 240 bp; 3: 500 bp and 4: 325 bp). Similar distributions of genotypes and alleles were observed in both groups (Table 2).

Linkage Disequilibrium (LD) between the SNPs and VNTR are low. Haplotype analysis was not performed as both rs16944 and rs2234663 are in different block and rs1143634 deviates from HWE. The distance between rs16944 and rs2234663 is 293Kbp.

3.2. Effect of genotypes of SNPs of IL1B and VNTR of ILRN on plasma IL-1β and IL-1Ra levels

Cytokines analysis was investigated in 354 patients with CL (264 males + 90 females) and 376 healthy individuals (254 males + 122 females). Plasma IL-1β was significantly higher in patients compared to controls ($p < 0.0001$). In contrast, IL-1Ra was higher among the controls ($p < 0.0001$) as depicted in Fig. 1a.

The effects of the different SNPs and VNTR on plasma levels of cytokines are shown in Fig. 1b–d for both healthy individuals and the patients with CL and in Fig. 2 for the total individuals (patients with CL plus Healthy individuals). The *IL1B* rs16944 genotypes among the

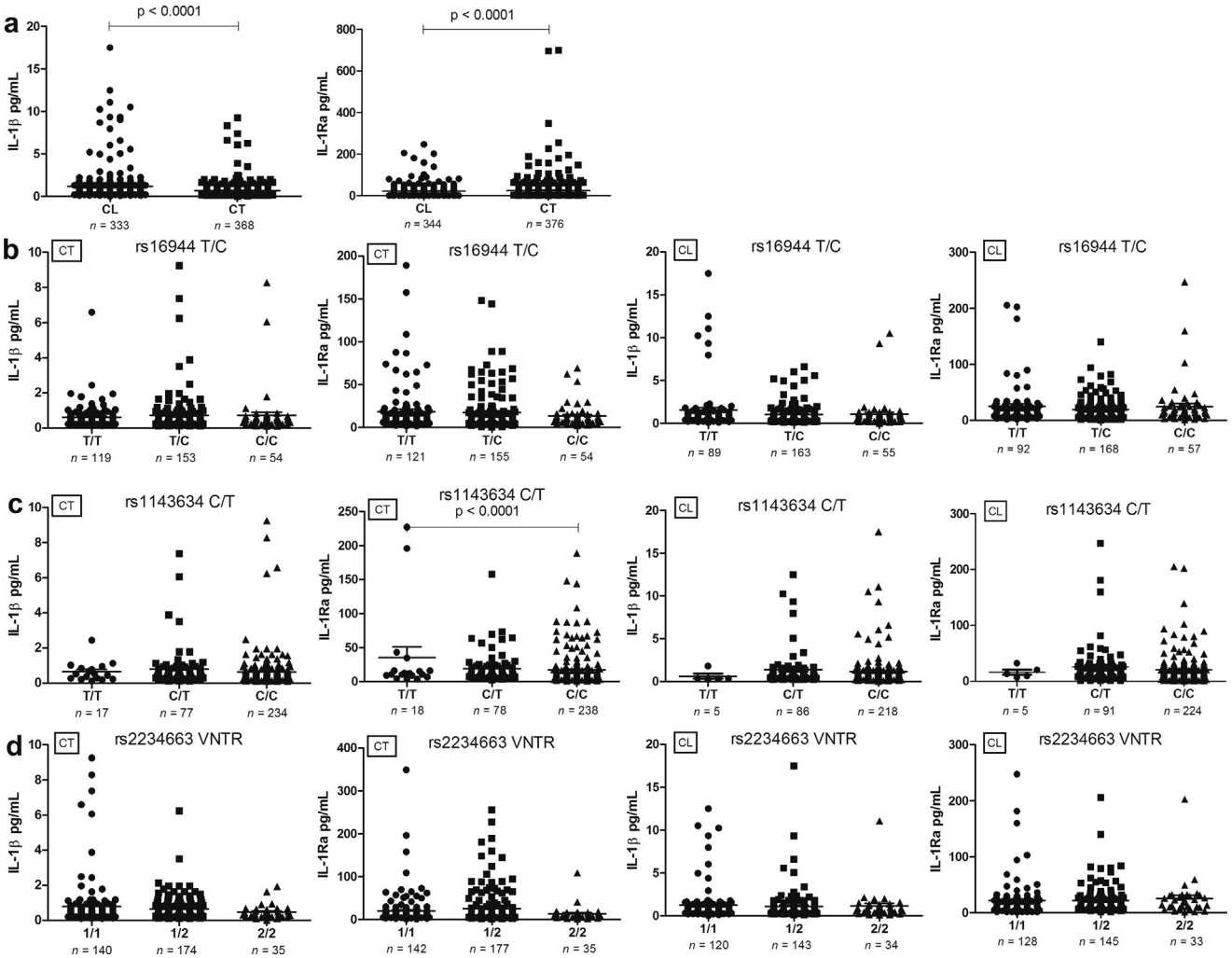

Fig. 1. Comparison of plasma cytokines levels between patients with Cutaneous Leishmaniasis and healthy subjects (a) and effects of the *IL1B* −511T/C (b); +3954C/T (c) and *IL1RN* VNTR (d) on plasma cytokines in healthy subjects and patients.

healthy individuals did not show any correlations with the levels of IL-1β. A tendency of correlation of the levels of IL-1Ra with rs16944 genotypes was observed. Carriers of the rs16944 T/T and T/C had higher levels (mean ± SD; 18.57 ± 27.46 pg/mL and 17.43 ± 23.02 pg/mL respectively) compared to the CC genotypes (13.33 ± 13.39 pg/mL) (Fig. 1b). The distribution profile of cytokines levels among the patient according to the rs16944 genotypes is completely different to the healthy individuals and there were not any correlations with genotypes and cytokines levels. Notably, the patients sought for treatment three to four weeks after infection when the lesions have already developed with an exacerbation of inflammation. Exacerbation of inflammation may mask the effect of the influence of the SNP. Pooling of the controls and patients with CL eliminates the correlation tendency of the rs16944 with IL-1Ra observed among the controls (Fig. 2a).

For the *IL1B* rs1143634, the genotype TT correlated with higher IL-1Ra levels (35.4 ± 64.95 pg/mL) compared to CC genotypes (16.7 ± 24.25 pg/mL) and CT genotypes (19.03 ± 22.75 pg/mL) (Kruskal-Wallis $p < 0.0001$) in the control group (Fig. 1c). The genotype TT still correlated with higher levels (31.3 ± 57.82 pg/mL) compared to CC genotypes (20.1 ± 40.23 pg/mL) and CT genotypes (22.6 ± 30.37 pg/mL) (Kruskal-Wallis $p < 0.0001$) when the samples were pooled (patients with CL + controls) as shown in Fig. 2b. This correlation was not shown among the patients with CL. (Fig. 2b).

The *IL1RN (VNTR)* showed a trend of correlation to the genotype 1/1 with higher levels of IL-1β (0.82 ± 1.4 pg/mL) followed with intermediate levels (0.62 ± 0.62 pg/mL) for 1/2 and lower levels (0.48 ± 0.39 pg/mL) for 2/2 genotypes among the healthy individuals (Fig. 1d). Homozygosity for allele 1 correlated with higher plasma levels of IL-1Ra (19.71 ± 37.6 pg/mL) compared to homozygosity for allele 2 (13.1 ± 18.01 pg/mL). No correlations were detected among the patients with CL (Fig. 1d). The trend of correlation disappeared when patients with CL were pooled with the controls (Fig. 2c).

4. Discussion

Despite a nearly 2.4 million disability-adjusted life years (DALYs) lost worldwide due to CL and VL [25] and the second highest cause of mortality and fourth leading cause of morbidity among tropical disease [26] there is no vaccine or an efficient treatment. Most of the chemotherapy provided is not without collateral effects and treatment success is hardly 53 to 70%. CL causes 41,700 DALYs [27].

The wide spectrum of clinical manifestations to *Leishmania-infection* and only a fraction of individuals living in area of endemicity to leishmaniasis develops clinical symptoms strongly suggests that the genetic background of the host play an important role in the outcome of infection. The identification of these genes that may be related to resistance or susceptibility to *Leishmania*-infection may provide a better understanding of the molecular mechanisms in the development of the disease and help in the designing of an efficient vaccine or in providing,

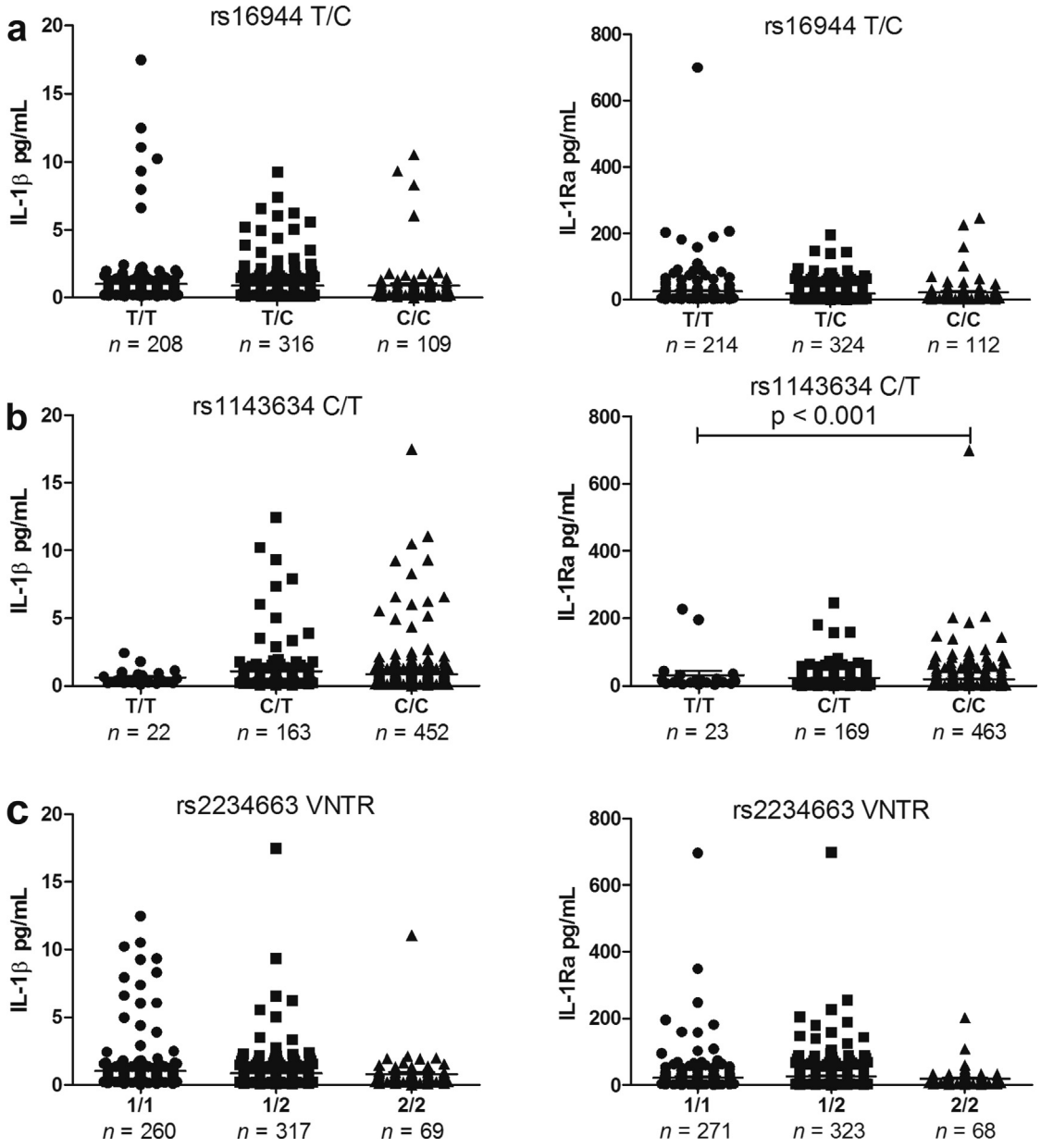

Fig. 2. Effects of the *IL1B* −511T/C (a); +3954C/T (b) and *IL1RN* VNTR (c) on plasma cytokines on total subjects (healthy subjects plus patients with CL).

a better chemotherapy combined with immunotherapy. Here, we show an association of the rs16944 C allele with susceptibility to CL.

The innate immune response is the first line of host defense to microbial infections. Pathogen recognition receptors such as Toll-Like receptors that recognized pathogen-associated molecular patterns or endogenous danger-associated molecular patterns activate the innate immune response [28] to trigger a cascade of signaling pathways culminating in the liberation of the transcription factor NFkB to enter the nucleus and transcribes pro-inflammatory cytokines to keep in check the invading pathogens. Another inflammatory pathway downstream of the PRR termed NLRP3 inflammasome, a multiprotein complex, activates Caspase 1 to proteolytically cleave pro-IL-1β and pro-IL-18 to liberate their biologically active forms to trigger the pro-inflammatory response to eliminate pathogens [29]. The activation of the NLRP3 inflammasome triggers the host defense against various infectious agents [30]. IL-1β production due NLRP3 plays a key role in immune responses during infections with *Leishmania* parasites [4–7].

SNPs in *IL1B* are cited to contribute to the severity of CL in Mexico and VL in Iran [9,31]. The rs16944 T/T genotype is associated with protection while the C/C genotype confers nearly thrice the chance of developing VL [31]. Our result is in line with this observation but contrasts with that carried in Mexico where heterozygous carriers (rs16944T/C) were associated with susceptibility to CL caused by *L. mexicana* with an OR of 3.7. The minor allele frequency (rs16944C 40%) is similar in both the Amazonian and Mexican population. Of note, in the Mexican study only 58 patients with CL and 123 controls were included. In patients with Chagas' disease caused by the intracellular protozoan parasite *Trypanozoma cruzi*, there was no association with any of the rs16944 genotypes [18]. In *Plasmodium falciparum* malaria in Gambia, severe malaria cases showed a lack of association with the rs16944 when were compared to healthy controls or mild malaria cases [32].

Concerning the *IL1RN* VNTR, no association with CL was detected. The lack of association of the VNTR has also been reported in other infectious diseases caused by other protozoan parasites [16,33]. The effect of this VNTR on plasma cytokines levels showed a tendency of homozygosity for allele 1 to correlate with higher levels of IL-1β and homozygosity for allele 2 with lower levels while heterozygosity with

intermediate levels. Similarly, homozogosity for the allele 1 correlated with higher plasma levels of IL-1Ra compared to allele 2. Our results differ from Santilla et al.[13] where stimulated mononuclear cells from bearers of allele 2 secreted higher levels of IL-1β. Another group studied the SNP rs579543 that is in strong LD to the VNTR and showed the effect is in contrast to our observation [34].

The effect of rs16944 on the levels of cytokines showed that individuals homozygous for the rs16944 C allele had non-significant lower levels of IL-1Ra compared to homozygosity for the rs16944 T allele. LPS-stimulated mononuclear cells from carriers of the allele C is reported to secrete reduce level of IL-1β in one study [35] and higher in another [36]. In this study, the rs16944 TT genotype correlated with slightly higher levels of plasma IL-1β. The conflicting results observed in different populations may suggest that these SNPs do not actually influence the expression of the gene but may be in LD with a yet unknown SNP.

The rs1143634 showed an effect on the levels of IL-1Ra. The genotype TT segregates with higher levels of IL-1Ra. Recently, it has been shown that the use of recombinant IL-1Ra, anakinra, decreases NLRP3 inflammasome dependent inflammation in murine and human cystic fibrosis [37] and in *L. braziliensis*-infection [7]. Furthermore, the IL-1Ra was associated with resistance to the development of CL in BALB/c mice [38,39].

Comparison of the levels of cytokines showed that IL-1Ra was higher among the controls. Interestingly, homozygosity for the TT genotypes of both SNP segregates with higher levels IL-1Ra among the healthy individuals. This may suggest that individuals with higher levels of IL-1Ra control the inflammatory process once eliminating the parasites and avoids tissue damage while high levels of IL-1β appear to exacerbate disease.

IL-1β upregulates CXCL1, CCL4 and CXCL2 that are known to be potent neutrophils-chemoattractant [40]. Neutrophil recruitment at the site of the phlebotomine bite enhanced by IL-1β may play an important role in the pathogenesis of murine *Leishmania* infection [41]. Neutropenic mice Genista is resistant to *L. major* Seidman strain [6]. Furthermore, IL-1β can deactivate pro-inflammatory Th1 response by reducing IL-12R and by impeding IL-6-induced phosphorylation of signal transducer and activator of transcription 1 [42]. A regulated Th1 response is necessary for efficiently controlled the parasites and to avoid tissue damage.

A primary limitation of the study is that the healthy individuals did not undergo the Montenegro test to be sure that all individuals were exposed to *L. guyanensis*. However, as all these individuals are from the same endemic areas and have lived there for more than five years, it is plausible that they share similar environments as the patients with CL. Of note, most of the participants in this study are agricultural workers and share the same risk of infection to the parasite *L. guyanensis*. Another limitation of the study is the deviation of the SNP rs1143634 from the HWE. One may suggest that there is bias in the selection of controls and there may be a degree of kinship among them or errors during genotyping by PCR-RFLP. To the best of our knowledge, all the healthy individuals were recruited with the care of asking if they have any parents living in the same endemic area to avoid the inclusion of any parent. Furthermore, the three polymorphisms studied are located on the same chromosome within 430 KB. As the rs16944 and the VNTR rs2234663 are in HWE among the controls, this rules out any bias in the selection. Concerning the PCR-RFLP technique used, the common allele is cleaved by the restriction enzyme *Taqa I* while the rare allele remains uncleave. To ensure there was no error in genotyping, all the individuals with the rare allele were retyped.

Although the data presented here may look suggestive due to observed low odd ratios despite the large sample size of the study, leishmaniasis is a complex disease involving the parasite, the vector, a favorable environment as well as the genetic background of the host. It is highly probable that many genes are involved in the development of the disease and each gene may contribute to a low input in the susceptibility or protection to the infection caused by *L. guyanensis*.

Altogether, our data show that polymorphism in the *IL1B* gene is associated with susceptibility to CL caused *L. guyanensis* and individuals with a propensity to be high producer of IL-1Ra seems to be resistant to *Leishmania*-infection.

5. Keypoint

Polymorphisms in the IL-1B but not the VNTR of the IL-1RN are associated with Cutaneous Leishmaniasis caused by *Leishmania guyanensis* in the Amazonas state of Brazil. A trend of correlation of the cytokines IL-1B and IL-1Ra is observed with the IL1B genotypes.

Declaration of Competing Interest

The authors declare no conflict of interest

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