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Association of Rs7217186 Polymorphism of Arachidonic Acid 15-Lipoxygenase (ALOX15) Gene with Susceptibility to Allergic Rhinitis

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Abstract

Background: Allergic rhinitis (AR) is an inflammatory disorder of the nasal mucosa, caused by exposure to environmental allergens. It is known that 15-lipoxygenase (15-LOX) is involved in the biosynthetic pathways of anti-inflammatory lipid mediators, including resolvins and protectins.

Methods: In this study, which was performed on 130 AR patients and 130 healthy controls, we aimed to investigate the association of susceptibility to AR with two selected single-nucleotide polymorphisms (SNPs), that is, rs2619112:A>G and rs7217186:C>T, in the intron regions of arachidonic acid 15-LOX (*ALOX15*) gene, using SNPinfo and Regulome DB tools.

Results: The results showed that the CT genotype of rs7217186: C>T was significantly associated with the increased risk of AR compared to the CC genotype (*P*= 0.037, OR=1.943, CI: 1.038-0.638). However, there was no strong evidence of the association of rs2619112: A>G with susceptibility to AR (*P*> 0.05).

Conclusions: The present results indicated that rs7217186 polymorphism of *ALOX15* gene might be a potential biomarker for susceptibility to AR.

Keywords: Allergic rhinitis, Arachidonate 15-lipoxygenase, Immunoresolvents, Single-nucleotide polymorphism, Specialized proresolving lipid mediators (SPMs).

Introduction

Allergic rhinitis (AR) is a chronic inflammatory disorder of the nasal mucosa, characterized by sneezing, nasal congestion, nasal itching, and rhinorrhea (1-4). Acute inflammation is an immediate physiological response of the body to tissue and cell damage, caused by pathogens and harmful agents (5, 6). Many pro-inflammatory mediators, such as prostaglandins, leukotrienes, amines, cytokines, and chemokines, are involved in the inflammatory process (6-8). The resolution of inflammation, also known as catabasis, is necessary for return to tissue homeostasis and prevention of acute inflammation progression to chronic inflammation (5).

Specialized proresolving lipid mediators (SPMs) or immunoresolvents, including resolvins, protectins, lipoxins, and maresins, are omega-6 or omega-3 fatty acid-derived metabolites produced by the cyclooxygenase and lipoxygenase pathways, with anti-inflammatory and pro-resolving effects (9-12). Immunoresolvents play anti-inflammatory and pro-resolving roles through several mechanisms, such as inhibition of infiltration and migration of polymorphonuclear neutrophils (PMNs),

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stimulation of the apoptosis of exhausted PMN cells, efferocytosis by macrophages, and counter-regulation of cytokines and chemokines. Moreover, they can lead to the induction of macrophage phenotype switching from classic macrophages (M1) to alternative ones (M2), which can ultimately cause tissue regeneration, healing, and homeostasis (13).

Arachidonate 15-lipoxygenase (ALOX15) is a member of the lipid peroxidizing enzyme family, involved in the biosynthetic pathway of some immunoresolvents. Arachidonic acid (AA)-derived lipoxin A4 and B4 are locally produced at inflammatory sites by LOX-mediated pathways (e.g., 5, 15, and 12 lipoxygenases) (14). Resolvin E3 is directly produced by 18R-hydroxyeicosapentaenoic acid (18R-HEPE) through the enzymatic activity of 15-LOX, expressed by eosinophils (15). In eosinophils, docosahexaenoic acid (DHA) is converted to intermediate 16,17-epoxy-protectin by 15-LOX and then, to resolvin D (RvD) through enzymatic hydrolysis (16). Human 15-LOX is encoded by the *ALOX15* gene, located on the short arm of chromosome 17 in the *LOX* gene cluster (17). Several studies on single-nucleotide polymorphisms (SNPs) have confirmed the association of *ALOX15* SNPs with various inflammatory disorders, including coronary artery disease. However, no research has yet investigated the polymorphisms of *ALOX15* gene in AR. This case-control study aimed to assess the association of rs2619112 and rs7217186 SNPs in the intron regions with the risk of AR.

Materials and Methods

Selection of SNPs

According to the NCBI database (http://www.ncbi.nlm.nih.gov/SNP/), *ALOX15* gene (NC_000017.11), with more than 3,000 known SNPs in coding and non-coding regions, is located on the short arm of chromosome 17. In this study, all SNPs of *ALOX15* gene with a minor allele frequency (MAF)> 0.05 were confirmed in the HapMap Project, 1000 Genomes Project, and multiple independent submissions. Next, the genotype data were analyzed with bioinformatics software tools, including SIFT, PolyPhen, PROVEAN, SNPGO, MutPred, I-Mutant, SNPinfo, Regulome DB, I-TASSER, Mutation 3D Tool, and Hope Project Tool. The analysis of intron and exon regions indicated 55 SNPs with (MAF)> 0.01 in the intron regions and three SNPs with MAF> 0.01 in the 3'UTR region of *ALOX15* gene as potentially high-risk SNPs. Finally, rs7217186 and rs2619112 polymorphisms were selected by analyzing the intron regions, using the SNPinfo and Regulome DB tools for further gene-associated studies.

Subjects

This case-control study was performed on 260 individuals in Kermanshah Province, Iran. A total of 130 patients with AR were diagnosed by an allergist, according to the clinical features and diagnostic criteria outlined in the Practical Guideline for Management of Allergic Rhinitis (18). Also, 130 healthy individuals without allergic diseases or any other infectious or inflammatory disorders were recruited as the control group; the two groups were matched in terms of sex and age. This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences (permission code: IR.KUMS.REC.1399.785). Written consent was obtained from all the patients, as well as the controls.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes of all participants, using the salting-out method (19). The quality and concentration of extracted DNA were determined using a NanoDrop spectrophotometer (NanoDrop 2000 UV-Vis Spectrophotometer, Thermo Scientific, USA), and samples were stored at - 20 °C until further use. A polymerase chain reaction (PCR) assay was performed using specific primers for *ALOX15* gene variants (rs2619112 and rs7217186) (Table 1) in a total volume of 20 µL, containing 10 µL of

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Master Mix, 1 µL of the designed primers, 0.5 µL of DNA, and 7.5 µL of sterile distilled water. The cycling conditions in a thermal cycler (T100 Thermal Cycler, Bio-Rad, USA) consisted of initial denaturation at 95 °C for three minutes, followed by 34 cycles of denaturation at 95 °C for 30 seconds, annealing at 61 °C for 20 seconds, extension at 72 °C for one minute, and final extension at 72 °C for two minutes.

Table 1. Primer sequences for *ALOX15* gene polymorphisms.

Gene	SNP	Primer	Nucleotide sequence	Amplicon size
ALOX15	Rs2619112	F	GCAGGGCTATAACCACGAAGGG	730 bp
		R	ACCAGGTTTGCCACTTTGTCACC	
	Rs7217186	F	GGGCAGAGATAGTGGCAGGCAAGAG	428 bp
		R	AGCGGGCAGGAAGGGGAGG	

The genotypes of rs2619112 and rs7217186 polymorphisms were determined by the restriction fragment length polymorphism (RFLP) method using restriction enzymes AlwNI and MbiI (Fermentase, Thermofisher Scientific, USA) for 16 hours at 37 °C, respectively. After incubation, the digested samples were resolved on 1% agarose gel. In case of *ALOX15* rs2619112:A>G, the 730-bp AA homozygote was resistant to the restriction enzyme, while the AG heterozygote produced three fragments (730, 563, and 167 bp), and the CC homozygote yielded two fragments (563 and 167 bp). In case of *ALOX15* rs7217186 C>T, the CC homozygote produced two fragments (388 and 40 bp), the CT heterozygote was digested in three fragments (428, 388, and 40 bp), and 428-bp TT homozygotes were resistant to the restriction enzyme.

Statistical analysis

Statistical analysis was performed in SPSS Version 24. The genotype and allele distributions were analyzed in the groups using Chi-square test. Comparison of numerical values between the groups was performed using Kruskal-Wallis and Mann-Whitney tests. A logistic regression model was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs). Moreover, the genotypic distribution of the healthy controls and AR patients was assessed based on the Hardy–Weinberg equilibrium (HWE) principle. Additionally, the online SHEsis software was used to calculate the haplotype frequency and linkage disequilibrium (LD) between genetically modified polymorphisms. A *P*-value less than 0.05 was considered statistically significant.

Results

A total of 130 patients with AR (60 males and 70 females; age: 35.81±10.75 years) and 130 healthy individuals as the control group (60 males and 70 females; age: 35.92±10.45 years) were recruited in this study.

Association of ALOX15 SNPs (rs7217186 and rs2619112) with AR susceptibility

The distribution of genotype and allele frequencies for two polymorphisms of *ALOX15* gene (rs7217186 and rs2619112) between AR patients and healthy controls is presented in Table 2.

The distribution of rs7217186 and rs2619112 genotypes in the patient and control groups was in accordance with the HWE principle. In case of rs7217186: C>T, the frequency of TT and CC genotypes was higher in the control group compared to the patient group, while the frequency of CT genotype was higher in the patient group, and the difference was significant (*P*=0.037). Additionally, the frequency of T mutant allele was higher in the patient group compared to the control group, while the frequency of C allele was higher in the control

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group; however, the difference was not significant (*P*= 0.292).

Regarding rs2619112:A>G, the frequency of G allele and GG and AG genotypes was higher in the patient group compared to the control group, while the frequency of A allele and AA genotype was higher in the control group compared to the patient group; however, the differences were not significant (*P*> 0.05). The images of the electrophoresis pattern of the RFLP-PCR product and the sequencing results of rs7217186 and rs2619112 polymorphisms are presented in Figures 1 and 2, respectively.

Table 2. Frequency distribution of genotypes and related alleles in AR patients and control individuals.

SNPs	Patients (n/%)	Controls (n/%)	P-value	OR (95% CI)
Rs7217186				
Allele frequency				
C	118 (47.5%)	130 (52.4%)	0.292	1.203 (0.853–1.698)
T	142 (52.2%)	130 (47.7%)		
CC	23 (17.7%)	36 (27.7%)	0.037*	1.943 (1.038–3.638)
CT	72 (55.4%)	58 (44.6%)		
TT	35 (26.9%)	36 (27.7%)	0.239	1.522 (0.756–3.065)
Rs2619112				
Allele frequency				
A	128 (49.2%)	142 (54.6%)	0.219	1.241 (0.879–1.752)
G	132 (50.8%)	118 (45.4%)		
Genotype frequency				
AA	31 (23.8%)	41 (31.5%)		
AG	66 (50.8%)	60 (46.2%)	0.207	1.455 (0.812–2.606)
GG	33 (25.4%)	29 (22.3%)	0.240	1.505 (0.760–2.980)

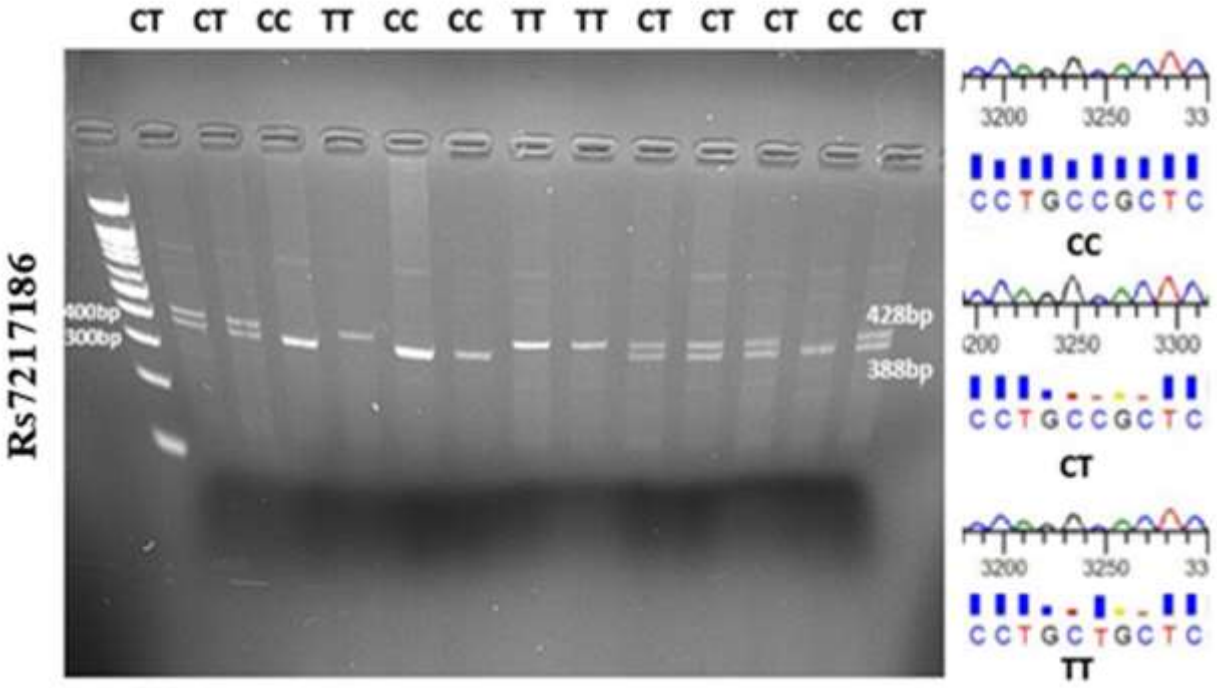

Fig. 1. Restriction digestion (MbiI) products of *ALOX15* rs7217186 polymorphism on 1% agarose gel: wild CC genotype (388 bp), heterozygous CT genotype (428 bp, 388 bp, and 40 bp), mutant TT genotype (428 and 40 bp), and their corresponding sequencing results.

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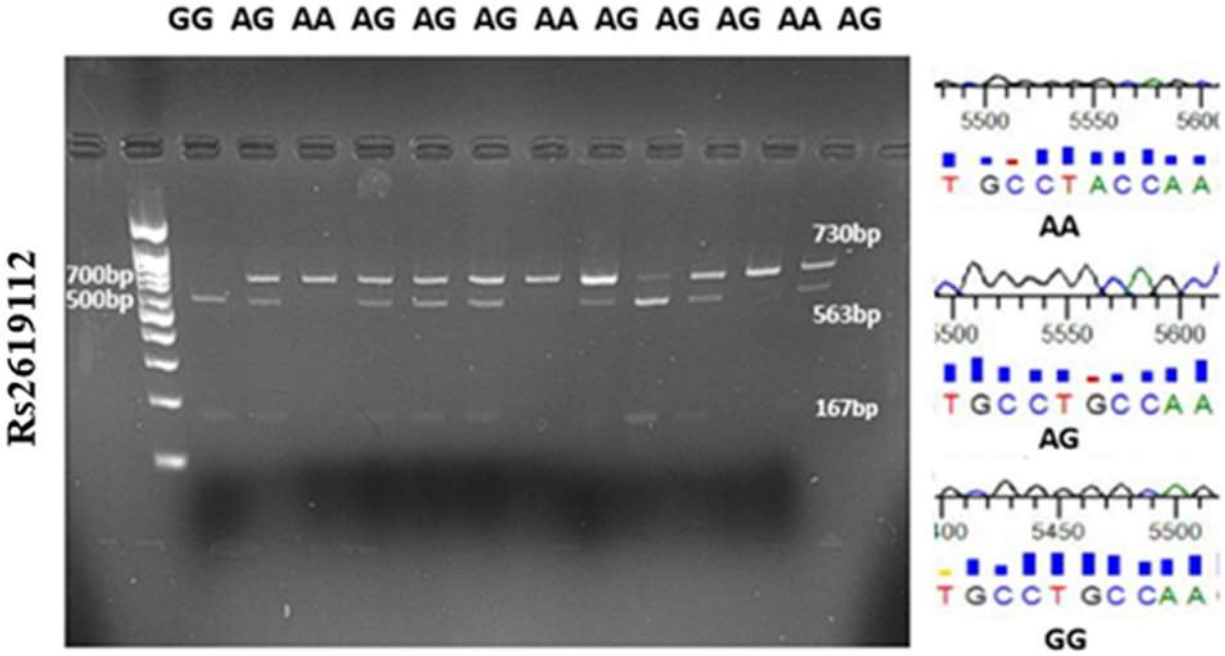

Fig. 2. Restriction digestion (AlwNI) products of *ALOX15* rs2619112 polymorphism on 1% agarose gel: wild AA genotype (730 bp), heterozygous AG genotype (730, 563, and 167 bp), mutant GG genotype (563 and 167 bp), and their corresponding sequencing results.

Haplotype frequency

The haplotype frequencies in the AR patients and healthy controls are shown in Table 3, indicating no significant relationship between haplotypes.

Table 3. Haplotype distribution of *ALOX15* gene SNPs in AR patients and control individuals.

Haplotypes	Case (frequency)	Control (frequency)	χ^2	Fisher's P-value	OR [95% CI]
AC	103.42 (0.398)	109.76 (0.422)	0.319	0.571941	0.904 [0.637~1.283]
AT	24.58 (0.095)	32.24 (0.124)	1.160	0.281593	0.738 [0.423~1.286]
GC	14.58 (0.056)	20.24 (0.078)	0.986	0.320654	0.704 [0.351~1.412]
GT	117.42 (0.452)	97.76 (0.376)	3.064	0.080084	1.367 [0.963~1.940]

Discussion

In this study, we investigated the association of rs7217186 and rs2619112 variants of *ALOX15* gene with the risk of AR. The present results revealed that rs7217186 CT genotype was significantly associated with an increased risk of AR compared to the CC genotype (*P*=0.037, OR=1.943, 95% CI: 1.038-3.638). In contrast, no significant association was found between rs2619112 polymorphism and AR. To the best of our knowledge, no research has yet investigated the association of rs2619112 and rs7217186 polymorphisms with AR, although the association of these SNPs with other inflammatory conditions has been explored. In this regard, Zhang et al. conducted a study to investigate the association of rs7217186:T>C and rs2619112:G>A polymorphisms of *ALOX15* gene with the risk of coronary artery disease (CAD) in a Chinese Han population (519 CAD patients and 608 healthy controls). They observed that the CC and CT genotypes

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of rs7217186:T>C were associated with a higher risk of CAD. In case of rs2619112:G>A, carrying the A allele was associated with a greater risk of CAD compared to GG homozygotes. Consistent with our results, they found an association between rs7217186 SNP and CAD in the Chinese Han population (20).

In another study by Zhao, the association of rs7217186 and rs2619112 SNPs of *ALOX15* gene with ischemic stroke was investigated in the Northern Chinese Han Population. They found that the frequencies of CC genotype and C allele of rs7217186 SNP and AA genotype and A allele of rs2619112 SNP were higher in patients with ischemic stroke compared to the control group (21). Moreover, in the north Indian population, Kaur et al. reported that GA and AA genotypes of *ALOX15* rs2619112 variant and CT and TT genotypes of *ALOX15* rs7217186 were associated with a significantly higher risk of CAD (22).

Additionally, KE et al. studied the association of five SNPs (rs9894225, rs748694, rs2619112, rs2619118, and rs916055) in *ALOX15* gene with obesity-related phenotypes in 1,296 cases from 427 Chinese nuclear families with male offspring, using quantitative transmission-disequilibrium test (TDT). They found that rs916055 SNP had a significant association with the percentage of fat mass in Chinese nuclear families who had male offspring (23). Moreover, Herlin et al. investigated the association between *ALOX15* variants (rs8074545, rs748694, rs9894225, rs916055, and rs2619112) with osteoporosis-associated phenotypes and incident fractures, including fractures of the hip, distal radius, vertebra, shoulders, pelvis, and proximal tibia, in young and elderly Swedish women. They reported that *ALOX15* rs2619112 SNP was associated with C-reactive protein (CRP) levels (P=0.004) and incident fractures of any type (P= 0.014) in elderly women. Besides, they showed that young women carrying common T alleles (*ALOX* 15 rs748694) had lower quantitative ultrasound values (P=0.002-0.006) (24).

In another study, Cheung et al. investigated the association of 10 *ALOX15* SNPs with the bone marrow density of premenopausal (n=225) and postmenopausal (n=401) Chinese women. Their findings showed that G allele in rs2619112 variant was related to a high bone mineral density (BMD) in the femoral neck of pre-menopausal women (OR=0.442, P=0.007); meanwhile, it was associated with an increased risk of lower BMD in postmenopausal women (OR=1.727, P=0.042). The haplotype analysis indicated the same results (25). In addition to a study conducted by E. Kleinstein et al., the results showed that rs261912 polymorphism (T>9562C) in *ALOX15* gene was associated with the rectal cancer risk (26).

Generally, we found that CT genotype of rs7217186 SNP was associated with susceptibility to AR; however, no significant association was found between rs2619112 SNP and the risk of AR. Although LOX-15 plays an important role in the biosynthesis of some anti-inflammatory lipid mediators, few studies have investigated the association of its genetic variants with inflammatory diseases, especially AR. Therefore, future studies with a large sample size and multiple SNPs of *ALOX15* gene, along with linkage research, are required to identify SNPs as markers of predisposition to AR.

Conflicts of interest

None of the authors has any potential financial conflicts of interest related to this manuscript.

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