

927T > C polymorphism of the cysteinyl-leukotriene type-1 receptor (*CYSLTR1*) gene in children with asthma and atopic dermatitis

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Asthma and atopic dermatitis share several common features and Cysteinyl-leukotrienes are mediators that participate in the pathogenesis of both diseases. Recently, a new polymorphism (927T > C) has been identified in cysteinyl-leukotriene type-1 receptor (*CYSLTR1*) gene.

This gene is found on the X chromosome. The aim of this study was to analyze this SNP in a population of children with asthma and atopic dermatitis. In this study, 166 individuals, 79 adult controls (CTR) and 87 children with asthma (AA) were included. Forty-one patients with asthma presented atopic dermatitis (AA-AD). Adults were chosen as controls to confirm lack of development of asthma and allergy during childhood. Standardized history, physical examination, skin prick tests, and lung function measurements were performed in all patients. The 927T > C *CYSLTR1* SNP was analyzed by direct sequencing after PCR amplification. In males (53 individuals), the C allele was significantly more common among AA-AD patients (47%) than in CTR (8%) (Fisher's $p < 0.005$; Monte Carlo $p < 0.008$; OR: 9.78; 95%CI: 1.73–55.30). When comparing AA-AD vs. AA-NAD (patients with asthma but not atopic dermatitis), significant differences were observed, (47% vs. 15%, Fisher's $p = 0.014$; Monte Carlo $p = 0.022$; OR: 4.97; 95%CI: 1.29–19.13). No differences in allele distribution were observed between these disease sub-groups in females. The 927T > C is a silent SNP; however, it could affect transcription or translation or may be linked to an unidentified, functional polymorphism and thus may pre-dispose male children to asthma and atopic dermatitis in our population. Further studies are needed to confirm these findings.

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Asthma is a pulmonary disorder characterized by reversible airflow obstruction, airway hyper-responsiveness and cellular inflammation. Its prevalence ranges from 2% to 32% and constitutes the main cause of hospitalization among children (1). Atopic dermatitis is a chronic inflammatory skin disease characterized by inflammation, dry skin, intense itching, and a course marked by exacerbations and remissions. In developed countries, atopic dermatitis can affect more than 10% of children (2). Both disorders are frequently associated. Thus,

asthma develops in approximately 30% of children with atopic dermatitis (3) and this entity has been associated with a poorer prognosis of asthma (4).

Environmental and genetic factors play a significant role in the development of both asthma and atopic dermatitis. The familial aggregation of atopic dermatitis and asthma is well established. Recent studies have identified some common chromosomal linkages between asthma and atopic dermatitis in several chromosomal regions (5, 6).

Asthma and atopic dermatitis share several common features: an inflammatory response to environmental triggers, the activation of Th2 cells, an increased production of IgE and the participation of eosinophils (7). Cysteinyl-leukotrienes are mediators that participate in the pathogenesis of allergic inflammation in lung and skin. Thus, patients with atopic dermatitis have enhanced spontaneous and stimulated production of cysteinyl-leukotrienes from leukocytes (8). Leukotrienes exert their effects through two subtypes of cysteinyl-leukotriene receptors, *CYSLTR1* and *CYSLTR2* (9). Activation of *CYSLTR1* receptor by LTD₄ results in proliferation and contraction of smooth muscle, edema and eosinophil migration to the lung. Cysteinyl-leukotriene type-1 receptor (*CYSLTR1*) gene has been cloned and mapped to chromosome X (Xq13-21) (9). Recently, a new polymorphism (927T>C) has been identified in the coding region of the *CYSLTR1* gene (10). Considering the relationship between asthma and atopic dermatitis and the paucity of information about this polymorphism, we decided to analyze this SNP in a population of Spanish children with asthma and atopic dermatitis.

Materials and methods

Subjects

In this study, 166 unrelated Caucasian individuals were included. Eighty-seven children with asthma (AA) and 79 adult controls (CTR) from the outpatient Allergy Department of the University Hospital of Salamanca were recruited. The study was performed following the recommendations of the Ethical Committee of the University Hospital of Salamanca.

Asthmatic children were recruited consecutively in the outpatient clinic if they had specialist physician-diagnosed asthma with at least two symptoms consistent with asthma (cough, wheeze, and dyspnoea) in the last 12 months and three crisis of asthma in their clinical records in absence of other conflicting pulmonary disorders. In all patients, lung function was measured by spirometry according to ATS standards and severity of asthma was classified following the Spanish Consensus Guidelines. Children were at least 6 yr old to perform spirometry.

Skin prick tests were performed according to EAACI recommendations, with a battery of common aeroallergens previously described (11). Total serum IgE levels were measured by

a fluoroenzymeimmunoassay with Pharmacia Cap System® (Pharmacia, Uppsala, Sweden), following manufacturer's instructions.

Atopic dermatitis was defined according to the clinical diagnostic criteria for atopic dermatitis of the Consensus Conference of the American Academy of Dermatology.

Control subjects were selected if they met all of the following criteria: (i) no symptoms or history of asthma or other pulmonary diseases, (ii) no symptoms or history of allergy, (iii) no symptoms or history of atopic dermatitis, (iv) negative skin prick tests to the abovementioned battery of common aeroallergens (<1 mm wheal greater than saline), (v) absence of first degree relatives with a history of asthma or atopy. To confirm lack of development of asthma and allergy during childhood, we selected a population of adult controls.

Genotype analysis

DNA purification from total blood was performed according to previously described procedure (11). To amplify the genomic region surrounding the SNP these primers were used: 5'-AAATCATGTTTTGGTCTTGC-3' and 5'-ATTTTCATTGGTTTGGACTG-3'. The reaction mixtures were performed in 25 µl including 20 ng of genomic DNA, 1.25 pmol primers and 12.5 µl of PCR Master Mix (Promega, WI, USA). Cycling conditions included one cycle of 95°C for 5 min; 40 cycles at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min; and a extension period at 72°C for 10 min. Laboratory procedures were performed according to the European Molecular Genetics Quality Network best practice guidelines. All fragments were visualized in a 2% agarose gel. GENE CLEAN Turbo kit (Q-BIOgene, CA, USA) was used for the clean up of the PCR products. The amplicons were sequenced in a 3100 Genetic Analyzer (Applied Biosystems, CA, USA) and the resulting chromatograms were aligned and viewed with Chromas 2.3 (Technelysium. Pty. Ltd. 1998–2004, Ewanton, Australia). The Genbank accession number for the reference genomic sequence used for *CYSLTR1* alignments was AY242130.1. The analysis of the sequences was performed blindly with respect to case-control status.

Statistical analysis

The allele and genotype frequencies in patients were compared with control population, for case-control studies. Hardy-Weinberg

equilibrium was tested by a χ^2 test (Pearson's p-value). Allele frequencies were computed taking into account that males provide only one copy of the X chromosome. Bivariate analysis used analysis of variance to compare continuous outcomes across the levels of each genotype. The dichotomous variables were analyzed using χ^2 , Fisher's exact test, Monte Carlo simulation after 10^4 iterations and odds ratio test (95% Confidence Interval) for comparisons of the distribution of categorical variables. To model the effects of multiple covariates on the continuous and dichotomous outcomes, Logistic Regression was used. Age was included as potential covariate in multivariate analysis. A p-value of less than 0.05 was considered statistically significant. The statistical power and the false positive report probability (FPRP) were calculated for this study. To manage and analyze the data SPSS version 12 and SHEsis software platform were used (12).

Results

Phenotypic characteristics of our population are presented in Table 1. Since *CYSLTR1* gene is found on the X chromosome, results for males and females subjects were calculated separately. Genotype frequencies did not differ significantly from Hardy-Weinberg equilibrium in the female group. Genotype frequencies of 927T>C *CYSLTR1* polymorphism are shown in Tables 2 and 3.

Male group

In the group of male individuals, the C allele of 927T>C SNP (Fig. 1) was certainly more common in allergic asthma patients (AA) patients (0.25) than in controls (CTR) (0.08), Fisher's p-value = 0.08; Monte Carlo p-value (after 10^4 simulations) = 0.012; OR, 3.86; 95% CI, 0.79–18.75 (Table 2).

Table 1. Demographic characteristics of subjects

Characteristic	Controls	Asthmatics
No. of subjects	79	87
Age (yr) $\pm$ s.d.	40.9 $\pm$ 17.9	10.34 $\pm$ 2.6
Sex (No.)		
Male	24	53
Female	55	34
Log ₁₀ IgE		
Geometric mean	1.5	2.4
95% confidence interval	1.4–1.6	2.3–2.6

Table 2. Genotype frequencies of 927T>C *CYSLTR1* in male patients according to asthma and atopic dermatitis

927T>C	Males n = 77			
	CTR (n = 24)	Asthmatics (n = 53)	AA-AD (n = 17)	AA-NAD (n = 36)
T	0.92	0.76	0.53	0.85
C	0.08	0.24	0.47*	0.15

CTR, controls; AA-AD, allergic asthma with atopic dermatitis, AA-NAD, allergic asthma but not atopic dermatitis.

*Fisher's p < 0.005; Monte Carlo p < 0.008; OR, 9.78; 95% CI, 1.72–55.30 for the comparison of patients with AA-AD to CTR. Fisher's p < 0.014; Monte Carlo p = 0.022; OR, 4.96; 95% CI, 1.29–19.13 for the comparison of patients with AA-AD to AA-NAD.

Table 3. Genotype frequencies of 927T>C *CYSLTR1* in female patients according to asthma and atopic dermatitis

927T>C	Females n=89			
	CTR (n = 55)	Asthmatics (n = 34)	AA-AD (n = 25)	AA-NAD (n = 9)
TT	0.66	0.59	0.60	0.60
TC	0.27	0.35	0.36	0.40
CC	0.07	0.06	0.04	0.00

CTR, controls; AA AD, allergic asthma with atopic dermatitis. AA-NAD, allergic asthma but not atopic dermatitis.

When atopic dermatitis was considered, the C allele was clearly more frequent in the group of patients with allergic asthma who presented atopic dermatitis (AA-AD) (0.47) than in CTR (0.08); Fisher's p < 0.005; Monte Carlo p-value (after 10^4 simulations) < 0.008; OR, 9.78; 95% CI, 1.72–55.30. The statistical power considering our own population as reference was 70% for a significance less than 0.05. Since there are no previous data, if we consider this OR, the FPRP would be 50% for a pre-test probability of 1%.

No differences were observed in the group of patients with allergic asthma and but not atopic dermatitis (AA-NAD) (0.15) compared to the CTR group (0.08), Monte Carlo p-value 0.68 (Table 2).

When comparing AA-AD patients (0.47) vs. AA-NAD patients (0.15), a significant difference was also observed, Fisher's p = 0.014; Monte Carlo p = 0.022; OR, 4.97; 95% CI, 1.29–19.13.

Female group

In the group of female subjects, no significant differences were observed between CTR and AA or AA-AD patients (Table 3).

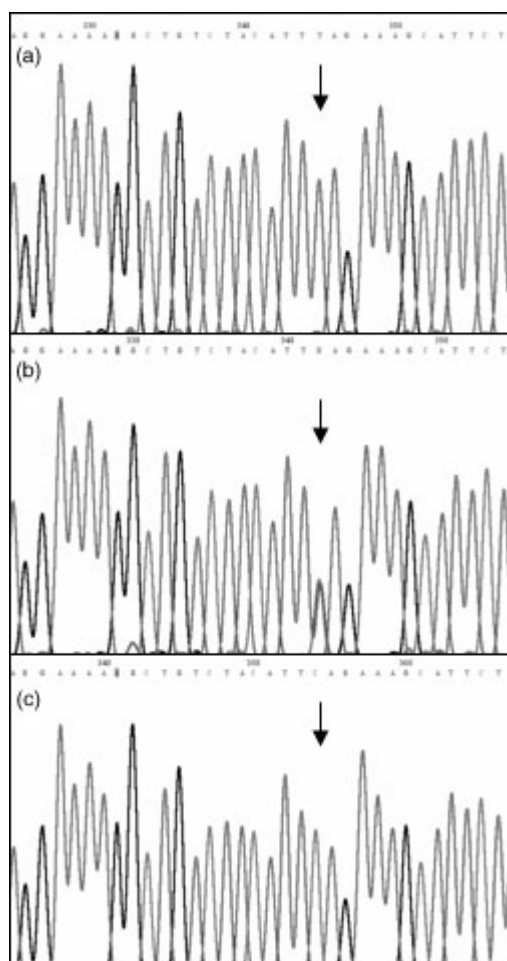


Fig. 1. Chromatogram sequences for the 927T>C polymorphism of *CYSLTR1*. (a) T/T genotype; (b) T/C genotype and (c) C/C genotype. Arrows indicate the polymorphic sites.

Discussion

In our population, the C allele of the 927T>C polymorphism was more frequent among male patients with allergic asthma and atopic dermatitis (47%) than in male controls (8%) and than in male patients with allergic asthma but not atopic dermatitis (15%). To the best of our knowledge, these findings have not been previously described. The distribution of the C allele did not differ between male patients with allergic asthma but not atopic dermatitis and male controls. Differences in females did not reach statistical significance. Allergic asthma and atopic dermatitis are clinical entities narrowly associated and there are clear epidemiological parallelisms. It has been demonstrated that leukotrienes participate in both asthma (13) and atopic dermatitis (14). This may partly explain why male patients with allergic asthma and atopic dermatitis could share a genetic background in some cases. In addition,

the treatment with antileukotrienes can improve both asthma and atopic dermatitis. Several studies have concluded that the leukotriene receptor antagonist montelukast is effective in pediatric patients with mild to moderate asthma and exercise and allergen induced asthma (15, 16). Capella et al. (17) concluded that montelukast is as effective as the comparison combined regimen to treat atopic dermatitis of adults; in this study more than 85% patients had rhinitis or asthma. It has also been reported that there is a significant alleviation of atopic dermatitis with the use of antileukotriene drugs (18–20), although recently Veien et al. did not confirm these results (21). Polymorphisms in *CYSLTR1* gene could modify the expression of this receptor and exert influence in the different response of these patients to antileukotriene therapy. Only a percentage of asthmatic patients have a significant clinical response to antileukotriene therapy in asthma (22). Furthermore, recent studies suggest that certain mutations in the promoter region of leukotriene synthesis pathway enzymes could contribute to the level of responsiveness. In this sense, polymorphisms of LTC4S appeared to be associated to the clinical response (23).

In our study, there is a clear difference between male and female patients. Gender influence has been previously reported in asthma, which is more common in boys than in girls during the early childhood. The prevalence equalizes between sexes during the adolescence and then switches to female predominance in adulthood (24). Gender aspects have also been reported in atopic dermatitis (2), however, the results of these studies are controversial. There are not genome-wide screens associating atopic dermatitis to X chromosome, only few studies report some genetic association. Thus, a relationship between atopic eczema and Xp11.23 has recently been established in patients with Wiskott–Aldrich syndrome (25) and other X-linked diseases such X ichthyosis and Hyper IgM syndrome have been related to atopy and eczema (26). *CYSLTR1* was mapped to X chromosome (Xq13-Xq21). 927C>T SNP has been firstly reported by Choi et al. (10), although no association was detected in a group of patients with aspirin induced asthma. In our population, there are differences in allele distribution related to gender. In this sense, male patients, who carried a single copy of C allele, have an increased risk (OR, 9.78; 95% CI, 1.72–55.30) of allergic asthma with atopic dermatitis. This increased risk is not observed in female patients, what could be directly related to the presence of another copy of the gene.

Although the 927C>T SNP is a synonymous change, it could affect the efficiency of transcription or translation. It also could be in tight linkage disequilibrium with another polymorphism in functionally important genomic elements of the *CYSLTR1* (10). This could be a promoter polymorphism able to alter the expression or turnover of the CysLT1 receptor on target tissues, or a coding polymorphism that may alter the binding affinity or efficacy of CysLT1 receptor ligands such as LTD4. If such SNP caused changes in CysLT1 protein structure it could modify the binding of CysLT1 receptor antagonists with implications for their therapeutic efficacy. These findings need to be also considered in the context of genetic variation reported in other enzymes and receptors of the cysteinyl-LT pathway, such as 5-LO, FLAP, LTC4S, and CysLT2 (10, 27). These have been linked to asthma and aspirin-intolerance (28, 29), although the results are still controversial; clinical responsiveness of asthmatics to antileukotriene therapy (22, 23); or predisposition to allergy in a Tristan da Cunha population (30).

Here, we present for the first time a polymorphism that seems to be associated with allergic asthma with atopic dermatitis that is clearly related to gender. Many factors may be influencing in positive results in genetic association studies, so results from these studies must be considered with caution. In this sense, stringent significant levels, and statistical quality control are required to assure reproducibility. This is a small study and its statistical power is limited, however, considering the OR detected, the FPRP seems to be enough to point out the trend of this association. Further studies are required to confirm these findings in order to enhance understanding of environmental, genetic and biological causes of these increasingly common conditions and to assess possible implications for severity, prevention, targeted early intervention and treatment of childhood atopic disease.

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References

1. ASHER MI, ANDERSON HR, STEWART AW, et al. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J* 1998; 12: 315–35.
2. WILLIAMS H, ROBERTSON C, STEWART A, et al. World-wide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. *J Allergy Clin Immunol* 1999; 103: 125–38.
3. LUOMA R, KOIVIKKO A, VIANDER M. Development of asthma, allergic rhinitis and atopic dermatitis by the age of five years. A prospective study of 543 newborns. *Allergy* 1983; 38: 339–46.
4. SPORIK R, HOLGATE ST, COGSWELL JJ. Natural history of asthma in childhood – a birth cohort study. *Arch Dis Child* 1991; 66: 1050–53.
5. HOWARD TD, MEYERS DA, BLEECKER ER. Mapping susceptibility genes for allergic diseases. *Chest* 2003; 123 (Suppl. 3): 363S–8S.
6. HOJAN S, EPPLER JT. The genetics of atopic dermatitis: recent findings and future options. *J Mol Med* 2005; 83: 682–92.
7. LEUNG DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000; 105: 860–76.
8. RUZICKA T, RING J. Enhanced releasability of prostaglandin E2 and leukotrienes B4 and C4 from leukocytes of patients with atopic eczema. *Acta Derm Venereol* 1987; 67: 469–75.
9. LYNCH KR, O'NEILL GP, LIU Q, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999; 399: 789–93.
10. CHOI JH, PARK HS, OH HB, et al. Leukotriene-related gene polymorphisms in ASA-intolerant asthma: an association with a haplotype of 5-lipoxygenase. *Hum Genet* 2004; 114: 337–44.
11. ISIDORO-GARCIA M, DAVILA I, LAFFOND E, MORENO E, LORENTE F, GONZALEZ-SARMIENTO R. Interleukin-4 (IL4) and Interleukin-4 receptor (IL4RA) polymorphisms in asthma: a case control study. *Clin Mol Allergy* 2005; 3: 15.
12. SHI YY, HE L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005; 15: 97–8.
13. HOLGATE ST, PETERS-GOLDEN M, PANETTIERI RA, HENDERSON WR Jr. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J Allergy Clin Immunol* 2003; 111 (Suppl. 1): S18–S34.
14. OYMAR K, AKSNES L. Increased levels of urinary leukotriene E4 in children with severe atopic eczema/dermatitis syndrome. *Allergy* 2005; 60: 86–9.
15. KNORR B, FRANCHI LM, BISGAARD H, et al. Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2 to 5 years. *Pediatrics* 2001; 108: E48.
16. STRAUB DA, MOELLER A, MINOCCHIERI S, et al. The effect of montelukast on lung function and exhaled nitric oxide in infants with early childhood asthma. *Eur Respir J* 2005; 25: 289–94.
17. CAPELLA GL, GRIGERIO E, ALTOMARE G. A randomized trial of leukotriene receptor antagonist montelukast in moderate-to-severe atopic dermatitis of adults. *Eur J Dermatol* 2001; 11: 209–13.
18. CARUCCI JA, WASHENIK K, WEINSTEIN A, SHUPACK J, COHEN DE. The leukotriene antagonist zafirlukast as a therapeutic agent for atopic dermatitis. *Arch Dermatol* 1998; 134: 785–6.

19. WOODMANSEE DP, SIMON RA. A pilot study examining the role of zileuton in atopic dermatitis. *Ann Allergy Asthma Immunol* 1999; 83: 548–52.
20. YANASE DJ, DAVID-BAJAR K. The leukotriene antagonist montelukast as a therapeutic agent for atopic dermatitis. *J Am Acad Dermatol* 2001; 44: 89–93.
21. VEIEN NK, BUSCH-SORENSEN M, STAUSBOL-GRON B. Montelukast treatment of moderate to severe atopic dermatitis in adults: a randomized, double-blind, placebo-controlled trial. *J Am Acad Dermatol* 2005; 53: 147–9.
22. MALMSTROM K, RODRIGUEZ-GOMEZ G, GUERRA J, et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group. *Ann Intern Med* 1999; 130: 487–95.
23. SAMPSON AP, SIDDIQUI S, BUCHANAN D, et al. Variant LTC(4) synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predicts clinical response to zafirlukast. *Thorax* 2000; 55 (Suppl. 2): S28–31.
24. BJORNSON CL, MITCHELL I. Gender differences in asthma in childhood and adolescence. *J Gend Specif Med* 2000; 3: 57–61.
25. BRADLEY M, SODERHALL C, WAHLGREN CF, LUTHMAN H, NORDENSKJOLD M, KOCKUM I. The Wiskott-Aldrich syndrome gene as a candidate gene for atopic dermatitis. *Acta Derm Venereol* 2001; 81: 340–2.
26. VIELUF D, KORTING HC, BELOHRADSKY BH. Eczematous skin lesions in X-linked immunodeficiency with hyper-IgM. *Curr Probl Dermatol* 1989; 18: 60–5.
27. FUKAI H, OGASAWARA Y, MIGITA O, et al. Association between a polymorphism in cysteinyl leukotriene receptor 2 on chromosome 13q14 and atopic asthma. *Pharmacogenetics* 2004; 14: 683–90.
28. SANAK M, PIERZCHALSKA M, BAZAN-SOCHA S, SZCZEKLIK A. Enhanced expression of the leukotriene C(4) synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000; 23: 290–6.
29. ISIDORO-GARCIA M, DAVILA I, MORENO E, LORENTE F, GONZALEZ-SARMIENTO R. Analysis of the leukotriene C4 synthase A-444C promoter polymorphism in a Spanish population. *J Allergy Clin Immunol* 2005; 115: 206–7.
30. THOMPSON MD, STORM VAN'S GK, GALCZENSKI H, et al. A cysteinyl leukotriene 2 receptor variant is associated with atopy in the population of Tristan da Cunha. *Pharmacogenetics* 2003; 13: 641–9.