NLRP3 polymorphism is associated with protection against human T-lymphotropic virus 1 infection

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Inter-individual heterogeneity in the response to human T-lymphotropic virus 1 (HTLV-1) infection has been partially attributed to host genetic background. The antiviral activity of the inflammasome cytoplasmic complex recognises viral molecular patterns and regulates immune responses via *the activation of interleukin (IL)-1 family (IL-1, IL-18 and IL-33) members. The association between polymorphisms in the inflammasome receptors* NLRP1 *and* NLRP3 *and HTLV-1 infection was evaluated in a northeastern Brazilian population (84 HTLV-1 carriers and 155 healthy controls).* NLRP3 *rs10754558 G/G was associated with protection against HTLV-1 infection (p = 0.012; odds ratio = 0.37). rs10754558 affects* NLRP3 *mRNA stability; therefore, our results suggest that higher NLRP3 expression may augment first-line defences, leading to the effective protection against HTLV-1 infection.*

Key words: NLRP3 - NLRP1 - single nucleotide polymorphisms - innate immunity - HTLV-1

The initial stage of human T-lymphotropic virus 1 (HTLV-1) infection involves cell-cell transmission of HTLV-1 from infected to uninfected T-lymphocytes, followed by the clonal expansion of infected cells, with almost undetectable levels of circulating virions (Pique & Jones 2012). The pathogenic outcome has been strongly associated with the clonal expansion and infiltration of infected lymphocytes into affected tissues (Lairmore et al. 2012), while efficient antigen presentation and cytolytic activity of HTLV-1-specific CD8^{+} cytotoxic T-lymphocytes have been associated with HTLV-1 proviral load (PVL) control. Several cytokine-coding genes and human leukocyte antigen variants were previously described as important restriction factors of PVL and HTLV-1 pathogenesis; therefore, the genetic backgrounds of several host immunity components have recently been investigated (Vine et al. 2002, Haddad et al. 2011, Rocha-Júnior et al. 2012).

The early innate response to several viruses such as influenza virus, Sendai virus, hepatitis C virus (HCV) and adenovirus consists of interleukin (IL)-1ß production *via* inflammasome activation. The inflammasome is a cytosolic complex that detects pathogen or danger-associated molecular patterns and leads to the activation of caspase-1, the enzyme responsible for pro-IL-1ß/IL-1ß conversion (Gram et al. 2012). Significant expression of IL-1ß has been reported to be induced by HTLV-1 transactivator protein in macrophages, microglial cells and astrocytes

(Banerjee et al. 2007, Takahashi et al. 2013), suggesting an innate response through inflammasome regulation. Furthermore, the HTLV-1-specific cytolytic response was shown to be modulated by IL-1 in mice because this cytokine enhanced the expansion and tissue localisation of antigen-driven CD8^{+} T cell (Ben-Sasson et al. 2013).

The inflammasome plays a major role in IL-1ß production and may also be involved in the cytoplasmic recognition of HTLV-1; therefore, we investigated the possible association between selected single nucleotide polymorphisms (SNPs) in the inflammasome receptor genes *NLRP3* and *NLRP1* and susceptibility to HTLV-1 infection in HTLV-1-infected patients and controls from northeastern Brazil.

Eighty-four HTLV-1-infected subjects (46.32% males, 53.68% females; mean age = 45.29 ± 2.60 years) were enrolled from the metropolitan area of Recife, state of Pernambuco (PE), Brazil. All participants were screened for the presence of plasma anti-HTLV-1 antibodies (ELISA: Murex Biotech Limited, UK) and positivity was confirmed by qualitative nested-polymerase chain reaction (PCR) analysis performed at the Hemope Foundation (blood center of PE). A total of 155 healthy controls (HCs) (46.46% male, 53.54% females; mean age = 21.72 ± 2.45 years) with negative HTLV-1 serology and negative qualitative PCR were recruited as control subjects from the same metropolitan area at Hemope. Written informed consent was obtained according to the Hemope Ethical Committee (protocol 016/2010).

The HTLV-1 carriers and HCs were admixed northeastern Brazilians. The ethnicity of the patients and HCs was analysed using ancestry markers as reported by Kosoy et al. (2009) and the ethnic background results demonstrated similar frequencies of approximately 58% Caucasian, 25% African and 17% Amerindian backgrounds.

DNA was obtained from the peripheral blood of uninfected HCs and HTLV-1 carriers using the "salting

TABLE

NLRP1 and *NLRP3* single nucleotide polymorphisms (SNPs) frequencies and association results in northeastern Brazilian cohort of human T-lymphotropic virus 1 (HTLV-1) patients and healthy controls (HCs)

SNPs ID	HC (n = 155) n (%)	HTLV (n = 84) n (%)	p and OR (minor allele)	p and OR (model of inheritance) Co-dominant	Dominant	Recessive	Over-dominant
rs12150220							
A	207 (0.67)	122 (0.73)	0.215	-	-	-	-
T	103 (0.33)	46 (0.27)	(0.76)				
A/A	74 (0.48)	46 (0.55)	-	0.530	0.300	0.431	0.579
A/T	61 (0.39)	30 (0.36)		(A/T vs. A/A: 0.45; T/T vs. A/A: 0.26)	(A/T+T/T vs. A/A: 0.44)	(A/A+A/T vs. T/T: 0.30)	(A/T vs. A/A+T/T: 0.49)
T/T	20 (0.13)	8 (0.09)					
rs2670660							
A	169 (0.55)	101 (0.60)	0.248	-	-	-	-
G	141 (0.45)	67 (0.40)	(0.80)				
A/A	48 (0.31)	31 (0.37)	-	0.549	0.353	0.386	0.846
A/G	74 (0.48)	39 (0.46)		(A/G vs. A/A: 0.45; G/G vs. A/A: 0.30)	(A/G+G/G vs. A/A: 0.44)	(A/A+A/G vs. G/G: 0.37)	(A/G vs. A/A+G/G: 0.56)
G/G	33 (0.21)	14 (0.17)					
rs10754558							
C	176 (0.57)	106 (0.63)	0.206	-	-	-	-
G	134 (0.43)	62 (0.37)	(0.77)				
C/C	57 (0.37)	30 (0.36)	-	0.025	0.871	**0.012ª**	0.046
C/G	64 (0.41)	46 (0.55)		(C/G vs. C/C: 0.76; G/G vs. C/C: 0.18)	(C/G+G/G vs. C/C: 0.60)	**(C/C+C/G vs. G/G: 0.37)**	(C/G vs. C/C+G/G: 1.01)
G/G	34 (0.22)	8 (0.09)					
rs35829419							
C	307 (0.99)	164 (0.98)	0.248	-	-	-	-
A	3 (0.01)	4 (0.02)	(2.49)				
C/C	152 (0.98)	80 (0.95)	-	0.229	-	-	-
C/A	3 (0.02)	4 (0.05)		(C/A vs. C/C: 2.53)			
A/A	0 (0)	0 (0)					

a: the most fitting model for rs10754558-G allele association was determined by the lowest value indicated by the Akaike information criterion (co-dominant: 308.5, dominant: 313.9, recessive: 307.6, over-dominant: 309.9). SNPs ID, allelic and genotypic counts and frequencies were reported as well as adjusted p values (adjusted for sex, age and ethnicity). Odds ratio (OR) was reported for alleles comparison and for all genotypes inheritance models analysed with SNPassoc software. Significant p values after Bonferroni correction (p < 0.013) are evidenced in bold.

out" protocol for genomic DNA extraction (Miller et al. 1988). SNPs in the *NLRP3* and *NLRP1* genes were selected considering their reported functional effects on inflammasome biology and IL-1ß secretion (Glinskii et al. 2009, Hitomi et al. 2009, Verma et al. 2012, Levandowski et al. 2013). Genotyping was performed using commercially available TaqMan assays (Applied Biosystems, USA). The TaqMan reactions were set up based on the manufacturer's protocol and the samples were run on an ABI7500 Real Time PCR System (Applied Biosystems). Allelic discrimination was performed using the SDS software (v.2.3) (Applied Biosystems).

R software (r-project.org) was used to perform Fisher's exact test and odds ratio (OR) calculations for alleles and haplotypes as well as genotype associations and inheritance modelling (Lewis 2002) (package SNPassoc v.1.5-2). The results are reported as p-values adjusted for sex, ethnicity and age. Formal Bonferroni's correction for the number of SNPs analysed required a significance threshold of p = 0.013 (P0/n, P0 = 0.05, n = 4 tests). The *post hoc* statistical power analysis was performed with the G*power software (v.3.0.5), with an alpha-error probability of 0.05. The Haploview software was used to investigate the association and linkage disequilibrium (LD) pattern and to derive the haplotypes (Barret et al. 2005). According to this software, haplotype frequencies were estimated using the expectation-maximisation (EM) algorithm and haplotype association was evaluated using the chi-square test.

The *NLRP1* rs12150220 and rs2670660 polymorphisms were not associated with susceptibility to HTLV-1 infection in our case-control cohort because the frequency distribution of these two SNPs was not significantly different among the HTLV-1 carriers and HCs (Table). The two *NLRP1* SNPs were in LD (D'/LOD = 94), confirming previous studies (Jin et al. 2007). However, the resulting rs12150220-rs2670660 haplotypes (A-A, T-G, A-G) did not appear to be differentially distributed between the cases and controls (p = 0.287, OR = 0.77; p = 0.147, OR = 1.40; p = 0.810, OR = 0.93; respectively) (Supplementary data).

The *NLRP3* rs35829419 allele and genotype frequency distributions were also similar within the HTLV-1 and HC groups, with no association observed (p = 0.248 and p = 0.229, respectively) (Table).

The allelic distribution of the *NLRP3* rs10754558 polymorphism did not vary significantly between the cases and controls (p = 0.206). Although the *NLRP3* rs10754558 genotype frequencies were not differentially distributed (p = 0.025) (Table) between HTLV-1 patients and HCs, a significant difference was found when a recessive model of rs10754558 heritage was utilised [lowest value of the Akaike information criterion according to Lewis (2002) and SNPassoc software], suggesting that homozygosity for the minor G allele confers protection against HTLV-1 infection (G/G vs. C/C + C/G: p = 0.016). No association was found using different inheritance models of rs12150220, rs2670660 (*NLRP1*) and rs35829419 (*NLRP3*) SNPs (p > 0.05).

The presence of the rs10754558 G allele has been reported to increase the stability of the 3'UTR structure of *NLRP3* mRNA, resulting in a higher expression level (Hitomi et al. 2009).

These data suggest that individuals presenting a more stable *NLRP3* expression pattern may be more effectively protected against HTLV-1 infection. Stable *NLRP3* expression may be the consequence of an increased number of NLRP3 viral-sensing receptors or augmented basal IL-1ß production due to the increased expression of NLRP3, which may activate the inflammasome through the recognition of several cell stress molecules (Gram et al. 2012) produced as a result of HTLV infection. We are aware that our study had two major limitations, specifically the extent of size of casuistic and the lack of functional assays, although the effect of the rs10754558 G allele was previously demonstrated (Hitomi et al. 2009). However, we speculate that NLRP3 may be able to sense HTLV-1 in the cytoplasm of infected cells, in a manner similar to the detection of influenza virus or HCV (Gram et al. 2012). According to this hypothesis, increased NLRP3 expression may confer significant protection against virus infection. However, even if NLRP3 is not involved in sensing HTLV, increased NLRP3 expression may affect the rate of inflammasome activation, leading to greater IL-1ß production. Inflammasome activation and IL-1ß are known to be involved in antigen presentation (Qu et al. 2009) and T-cell activation (Liu et al. 2013); therefore, we propose that heightened inflammasome activation may contribute to a better anti-HTLV immunologic response. We would also like to emphasise that despite the differences in virus type and infection cycle, our group recently showed that *NLRP3* rs10754558 was similarly associated with HIV-1 infection (Pontillo et al. 2010), with the G/G genotype detected less frequently in HIV+ patients. This finding suggests that through a currently unidentified mechanism, rs10754558 carriers may be protected against viral infection. However, further studies are required to confirm these data in other cohorts and to investigate the functional role of this variation in inflammasome/virus interplay.

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NLRP1 rs12150220-rs2670660 haplotype frequencies and association results

Haplotype	HTLV, HC frequencies	p
A-A	0.588, 0.538	0.287
T-G	0.261, 0.325	0.147
A-G	0.138, 0.130	0.810

frequencies and p-values were reported for resulting haplotypes in human T-lymphotropic virus 1 (HTLV-1) and health controls (HC) groups (according to Haploview software, haplotype frequencies were estimated by the expectation-maximization algorithm and haplotype association was evaluated by chi-square test).