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New variants in NLRP3 inflammasome genes increase risk for asthma and *Blomia tropicalis*-induced allergy in a Brazilian population

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ABSTRACT

Atopic asthma is a chronic lung disease of lower airways caused mainly due to action of T-helper (Th) 2 type cytokines, eosinophilic inflammation, mucus hypersecretion and airway remodelling. Interleukin (IL)-33 increases type 2 immunity polarization in airway playing critical role in eosinophilic asthma. On the other hand, NLRP3 inflammasome activation results in the release of caspase-1 (Casp-1) which, in its turn, promotes IL-33 inactivation. Recent studies have shown associations between *NLRP3* variants and inflammatory diseases. However, no study with genes in NLRP3 inflammassome route has been conducted so far with asthma and atopy in any population to date. Blood samples were collected from 1246 asthmatic and non-asthmatic children. Associations were tested for single nucleotide polymorphism (SNP)s in *NLRP3* and *CASP1* with asthma and markers of atopy and in cultures stimulated with *Blomia tropicalis* (Bt) mite crude extract. The T allele of rs4925648 (*NLRP3)* was associated with increased asthma risk (OR 1.50, P = 0.005). In addition, the T allele of rs12130711 polymorphism, whithin the same gene, acted as a protector factor for asthma (OR 0.78, P = 0.038). On the other hand, the C allele of rs4378247 *NLRP3* variant was associated with lower levels of IL-13 production when peripheral blood cells were stimulated with Bt (OR 0.39, P = 4E-04). In addition, the greater the number of risk alleles in *IL33/NLRP3/CASP1* route the greater was the risk for asthma. The T allele of rs7925706 *CASP1* variant was also associated with increased risk for asthma (OR 1.47, P = 0.008). In addition, this same allele increased the eosinophil counts in blood (mm3) in asthmatic individuals compared with non-asthmatic (P = 0.0004). These results suggest that *NLRP3* and *CASP1* polymorphisms may be associated with susceptibility for asthma and markers of atopy in our population.

1. Introduction

Asthma is a disease that affect the life of people, being more prevalent in childhood [1]. *Blomia tropicalis* (Bt) mite rapidly settles in homes of climates countries such as Brazil [2], being the main asthma-induced allergen in the tropics [3–6]. Atopic asthma is characterized by inflammation of airways, with predominance of Th2-type cytokines, as IL-13, and eosinophilic inflammation [7]. These cytokine may induce airway hyperreactivity via direct effects on several cells that form and make up the airway [8].

IL-33 has the ability to polarize the antigen-driven atopic asthma response [9]. This cytokine is commonly expressed in epithelial cells and acts as one of the signals in response to damage [10,11]. IL-33 release activates several innate and adaptative immune cells through its membrane receptor (ST2L) and induces Th2-type cytokines, polarizing the inflammation for a type 2 immune response [7,12].

Interestingly, inflammasomes are a variety of intracellular of multiprotein complexes that can be activated by several stimuli resulting in pathogens invasion through the airway epithelium constantly exposed, playing an important role in both innate and adaptive immune

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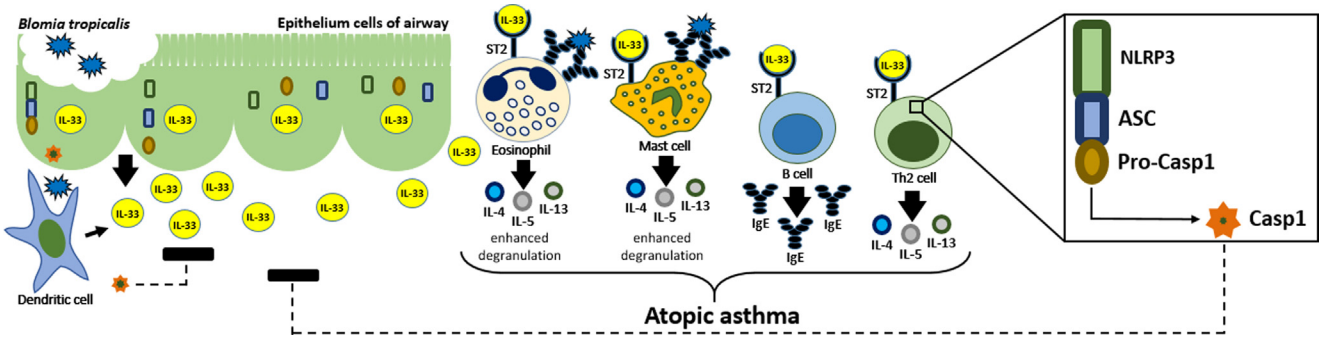

Fig. 1. Immunopathogenesis of Bt-induced atopic asthma in the IL-33/NLRP3/CASP1 route. The contact of Bt with cells of the aerial epithelium results in damage and consequent release of IL-33. This, in turn, favors the Th2 response profile by activating several innate and adaptive immune cells, with high production of proinflammatory cytokines, also increasing degranulation and specific IgE production. On the other hand, activation of the intracellular NLRP3 receptor, present in the cytoplasm of the cells involved in this response, results in NLRP3 inflammasome formation and Casp-1 release. Casp-1 acts by inhibiting the IL-33 action and, consequently, attenuates the inflammation caused by this cytokine in airway.

responses. Among the inflammasomes AIM2, IPAF, NLRP1 and NLRP3, this last one has prominent role in various inflammatory diseases [13–16]. In this context, the airway invasion by potentially antigens becomes an enabling environment for activation of the NLRP3 complex (NLRP3, ASC and Pro-caspase-1), that culminates in the release of Casp-1. Casp-1, in its turn, promotes inactivation of IL-33 [15,17,18].

One recent study showed that activation of NLRP3 inflamassome and release of caspase-1 reduced the allergic airway inflammation induced by mite and orchestrated by IL-33 [19]. Fig. 1 shows the immunopathogenesis of Bt-induced atopic asthma and key role of IL-33/NLRP3 inflamassome route activation.

The immunopathogenesis of asthma is multi-faceted, involving genetic regulation of gene related to cytokines, transcription factors as well as epigenetic regulators [20,21]. Moreover, it is known that genetic variations can be differentially distributed according to ethical and geographical origins [22]. Thus, genetic variants in NLRP3/CASP1 genes may affect the susceptibility to asthma and atopy in certain populations [23].

Recent genetic studies have shown polymorphisms in *NLRP3* associated with autoimmune and inflammatory diseases [24], such as type 1 diabetes and inflammatory bowel disease [25,26], primary gout [27], type-2 diabetes mellitus and insulin resistance [28,29] and ankylosing spondylitis [30]. However, associations between variants in *NLRP3* have never been explored before in the context of allergic diseases [24].

Thus, considering the key role of such genes on the inactivation of IL-33 and decreased Th2 immune response, our goal was to verify if genetic variations on *NLRP3* and *CASP1* genes affect asthma symptoms and allergy, which from the best of our knowledge, was never reported before.

2. Materials and methods

2.1. Study populations and ethical approvals

This is a population-based study where the data presented herein were obtained from 1246 individuals from 5 to 11 years old enrolled in SCAALA Program, in Salvador city, northeastern Brazil [7,31–35], as previously described. The complete information of study design can be found elsewhere (Barreto at al 2006). In SCAALA-Salvador cohort, asthma was defined according to the International Study of Allergy and Asthma in Childhood (ISAAC) and only children without any other respiratory and/or chronic condition were assessed. In addition to that, since some consanguineous relatives were included in the original cohort and those individuals genetically identified as relatives (degree of kinship), they were also excluded of this analysis. Also, we excluded individuals with genotyping call rate bellow than 10%. Thus, from originally 1445 individuals from SCAALA-Salvador cohort, a total of 1246 individuals with complete dataset were included in this work. The free and informed consent form as well as the ISAAC phase II questionnaire, was signed and answered by the legal guardian of each children. The Ethics Committee of the Collective Health Institute approved this study (register 003-05/CEP-ISC).

2.2. Asthma definition

We considered asthmatic children as those with information of diagnostic of asthma or wheezing in the past 12 months and at least one of the following clinic conditions: sleep interrupted by the wheezing; wheezing > 4 times; wheezing during physical activity. All the other children belonged to the non-asthmatics group.

2.3. Obtention of blood sample and markers of atopy

Heparinized blood was colleted and the serum frozen until the moment of use. The measurement of anti-Bt IgE was performed according to the manufacturer's instructions (Phadia Diagnostics AB, Uppsala, Sweden). Skin prick test (SPT) using extract of Bt was applied on forearm of each subject [7] as described previously. In addition, we have used IL-13 as a marker of Th2 immune response. To this end, whole blood cultures were performed and IL-13 accumulation in culture upon Bt (40 μg/ml) extract stimulation was measured in supernatants [7] as previously described.

2.4. IL-13 levels in whole blood cultures

The concentration of IL-13 in supernatants in pg/mL was performed by sandwich ELISA, following all manufacturer's instructions (BD PharMingen, San Diego, CA, USA). Regarding IL-13 levels, children who presented variation in concentration between the lowest and highest points of the standard curve (62.5/4000) we considered as responders for IL-13 [7], as previously described.

2.5. DNA extraction and genotyping analysis

White blood cells from each children were used for DNA extration, according to the Flexigene@DNA Kit (Qiagen, Hilden, Germany) protocol. For genotyping, we used a BeadChip panel from Illumina with 2.5 Million of variants [7], as described previously. After genotyping, we proceeded with extraction of all SNPs from the region of the two genes of interest, *NLRP3* and *CASP1*. For this, we delimit the start and end positions of both genes as show in assembly GRCh37.p13 as follows: in chromosome 1, of 1,247,579,247 to 247,612,410 on *NLRP3* and in chromosome 11, of 104,896,235 to 104,905,884 on *CASP1* (ncbi.nlm.nih.gov). After this stage, we obtained a total of 66 SNPs in the NLRP3

gene and 8 SNPs in the CASP1 gene. We then proceed with the application of the following filters for quality control: genotyping error rate ≤ 10%; Hardy-Weinberg equilibrium p < 0.05 and minor allele frequency (MAF) p < 1% [36]. After applying those filters, we obtained 56 markers in *NLRP3* and 6 in *CASP1*, which were included in the analysis. Of these, 17 were statistically significant for *NLRP3* and 2 for *CASP1*. We did not have any significant associations for *ASC* (data not shown).

2.6. Functional in silico analysis

General information regarding each variant such as position, function and chromosome were accessed in (ncbi.nlm.nih.gov) and 1000 genomes (internationalgenome.org). In addition, we also used the RegulomeDB (http://www.regulomedb.org) which attributes a score of 1 to 6 for each SNP and the lower the score the greater the probability of a certain SNP being involved in regulatory processes. The scores of 1a to 1f, 2a to 2c and 3a to 3b are likely to affect binding. The 4 to 6 scores have less binding evidence [37] and 7 score has no available information. In rSNPBase (http://rSNP.psych.ac.cn/) we accessed information about regulation in terms of proximal, distal and post-transcriptional regulation of the SNPs included in this study.

2.7. Statistical analysis

Analysis of association using logistic regressions by means of multivariated models adjusted by sex, age, helminth infection and individual ancestry (PC1 and PC2 – which classify subjects according to their ethnic characteristics [38], was performed between variants in *NLRP3* and *CASP1* and asthma, severity of asthma symptoms, positivity to SPT and specific IgE, using PLINK 1.9 software. We used the additive model for all the genetic analyses presented herein. The additive model very well represents the dominant model, and we do not see much advantage in making the recessive model in our population due to the low number of individuals having the rare allele in homozygosis in certain analysis [39], thus we decided to present results using the additive model while both, the recessive and dominant models, are presented in the supplementary material (Tables 1 and 2, supplementary material). In addition, power analyzes were conducted using PGA v 2.0 software [40] considering a Relative Risk (RR) of 1.5, which is consistent with an expected moderate effect size for SNPs associated with complex outcomes, and different genetic models (additive, recessive, and dominant) (see Supplementary Table 3). To create LD plot we use Haploview software. GraphPad 6 software was used for statistical analysis with eosinophil number using Mann–Whitney test. For haplotype analysis we used SNPStats platform (https://www.snpstats.net/start.htm) [41]. For genetic risk score analysis, we used alleles of variants in IL-33/inflammasome NLRP3 route that presented risk for asthma in our population. The A, T and T alleles of rs12551256 (*IL33*), rs4925648 (*NLRP3*) and rs7925706 (*CASP1*), respectively. We assigned a score from 0 to 4 for each individual according to the number of risk alleles and proceeded to logistic regression of each group with risk alleles compared to individuals without any risk alleles. P < 0.05 was considered as significant.

3. Results

The descriptive analysis of 942 controls and 274 asthmatics enrolled in the present study is shown in Table 1. Statistically significant differences were observed for all analyzed phenotypes, with p-value < 0.001. The asthmatic group was younger and had more males. Also, they had high levels of specific IgE for Bt and were more reactive to SPT to Bt and upon Bt stimulation of peripheral blood cells, also produced more IL-13 than non-asthmatics (Table 1).

Table 2 presents characteristics of all polymorphisms in *NLRP3* and *CASP1*, explored in our study. All SNPs had MAF > 1% and the majority, intron variant. In the same Table 2, the attributable RegulomeDB score is also presented where 2.86% of the studied SNPs were classified as 1f score; 8,57% as 3a; 65,71% as having 4 to 6 and 20% with no information. From now on, only variants with significant associations with the phenotypes explored herein, will be presented bellow.

Table 1
Population of study and demographic characteristics.

	Children group (1246)				
Variables	Control	%	Asthma	%	P-value
	942*	75.90%	274*	22%	
Age					
≤5	314	33.30%	132	48.20%	< 0.001
6–7	337	35.80%	88	32.10%	
≥8	291	30.90%	54	19.70%	
Sex					
Male	506	53.50%	151	55.10%	< 0.001
Female	436	46.10%	123	44.90%	
Specific IgE for					
Bt	288	30.60%	128	46.70%	< 0.001
SPT for					
Bt	192	20.40%	76	27.70%	< 0.001
Cytokine production by Bt stimulated peripheral blood cells					
IL-13	53	5.60%	18	6.60%	< 0.001

* 30 subjects presented data miss. For this analysis was used chi-squared test.

Polymorphic alleles of variants in *NLRP3* and *CASP1* showed significant associations with asthma, asthma severity, IL-13 levels, specific IgE and SPT for Bt (see Table 3). The majority of *NLRP3* and *CASP1* SNPs were associated with protection rather than with risk for asthma and its severity, as well as, atopy markers. The T, A, G, T and T alleles of rs12130711, rs36021952, rs72771992, rs56383829 and rs74154644, respectively, were negatively associated with asthma (OR 0.78, 95% CI: 0.62–0.98, P = 0.038), (OR 0.69, 95% CI: 0.49–0.98, P = 0.038), (OR 0.67, 95% CI: 0.48–0.94, P = 0.019), (OR 0.82, 95% CI: 0.67–0.99, P = 0.046) and (OR 0.69, 95% CI: 0.49–0.98, P = 0.038). The A and T alleles of rs4925654 and rs12728998 were associated with mild asthma symptoms (OR 0.62, 95% CI: 0.44–0.88, P = 0.009) and (OR 0.68, 95% CI: 0.48–0.98, P = 0.042), respectively. Also the C, T, A and T alleles of rs4378247, rs12565738, rs45624634, and rs7525979, respectively, were negatively associated with IL-13 production (OR 0.39, 95% CI: 0.22–0.67, P = 0.0004), (OR 0.24, 95% CI: 0.09–0.65, P = 0.004), (OR 0.41, 95% CI: 0.24–0.70, P = 0.001) and (OR 0.38, 95% CI: 0.19–0.75, P = 0.006) when peripheral blood cells were stimulated with Bt. In addition, the A allele of rs79180143 was associated with a lower SPT reactivity (OR 0.79, 95% CI: 0.63–0.99, P = 0.046) against the Bt crude extract. On the other hand, the C, T, G, A and T alleles of polymorphisms rs12137901, rs4925648, rs72553860, rs55914518 and rs7925706, respectively, were positively associated with asthma (OR 1.28, 95% CI: 1.04–1.57, P = 0.016), (OR 1.50, 95% CI: 1.14–1.98, P = 0.005), (OR 1.38, 95% CI: 1.11–1.72, P = 0.004), (OR 1.85, 95% CI: 1.04–3.29, P = 0.034) and (OR 1.47, 95% CI: 1.11–1.96, P = 0.008). Also, both A alleles of rs116096527 and rs4925659 were positively associated with sIgE levels (OR 2.25, 95% CI: 1.13–4.48, P = 0.043) and SPT positivity (OR 1.30, 95% CI: 1.05–1.61, P = 0.021), both against Bt whole extract.

Table 4 presents polymorphisms in *NLRP3* and *CASP1* and data on the impact of such SNPs in gene expression based in experimental studies, according to rSNPBase. Regarding variants in *NLRP3*, the SNPs rs12137901, rs4925659, rs72553860, rs4925648, rs36021952, rs72771992, rs74154644 were related with proximal regulation only, while the rs56383829 was related with distal regulation. Regarding variants in *CASP1*, the SNPs rs79180143 and rs7925706 were related with proximal regulation and with RNA binding protein-mediated

Table 2
Characterization of all polymorphisms in *NLRP3* and *CASP1*, included in analysis of study.

Gene	CHR	SNP	A1	A2	MAF	FUNCTION	RegulomeDB
NLRP3	1	rs56383829	T	C	0.49	intron variant	5
NLRP3	1	rs3806265	C	T	0.47	intron variant	5
NLRP3	1	rs10159239	G	A	0.39	intron variant	5
NLRP3	1	rs12137901	C	T	0.30	intron variant, upstream variant 2 KB	4
NLRP3	1	rs12130711	T	C	0.28	intron variant	1f
NLRP3	1	rs4925659	A	G	0.28	intron variant	4
NLRP3	1	rs10925023	T	G	0.28	intron variant	7
NLRP3	1	rs200927356	G	A	0.27	intron variant	5
NLRP3	1	rs72553860	G	A	0.26	intron variant, upstream variant 2 KB	4
NLRP3	1	rs4925650	A	G	0.26	intron variant	7
NLRP3	1	rs4378247	C	T	0.24	intron variant	3a
NLRP3	1	rs12565738	T	C	0.24	intron variant	5
NLRP3	1	rs4925654	A	G	0.21	intron variant	6
NLRP3	1	rs12728998	T	C	0.18	intron variant	7
NLRP3	1	rs7525979	T	C	0.15	synonymous codon	5
NLRP3	1	rs79796552	G	A	0.14	intron variant	6
NLRP3	1	rs4925648	T	C	0.13	intron variant, upstream variant 2 KB	7
NLRP3	1	rs10925022	G	A	0.12	intron variant	5
NLRP3	1	rs36021952	A	G	0.11	intron variant	3a
NLRP3	1	rs72771992	G	T	0.11	utr variant 5 prime	7
NLRP3	1	rs74154644	T	C	0.11	intron variant	5
NLRP3	1	rs45624634	A	C	0.11	intron variant	4
NLRP3	1	rs4925651	T	G	0.10	intron variant	5
NLRP3	1	rs4925543	A	G	0.10	synonymous codon	5
NLRP3	1	rs7524914	C	T	0.09	intron variant	6
NLRP3	1	rs73136263	T	G	0.03	utr variant 5 prime	7
NLRP3	1	rs55914518	A	G	0.02	intron variant	6
NLRP3	1	rs35433972	T	C	0.02	intron variant	5
NLRP3	1	rs116096527	A	G	0.01	intron variant	5
CASP1	11	rs79180143	A	C	0.28	intron variant, utr variant 3 prime	7
CASP1	11	rs531542	A	G	0.14	intron variant	3a
CASP1	11	rs580253	A	G	0.14	intron variant, synonymous codon	7
CASP1	11	rs7925706	T	C	0.13	intron variant, utr variant 5 prime	4
CASP1	11	rs556205	G	T	0.08	intron variant	6
CASP1	11	rs75775137	G	A	0.06	intron variant	5

CHR, chromosome; SNP, single nucleotide polymorphism; A1, minor allele; A2, ancestral allele; MAF, minor allele frequency; utr, untranslated region.

regulation. We performed haplotypes analysis in NLRP3 gene. The following haplotypes for SNPs rs72553860, rs12137901, rs36021952 and rs74154644 have shown significant associations with asthma (H1-GCGC; OR 1.43, 95% CI: 1.09–1.87, P = 0.011); (H2-GCC; OR 1.39, 95% CI: 1.09–1.77, P = 0.008); (H3-GCG; OR 1.38, 95% CI: 1.08–1.76, P = 0.009); (H4-GC; OR 1.37, 95% CI: 1.10–1.70, P = 0.005) (see Table 5). On the other hand, the following SNPs rs45624634, rs12565738, rs7525979 and rs4378247 were associated with IL-13 production in Bt-stimulated blood cell cultures in haplotype analysis (H1-CTC; OR 0.53, 95% CI: 0.29–0.98, P = 0.042); (H2-CCT; OR 0.41, 95% CI: 0.21–0.81, P = 0.011); (H3-ATC; OR 0.26, 95% CI: 0.09–0.71, P = 0.009); (H4-CCTT; OR 0.13, 95% CI: 0.03–0.56, P = 0.006); (H5-ATCT; OR 0.11, 95% CI: 0.01–0.80, P = 0.030) (see Table 5).

In a previous study with this same population, the G allele of rs12551256 (*IL33*) was associated with protection to asthma [7]. Therefore, the most common allele for this particular SNP (the A allele) in our population was associated to asthma risk (OR 1.41, 95% CI: 1.06–1.88, P = 0.017). In order to perform a risk score analysis [42], we analyzed the A allele of rs12551256 (*IL33*) along with the T allele of rs4925648 (*NLRP3*) and the allele and T allele of rs7925706 (*CASP1*) to check if increasing the number of risk allele, the greater is the risk for asthma (see Table 6). And indeed, we were able to confirm that hypothesis where the greater the number of risk alleles in those genes all together, the greater is the risk for asthma in our population. So as can be seen in Table 6, subjects with one risk allele had more than the double of risk for asthma. In the individuals with two or three risk alleles, the risk for asthma is considerable increased. The maximum risk was found in individuals with four risk alleles which represented a risk five times greater for asthma in relation to the reference group (which subjects have no risk alleles). In addition, when we looked for the effect of variants in both *NLRP3* and *CASP1* on eosinophils in blood from asthmatics patients, only rs7925706 variant from *CASP1* was able to modulate eosinophil counts in blood from asthmatics subjects (Fig. 3).

4. Discussion

NLRP3 receptor is one of the proteins that make up the multiprotein complex that forms the NLRP3 inflammasome, that in their state of activation, results in activation of Casp-1. This protein complex also acts as a key role in both innate and adaptive immunities. In addition, in Th2 cells from mice, NLRP3 inflammasome positively regulates Th2 programming, promoting Th2 differentiation as a transcription factor [14,43].

A model of mice-induced pulmonary inflammation in *NLRP3* and *CASP1*-deficient showed an increased airway inflammation, with influx of eosinophils, Th2 cytokines profile and IL-33, thus, demonstrating that the Th2 response generated by IL-33 was regulated by Casp-1. Casp-1 activation via NLRP3 inflammasome leading to, downregulation of IL-33 and, thus, Th2 response [19].

In this present study, we reported several polymorphisms in *NLRP3* and *CASP1* never described before to be associated to asthma and Bt-induced allergy. Regarding variants in *NLRP3*, the G, C and T alleles of rs72553860, rs12137901, and rs4925648, respectively, and the T allele of *CASP1* SNP rs7925706 were associated with susceptibility to asthma. The first two SNPs (rs72553860 and rs12137901) are in high LD (r^2 = 0.81) (Fig. 2). The *CASP1* polymorphism, rs7925706 (T allele), besides of being associated with asthma was also associated with an increased number of eosinophils (mm^3) in blood among asthmatic subjects (see Fig. 3).

In the same direction, the A and A alleles of rs4925659 and

Table 3
Significant associations between variants on *NLRP3* and *CASP1* with asthma, severity asthma symptoms, IL-13 production, anti-Bt IgE and SPT for Bt.

Gene	SNP	Model	Geno	Aff	Unaff	OR[a]	CI 95%	P-value*
Asthma								
NLRP3	rs12137901	Additive	C/C	33	79	1.28	1.04–1.57	0.016
			C/T	111	359			
			T/T	116	457			
NLRP3	rs4925648	Additive	T/T	11	11	1.5	1.14–1.98	0.005
			T/C	63	183			
			C/C	186	696			
NLRP3	rs72553860	Additive	G/G	28	51	1.38	1.11–1.72	0.004
			G/A	104	335			
			A/A	129	509			
NLRP3	rs55914518	Additive	A/A	1	0	1.85	1.04–3.29	0.034
			A/G	17	35			
			G/G	243	860			
NLRP3	rs12130711	Additive	T/T	12	75	0.78	0.62–0.98	0.038
			T/C	101	369			
			C/C	148	451			
NLRP3	rs36021952	Additive	A/A	2	12	0.69	0.49–0.98	0.038
			A/G	42	190			
			G/G	216	688			
NLRP3	rs72771992	Additive	G/G	3	18	0.67	0.48–0.94	0.019
			G/T	37	174			
			T/T	219	697			
NLRP3	rs56383829	Additive	T/T	52	236	0.82	0.67–0.99	0.046
			T/C	134	436			
			C/C	75	223			
NLRP3	rs74154644	Additive	T/T	2	12	0.69	0.49–0.98	0.038
			T/C	42	190			
			C/C	217	692			
CASP1	rs7925706	Additive	T/T	8	9	1.47	1.11–1.96	0.008
			T/C	65	185			
			C/C	177	675			
Severity asthma symptoms								
NLRP3	rs4925654	Additive	A/A	3	45	0.62	0.44–0.88	0.009
			A/G	37	357			
			G/G	101	613			
NLRP3	rs12728998	Additive	T/T	2	34	0.68	0.48–0.98	0.042
			T/C	35	321			
			C/C	104	660			
IL-13 production in *Blomia tropicalis*-stimulated blood cell cultures								
NLRP3	rs4378247	Additive	C/C	0	59	0.39	0.22–0.67	0.0004
			C/T	15	394			
			T/T	50	570			
NLRP3	rs12565738	Additive	T/T	1	67	0.41	0.24–0.70	0.001
			T/C	14	385			
			C/C	53	588			
NLRP3	rs45624634	Additive	A/A	0	10	0.24	0.09–0.65	0.004
			A/C	4	209			
			C/C	64	821			
NLRP3	rs7525979	Additive	T/T	0	33	0.38	0.19–0.75	0.006
			T/C	9	268			
			C/C	59	739			
Anti-*Blomia tropicalis* IgE								
NLRP3	rs116096527	Additive	A/A	0	2	2.25	1.13–4.48	0.043
			A/G	17	10			
			G/G	383	756			
SPT for *Blomia tropicalis*								
NLRP3	rs4925659	Additive	A/A	25	69	1.3	1.05–1.61	0.021
			A/G	117	334			
			G/G	118	495			
CASP1	rs79180143	Additive	A/A	21	74	0.79	0.63–0.99	0.046
			A/C	81	355			
			C/C	154	446			

Geno, genotype; Aff, affected; Unaff, no affected; OR, odds ratio; CI, confidence interval.
[a] Adjusted by gender, age, helminth infection, ancestry markers.
* P-value corrected.

rs116096527 implied in increased Bt-specific IgE and SPT reactivity. Thus, we hypothesize that a greater release of IL-33 in response to Bt in the airways may occur, contributing to Th2 inflammation.

NLRP3 acts as a transcription factor in T helper cells, polarizing them to a Th2 profile [14]. Thus, NLRP3 inflammasome and Casp-1 may be activated upon activation of IL-33/ST2 pathway. Although we cannot confirm that based on our findings, our hypothesis is that SNPs in NLRP3/CASP1 pathway may lead to a low binding of transcription factor inducing a lower expression of the *NLRP3* and *CASP1*. Decreased activation such genes, results in lower inactivation of IL-33 and thus larger Th2-inflammation with increased release of markers of allergy such as IgE and IL-13 studied herein and increasing the risk for asthma.

Table 4
Possible mechanisms where by SNPs on *NLRP3 and CASP1* may affect these genes expression, according to rSNPBase.

Gene	CHR	SNP	PR	DR	RNA BPMR
NLRP3	1	rs12137901	Yes	No	No
NLRP3	1	rs4925659	Yes	No	No
NLRP3	1	rs72553860	Yes	No	No
NLRP3	1	rs4925648	Yes	No	No
NLRP3	1	rs36021952	Yes	No	No
NLRP3	1	rs72771992	Yes	No	No
NLRP3	1	rs74154644	Yes	No	No
NLRP3	1	rs56383829	No	Yes	No
CASP1	11	rs79180143	Yes	No	Yes
CASP1	11	rs7925706	Yes	No	Yes

CHR, chromosome; SNP, single nucleotide polymorphism; PR, Proximal regulation; DR, Distal regulation; BPMR, binding protein mediated regulation.

Recent studies have shown that IL-33 cytokine is the key element that links early events after lung epithelial cells contact with the allergen and Th2 response, resulting on asthma [9,44]. An experimental mice model receiving CD4+ effector Th2 cells, exposed to Bt antigens have developed lung eosinophilia, hyperplasia of smooth muscle cells and goblet cell, as well as overproduction of IgE and mucus production through IL-13 [6].

On the other hand, regarding variants in *NLRP3*, the A, T, T, G and T alleles of rs36021952, rs74154644, rs12130711, rs72771992 and rs56383829 respectively, were protector factors for asthma in our population. The first and the second SNPs (rs36021952 and rs74154644) are in total LD ($r^2 = 1$) (see Fig. 2). Both the A and T alleles of *NLRP3* polymorphisms rs4925654 and rs12728998 respectively, were associated with decreasing severity of asthma symptoms. The C, T and A alleles of *NLRP3* SNPs rs4378247, rs12565738, rs45624634 and rs7525979 respectively, were negatively associated with IL-13 production in peripheral blood cells when stimulated with Bt and whereas the first two SNPs (rs4378247 and rs12565738) are in moderate LD ($r^2 = 0.76$) (see Fig. 2). Carriers of A allele of *CASP1* variant rs79180143 were less likely to have a positivity SPT reaction for Bt.

Interestingly, in the present study most of the observed associations between analyzed SNPs and the investigated outcomes were related to protection to Bt-induced allergy/asthma. In other words, the most common alleles for these SNPs in our population were risk alleles. These results are in some way in agreement with epidemiological findings that demonstrate high prevalence of asthma and other allergic conditions in our population [34,45,46]. However, these are complex diseases and likely influenced by dozens or hundreds of genes [47,48]. Thus, these results need to be interpreted with caution, so that they do not lead to mistaken conclusions regarding the genetic predisposition of our population to these conditions.

Table 6
Genetic risk score with the A allele of rs12551256 (*IL33*) + T allele of rs4925648 (*NLRP3*) + T allele of rs7925706 (*CASP1*) both association with risk for asthma.

	Asthma			
Risk allele	Controls	Cases	Odds Ratio[a]/95% Confidence interval	P-value
0	55 (17.6%)	6 (8%)	1.00	–
1	257 (82.4%)	69 (92%)	2.47 (1.01–6.04)	0.029
2	368 (87%)	113 (95%)	3.00 (1.24–7.24)	0.006
3	150 (73.2%)	50 (89.3%)	3.15 (1.27–7.86)	0.006
4	32 (36.8%)	16 (72.7%)	5.77 (1.89–17.60)	9E-04

[a] Adjusted for confounding variables.

For the *NLRP3* gene, the nine SNPs originally identified as associated with asthma symptoms were reduced to four independent regions after a clumping analysis, two of which were positively associated and the other two negatively associated with asthma outcome (see Supplementary Table 4). This suggests that different variants within this gene may have antagonistic effects in relation to the risk of asthma, which is a matter for future investigation.

We also conducted a haplotype analysis in the *NLRP3* gene for asthma and IL-13 production. We showed that haplotypes with two, three or four alleles increased risk of asthma (see Table 5). On the other hand, we also have shown a high protection for IL-13 production upon Bt stimulation in addition of three or four alleles in another haplotype described here (see Table 5). No significant result was obtained after the haplotypes analysis in *CASP1* (data not shown). In addition, we performed a polygenic risk score with A allele of *IL33* rs12551256 variant previously described [7], as well as T and T alleles of *NLRP3* rs4925648 and *CASP1* rs7925706 polymorphisms, respectively, both risk alleles described here for asthma, and again, the greater the number of the risk alleles in *IL33/NLRP3/CASP1* route, the greater the risk for asthma (see Table 6). No previous work was found in literature taking into account variants occurring together in such genes for asthma or allergy.

Table 5
Haplotype analysis between rs72553860, rs12137901, rs36021952 and rs74154644 on asthma risk and between rs45624634, rs12565738, rs7525979 and rs4378247 in decreasing IL-13 production Bt-stimulated, both in NLRP3 gene.

					Frequencies			
Haplotype	rs72553860	rs12137901	rs36021952	rs74154644	Case	Control	Odds Ratio[a]/95% Confidence interval	P-value
Reference	A	T	G	C	0.57	0.55	1	–
Haplotype association with risk for asthma								
1 asthma	G	C	G	C	0.19	0.25	1.43 (1.09–1.87)	0.011
2 asthma	G	C	–	C	0.23	0.21	1.39 (1.09–1.77)	0.008
3 asthma	G	C	G	–	0.23	0.22	1.38 (1.08–1.76)	0.009
4 asthma	G	C	–	–	0.26	0.24	1.37 (1.10–1.70)	0.005
Happlotype	rs45624634	rs12565738	rs7525979	rs4378247	Case	Control	Odds Ratio[a]/95% Confidence interval	P-value
Reference	C	C	C	T	0.48	0.47	1	–
Haplotype association with IL-13								
1 IL-13	C	T	C	–	0.12	0.13	0.53 (0.29–0.98)	0.042
2 IL-13	C	C	T	–	0.12	0.13	0.41 (0.21–0.81)	0.011
3 IL-13	A	T	C	–	0.08	0.09	0.26 (0.09–0.71)	0.009
4 IL-13	C	C	T	T	0.09	0.1	0.13 (0.03–0.56)	0.006
5 IL-13	A	T	C	T	0.06	0.07	0.11 (0.01–0.80)	0.030

[a] Adjusted for confounding variables.

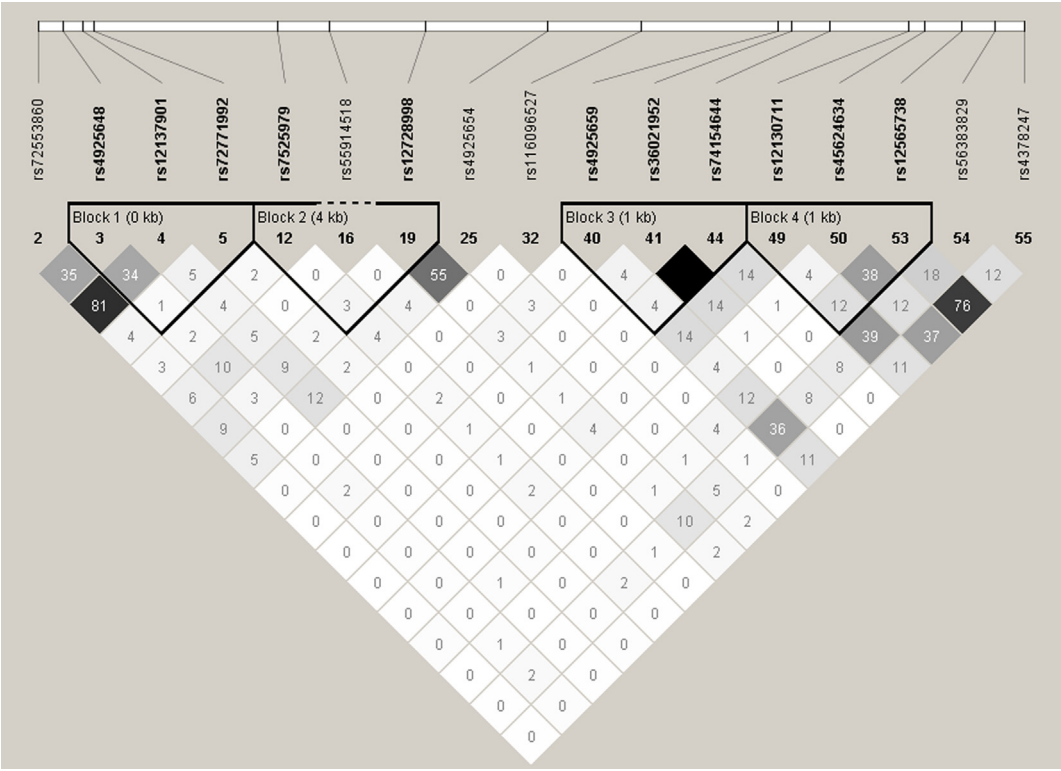

Fig. 2. LD plot of significance analysis of 17 SNPs on *NLRP3*, analyzed in our population. Top bar indicates the physical location of each variant. The colors of each square vary according to the degree of LD, being the total equilibrium in color white with value of r^2 equal to 0 and total unbalance in color black with value of r^2 equal to one. The different shades of gray show an intermediate imbalance with a value of $r^2 > 0$ and < 0.8. This analysis was perfomed with Haploview software.

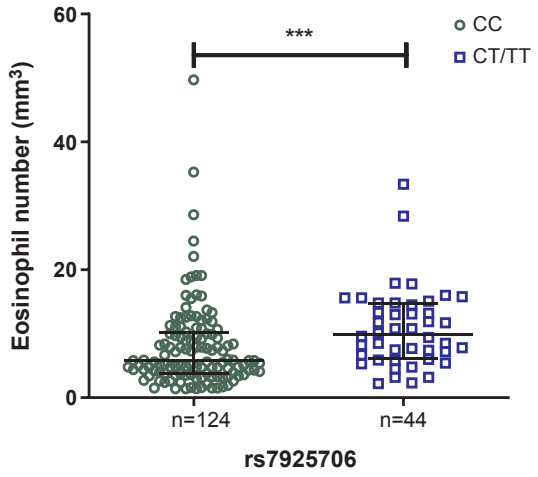

Fig. 3. Eosinophil number per mm^3 in blood of asthmatic subjects according to genotype of *CASP1* SNP rs7925706. Bars represent the median of eosinophils number according with each group genotyp, represented by its holders. CC: median = 5.800 and CT/TT: median = 9.850. The analyis of the data was by Manny-Whitney test. ***P < 0.001.

5. Conclusion

In summary, our findings demonstrate associations between polymorphisms in NLRP3 inflammassome pathway with asthma and allergy and that variants in *IL33/NLRP3/CASP1* route, together, increase asthma risk. We also have shown the effect of *CASP1* variant rs7925706 (T allele) in eosinophil counts in blood among asthmatic individuals. Our data reinforce a critical role of *NLRP3* and *CASP1* on asthma and allergic inflammation. Additional studies are need to better understand the role and the impact of such polymorphisms on asthma and allergy as means of potential targets for therapeutical intervetions.

CRediT authorship contribution statement

Gerson A. Queiroz: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft. **Raimon R. Silva:** Validation, Writing - original draft. **Anaque O. Pires:** Validation, Writing - original draft. **Ryan S. Costa:** Writing - review & editing. **Neuza M. Alcântara-Neves:** Resources, Writing - review & editing. **Thiago M. Silva:** Formal analysis, Validation. **Mauricio L. Barreto:** Supervision, Resources, Writing - review & editing. **Sergio C. Oliveira:** Writing - review & editing. **Camila A. Figueirêdo:** Supervision, Resources, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics

The Ethics Committee of Federal University of Bahia approved this study (register 003-05/CEP-ISC).

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Appendix A. Supplementary data

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