



Variants in the *CYSLTR2* are associated with asthma, atopy markers and helminths infections in the Brazilian population

Talita dos S. Jesus^a, Ryan dos S. Costa^a, Neuza Maria Alcântara-Neves^a, Maurício L. Barreto^b, Camila Alexandrina Figueiredo^{a,*}

^a Departamento de Biorregulação, Laboratório de Imunofarmacologia e Biologia Molecular, Universidade Federal da Bahia, Instituto de Ciências da Saúde, Av. Reitor Miguel Calmon, s/n - Canela, Salvador, Bahia, Brazil

^b Fundação Oswaldo Cruz, Salvador, Bahia, Brazil

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ABSTRACT

Background: Asthma is a chronic disease of the airways and its most common phenotype is characterized by a T2 type response with IgE production and inflammatory mediators in response to common allergens. Cysteinyl leukotrienes (CysLTs), LTC₄, LTD₄ and LTE₄, are mediators known to possess important proinflammatory action. CysLTs can bind to the Cysteinyl leukotriene receptor type 2 (CysLTR2) and activate an inflammatory. Polymorphisms in CysLTR2 have been associated with asthma and atopy, although the mechanism is not clear. **Objective:** To evaluate the association between genetic polymorphisms in *CYSLTR2* with asthma phenotypes, atopy markers and helminth infection.

Methods: Genotyping was performed using a panel Illumina and carried out in 1245 participants of SCAALA program (Social Change, Asthma, Allergy in Latin American). Logistic regressions for asthma, helminth infections (*Trichuris trichiura* and *Ascaris lumbricoides*) and allergy markers (skin tests and IgE production) were performed using PLINK 1.9 software adjusted for sex, age, helminth infection and ancestry markers.

Results: The G allele of rs1323556 was negatively associated with asthma in the additive model (OR 0.74, 95% CI 0.59–0.93) and in the dominant model (OR 0.71, 95% CI 0.53–0.74). The G allele of rs1575464 was also negatively associated with asthma in two genetic models, additive (OR 0.77, 95% CI 0.62–0.96) and dominant (OR 0.73, 95% CI 0.55–0.97). The G allele of rs61735175 was positively associated with asthma severity in the additive model (OR 1.72, 95% CI 1.07–2.77) and in the dominant model (OR 1.77, 95% CI 1.09–2.85). Five SNVs were associated with atopy markers and four SNVs were associated with helminth infections.

Conclusion: Polymorphisms in the *CYSLTR2* gene are associated with asthma, atopy markers and helminth infection in Brazilian individuals, which may lead to protection or risk for such conditions, however, more studies are needed to evaluate the functional of this variants here in described.

1. Introduction

Prevalence of allergic disorder is increasing worldwide and it is estimated that such conditions may affect 30–35% of people in the world at some point in their lives [1,2]. Within allergic diseases atopic asthma is highlighted due to its impact on the individuals' quality of life and also because of its highly prevalent. Asthma may affect as many as 334 million people in the world and its prevalence is also rising [3].

Asthma is defined as a complex disorder of airways characterized by lower airway hyperreactivity, mucus overproduction and airflow limitation [4]. The immunopathology of allergic disorders such as asthma is mainly lead by T2-type molecules and cytokines [5]. Contact of allergens with Th2 cells elicits physiological responses that include the production of specific cytokines such as IL-4, IL-5, IL-9 and IL-13, these cytokines are related to the production of immunoglobulin E (IgE) antibodies, eosinophils recruitment to the site of inflammation as well as

Abbreviations: CI, confidence interval; IgE, immunoglobulin E; SPT, skin prick test; LD, linkage disequilibrium; OR, odds ratio; PC, principal component; NCBI, national center for biotechnology information; eQTLs, expression quantitative trait loci; SCAALA, social change, asthma, allergy in Latin American; ISAAC, II international study of asthma and allergies in childhood; HWE, hardy-weinberg equilibrium; GTEx, the genotype-tissue expression; MAF, minor allele frequency; GENO, genotype call rate; IL, interleukin; Th2, T helper 2; SNV, single nucleotide variant; CysLT, cysteinyl leukotriene; CysLTR2, cysteinyl leukotrienes type 2 receptor; *CYSLTR2*, cysteinyl leukotrienes type 2 receptor gene; ADD, additive model; DOM, dominant model

* Corresponding author.

E-mail address: camilavf@ufba.br (C.A. Figueiredo).

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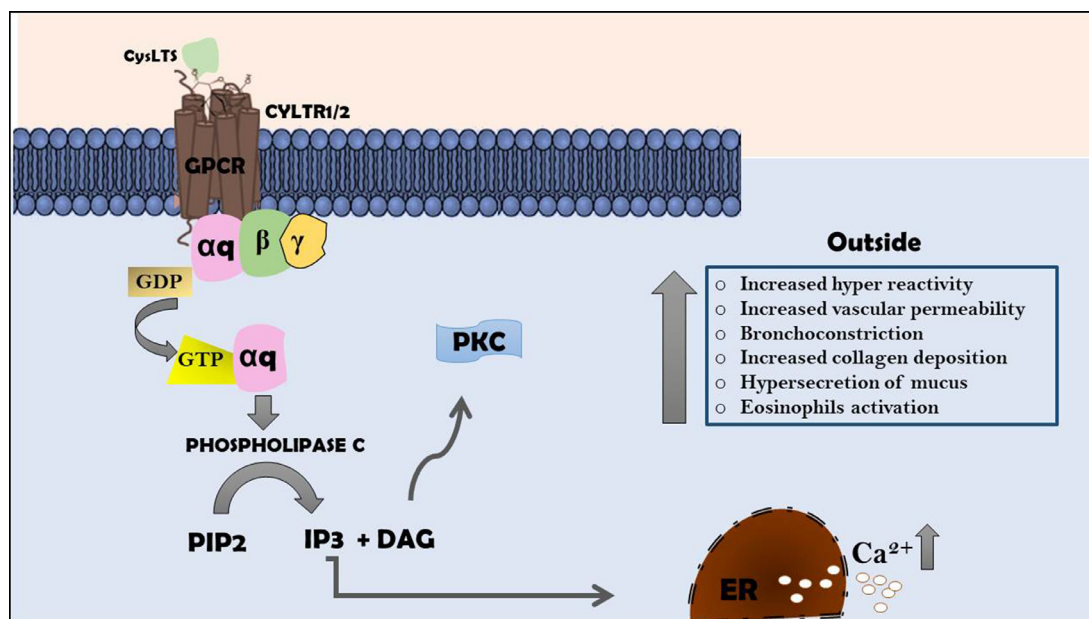


Fig. 1. Signaling pathway and the biological effects mediated by CysLTs (LTC 4, LTD 4 and LTE 4) and its binding with CysLT1/2 receptor. CysLTs act through GPCR coupled to G q protein and thereby increase intracellular calcium. PIP2 = Phosphatidylinositol biphosphate; IP3 = inositol triphosphate; DAG = diacylglycerol; ER = endoplasmic reticulum; PKC = protein kinase C.

mucus production and release of histamine and other mediators such as leukotrienes from mast cells and basophils [6].

On the other hand, host defense against helminth infections has the same T2-type immunological mechanisms of allergic diseases [2], however, during chronic helminth infection, the activation of a regulatory network which has been linked such infections during childhood with the protection of atopic conditions [5,7,8].

Cysteinyl leukotrienes (CysLTs) which comprises LTC₄, LTD₄ and LTE₄, all with high affinity to Cysteinyl leukotriene receptor 1 and 2 (CysLTR1/CysLTR2) have their biological action through the activation of G-protein with GDP-GTP exchange, hydrolysis of GTP and intracellular signaling events [9,10] leading symptoms such as bronchoconstriction, airway hyperresponsiveness, increased vascular permeability and eosinophilia, as can be seen in Fig. 1. Those mediators are prominent products of activated eosinophils, basophils, mast cells (MCs), and macrophages [11] and they are derived from arachidonic acid after its degradation by the 5-lipoxygenase [12,10].

CYSLTR2 is located on chromosome 13 and encodes a protein of 346 amino acids, with an amino acid sequence 38% identical to that of the CysLT1 receptor [13]. The CysLTR2 is a member of G-protein receptors family and an increasing number of studies has been conducted in the last years since it can play a role in diseases such asthma, rhinosinusitis, anaphylaxis and cardiovascular disorders. The *CYSLTR2* is expressed in the spleen and peripheral blood leukocytes, lung smooth muscle cells, alveolar macrophages and other tissues [10,14] and it has been identified on the mucus gland and nasal mucosal epithelium of patients with chronic rhinosinusitis, or allergic nasal vascular smooth muscles [11]. The CysLT receptors are regulated immunologically, mainly several local signs of inflammation stimulated by allergens and some studies have shown that CysLTR2 can have an important role in allergic inflammation [15,16,17,14]. One study has demonstrated upregulation of CysLTs receptors by T2-type cytokines, appearing to be up-regulated by IL-4 in human umbilical vein endothelial cells, demonstrating a relationship between CysLTs and T2-type immune response [18].

Considering the important role of CysLTs receptors in inducing the downstream pathways related to bronchoconstriction observed in allergic conditions such as asthma, genetics alteration in the gene encoding this receptor could play a role in the pathogenesis of inflammatory response. Polymorphism in *CYSLTR2* has been associated

with allergic diseases such as asthma and atopy in several European countries [19,20,21,16] but no genetic study on *CYSLTR2* was performed in Brazil, a mixed population that has one of the highest prevalence of asthma among children [22]. Also, there is no study evaluating the association between *CYSLTR2* polymorphism and helminth infections. For this reason, our study aimed to evaluate possible associations between polymorphism in *CYSLTR2* with asthma, atopy markers and helminth infections in a Latin American cohort.

2. Methods

2.1. Study population

The study population was previously described [23]. It is a population-based study which originally evaluated the impact of the sanitation program on the occurrence of childhood diarrhea. This first study recruited children from 24 small geographical areas in the city of Salvador selected to represent the population without sanitation in Salvador, called sentinel-areas. Standardized questionnaires were applied during the baseline surveys to obtain data on demographic characteristics and living conditions of the families, the children who were aged 4–11 years and had complete follow-up information (e.g. stool examination conducted at 3 different time points) were selected for our study. Data were collected from standardized questionnaires that were applied to each child legal guardian in 2005 to collect data on demographic and social variables, as well as on the domestic environment and to obtain blood samples for laboratory tests and isolation of genomic DNA. This work was approved by the National Research Ethics Committee (120.616) and free informed consent was properly obtained from the legal guardian of each child.

2.2. Asthma definition

Asthma was defined according to the ISAAC studies as the occurrence of wheezing in the last 12 months (ISAAC phase II). The children were considered asthmatic when they presented wheezing in the past 12 months and at least one of the following symptoms: sleep disturbed due to wheezing, four or more wheezing episodes, wheezing during the practice of physical activity, both in the last 12 months or asthma

diagnosed by physician once in life.

2.3. Markers of atopy: specific IgE and skin tests

All individuals were submitted to skin tests to common inhalant environmental allergens in our region (mites - *D. pteronyssinus*, *D. farinae*, *B. tropicalis*; cockroach - *P. american* and *B. germanica*; fungi - *A. fumigatus* and *P. notatum*) on the anterior forearm. After 15 min, wheal size was measured. The test was considered positive if the mean of the largest perpendicular diameters (excluding pseudopodia) was at least 3 mm greater than the negative control.

To determine specific IgE levels, tests were conducted to airborne allergens such as *B. tropicalis* and *D. pteronyssinus* mites and cockroaches, *B. germanica* and *P. americana*, using ImmunoCAP assay (Phadia Diagnostics AB, Uppsala Sweden).

Children who had specific IgE levels greater than or equal to 0.70 kU/L and/or positive SPT, for at least one aeroallergen tested, was defined as atopic.

2.4. Detection of helminth infection

We performed test to detect helminth infection using Kato–Katz technique [23]. Stool samples were collected in two different days for each child, 2 days apart. Also, samples were analyzed using Hoffman, Pons & Janner sedimentation technique of to detect helminth eggs, protozoan cysts and oocysts. Two slides were examined for each stool sample [5,24].

2.5. Extraction and genotyping

DNA was extracted from peripheral blood samples using the protocol Flexigene DNA Kit (Qiagen, Hilden, Germany). The genotyping was performed using standardized commercial panel 2.5–8 Human Omni Bead chip and currently available from Illumina (www.illumina.com) through the Epigen-Brazil Consortium. Most of these SNVs were selected from the 3.1 Million genotyped SNVs in the 1000 Genome Project data. The *CYSLTR2* genetic information were extracted from NCBI database (location: NC_NC_000013.10) position at the chromosome 13. A total of 23 markers were analyzed for *CYSLTR2*.

2.6. Data analysis

Before genetic association analysis, all SNVs were submitted to quality control tests that include parameters as percentage of obtained genotypes, Hardy–Weinberg equilibrium ($HWE > 0.05$) and minor allele frequency ($MAF > 0.05$). Such parameters were evaluated using the PLINK (version 1.9) software. From 39 SNVs typed in *CysLTR2* gene, 16 were excluded based on the quality control parameters applied, mainly MAF. A total of 24 SNVs were included in the logistic regression analysis using asthma, asthma severity, skin prick test reactivity, specific IgE against common allergens and for *Trichuris trichiura* and *Ascaris lumbricoides* infection as main outcomes in two genetic models, additive (ADD) and dominant (DOM). To eliminate false associations, the analyzes were adjusted for sex, age, and principal component (PC) 1 and 2 (PC1 for African contribution and PC2 for European contribution) and helminth infection (when appropriated). To obtain *p* value a simple allelic test of χ^2 was applied to SNVs, and after categorization of chosen phenotype, *p* value < 0.05 was considered statistically significant. The genetic model chosen for analysis was additive and dominant. Correction for multiple test using permutation analysis strategy was also applied (PERM *p* value in tables). Pairwise LD was created using Haploview 4.2 to calculate the degree of confidence in the *D'* value and Regulome Db Score was search in regulomedb.org [25].

2.7. In silico analysis

The National Center for Biotechnology Information (NCBI) was used to obtain information about the function of each SNV used in the present study (www.ncbi.nlm.nih.gov). RegulomeDB is a database containing information about thousands of SNVs across the genome and also contains information on the impact of SNVs on gene expression through regulatory elements known in the regions of the genomes [25]. To do that, it uses databases such as ENCODE as well as published literature. RegulomeDB provides a score ranging from 1 to 6 and the lower the score, the stronger is the impact of SNV on the expression of certain gene [25]. We used The Genotype-Tissue Expression Project (GTEx) (www.gtexportal.org) of the National Institutes of Health Common Fund which establishes a relationship between gene expression, genetic variations and other molecular phenotypes in multiple human reference tissues [26]. By using this platform, we can have information of the expression quantitative trait loci (eQTL) locus offers a powerful approach to elucidate the genetic component underlying the altered expression of the gene. Genetic variation may also influence gene expression through splicing changes, non-coding RNAs, and RNA stability. From this project were extracted information on the functional impact of each SNV in different tissues, such as lung and whole blood. A haplotype analysis was performed on the SNPStats online platform for SNVs associated with the same phenotype (www.snptest.net).

3. Results

3.1. Study population

The Table 1 summarizes the clinical and demographic characteristics of the study population. From a total of 1245 children participating in the cohort, analyzes were performed on those who had complete genotype and phenotype data. For the categorization of the study population the subjects were divided between asthmatics and non-asthmatics, 25 subjects were lost in the analyzes due to missing genotype or phenotype data.

In this study, 39 SNVs in the *CYSLTR2* were extracted from the Illumina genotyping chip. After quality control 15 SNVs with frequency less than 5% were excluded and 1 SNV failed in the HWE test, leaving 24 SNVs to be analyzed. Of the 24 SNVs analyzed with asthma, atopy markers and helminth infection, 19 were associated with one or more phenotypes and are summarized in Table 2. Among the analyzed SNVs most of them have a minor allele frequency (MAF) higher than 10% and nearly all are located in intronic region of gene. RegulomeDB is a database that annotates SVs with known and predicted regulatory elements in the intergenic regions (<http://www.regulomedb.org>), platform results showed scores between 5–6, the score 5 means that the SNV may be related to transcription factors and peak DNases and 6 that there is no information. We present association results of variants associated in two or more phenotype or those with an association plus association in Gtex.

3.2. Variants in *CYSLTR2*, asthma and asthma severity

The G allele of rs1323556 was negatively associated with asthma in the additive model (OR 0.74, 95% CI 0.59–0.93) and in the dominant model (OR 0.71, 95% CI 0.53–0.74). The G allele of rs1575464 was also negatively associated with asthma in two genetic models ADD (OR 0.77, 95% CI 0.62–0.96) and DOM (OR 0.73, 95% CI 0.55–0.97). These SNVs are in high linkage disequilibrium (Fig. 2). The C allele of rs9595950 was negatively associated with asthma in the ADD model (OR 0.80, 95% CI 0.65–0.98). The T allele of rs61735175 was positively associated with asthma severity in the ADD model (OR 1.72, 95% CI 1.07–2.77) and in the DOM model (OR 1.77, 95% CI 1.09–2.85) and the G allele of rs7996072 was also positively associated with the same phenotype in ADD model (OR 1.65, 95% CI (1.01–7.71) and they SNVs

Table 1

Characteristics of the social changes asthma and allergy in Latin America population according to allergic status and variables included in this study.

	Non-asthma N (77.5%)	Asthma N (22.5%)	p value*
n ^a	946	274	
Sex (1203)			
Male	501 (76.4)	154(23.6)	0.233
Female	429 (76.1)	119 (21.1)	0.162
Age group (1203)			
≤5 years	342 (76.7)	96 (21.5)	0.545
6–7 years	316 (74.0)	103 (24.1)	0.466
≥8 years	272 (76.6)	74 (20.8)	0.419
Positive IgE production (1.246)			
Specific IgE for ≥1 allergen (0.70 kU/L)	325(70.2)	133(28.7)	<0.001*
Specific IgE for <i>D. pteronyssinus</i>	179(65.3)	91 (32.9)	<0.001*
Specific IgE for <i>B. tropicalis</i>	288(68.4)	128(30.4)	<0.001*
Specific IgE for <i>B. germanica</i>	117(70.1)	47(28.1)	<0.001*
Specific IgE for <i>P. americana</i>	81(71.1)	31(27.2)	<0.001*
Positive skin prick test response (1.103)			
Specific skin test for >3 mm	272 (72.1)	100(26.5)	<0.001*
Specific skin test for <i>D. pteronyssinus</i>	131(67.2)	62(31.8)	<0.001*
Specific skin test for <i>B. tropicalis</i>	192(70.8)	76(28.0)	<0.001*
Specific skin test for <i>B. germanica</i>	70(68.0)	31(30.0)	<0.001*
Specific skin test for <i>P. americana</i>	120(69.4)	49(28.3)	<0.001*

* p < 0.05.

Table 2

Base pairs, allele, Minor allele frequency, Hardy–Weinberg equilibrium, function and

RegulomeDB score of *CYSLTR2* SNVs explored in this study.

SNV	A1	A2	MAF	HWE	FUNCTION	REGULOME DB
rs1323556	G	A	0.29	0.23	INTRON	–
rs1575464	G	T	0.30	0.17	INTRON	–
rs9595950	C	T	0.44	0.40	INTRON	5
rs61735175	T	C	0.05	0.12	MISSENSE	–
rs7996072	G	A	0.05	0.17	INTRON	–
rs9595965	A	G	0.14	0.58	INTRON	–
rs7335086	G	A	0.06	0.11	INTRON	5
rs9568079	G	A	0.11	0.20	INTRON	6
rs1323555	T	C	0.17	0.60	INTRON	–
rs12875723	A	G	0.38	0.59	INTRON	6
rs78149855	A	G	0.07	0.83	INTRON	5
rs7330144	T	C	0.12	0.80	INTRON	6
rs34494076	T	C	0.12	0.80	UTR VARIANT	5
rs1359108	C	T	0.43	0.43	INTRON	6
rs11843756	G	T	0.20	0.86	INTRON	–
rs9285169	T	C	0.28	0.24	INTRON	–
rs9591194	G	A	0.31	0.10	INTRON	5
rs9595960	T	C	0.44	0.21	INTRON	–
rs17072059	T	C	0.15	0.75	INTRON	6

A1- Polymorphic allele; A2- Ancestral allele; MAF- Minor Frequency Allele; HWE- Hardy-Weinberg equilibrium.

are in high linkage disequilibrium (Fig. 2).

3.3. Variants in *CYSLTR2* and atopy markers

The A allele of rs9595965 was positively associated with IgE for at least one allergen in DOM model (OR 1.34, 1.03–1.75), in ADD model for SK test for *Dermatophagoides pteronyssinus* (OR 1.37, 95% CI 1.01–1.85) and also with skin test for at least one allergen in the ADD model (OR 1.30, 95% CI 1.19–1.67) while the G allele of rs1575464 was negatively associated for the same phenotype (OR 0.71, 0.53–0.74).

The allele G of rs7335086 was negatively associated in the two genetic models for SK test for *Blomia tropicalis* (ADD: OR 0.52, 95% CI 0.32–0.85; DOM: OR 0.52 95% CI 0.32–0.86). The A allele of rs78149855 was positively associated with skin test for *Periplaneta Americana* in DOM model (OR 1.58, 95% CI 1.03–2.42) while the A allele of rs12875723 was negatively associated with the same

phenotype (OR 0.65 95% CI 0.46–0.91).

3.4. Variants in *CYSLTR2* and helminth infection

The allele A of rs78149855 (ADD: OR 0.41, 0.22–0.76; DOM: 0.41, 0.22–0.76) and the A allele of rs12875723 (DOM: OR 0.67, 0.47–0.95) were negatively associated with infection by *Trichuris trichiura* (Table 5). In addition, the G allele of rs11843756 (ADD: OR 1.38 95% CI 1.04–1.84; DOM: OR 1.49 95% CI 1.05–2.10) and the C allele of rs1359108 were positively associated with *T. trichiura* infection. The G allele of rs11843756 was positively associated with *A. lumbricoides* infection in both genetic models ADD: OR 0.08 95% CI 0.011–0.015; DOM: OR 0.009 95% CI 0.009–0.18) (Table 5). The C allele of rs9595950 was negatively associated with helminth infection in additive model (OR 0.80 95% CI 0.65–0.97), while the SNVs rs11843756 (ADD: OR 1.28 95% CI 1.02–1.63; DOM OR 1.4 95% CI 1.54–1.85) and rs1359108 (DOM: 1.55 95% CI 1.14–2.13) were positively associated with helminth infection (Table 5), analysis on the SNPStats platform showed that these two SNVs form a haplotype of risk alleles (G/C, respectively) with a frequency of 20% in this population, further increasing the risk for helminth infection (OR 1.62 CI 1.20–2.18, p value: 0.0015) (data not show).

3.5. The linkage disequilibrium

Analysis of linkage disequilibrium (LD) was performed using HaploView program where it was observed that few SNVs in *CYSLTR2* are in high or complete linkage disequilibrium, as shown in Fig. 2.

3.6. In silico analysis showed that variants in *CYSLTR2* alter gene expression in the lung and whole blood

The eQTL was examined for expression of the gene depending on the genotype in lung and whole blood. The genotype (T/T) of rs1575464, (A/A) of rs9595965 and (C/C) of rs9595960 and (A/A) of rs78149855 significantly increased the expression of *CYSLTR2* in the lung (Fig. 3).

The genotype (T/T) of rs9595950, (C/T) of rs61735175 and (A/G) of rs7996072 significantly increased expression of *CYSLTR2* in the whole blood (Fig. 4).

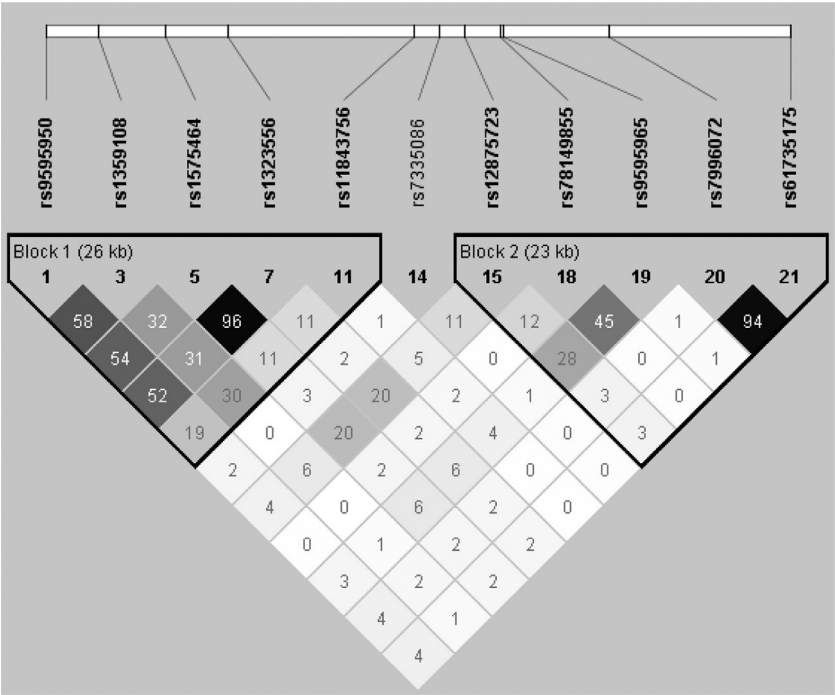


Fig. 2. Pairwise LD within Haploview by using the R^2 squared statistic for the associated SNVs in CysLTR2 gene. Color intensity and value in square indicates the degree of confidence in the R^2 value and how much the SNVs are in linkage disequilibrium.

4. Discussion

The gene encoding the human CysLTR2 receptor is on chromosome

13q14 and this locus has been associated with asthma in previous studies [20,27,16]. This receptor is directly involved in allergic inflammation and this corroborates the importance of studying

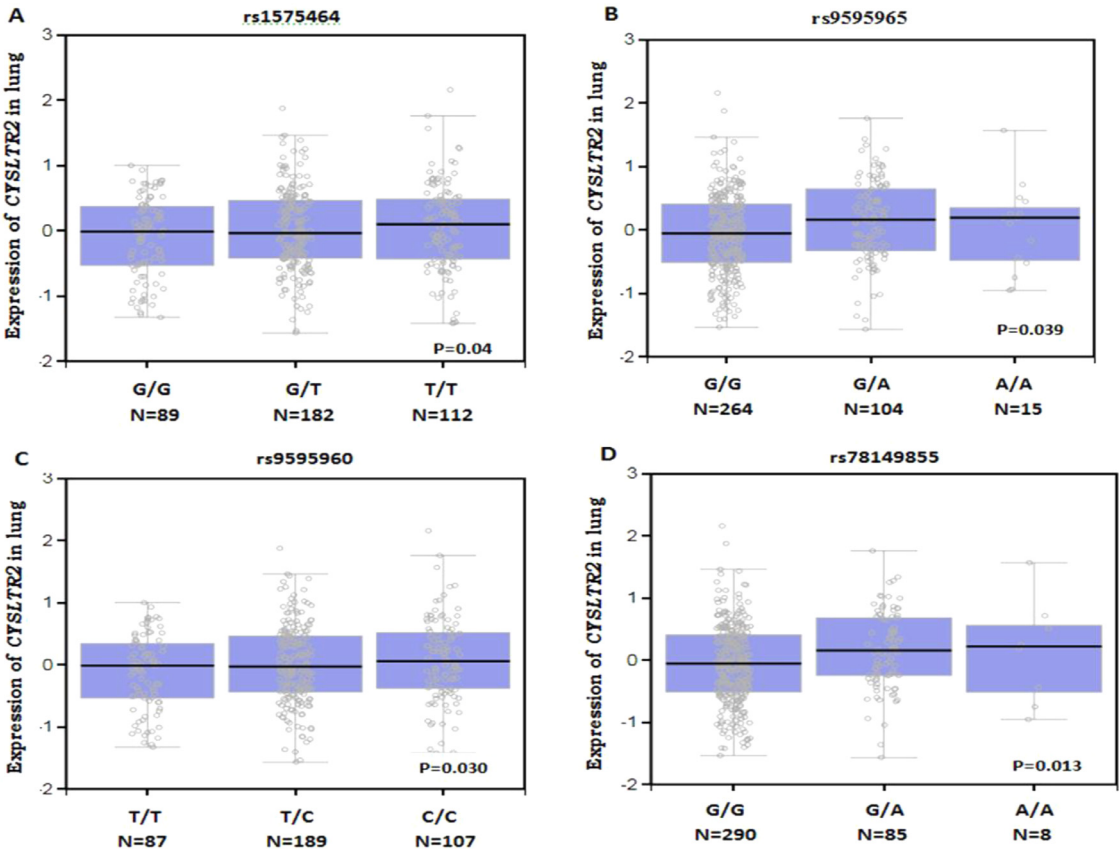


Fig. 3. Levels of CYSLTR2 expression in the lung grouped by GTEx genotypes: A. rs1575464 ($P = 0.04$); ($P = 0.037$); B. rs9595965 ($P = 0.03$); C. rs9595960 ($P = 0.030$)D. rs78149855 ($P = 0.013$).

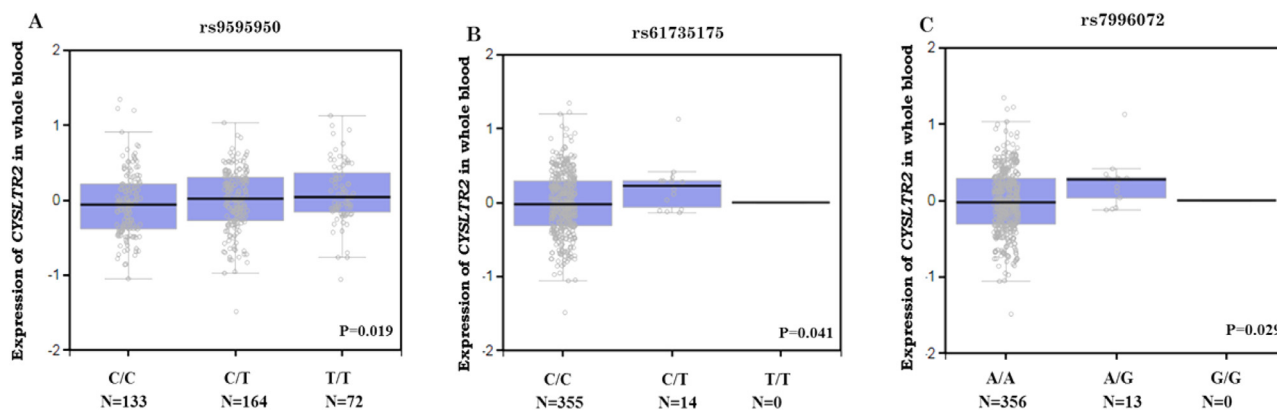


Fig. 4. Levels of *CYSLTR2* expression in the whole blood grouped by GTEx genotypes: A. rs9595950 ($P = 0.019$); B. rs61735175 ($P = 0.041$); C. rs7996072 ($P = 0.029$).

polymorphisms in this gene and its impact on T2-type immune response. Several publications have described the main tissues where the *CysLTR2* is more expressed and how polymorphisms in this gene could have an impact in different illnesses, such as asthma [28,12,14].

In our study the G allele of rs1575464 was negatively associated with asthma and atopy. It also is in complete linkage disequilibrium with the rs1323556 which was also negatively associated with asthma. A study in Japan has demonstrated that the expression of *CysLTR2* in the lung of asthmatic subjects, and they assume that the increased expression of *CysLTR2* receptors may contribute to antigen-induced bronchoconstriction in certain cases of asthma [12]. The in silico analysis for gene expression according to rs1575464 genotypes, the T allele increased the expression of *CYSLTR2* in the lung (Fig. 3A). This corroborates our findings whereas the polymorphic G allele, which has presented a lower expression of the receptor, could lead to less bronchoconstriction and therefore individuals with allele G would have a lower chance of developing asthma. Moreover the C allele of SNV 9,595,950 which has also been negatively associated with asthma, is in high LD with the above-mentioned variants and appears to decreased the gene expression in the Gtex database but in whole blood. This means that all of these variants may be acting together as a protective factor for the development of asthma, although the exact mechanism is not fully understood.

The G allele of rs61735175 of missense variant was positively associated with asthma severity in our study, furthermore it is in linkage disequilibrium with rs7996072 which was also positively associated

with the same phenotype in our study (Table 3). This can be corroborated by the findings in GTEx where the polymorphic alleles of these two variants were associated with increased expression of *CYSLTR2* in whole blood (Fig. 4B and C). Increased expression of the *CysLTR2* receptor may result in a greater activation of the cysteines leukotrienes pathway, leading to an exacerbation of the symptoms caused by these mediators such as bronchoconstriction and decrease in airflow [13] which could explain the association of these variants with asthma severity (Table 3).

The allele A of the variant rs9595965 was positively associated with three different allergic markers (Table 2) and the A allele of rs78149855 was positively associated with a marker of atopy, both variants demonstrated to increase expression of the gene in the lung (Fig. 3B and D) when compared to the reference alleles and a higher receptor expression probably causes greater activation by agonist which could explain the increased risk of atopy related to these SNVs.

In our study some polymorphisms were positively associated with helminth infection (rs78149855, rs78149855, rs12875723, rs9595950) and others were negatively associated (rs11843756, rs11843756, rs1359108). Although the response to helminths is similar to that for allergies, Th2 response with IgE production, recruitment of leukocytes and production of mediators, it is not yet known the exact role of *CysLTRs* in the helminth infection. It has already been demonstrated that these mediators can act both in the expulsion of the helminth as well as being an evasion mechanism of the parasites from the immune system [29]. Therefore further studies are needed in order to elucidate how

Table 3

Significant associations between polymorphism in the *CYSLTR2* with asthma and severity by logistic regression adjusted for sex, age, helminth infection and ancestry markers.

SNV	Genotype	Control	Case	Model	OR (95% CI)	P value	Perm
Asthma							
rs1323556	AA	428 (48%)	146 (55.9%)	Additive	0.74 (0.59–0.93)	0.009	0.009
	AG	370 (41.5%)	97 (37.1%)	Dominant	0.71 (0.53–0.74)	0.017	0.015
	GG	93 (10.4%)	18 (6.89%)	Recessive	0.62 (0.36–1.05)	0.07	0.07
rs1575464	TT	423 (47%)	142 (54.4%)	Additive	0.77 (0.62–0.96)	0.022	0.025
	TG	376 (42%)	99 (37.9%)	Dominant	0.73 (0.55–0.97)	0.033	0.035
	GG	96 (10.7%)	20 (7.66%)	Recessive	0.68 (0.41–1.14)	0.14	0.15
rs9595950	TT	267 (29%)	94 (36%)	Additive	0.80 (0.65–0.98)	0.033	0.049
	TC	436 (48.7%)	124 (47.5%)	Dominant	0.74 (0.55–1.00)	0.054	0.054
	CC	191 (21.3%)	43 (16.4%)	Recessive	0.74 (0.51–1.08)	0.12	0.13
Severity							
rs61735175	CC	902 (88%)	116 (82.2%)	Additive	1.72 (1.07–2.77)	0.023	0.017
	CT	112 (11%)	25 (17.7%)	Dominant	1.77 (1.09–2.85)	0.019	0.021
	TT	1 (0.09%)	0 (0%)	Recessive	NS	NS	NS
rs7996072	AA	906 (89%)	118 (83.6%)	Additive	1.61 (0.99–2.63)	0.053	0.058
	AG	108 (10.6%)	23 (16.3%)	Dominant	1.65 (1.01–7.71)	0.045	0.048
	GG	1 (0.09%)	0 (0%)	Recessive	NS	NS	NS

OR- odds ratio; Perm- permutational p value; statistical significance $p < 0.05$.

Table 4Significant associations between polymorphism in the *CYSLTR2* and atopy markers by logistic regression adjusted for sex, age, helminth infection and ancestry markers.

SNV	Genotype	Control	Case	Model	OR (95% CI)	P value	Perm
IgE production for at least one allergen							
rs9595965	GG	542 (74%)	302 (68.7%)	Additive	1.26 (0.99–1.60)	0.052	0.063
	GA	170 (23.4%)	129 (29.3%)	Dominant	1.34 (1.03–1.75)	0.029	0.033
	AA	14 (1.92%)	8 (1.82%)	Recessive	0.62 (0.36–1.05)	0.92	1
rs1575464	TT	357 (49%)	217 (49.3%)	Additive	0.96 (0.80–1.15)	0.70	1
	TG	297 (40.7%)	180 (40.9%)	Dominant	0.71 (0.53–0.74)	0.017	0.015
	GG	74 (10.1%)	43 (9.77%)	Recessive	0.93(0.62–1.40)	0.75	0.58
Positive Skin Test for at least one allergen							
rs9595965	GG	599 (74%)	245 (68.4%)	Additive	1.37(1.01–1.85)	0.040	0.046
	GA	196 (24.2%)	103 (28.7%)	Dominant	1.77 (1.09–2.85)	0.019	0.021
	AA	12 (1.48%)	10 (2.79%)	Recessive	1.30 (0.98–1.71)	0.059	0.069
rs7335086	AA	702 (86%)	321 (89.6%)	Additive	0.52 (0.32–0.85)	0.009	0.009
	AG	102 (12.6%)	37 (10.3%)	Dominant	0.52 (0.32–0.87)	0.012	0.014
	GG	5 (0.61%)	0 (0%)	Recessive	NS	NS	NS
Positive Skin test for <i>Dermatophagoides pteronyssinus</i>							
rs9595965	GG	720 (73%)	124 (66.6%)	Additive	1.37 (1.01–1.85)	0.040	0.046
	GA	243 (24.8%)	56 (30.1%)	Dominant	1.37 (0.97–1.93)	0.068	0.062
	AA	16 (1.63%)	6 (3.22%)	Recessive	2.04 (0.78–5.33)	0.14	0.072
Positive Skin test for <i>Periplaneta americana</i>							
rs78149855	GG	870 (86%)	131 (79.8%)	Additive	1.48 (0.98–2.23)	0.059	0.081
	GA	130 (12.9%)	33 (20.1%)	Dominant	1.58 (1.03–2.42)	0.034	0.029
	AA	4 (0.39%)	0 (0%)	Recessive	NS	NS	NS
rs12875723	GG	373 (37%)	78 (47.5%)	Additive	0.80 (0.62–1.03)	0.086	0.10
	GA	490 (48.9%)	63 (38.4%)	Dominant	0.65 (0.46–0.91)	0.012	0.013
	AA	138 (13.7%)	23 (14%)	Recessive	1.04 (0.64–1.68)	0.86	1
Positive Skin test for <i>Blomia tropicalis</i>							
rs7335086	AA	783 (86%)	240 (92.3%)	Additive	0.52 (0.32–0.85)	0.009	0.009
	AG	119 (13.1%)	20 (7.69%)	Dominant	0.52 (0.32–0.87)	0.012	0.014
	GG	5 (0.55%)	0 (0%)	Recessive	NS	NS	NS

OR- odds ratio; Perm- permutational p value; statistical significance $p < 0.05$.

polymorphisms in genes receptors could influence the immune response against helminths.

Other SNVs in *CYSLTR2* has been associated in other studies with asthma and atopy. Thompson and collaborators [17] found a new genetic variant in *CYSLTR2* in an isolated population in the island of Tristan da Cunha (Met201Val) a missense polymorphism that altered the CysLT receptor and inactivated it causing high rates of atopy in this population [16]. The same variant was later associated with a decrease in LTD4 affinity to its receptor described in the functional part of a

genome wide association study with participants from several European countries and USA [30,21]. These findings confirm the importance of replicating studies of genetic polymorphisms in different populations.

In conclusion, genetic polymorphisms in *CYSLTR2* gene may play a role in the immunopathology of T2-type immediate hypersensitivity, especially considering the role of the leukotriene pathway in asthma, allergies and helminth infections. However, more studies are needed to verify the real functional impact of these SNVs on the immunopathology of atopic diseases as well as on the defense against

Table 5Significant associations between polymorphism in the *CYSLTR2* gene and helminth infection by logistic regression adjusted for sex, age and ancestry markers.

SNV	Genotype	Control	Case	Model	*OR (95% CI)	P value	Perm
<i>Trichuris trichiura</i> (egg/g)							
rs78149855	GG	853 (84%)	148 (92.5%)	Additive	0.41 (0.22–0.76)	0.005	0.003
	GA	151 (14.9%)	12 (7.5%)	Dominant	0.41 (0.22–0.76)	0.005	0.003
	AA	4 (0.39%)	0 (0%)	Recessive	NS	NS	NS
rs12875723	GG	379 (37%)	72 (45.2%)	Additive	0.83 (0.64–1.07)	0.15	0.12
	GA	490 (48.7%)	63 (39.6%)	Dominant	0.67 (0.47–0.95)	0.026	0.025
	AA	137 (13.6%)	24 (15%)	Recessive	1.08 (0.66–1.74)	0.75	0.77
rs11843756	TT	642 (63%)	89 (55.6%)	Additive	1.38 (1.04–1.84)	0.023	0.025
	TG	324 (32.1%)	62 (38.7%)	Dominant	1.49 (1.05–2.10)	0.023	0.022
	GG	42 (4.16%)	9 (5.62%)	Recessive	1.47 (0.69–3.13)	0.31	0.43
rs1359108	TT	335 (33%)	40 (25%)	Additive	1.08(0.85–1.38)	0.51	0.72
	TC	487 (48.3%)	98 (61.2%)	Dominant	1.56 (1.06–2.31)	0.023	0.028
	CC	186 (18.4%)	22 (13.7%)	Recessive	0.67 (0.41–1.1)	0.11	0.10
Helminth infection (<i>Trichuris trichiura</i> and <i>Ascaris lumbricoides</i>)							
rs9595950	TT	274 (30%)	94 (34.9%)	Additive	0.80 (0.65–0.97)	0.029	0.033
	TC	429 (47.7%)	134 (49.8%)	Dominant	0.82 (0.61–1.10)	0.068	0.20
	CC	195 (21.7%)	41 (15.2%)	Recessive	0.63 (0.43–0.92)	0.01	0.03
rs11843756	TT	870 (86%)	131 (79.8%)	Additive	1.40 (1.54–1.85)	0.02	0.022
	TG	130 (12.9%)	33 (20.1%)	Dominant	1.55 (1.14–2.13)	0.005	0.004
	GG	4 (0.39%)	0 (0%)	Recessive	1.17(0.61–2.25)	0.63	0.52
rs1359108	TT	373 (37%)	78 (47.5%)	Additive	0.80 (0.62–1.03)	0.086	0.10
	TC	490 (48.9%)	63 (38.4%)	Dominant	1.55 (1.14–2.13)	0.005	0.004
	CC	138 (13.7%)	23 (14%)	Recessive	1.04 (0.73–1.5)	0.80	1

OR- odds ratio; Perm- permutational p value; statistical significance $p < 0.05$.

helminths parasites, This could highlight a new pharmacological mechanism of blocking inflammation as a possible new strategy for the treatment of allergic diseases.

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