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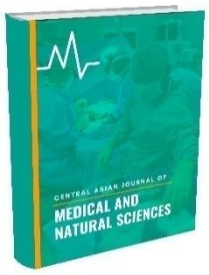

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Polymorphisms of *444A > C Leukotriene C4 Synthase (LTC4S)* in Asthmatic Iraqi Patients

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Abstract: The aim of this study was to investigate the association of the LTC4S rs730012 C/A polymorphism with asthma susceptibility in Iraqi patients. Forty-five asthmatic patients and 35 healthy controls were genotyped for the rs730012 C/A gene polymorphism. The distribution frequencies of genotypes and alleles of the rs730012 gene were in Hardy-Weinberg equilibrium in both patients and control groups. The most common genotype in both control and asthma patients was the heterozygous genotype CA with a percentage of 80% and 57.78% respectively. The genotype CC was higher in the asthmatic group (40%) compared to the control group (11.43%). In contrast, genotype AA, the less predominant genotype, was less in the asthmatic group were less in asthmatic group (2.22%) compared control group (8.57%). The C allele was more predominant compared to the A allele with percentages of 51.43% and 68.89% for C, 48.57% and 31.11% for A in the control and patient groups respectively with significant differences (p =0.02451). The association analysis displayed that the individuals carrying the homozygous CC genotype were more likely to have a significantly increased risk of asthma with OR=5.1667 (Cl95%1.5562 to 17.1541) (p= 0.0073). The heterozygous CA and homozygous AA genotypes decrease the association with asthma OR=0.3421 (CI95%0.1236 to 0.9467) and OR=0.2424 (CI95%0.0241 to 2.4388) respectively. However, the difference between the control and asthma groups of the CA genotype was significant (p=0.0389) but not for the AA genotype (P=0.2289). These results suggested that the C allele might play a risk factor for asthma whereas the A allele might consider a protective role against asthma.

The subgroup analysis revealed that the asthma risk of females with LTC4S CC genotype was 13.33 times higher than that in controls OR=13.3333 (CI95% 1.4957 to 118.8614), with significant

difference (p=0.0203) between females of control and asthmatic group. CA genotype decreases the probability of contracting the disease significantly with OR=0.2143 (CI 95% 0.0478 to 0.9615), p=0.0443. Similarly, the AA genotype decreases the probability of contracting the disease with OR=0.3571 (CI 95%0.0296 to 4.3088), p=0.4177. Among males, of the LTC4S CC genotype increased asthmatic risk among patients males OR=2.6667 (CI0.5905 to 12.0427) p=0.2023. The homozygous CA genotype decreases the association with the disease OR=0.5357 (CI0.1316 to 2.1811) p=0.3836. Similarly, the homozygous AA genotype decreases the association with the disease OR=0.2482 (CI0.0095 to 6.4659) p=0.4022.

In conclusion, the polymorphism of LTC4S rs730012 is associated with susceptibility to asthma in Iraqi patients.

Introduction

Asthma, commonly referred to as bronchial asthma, is a chronic lung inflammatory disease (1). Over 300 million individuals worldwide suffer from asthma, a widespread and diverse non-communicable illness (2). For early detection and preventative care, prognostic indicators to identify high-risk people are critically needed. Genetic susceptibility to asthma is one of the key study concerns in the scientific community (3). Several single nucleotide polymorphisms (SNPs) in these genes have been identified to be associated to asthma risk in various populations as a result of the recent decade's effort on understanding the susceptibility genes for asthma (4, 5). Gene-environment interactions influence the initiation and severity of asthma propensity. Asthma has a genetic component to its pathogenesis, and between 36% and 77% of cases are heritable. The pathophysiology of asthma and other disorders is thought to be influenced by more than 100 genes (6).

Cysteine leukotrienes (Cys-LTs) are significant mediators of eosinophilia and airway constriction in bronchial asthma (7). They are produced by the 5-lipoxygenase pathway, which uses a variety of different enzymes. Cytosolic phospholipase A2, 5-lipoxygenase (5-LO), and 5-lipoxygenase activating protein (FLAP) govern the release of arachidonic acid from membrane phospholipids, which is then converted into the unstable intermediate LTA4. The terminal enzyme LTC4 synthase combines LTA4 and glutathione to produce LTC4 (8, 9). After carrier-mediated export (10, 11), glutamic acid and glycine are successively released from the glutathione moiety of LTC4 to create LTD4 and LTE4 (12, 13). The pace at which cysteinyl LTs are synthesized is constrained by the enzyme *LTC4S*. Additionally, it has been shown that blood eosinophils from asthmatic patients express more *LTC4S* mRNA than do those from control individuals. (14).

The biological effects of Cys-LTs are most likely brought about by their interaction with the *CYSLTR1 and CYSLTR2* receptors on target cells (15). The activation of *CYSLTR1* by LTD4 results in the growth and contraction of smooth muscle, oedema, and the migration of eosinophils to the lung (16, 17). The involvement of the cys-LT receptors in bronchial asthma has been shown by the therapeutic effectiveness of biosynthesis inhibitors and targeted *CYSLTR1* blockers (18).

The present study was designed to analyse the polymorphism of *444A > C Leukotriene C4 Synthase*, and to determine whether there is an association between this polymorphism and the asthma phenotype in an Iraqi population from Wasit province.

Materials and Methods

This study is a case –control study, The participants included 45 confirmed asthmatic patient (23 males and 22 females) their age 19 to 70 years (mean ± standard deviation: 40 ± 12.51 years, median= 40 years). The control group comprised of 35 healthy individuals (18 males and 17 females), their age 18-71 years (mean ± standard deviation: 32.38 ± 13.68 years, median=28 years). Data collection

encompassed a range of factors, including demographic details, medical history, and sample collection date, gathered from participants who met global diagnostic criteria. Samples were collected from Alzahraa Teaching Hospital, Chest Diseases Centre, and the blood bank in Kut, Iraq. For blood sample collection, 3 ml of blood was obtained via vein puncture from each participant, with the collected blood then transferred to sterile ethylenediaminetetraacetic acid –k3 (EDTA) tubes, labelled, and stored at -20°C for subsequent DNA extraction and genotyping.

Genomic DNA Extraction:

Genomic DNA was extracted from whole blood utilizing the Quick-DNA™ Blood MiniPrep kit (Zymo, USA) Catalogue Nos. D3024 & D3025. The quality of the extracted genomic DNA was assessed via Nanodrop, measuring the A260/A280 absorbance ratio within the range of 1.8 to 2.0, indicative of high quality.

Agarose Gel Electrophoresis:

A 1.5% agarose gel was prepared according to established protocols. The gel, containing DNA samples, was subjected to electrophoresis for detection and sizing of DNA fragments.

SNPs Genotyping:

The TaqMan custom SNP genotyping assay from Thermo Fisher Scientific was utilized for genotyping the SNP *444A > C Leukotriene C4 Synthase (LTC4S)* gene. Real-time PCR was employed for the allele-specific discriminating approach. The reference and alternative alleles for *rs730012* were referred to from NCBI.

Statistical Analysis:

The data analysis was carried out using SPSS 21.0 software. The significance level (*P*-value) was categorized as follows: Sig. denoting Significant (P<0.05), and NS representing non-Significant. Analysis of variance (ANOVA) was employed to assess group differences.

Results

Forty-five asthmatic patients (23 males and 22 females) and 35 healthy controls (18 males and 17 females) were genotyped for of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* gene polymorphism. In both patients and control groups, the distribution frequencies of genotypes and alleles of the *rs730012* C/A gene were the Hardy-Weinberg equilibrium (P < 0.05) indicating that the gene frequencies did not reach the genetic equilibrium (Table 1). The allele and genotype frequencies of *rs730012* C/A gene polymorphisms were used to estimate the odds ratio (OR), confidence intervals (95% CIs), χ 2, and p-value. The results showed that the most common genotype in both control and asthma patients was the heterozygous genotype CA with a percentage of 80% and 57.78% respectively. The genotype CC was higher in the asthmatic group (40%) compared to the control group (11.43%). In contrast, genotype AA, the less predominant genotype, was less in the asthmatic group (2.22%) compared control group (8.57%). The C allele was more predominant compared to the A allele with percentages of 51.43% and 68.89% for C, 48.57% and 31.11% for A in the control and patient groups respectively with significant differences (p =0.02451).

Table 1: Distribution of genotypes and allele frequency of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* in asthmatic patients and control

Groups	Genotype no. (%)			Allele frequency no. (%)	
	CC	CA	AA	C	A
Control	4(11.43)	28(80.00)	3(8.57)	36(51.43)	34(48.57)
Patients	18(40.00)	26(57.78)	1(2.22)	62(68.89)	28(31.11)

Chi-square	8.8718	5.058	
P-value	0.011844	0.02451	
Significance	*		

*p<0.05

Susceptibility analysis of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* polymorphism with asthma

Table (2) shows the association of each genotype of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* with susceptibility to asthma. This further analysis showed that the individuals carrying the homozygous CC genotype were more likely to have a significantly increased risk of asthma with OR=5.1667 (Cl95%1.5562 to 17.1541) (p= 0.0073). The heterozygous CA and homozygous AA genotypes decrease the association with asthma OR=0.3421 (CI95%0.1236 to 0.9467) and OR=0.2424 (CI95%0.0241 to 2.4388) respectively. However, the difference between the control and asthma groups of the CA genotype was significant (p=0.0389) but not for the AA genotype (P=0.2289). These results suggested that the C allele might play a risk factor for asthma whereas the A allele might consider a protective role against asthma.

Table 2: Odds ratio of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* in asthmatic patients and control

Genotypes	Control no. (%)	Patients no. (%)	OR	OR95%CI	*P* value	Statistical significance
CC	4(11.43)	18(40.00)	5.1667	1.5562 to 17.1541	0.0073	*
CA	28(80.00)	26(57.78)	0.3421	0.1236 to 0.9467	0.0389	*
AA	3(8.57)	1(2.22)	0.2424	0.0241 to 2.4388	0.2289	Ns.

*p<0.05, Ns. Non-significant (P>0.05)

The odds ratio among females of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* in studied groups

Association analysis showed that the asthma risk of females with *LTC4S* CC genotype was 13.33 times higher than that in controls OR=13.3333 (CI95% 1.4957 to 118.8614), with significant difference (p=0.0203) between females of control and asthmatic group, table (3). CA genotype decreases the probability of contracting the disease significantly with OR=0.2143 (CI 95% 0.0478 to 0.9615), p=0.0443. Similarly, the AA genotype decreases the probability of contracting the disease with OR=0.3571 (CI 95%0.0296 to 4.3088), p=0.4177

Table 3: Odds ratio of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* in females asthmatic patients and control

Genotypes	Control no. (%)	Patients no. (%)	OR	OR95%CI	*P* value	Statistical significance
CC	1(5.88)	10(45.45)	13.3333	1.4957 to 118.8614	0.0203	*
CA	14(82.35)	11(50.00)	0.2143	0.0478 to 0.9615	0.0443	*
AA	2(11.76)	1(4.55)	0.3571	0.0296 to 4.3088	0.4177	Ns.

*p<0.05, Ns. Non-significant (P>0.05)

The odds ratio among males of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* in studied groups

The association analysis showed that the polymorphism *rs730012* C/A of the *LTC4S* CC genotype increased asthmatic risk among patients males OR=2.6667 (CI0.5905 to 12.0427) p=0.2023. The

homozygous CA genotype decreases the association with the disease OR=0.5357 (CI0.1316 to 2.1811) p=0.3836. Similarly, the homozygous AA genotype decreases the association with the disease OR=0.2482 (CI0.0095 to 6.4659) p=0.4022 (Table 4).

Table 4: Odds ratio among males of *444A > C Leukotriene C4 Synthase (LTC4S) rs730012* in studied groups

Genotypes	Control no. (%)	Patients no. (%)	OR	OR95%CI	*P* value	Statistical significance
CC	3(16.66)	8(34.78)	2.6667	0.5905 to 12.0427	0.2023	Ns.
CA	14(77.77)	15(65.22)	0.5357	0.1316 to 2.1811	0.3836	Ns.
AA	1(5.55)	0(0.00)	0.2482	0.0095 to 6.4659	0.4022	Ns.

Ns. Non-significant(P>0.05)

Discussion

One of the syntropic genes of allergy disorders is the *LTC4S* gene. The *LTC4S* gene is a functional member of the network of allergic inflammatory genes through a syntropic impact (19). In a previous study of Iraqi asthmatic patients from Wasit province, Jiad and Ahmed ,2022 found that cell free nuclear DNA and cell free mitochondrial DNAcould be significant biomarkers for asthma (20).

The results showed that the most common genotype in both control and asthma patients was CA. The genotype CC was higher in the asthmatic group than in to control group. In contrast, genotype AA, the less predominant genotype, was less in the asthmatic group. The C allele was more predominant compared to the A allele. The results revealed that the individuals carrying the homozygous CC genotype were more likely to have a significantly increased risk of asthma. The association of 444 A > C *LTC4S* CC genotype with asthma was also evident in females and males, and it was more evident in females than in males.

These findings support the hypothesis that the allele C tended to be more prevalent in the sick population as compared to controls. These findings don't line up with Berghea *et al*., 2015 (21) and Kmyta et al., 2018 (22). The findings of the present investigation with statistical significance point to a potential function of this SNP in the development of asthma in Iraqi patients. It is unclear how *LTC4S* gene polymorphisms contribute to asthma pathophysiology, and the findings are debatable. A meta-analysis looking at the relationship between the -444A/C polymorphism of the *LTC4S* gene and asthma risk, which included 3042 cases and 1902 controls from 13 case-control studies, revealed a significant association for Caucasians but not for Asians or African-Americans, highlighting the significance of the genetic background for this association (23).

There is a lot of data to support the idea that the amount of cysteinyl-leukotrienes in sputum has grown (24, 25), lung lining fluid (26), blood, and urine (27), comparing asthmatics to healthy people, linking these substances to the pathogenesis of bronchial asthma. Asthma that is resistant to aspirin is connected with the (444)A>C SNP in the *LTC4S* gene (28). Due to the creation of a putative H4TF-2 transcription factor binding site brought on by the sequence change in the (444)C promoter region, the production of the inflammatory molecules *LTC4*, LTD4, and LTE4 is elevated (29). Aspirin-intolerant asthmatics compared to aspirin-tolerant asthmatics or when compared to healthy persons have been shown to have increased bronchial expression of LTC4S, which causes substantial overproduction of cysteinyl-leukotrienes and concomitant bronchoconstriction (30). Thus, it has been proven that the *LTC4S* (444)C variant has a role in the inflammatory process of asthma, probably by increased production of inflammatory leukotrienes.

Furthermore. The *LTC4S* gene is situated on chromosome 5's long arm, which also contains several genes for cytokines, growth factors, and receptors related to the asthmatic phenotype. These comprise

the cytokines granulocyte-macrophage colony-stimulating factors, IL-3, IL-4, and IL-5, all of which are crucial in allergic inflammation (31). *LTC4S* and Th2-type cytokines are related in terms of function. As a result, it was discovered that human mast cells produce *LTC4S* mRNA and protein when exposed to IL-4 (32), increasing the amount of enzyme activity available for cysLT production. The production of cysLT by mast cells through FcERI is significantly increased when either IL-3 or IL-5 are combined with IL-4 (33).

Conclusion

The present study provides an evidence that single nucleotide polymorphism *rs730012* in *leukotriene C4 synthase* is associated with asthma in an Iraqi population and that this effect is comparable in males and females. This Polymorphism is important risk factors for asthma and can be useful in understanding the pathways of asthma susceptibility. The genotype CC of 444A > *LTC4S rs730012* significantly increases the risk of asthma and the C allele is a risk factor in asthma.

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