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Original Article

Correlation between IL-27p28 Genetic Polymorphisms and Risk of Allergic Rhinitis

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Abstract

Background and objectives: Interleukin 27 (IL-27) is a cytokine consisting of two subunits, p28 and EBI3, and is a key mediator in regulating the differentiation of TCD4 + cells while playing a crucial role in immune-related disorders. This study aims to elucidate the possible association between IL-27p28 single nucleotide polymorphisms (SNPs), IL-27 serum levels, and the risk of allergic rhinitis (AR).

Materials and methods: Blood samples were collected from 130 patients with AR and 130 healthy individuals, and DNA and serum were separated. The relationship between IL-27p28 SNPs (rs153109 and rs181206) and the risk of AR was evaluated using the polymerase chain reaction-restriction fragment length polymorphism method. The serum levels of IL-27 in the participants were determined using enzyme-linked immunosorbent assay.

Results: Our results did not show a significant relationship between IL-27p28 SNPs (rs153109 and rs181206) and the risk of AR or serum IL-27 levels. However, our results showed a significant decrease in the serum level of IL-27 in patients with AR (342 ± 299 pg/mL) compared to healthy subjects (455 ± 274 pg/mL) (p = 0.02).

Conclusion: Our results suggest that IL-27p28 SNPs (rs181206 and rs153109) are not associated with susceptibility to AR, but that decreased serum IL-27 levels may be associated with the development of AR.

Introduction

Allergic rhinitis (AR) is considered an inflammatory disorder of the upper respiratory tract that is mediated by immunoglobulin E. AR is the most common type of rhinitis, affecting approximately 20–30% of adults and 40% of children, and is characterized by symptoms of nasal congestion, itchy nose, sneezing, and rhinorrhea Helper T (Th) cells and related cytokines play key roles in the development and control of allergic diseases.[1–3] For example, Th2 and Th17 cells and their related cytokines are key regulators of the development of allergic reactions, while regulatory T cells and Th1 cells act as suppressors of allergic reactions.[4] Interleukin (IL)-27 is a pleiotropic cytokine that regulates the differentiation of Th subsets.[5] IL-27 increases the differentiation of Th1 and Treg cells but suppresses the polarization of Th2 and Th17 cells.[6,7] IL-27, which belongs to the IL-12/IL-6 family, is composed of two subunits: Epstein-Barr virus-induced gene 3 (EBI3) and IL-27p28. The human IL-27p28 gene contains five exons and four introns, and is located on chromosome 16p11.[8] This cytokine is mainly produced by macrophages, inflammatory monocytes, dendritic cells, microglia, and dendritic cells.[9]

Although the true cause of allergic rhinitis remains unknown, studies suggest that genetic and environmental factors play a decisive role in its development.[10] Several studies have been conducted

Keywords: Allergic rhinitis; Single nucleotide polymorphism; IL-27p28; Polymerase chain reaction-restriction fragment length polymorphism; Enzyme-linked immunosorbent assay.

Abbreviations: AR, allergic rhinitis; CI, confidence interval; EBI3, Epstein-Barr virus-induced gene 3; ELISA, enzyme-linked immunosorbent assay; IL-27, interleukine-27; IL-27R, IL-27 receptor; OR, odds ratio; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, Single nucleotide polymorphism; Th, Helper T; Th cell, helper T cell; Treg cell, regulatory T cell.

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Table 1. Primer sequences used for PCR–RFLP analysis of the IL-27p28 polymorphisms

SNP ID	Regions	Primer sequence	Amplified size
Rs181206	Exon 4	F: GGAAGAGCTACAGGGATGGACTG	472bp
		R: CTCACTCTCCCACACTTACGGAC	
Rs153109	Promoter	F: GAGGAGGCAGAGAGCAGGAAG	691bp
		R: CTCAGTTTGTAACTAGGGTCAGGGC	

IL-27, interleukine-27; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

to understand the genetic basis of AR disease, and in recent years, a relatively large number of troth genes and genetic polymorphisms associated with AR have been identified. In fact, evidence suggests that polymorphisms in some cytokine genes, such as IL-1, IL-4, IL 12, and IL-17, may play a role in the development and severity of allergic rhinitis.[11–14]

Single nucleotide polymorphisms (SNPs) are the most common genetic variation in the human genome. They are particularly important because they may affect protein function, gene splicing, and gene expression.[15] Recent studies have reported that some SNPs of IL-27p28, including rs153109 and rs17855750, are associated with the pathogenesis of certain immune-related diseases such as asthma, AR, autoimmune diseases, chronic obstructive pulmonary disease, and tuberculosis.[16–22] Several studies have shown that decreased IL-27 levels in AR patients are associated with increased levels of IL-17 and IL-23 cytokines, increased proliferation of type 2 innate lymphoid cells, and increased secretion of type 2 cytokines, which may lead to the exacerbation of AR.[23–25] Therefore, recombinant IL-27 has been proposed as a new therapeutic target to reduce the pathogenesis of AR and Th2-related diseases through interaction with IL-27R.[25] Considering the importance of IL-27 in the pathogenesis of AR, this study aimed to determine the association between IL-27p28 SNPs (rs181206 and rs153109) with susceptibility to allergic rhinitis and also the effect of these SNPs on IL-27 serum levels.

Materials and methods

Selection of IL-27p28 single nucleotide polymorphisms (SNPs)

IL-27 contains a large number of SNPs. In our previous study, we performed a bioinformatics analysis to predict which IL-27 SNPs have a deleterious role in gene function or are likely to be associated with disease.[26] Briefly, we retrieved human IL-27 SNPs from the NCBI dbSNP database. 114 human IL-27 SNPs with minor allelic frequency > 0.01 were analyzed using nine different bioinformatics prediction tools, and 22 SNPs were identified as deleterious variants. In the present study, two of them (rs181206T>C and rs153109A>G) were selected to investigate their association with AR.

Study participants

This study was conducted on Kurdish people with AR who were referred to the Allergy Clinic of Dr. Mohammad Kermanshahi Hospital from August 2018 to September 2018, as well as healthy Kurdish people from Kermanshah province, Iran.

In total, 130 individuals with AR and 130 healthy individuals (matched for sex, age, and ethnicity) participated in this study. Inclusion criteria for patients with AR were as follows: diagnosis of allergic rhinitis by an allergist according to practice guidelines for allergic rhinitis management and no use of systemic corticosteroids in the past three months, except topical nasal steroids and/or anticonvulsants.[27] The inclusion criteria for healthy people were as follows: no history of allergy, no inflammatory or infectious disease at the time of registration, resident of Kermanshah province, and Kurdish. Exclusion criteria were as follows: suffering from any other comorbidity such as asthma, suffering from infectious diseases, using steroid drugs for any purpose in the last three months, being a non-native of Kermanshah province, pregnant, and being related to another participant. It is worth mentioning that in this study only the Kurdish population living in Kermanshah province was included; other ethnic groups were excluded from the study. This study conformed to the ethical guidelines of the Helsinki Declaration (as revised in 2013) and has been confirmed by the ethics committee of Kermanshah University of Medical Sciences with agreement code: IR.KUMS.REC.1397.159. After being informed about the aims and conditions of the study, all participants signed the written consent form. This is an observational study, and thus has not been registered on the WHO platform.

Blood samples and DNA extraction

Five milliliters of blood were taken from each participant; part of it was transferred to the tube containing ethylenediaminetetraacetic acid and the other part to the clot tube. Using the salting-out method, genomic DNA was separated from the tube containing ethylenediaminetetraacetic acid and kept at −20°C until use.[28] The serum was separated from the clot tube and stored at −80°C until use.

Determination of IL-27p28 gene genotype

Genotyping of the rs181206 T>C SNP in exon 4 of the IL-27P28 gene and rs153109 A>G SNP in the IL-27P28 gene promoter was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR was performed in a total volume of 20 µL including 0.5 µL of genomic DNA, 1 µL of each primer, 10 µL of master mix, and 7.5 µL of nuclease-free water. The sequence of primers is shown in Table 1. PCR reaction for both SNPs was performed as follows: first 3 min of incubation at 95°C, then 30 cycles of 30 s at 95°C, 20 s at 61°C, and 1 min at 72°C, and finally 2 min at 72°C to complete the expansion. PCR-amplified DNA samples were incubated with restriction enzymes according to the manufacturer's recommendations. FauI (New England Biolabs) and XhoI (Thermo Fisher Scientific) restriction enzymes were used to determine the genotype of rs181206 T>C and rs153109 A>G SNPs, respectively. Enzymatic digestion products were stained with a safe stain and observed on 1% agarose gel.

Determination of IL-27 serum levels

Serum levels of IL-27 were determined using an enzyme-linked immunosorbent assay (ELISA) kit (EAST BIOPHARM, HANGZHOU, China). The sensitivity and detection limits of the kit were 2.57 pg/mL and 5 pg/mL to 2,000 pg/mL, respectively. The range of detection and sensitivity of the kit were 5 – 2,000 pg/mL and

Table 2. Demographic characteristics of study subjects

Characteristics	AR	Control
Number	130	130
Gender[male/female]	60 (46.2%) / 70 (53.8%)	60 (46.2%) / 70 (53.8%)
Age (years)	Mean ± SD = 35.80 ± 10.77	Mean ± SD = 35.87 ± 10.67
Nasal rhinorrhea	123 (94.6%)	–
Sneezing	119 (91.5%)	–
Eye itching	80 (61.5%)	–
Nasal congestion	119 (91.5%)	–
Nasal itching	113 (86.9%)	–

AR, allergic rhinitis.

2.57 pg/mL, respectively.

Statistical analysis

The analysis of all data was performed using SPSS version 18.0 software. Continuous data are presented as mean ± standard deviation. The Hardy-Weinberg equilibrium in allele frequencies in the patient and control groups was checked using the Chi-Square ($\chi 2$) test. The frequency of the genotypes and alleles in the patient and control groups was also checked by the Chi-square ($\chi 2$) test. In addition, the correlation between IL-27 polymorphism genotypes and AR risk was evaluated by calculating odds ratio (ORs) and 95% confidence interval (CIs). The IL-27 serum level was evaluated for normality by the Kolmogorov-Smirnov test and non-normal distribution was considered, which was further evaluated by the Mann-Whitney test. Furthermore, One-way analysis of variance was used to determine the correlation between serum IL-27 levels and genotype distribution of polymorphisms. *P*-values less than or equal to 0.05 were considered statistically significant.

Results

Demographic characteristics of the studied subjects

The basic traits of AR patients and healthy subjects are shown in Table 2. No significant difference was observed in gender and age distribution between AR patients and healthy subjects.

Association of IL-27 genetic polymorphisms with AR risk

The genotype and allelic distribution of IL-27p28 SNPs in the study participants are shown in Table 3. The genotype distribution of IL-27p28 SNPs (rs181206 T>C and rs153109 A>G) in AR patients and healthy individuals was in Hardy-Weinberg equilibrium. The image of electrophoresis bands of PCR-RFLP technique products on agar gel is shown in Figures 1 and 2. Our results indicated that there is no statistically significant difference in rs181206 T>C genotype distribution and allele frequency between AR patients and healthy subjects ($p > 0.05$). In addition, although the frequency of the GG genotype ($p = 0.086$, OR 0.548, 95% CI: 0.274–1.095) and G allele ($p = 0.055$, OR 0.703, 95% CI: 0.491–1.008) of rs153109 A>G was lower in AR patients compared to healthy individuals, however, it was not statistically significant ($p > 0.05$). Furthermore, the association between IL-27p28 SNPs (rs153109, rs17855750) and clinical features of AR patients was analyzed. However, there was no association between the clinical manifestations of patients with AR and IL-27p28 SNPs ($p > 0.05$).

IL-27 serum level and risk of AR

The serum level of IL-27 in the study participants was measured by the ELISA method. The results showed that the serum level of IL-27 in patients with AR (342 ± 299 pg/mL) was significantly

Table 3. The genotype and allele frequencies of IL-27p28 SNPs in AR patients and healthy controls

SNP	Genotype allele	AR (%) (N = 130)	Control (%) (N = 130)	χ2	*p value*	Unadjusted OR (95%CI)
RS181206	TT	73 (56.2%)	81 (62.3%)	1.019	0.313	0.775 (0.472–1.272)
	TC	47 (36.2%)	36 (27.7%)	2.141	0.143	1.479 (0.875–2.499)
	CC	10 (7.7%)	13 (10%)	0.429	0.512	0.750 (0.316–1.777)
	T	193 (74.2%)	198 (76.2%)	0.258	0.612	0.902 (0.606–1.343)
	C	67 (25.8%)	62 (23.8%)	0.258	0.612	1.109 (0.744–1.651)
RS153109	AA	62 (47.7%)	51 (39.2%)	1.894	0.169	1.412 (0.863–2.311)
	AG	53 (40.8%)	54 (41.5%)	0.016	0.9	0.969 (0.591–1.588)
	GG	15 (11.5%)	25 (19.2%)	2.955	0.086	0.548 (0.274–1.095)
	A	177 (68.1%)	156 (60%)	3.683	0.055	1.422 (0.992–2.038)
	G	83 (31.9%)	104 (40%)	3.683	0.055	0.703 (0.491–1.008)

AR, Allergic rhinitis; CI, confidence interval; OR, odds ratio; IL, interleukin; SNP, Single nucleotide polymorphism.

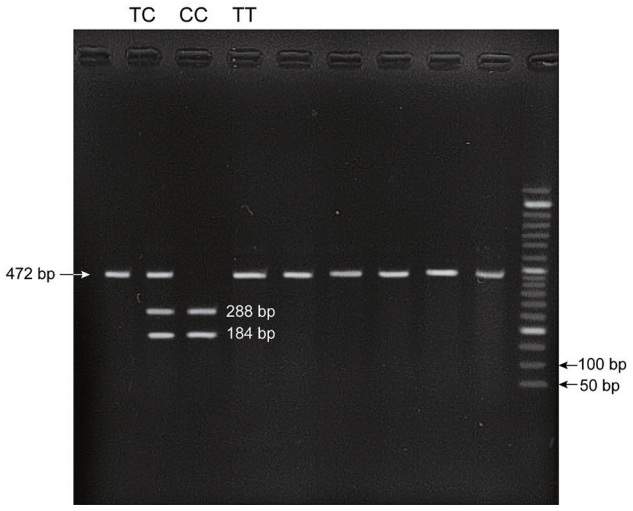

Fig. 1. IL-27p28 (rs181206T>C) PCR product after digestion with faul enzyme. Homozygotes wild TT genotype (472bp); heterozygous TC genotype (472bp, 288bp, and 184bp); mutant CC genotype (288bp, 184bp). IL, interleukine; PCR, polymerase chain reaction.

lower than that of healthy subjects (455 ± 274 pg/mL) (p = 0.02) (Fig. 3). However, no association was found between IL-27 serum levels and IL-27p28 SNPs (rs181206 T>C and rs153109 A> G) (Table 4).

Discussion

AR is known as the most prevalent allergic disease and is caused by a complex interaction between several environmental and genetic agents.[29] IL-27 is a heterodimeric cytokine composed of EBI3 and IL-27p28 chains and has a modulating effect on AR and asthma. While IL-27 receptor deficiency (IL-27R-/-) has been shown to increase asthmatic phenotypes in mice challenged with ovalbumin, administration of IL-27 reduces airway inflammation and improves airway hyperresponsiveness in a mouse model of chronic asthma.[30,31] This study aimed to investigate the effect of two selected IL-27p28 SNPs (rs181206 and rs153109) on IL-27 serum levels and the risk of AR in an Iranian population.

Our results indicated that there is no significant relationship be-

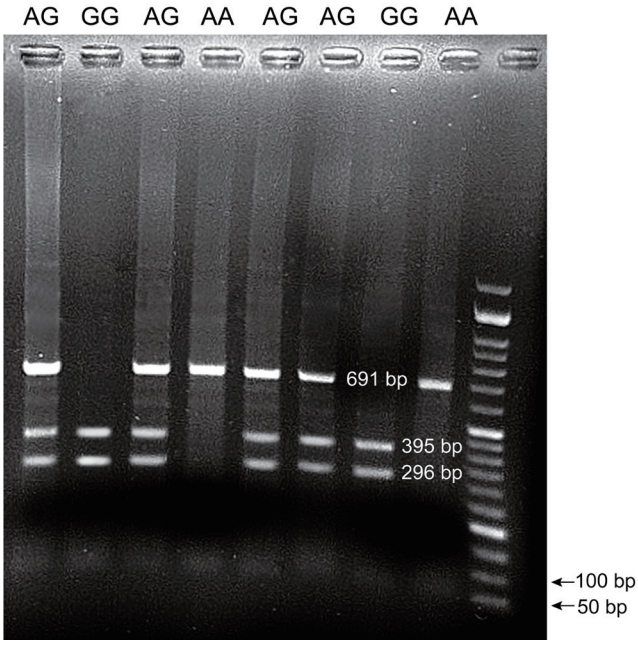

Fig. 2. IL-27p28 (rs153109A>G) PCR product after digestion with XhoI enzyme. Homozygotes wild AA genotype (691bp); heterozygous AG genotype (691bp, 395bp, and 296bp); mutant GG genotype (395bp, 296bp). IL, interleukine; PCR, polymerase chain reaction.

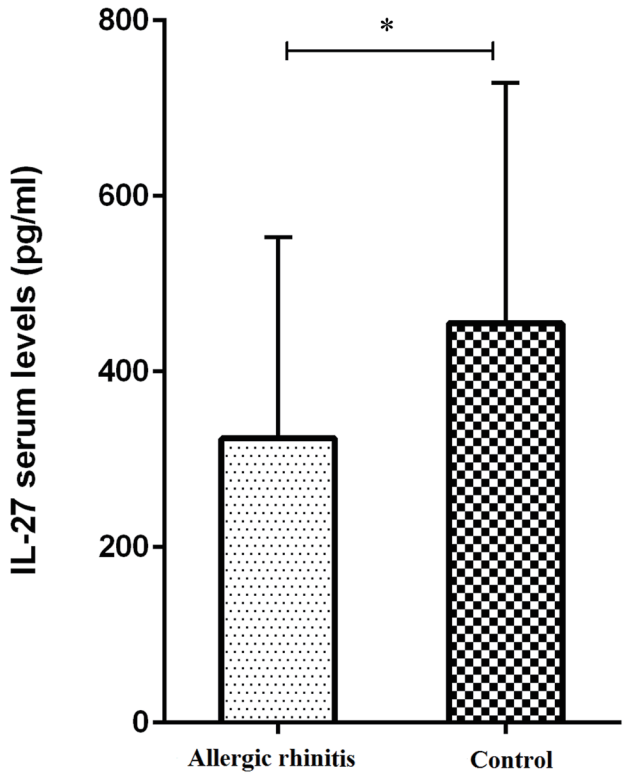

Fig. 3. Serum levels of IL-27 in patients with AR and healthy individuals. Measurement of serum IL-27 levels by ELISA method showed that serum IL-27 levels in patients with AR (342 ± 299 pg/ml) were significantly lower than in healthy controls (455 ± 274 pg/ml) (p = 0.02). *p < 0.05. AR, allergic rhinitis; ELISA, enzyme-linked immunosorbent assay; IL-27, interleukine-27.

Table 4. Association of serum IL-27 level with IL27p28 SNPs in AR patients and healthy individuals

SNP	Genotype	IL-27 (pg/mL)			*p value*
		N	Mean	SD	
	TT	32	374	260	
Rs181206	TC	23	380	247	0.838
	CC	11	426	254	
	AA	22	351	281	
Rs153109	AG	26	368	234	0.442
	GG	18	449	239	

AR, allergic rhinitis; IL-27, interleukine-27; SNP, single nucleotide polymorphism.

tween IL-27p28 SNPs (rs181206 and rs153109) and the risk of AR in the Kurdish population of Kermanshah. On the other hand, our results revealed that the serum level of IL-27 was significantly lower in patients with AR than in the control group. However, no significant association was observed between IL-27p28 SNPs and serum IL-27 levels.

The genetic variants rs181206 T>C and rs153109 A>G of the IL-27p28 have recently been identified and the correlation between these genetic polymorphisms and the risk of allergic diseases has been investigated in several ethnic groups. In a study conducted by Yang Shen *et al.*, they showed that there was a considerable difference in the genotype and allele distribution of the rs153109 polymorphism between AR patients and healthy subjects in a Chinese Han population. Nevertheless, they found no association between the rs181206 polymorphism and the risk of AR.[17] A study by Soo-Cheon Chae *et al.* also revealed a significant relationship between rs153109 SNP and asthma in a Korean population. However, they found no significant association between the rs181206 polymorphism and asthma.[16] On the other hand, in accordance with the findings of our study, Ji-In Yu *et al.* found that the frequencies of alleles and genotypes of rs181206 and rs153109 SNPs in AR patients and healthy controls in a Korean population were not significantly different.[32] Therefore, the results of this study, consistent with our findings, indicate that IL-27p28 SNPs (rs181206 and rs153109) may not be associated with AR susceptibility in some populations.

IL-27, a new member of the IL6/IL12 family, plays a crucial role in attenuating airway and pulmonary inflammation during the development of allergic asthma by its inhibitory effects on Th2 cytokine production.[30] In our study, the results showed that the serum level of IL-27 was significantly lower in patients with AR compared to the control group. In line with our findings, it has already been shown that the serum level of IL-27 and its mRNA level are decreased in AR patients compared to healthy individuals and have a negative correlation with Th2 cytokine.[24] In addition, another study has shown that the serum level of IL-27 is decreased in AR patients and is negatively correlated with the levels of IL-17 and IL-23.[23] Furthermore, Xi Luo *et al.* showed that serum IL-27 protein expression was significantly lower in patients with AR compared to controls.[25] In vitro studies have shown that although IL-27 plays a pro-inflammatory role when administered alone and when administered in combination with IL-4, it regulates IL-4-induced chemokine production in human bronchial epithelial cells and down-regulates bronchial epithelial cell activation.[33]

Therefore, the results of our study together with the results of other studies show that low levels of serum IL-27, due to the increased secretion of cytokines of Th2, type 2 innate lymphoid cells, and Th17 cells such as IL-5, IL-4, IL-13, IL-23 and IL-17 may be associated with the development of AR.

On the other hand, in our study, no association was found between serum IL-27 level and IL-27p28 SNPs (rs181206 and rs153109). Although IL-27 serum levels have not been measured in the few studies that have been conducted on the association of IL-27 polymorphisms and the risk of AR, in studies related to the relationship between IL-27 polymorphisms and tumor risk, IL-27 levels have been measured and the relationship between IL-27 polymorphisms and IL-27 serum levels has been investigated. Most of these studies have shown that the IL-27 rs153109 polymorphism significantly increases the risk of developing cancer.[34] However, no association was found between serum IL-27 levels and IL-27 polymorphisms. In a study conducted by Yu Jin Tong in patients with osteosarcoma, no significant relationship was observed between rs181206 and rs153109 polymorphisms and IL-27 serum levels.[19] Also, in Alireza Ghavami's study in Iran, no correlation was observed between serum IL-27 levels and rs153109 polymorphism in patients with acute lymphocytic leukemia.[35] Furthermore, Bin Zhu *et al.* showed that plasma levels of IL-27 in bladder cancer are not associated with rs153109 polymorphism.[36]

These results suggest that rs181206 and rs153109 SNPs of IL-27P28 may not affect IL-27 production. However, it is worth mentioning that in the current study, we faced some limitations. First, due to financial and equipment limitations, the sample size was considered small. Secondly, this study was conducted on the population of one of Iran's regions, and due to ethnic diversity in Iran, these results may not be generalizable to the whole of Iran. Therefore, more studies with larger sample sizes as well as studies on other Iranian ethnic groups are needed to confirm our findings. Third, the present study does not cover all genetic polymorphisms of IL-27p28. In addition, the patients were not examined by an otolaryngologist for chronic rhinosinusitis or nasal polyps. Thus, we cannot provide a comprehensive view of the possible association of genetic variation in IL-27p28 with IL-27 serum level and AR risk.

Conclusion

In summary, our study, despite limitations such as a small number of samples and a selection of samples from a specific Iranian ethnicity, did not show a significant relationship between the SNPs tested in IL-27 and AR disease. Nevertheless, our study showed a significant association between decreased serum IL-27 levels and an increased risk of AR, although no association was found between the targeted SNPs and serum IL-27 levels.

These findings show that there is a relationship between IL-27 serum level and AR sensitivity in this Iranian population, although the tested SNPs did not show a relationship with AR or IL-27 levels. Therefore, more studies using a larger number of IL-27 SNPs and a larger number of samples in the Kurdish population of Kermanshah as well as other Iranian ethnic groups are needed to reveal the relationship between IL-27 SNPs and AR.

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Funding

There is nothing to declare.

Conflict of interest

The authors declare that they have no potential financial conflict of interests related to this manuscript.

Author contributions

GKA: planned and supervised the study; KF: performed the experiments. FS and FK: did the sampling and wrote the first draft of the manuscript; MSHR: diagnosed and introduced allergic rhinitis patients; SF and RA: performed data analysis and data interpreta-

tion. All authors participated in preparing and editing the final version of the manuscript.

Ethical statement

This study conformed to the ethical guidelines of the Helsinki Declaration (as revised in 2013) and has been approved by the ethics committee of Kermanshah University of Medical Sciences (agreement code: IR.KUMS.REC.1397.159). Informed consent was obtained from all participants in the study. This is an observational study, and thus has not been registered on the WHO platform.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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