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Association between Two Single Nucleotide Polymorphisms of Thymic Stromal Lymphopoietin (TSLP) Gene and Asthma in Iranian Population

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ABSTRACT

Thymic stromal lymphopoietin (TSLP) is a cytokine similar to IL-7, which is released by airway epithelial cells in response to injury and inflammation. Current literature is contradictory about the association between different single nucleotide polymorphisms (SNPs) of the *TSLP* gene and asthma development in different countries.

We aimed to evaluate the association between two common *TSLP* SNPs (rs2289276 and rs2289278) and the risk of asthma in the Iranian population. Genotyping of the *TSLP* gene was performed in 126 adult asthmatic patients and 300 controls; using the TaqMan genotyping assay. Moreover, total serum IgE level and eosinophil count were assessed.

The results indicated that the TT genotype of rs2289276 was inversely associated with the risk of asthma (p=0.002). A similar inverse association was detected in subgroups of atopic (p=0.001) and non-atopic (p=0.005) asthma. Moreover, the TT genotype of this SNP was more prevalent in severe and late-onset categories of asthma. In subgroup analysis, a significant sex-specific association between rs2290276 and asthma was observed in women (p=0.004). The prevalence of rs2289276 was extremely low, which made it infeasible to perform any further analysis.

Overall, our findings indicated that rs2290276 SNP of the *TSLP* gene has a protective phenotype against asthma development in the Iranian population.

Keywords: Asthma; Atopic; Single nucleotide polymorphism; Thymic stromal lymphopoietin

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INTRODUCTION

Asthma is one of the most common chronic respiratory diseases associated with the inflammation of airways. The interaction of genetic and environmental factors play a critical role in the pathogenesis of this complex disease.[1] It has been estimated that over 300 million people suffer from this chronic disorder and its prevalence is increasing in both developed and developing countries.[2]In Iran, the prevalence of asthma has increased from 5.1% to 8.9% from 2004 to 2018.[3] Economic burden, and increasing prevalence has urged researchers around the world to study the different aspects of this disease.

Bronchial epithelial cells release a variety of epithelium-derived cytokines in response to the damages caused by allergens. Thymic stromal lymphopoietin (TSLP) is one of these epithelial cytokines which is encoded by the *TSLP* gene located in 5q22.1 chromosome. TSLP is secreted by different cell types such as epithelial cells, mast cells, basophils, keratinocytes, and dendritic cells (DCs).[4] There are two possible contradictory functions for TSLP. It either acts as an inflammatory or homeostatic cytokine. These different functions are mediated by two distinct isoforms, which are produced as a result of activation of different promoter regions in the *TSLP* gene.[5]

In asthma, TSLP increases the inflammatory responses by binding to its specific receptor (TSLP receptor), which is expressed by cells of the innate immune system such as eosinophils.[6] Moreover, TSLP-activated DCs leads to proliferation and differentiation of naive T cells into T helper 2 (Th2) cells and eventually, develop the general manifestations of asthma.[7] However, several studies have shown that certain single nucleotide polymorphisms (SNPs) in *TSLP* gene may result in lower risk and decreased severity of asthma.[8-10]

Given the paradoxical roles of TSLP, several studies have reported positive, negative, or null associations of *TSLP* gene polymorphisms and asthma disease.[9-14] Moreover, a recent study has reported an inverse sex-specific association between SNPs of *TSLP* and asthma in different populations.[10]

Considering the differences in the prevalence of polymorphisms among ethnic groups,[15] it is essential to conduct studies of gene polymorphisms in different races. Although a few studies have been conducted on populations in the Middle East,[8,9,14,16] there are no studies on the associations of *TSLP* SNPs and asthma in the Iranian population. Moreover, it is shown that environmental factors are related to the development of asthma and could differ across different areas. Therefore, we designed a case-control study to evaluate the attribution of two SNPs (rs2289276 & rs2289278) of the TSLP gene and risk of asthma with adjusting for related environmental factors in the Iranian population.

MATERIALS AND METHODS

Patients and Healthy Controls

We enrolled 130 adult asthmatic patients (18 to 64 years) with confirmed asthma diagnosis from Firoozabadi hospital of Tehran, Iran from 2018 to 2019. The control group included 300 individuals who had attended the hospital for regular check-ups with low IgE levels and no record of asthma or pulmonary diseases. Moreover, they were matched for sex and age with the cases to prevent confounding factors. Higher levels of total serum IgE (above 100 IU/mL), allergic symptoms, or chronic respiratory diseases were considered as exclusion criteria for the control group. This study has been approved by the ethics review committee of Iran University of medical sciences (approval ID: IR.IUMS.FMD.REC.1397.004).

All participants in this study have completed a questionnaire, which was adapted from the European community respiratory health survey,[17] Behavioral Risk Factor Surveillance System (BRFSS), and asthma survey adult questionnaire.[18] The job was categorized into low and high-risk groups based on Job Exposure Matrix (JEM).[19] We also categorized the study population based on the age of asthma onset. The onset of asthma at ages younger than 16 was defined as childhood-onset, and onset at ages of 16 or above was considered as later-onset asthma.[20]

Allergy Testing and Spirometry

Total serum IgE levels were measured for both patients and the control group; using the ELISA kit (EUROIMMUN, Germany). Based on the upper limit of the normal range for total serum IgE (100 IU/mL as stated by the kit manufacturer) and skin prick test results, patients were classified into atopic (above 100 IU/mL and/or positive test) and non-atopic (below 100 IU/mL and/or negative test) groups. The inclusion

criteria for the control group was a total serum IgE<100 IU/mL.

Skin prick test was performed for all patients based on the European standards[21] using common aeroallergens in Iran [22], including *Russian thistle*, tree mix, grass mix, weed mix, mite mix, cockroach, Mugwort, and mold (Hollisterstier, LA, USA). After 15 minutes, the diameter of the wheal induced was measured and compared with positive (histamine 0.1%) and negative (glycerin) controls. Spirometry test was performed for all patients based on American Thoracic Society guidelines.[23] Afterward, patients were classified into 3 groups of mild, moderate, and severe asthma using GINA guidelines[24] and FEV_1 (Forced Expiratory Volume in the first second), FVC (forced volume vital capacity), FEV_1/FVC data.

SNP Genotyping

Peripheral blood was collected from the patient and control group in EDTA-containing tubes. Genomic DNA was extracted from whole blood; using the salting-out method.[25] DNA samples were diluted at the concentrations of 10 ng/µL for ideal genotyping results. SNP genotyping analysis for two SNPs (rs2289276 & rs2289278) was performed; using TaqMan SNP genotyping assay kit (Applied Biosystems. Foster City, CA, USA) and Qiagen Rotor-Gene Q system (Qiagen, Hilden, Germany).of the many single nucleotide polymorphisms in the *TSLP* gene,[26] there are 5 SNPs that are shown to have associations with asthma[8-11,13,14,16]. These two most commonly studied SNPs in asthma (rs2289276 & 2289278) were chosen to be assessed in our study to obtain statistically comparable results.

Statistical Analysis

Associations between categorical parameters of interest were analyzed by chi-square or fisher's exact test. Independent T-test or its non-parametric counter-part, Mann-Whitney U test, were utilized for comparing numerical parameters between two subgroups of categorical parameters.

We used binary and multinomial logistic regressions to evaluate the adjusted correlation among *TSLP* polymorphisms and the risk of asthma. Covariates adjusted were job status (low or high risk), smoking status, type of delivery (vaginal or C-section), neonatal nutrition (breast milk or formula), attendance at kindergarten, and type of elementary school (public or private school).

The genotype frequencies of each SNP were tested for concordance with Hardy-Weinberg equilibrium using Pearson's χ 2 test. Two-tailed *p*-value <0.05 was considered statisticallysignificant.The analysis was conducted using IBM SPSS advanced statistics (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp).

RESULTS

Demographic Characteristics of Participants

The demographic characteristics of all participants were described in table 1. In the patients' group, 4 of 130 cases were excluded from the study due to failure in DNA extraction. Finally, 126 patients and 300 healthy controls were included in the analysis. The mean (±SD) age of patients and healthy controls were 35.08 (±10.49) and 36.00 (±11.21), respectively (p=0.188). Two groups were matched for sex with the female gender frequency being 74.6% and 78.0% in patient and control groups, respectively (p=0.447). Two groups were significantly different in terms of job status (p<0.001), smoking status (p<0.001), kindergarten attendance (p=0.016), and types of elementary school (p=0.012).

Patients had expectedly higher IgE levels compared to the control group (p<0.001. Likewise, eosinophil count was significantly higher in patients (4.639±2.32% vs 1.216±0.61 %, p<0.001).

Association of rs2289276 and rs2289278 in TSLP Gene with Risk of Asthma

Table 2 shows the results of univariate and multivariable models for the association between *TSLP* SNPs and asthma. There was a significant inverse association between the TT genotype of rs2289276 and the risk of asthma (OR=0.30, 95% CI=0.14-0.65, p=0.002). In contrast, we could not find any association between the GG genotype of rs2289278 and the risk of asthma. The GT genotype of this SNP did not show any significant association with the risk of asthma (OR=1.425, 95% CI=0.53-3.83, p=0.482).

We also conducted subgroup analysis to investigate associations of different SNP genotypes with the risk of asthma stratified by type (atopic and non-atopic) and severity of the disease (Tables 3 and 4).

Table 1. Clinical and demographic characteristics of patients and control subjects

Characteristics		Asthmatics N=126	Control subjects N=300	*p*
Age (year)	Mean (±SD)	35.08 (±10.49)	36.00 (±11.21)	0.188[a]
Sex	Female	94 (74.6)	234 (78.0)	0.447[b]
	Male	32 (25.4)	66 (22.0)	
BMI index (kg/m^2)	Mean (±SD)	25.92 (±3.902)	23.41 (±3.22)	0.190[a]
Education level	Under diploma	44 (34.9)	89 (33.6)	0.097[b]
	Diploma	55 (43.7)	93 (35.1)	
	Upper diploma	27 (21.4)	83 (31.3)	
Place of birth	City	93 (73.8)	176 (66.4)	0.140[b]
	Country side	33 (26.2)	89 (33.6)	
Type of delivery	Vaginal delivery	105 (83.3)	225 (84.9)	0.689[b]
	Caesarean delivery	21 (16.7)	40 (15.1)	
Neonatal nutrition	Breast milk	113 (89.7)	237 (89.4)	0.940[b]
	Formula	13 (10.3)	28 (10.6)	
Kindergarten	No	112 (88.9)	209 (78.9)	**0.016[b]**
	Yes	14 (11.1)	56 (21.1)	
Elementary school	Public school	124 (98.4)	239 (90.2)	**0.012[b]**
	Private school	2 (1.6)	26 (9.8)	
Job	Low risk	85 (67.5)	262 (98.9)	**<0.001[b]**
	High risk	41 (32.5)	3 (1.1)	
Smoking status	Smoker	11 (8.7)	7 (2.6)	**<0.001[b]**
	Expose to smoke	57 (45.2)	42 (15.8)	
	None	58 (46.0)	216 (81.5)	
Serum IgE (IU/mL)	Mean(±SD)	166.15 (±148.72)	38.51 (±27.19)	**<0.001[c]**
Eos count (%)	Mean(±SD)	4.639 (±2.32)	1.216 (±0.61)	**<0.001[a]**
FVC[d] (%)	Mean (min-max)	79.13 (36-156)	-	-
FEV1[e] (%)	Mean (min-max)	72.55 (30-126)	-	-
FEV1/FVC (%)	Mean (min-max)	89.45 (56-124)	-	-
Skin prick test	Positive	64 (50.79)	-	-
	Negative	62 (49.20)	-	-

N, number; EOS, Eosinophil. Bold type indicates a significant association

[a] Independent t-test; [b] Pearson Chi-Square; [c] Nonparametric Tests (Mann-Whitney U Test) ;[d] Forced vital capacity; [e] Forced expiratory volume in 1 second. n (%) are given unless stated otherwise.

There was significant inverse association between TT genotype of rs2289276 in both atopic and non- atopic asthma (OR= 0.16, 95% CI= 0.05-0.49, *p*=0.001; and OR=0.25, 95% CI=0.09-0.65, *p*=0.005; respectively). We found similar results for the summation of CT and TT genotypes of rs2289276 SNP (OR=0.48, 95% CI=0.24-0.95, *p*=0.04 and OR=0.42, 95% CI=0.22-0.81, *p*=0.009).

Regarding asthma severity, the TT genotype of rs2289276 was inversely associated with the risk of mild and moderate asthma. However, rs2289278 did not show significant association in any of the subgroup analyses.

In subgroup analysis based on gender, rs2289276 was inversely associated with risk of asthma (OR=0.27, 95% CI=0.11-0.65, *p*=0.004), and high level of eosinophil count (OR=0.81, 95% CI=0.66-0.99, *p*=0.04) in women (Table 5). Inverse correlation was also observed between rs2289276 SNP and the risk of childhood (OR=0.20, 95% CI=0.06-0.70, *p*=0.011) and later-onset asthma (OR= 0.20, 95% CI= 0.08-0.53, *p*=0.001) (Table 6). We performed further analysis to investigate associations between skin prick test results and the SNP genotypes. However, no significant correlation was spotted (data not shown).

Table 2. Overall association between thymic stromal lymphopoietin (*TSLP)* single nucleotide polymorphisms and the risk of asthma in the Iranian population

TSLP SNPs	Genotype & Alleles	Population		χ2	Univariate analysis		Multivariable analysis*	
		Total=126 N (%)	Total=300 N (%)		OR (95% CI)	*p*	OR (95% CI)	*p*
rs2289276	C/C[a]	47 (37.3)	88 (29.3)	**3.79**	1.00	-	1.00	-
	C/T	59 (46.8)	133 (44.3)		0.83 (0.52-1.33)	0.437	0.696 (0.41-1.23)	0.209
	T/T	20 (15.9)	79 (26.3)		**0.47 (0.26-0.87)**	**0.016**	**0.300 (0.14-0.65)**	**0.002**
	C/T+T/T	79 (62.7)	212 (70.7)		0.70 (0.45-1.08)	0.108	**0.547 (0.32-0.93)**	**0.027**
	C[a]	153 (60.7)	309 (51.5)		1.00	-	-	-
	T	99 (39.3)	291 (48.5)		**0.69 (0.51-0.93)**	**0.014**	-	-
rs2289278	C/C[a]	117 (92.9)	279 (93.0)	**0.95**	1.00	-	1.00	-
	C/G	9 (7.1)	20 (6.7)		1.07 (.48-2.43)	0.865	1.425 (0.53-3.83)	0.482
	G/G	0 (0.0)	1 (0.3)		0.00	1.00	0.00	1.00
	C/G+G/G	9 (7.1)	21 (7.0)		0.98 (0.44-2.20)	0.958	1.36 (0.61-3.61)	0.541
	C[a]	243 (96.4)	578 (96.3)		1.00	-	-	-
	G	9 (3.6)	22 (3.7)		0.97 (0.44-2.14)	0.946	-	

[a], reference; OR, odds ratio; N, number; CI, confidence interval;

* Multivariable analysis adjusted by job and smoking status, type of delivery, Neonatal nutrition, kindergarten, and elementary school.

Bold type indicates statistically significant results

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Table 3. Association between thymic stromal lymphopoietin (TSLP) single nucleotide polymorphisms and the risk of atopic/non-atopic asthma in the Iranian population

TSLP SNPs	Genotype	Population			Multivariable analysis*					
		Control subjects Total= 300 N (%)	Atopic Total= 64 N (%)	Non-atopic Total= 62 N (%)	Atopic asthma vs. Control		Non-atopic asthma vs. Control		Atopic vs. Non-atopic asthma	
					OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95% CI)	*p*
rs2289276	C/C[a]	88 (29.3)	22 (34.4)	25 (40.3)	1.00	-	1.00	-	1.00	-
	C/T	133 (44.3)	34 (53.1)	25 (40.3)	0.69 (0.33-1.41)	0.305	0.51 (0.25-1.02)	0.058	0.72 (0.23-2.25)	0.566
	T/T	79 (26.3)	8 (12.5)	12 (19.4)	**0.16 (0.05-0.49)**	**0.001**	**0.25 (0.09-0.65)**	**0.005**	1.37 (0.62-3.07)	0.439
	C/T+T/T	212 (70.7)	42 (65.6)	37 (59.7)	**0.48 (0.24-0.95)**	**0.036**	**0.42 (0.22-0.81)**	**0.009**	1.19 (0.55-2.55)	0.661
rs2289278	C/C[a]	279 (93.0)	58 (90.6)	59 (95.2)	1.00	-	1.00	-	1.00	-
	C/G	20 (6.7)	6 (9.4)	3 (4.8)	2.01 (0.61-6.60)	0.252	0.81 (0.20-3.29)	0.770	2.51 (0.57-11.10)	0.223
	G/G	1 (0.3)	0 (0.0)	0 (0.0)	NS	-	NS	-	-	-
	C/G+G/G	21 (7.0)	6 (9.4)	3 (4.8)	1.92 (0.59-6.26)	0.280	0.77 (0.19-3.11)	0.716	2.51 (0.57-11.10)	0.223

[a], reference; OR, odds ratio; N, number; CI, confidence interval; NS; not significant data * Multivariable analysis adjusted by job and smoking status, type of delivery, Neonatal nutrition, kindergarten, and elementary school. **Bold type** indicates statistically significant results

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Table 4. Association between thymic stromal lymphopoietin (*TSLP*) single nucleotide polymorphisms and the risk of different severities of asthma in the Iranian population

TSLP SNPs	Population					Multivariable analysis*					
						Mild asthma vs. Control		Moderate vs. Control		Severe vs. Control	
	Control subjects total=300 N (%)	Mild Total=38 N (%)	Moderate Total=55 N (%)	Severe Total=33 N (%)		OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
rs2289276	C/C[a]	88 (29.3)	23 (60.5)	18 (32.7)	6 (18.2)	1.00	-	1.00	-	1.00	-
	C/T	133 (44.3)	12 (31.6)	27 (49.1)	20 (60.6)	0.30 (0.13-0.65)	0.003	0.87 (0.41-1.83)	0.708	1.72 (0.54-5.47)	0.360
	T/T	79 (26.3)	3 (7.9)	10 (18.2)	7 (21.2)	0.10 (0.03-0.38)	0.001	0.33 (0.12-0.95)	0.039	0.42 (0.09-1.87)	0.253
	C/T+T/T	212 (70.7)	15 (39.5)	37 (67.3)	27 (81.8)	0.22 (0.11-0.47)	<0.001	0.67 (0.33-1.37)	0.271	1.17 (0.39-3.53)	0.781
rs2289278	C/C[a]	279 (93.0)	37 (97.4)	51 (92.7)	29 (87.9)	1.00	-	1.00	-	1.00	-
	C/G	20 (6.7)	1 (2.6)	4 (7.3)	4 (12.1)	0.48 (0.06-3.87)	0.493	1.54 (0.43-5.49)	0.504	4.12 (0.87-19.45)	0.074
	G/G	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	NS	0.996	NS	0.996	-	0.997
	C/G+G/G	21 (7.0)	1 (2.6)	4 (7.3)	4 (12.1)	0.46 (0.06-3.66)	0.463	1.47 (0.42-5.19)	0.550	3.95 (0.84-18.56)	0.082

[a], reference; OR, odds ratio; N, number; CI, confidence interval; NS; not significant data * Multivariable analysis adjusted by job and smoking status, type of delivery, Neonatal nutrition, kindergarten, and elementary school.

Bold type indicates statistically significant results

Table 4. Continue

Population	Multivariable analysis*					
	Mild VS Moderate		Mild vs. Severe		Moderate vs. Severe	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
TSLP SNPs	1.00	-	1.00	-	1.00	-
	0.31 (0.12-0.83)	0.020	0.18 (0.05-0.69)	0.012	0.55 (0.17-1.76)	0.311
	0.35 (0.07-1.63)	0.181	0.34 (0.05-2.25)	0.263	0.65 (0.15-2.78)	0.560
	0.32 (0.13-0.79)	0.014	0.21 (0.06-0.72)	0.013	0.57 (0.19-1.76)	0.329
rs2289278	1.00	-	1.00	-	1.00	-
	0.33 (0.03-3.19)	0.338	0.07 (0.06-0.92)	0.043	0.41 (0.08-1.96)	0.262
	-	-	-	-	-	-
	0.33 (0.03-3.19)	0.338	0.07 (0.06-0.92)	0.043	0.41 (0.08-1.96)	0.262

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Table 5. Sex-stratified association between thymic stromal lymphopoietin (*TSLP)* single nucleotide polymorphism rs2289276 and the risk of asthma in the Iranian population

rs2289276	Population		Univariate analysis		*Multivariable analysis	
	Asthmatics	Control subjects				
	Total=126 N (%)	Total=300 N (%)	OR (95% CI)	*p*	OR (95% CI)	*p*
Female						
C/C [a]	39 (41.5)	71 (30.3)	1.00	-	1.00	-
C/T	43 (45.7)	104 (44.4)	0.75 (0.44-1.28)	0.292	0.69 (0.38-1.27)	0.234
T/T	12 (12.8)	59 (25.2)	0.37 (0.18-0.77)	0.008*	0.27 (0.11-0.65)	0.004
Male						
C/C [a]	8 (25.0)	17 (25.8)	1.00	-	1.00	-
C/T	16 (50.0)	29 (43.9)	1.17 (0.42-3.31)	0.76	0.53 (0.09-3.28)	0.497
T/T	8 (25.0)	20 (30.3)	0.85 (0.26-2.75)	0.79	0.89 (0.13-5.93)	0.906

[a], reference; OR, odds ratio; N, number; CI, confidence interval; * Multivariable analysis adjusted by job and smoking status, type of delivery, Neonatal nutrition, kindergarten, and elementary school.

Bold type indicates statistically significant results

Table 6. Association between thymic stromal lymphopoietin (*TSLP)* single nucleotide polymorphism rs2289276 and the risk of different types of asthma by the timing of onset in the Iranian population

TSLP SNP	Genotype	Population			*Multivariable analysis					
		Control Total= 300 N (%)	Childhood-onset Total= 39 N (%)	Late-onset Total= 87 N (%)	Childhood-onset asthma vs. control		Late-onset asthma vs. control		Childhood-onset vs. Late-onset Asthma	
					OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
rs2289276	C/C [a]	88 (29.3)	14 (35.9)	33 (37.9)	1.00	-	1.00	-	1.00	-
	C/T	133 (44.3)	18 (46.2)	41 (47.1)	0.57 (0.24-1.35)	0.198	0.60 (0.32-1.12)	0.106	0.99 (0.41-2.39)	0.986
	T/T	79 (26.3)	7 (17.9)	13 (14.9)	**0.20 (0.06-0.70)**	**0.011**	**0.20 (0.08-0.53)**	**0.001**	1.10 (0.33-3.72)	0.875
	C/T+T/T	212 (70.7)	25 (64.1)	54 (62.1)	**0.43 (0.19-0.99)**	**0.046**	**0.45 (0.25-0.82)**	**0.009**	1.02 (0.44-2.34)	0.970

[a], reference; OR, odds ratio; N, number; CI, confidence interval; *Multivariable analysis adjusted by job and smoking status, type of delivery, Neonatal nutrition, kindergarten, and elementary school.

Bold type indicates statistically significant results

DISCUSSION

In this research, we found a significant inverse association between rs2289276 and the risk of asthma. This protective association was more significant in the female gender. However, we failed to detect any association of SNP rs2289278 with asthma, due to the extremely low frequency of this SNP in the studied population.

Along with the genetic factors, we identified that there are also environmental factors such as attendance at kindergarten, types of elementary school, and the status of smoking and job status as important governing factors in susceptibility to asthma.

Our study demonstrated that the TT genotype of rs2289276 was 70% less prevalent in asthmatic patients compared to the control group. This inverse association has also been obtained by some of the studies conducted in other populations.[9,10] However, some studies have reported contradictory results.[11,13] To justify the protective role of rs2289276 in asthma, Harada et al have proposed two possible mechanisms.[11]According to Harada et al, the affinity of activating enhancer-binding protein 2-alpha (AP-2α)to *TSLP* promoter is diminished when the T allele of rs2289276 is replaced with the C allele in the gene sequence. AP-2α is a transcription factor, which activates the transcription of different inflammatory-associated genes.[27] Another explanation refers to the two recently identified isoforms of TSLP. The short isoform of TSLP plays a homeostatic role by downregulating inflammatory mechanisms while the long isoform the opposite effects.[5] It seems that rs2289276 results in a decreased expression of long isoform TSLP; therefore, it eventually contributes to the protective phenotype. Considering these pieces of evidence, we assumed that the presence of the T allele in the promoter of the *TSLP* gene leads to a low level of *TSLP* gene expression, which might be the reason for the protective manner of this SNP.

This study also provides evidence for a high prevalence of rs2280276 SNP in non-atopic asthma. non-atopic asthma tends to be more severe than atopic asthma and may be less responsive to standard therapy.[28] This was supported in our analysis which demonstrated that the TT genotype of this SNP was more prevalent in severe and late-onset forms of the disease. This finding was not reported in previous studies.

Although asthma is more prevalent in adult women, we have found that the TT genotype of SNP rs2280276 protects against asthma in the female population. This finding has been first reported by Hunninghake et al [10]. Considering the effect of estrogen on allergic inflammation through inducing the production of TSLP,[29] it can be concluded that the TT genotype of rs2289276 which is located in the promoter region, might decrease the transcriptional activity of *TSLP* gene in women. Moreover, we observed similar sex-specific associations in the blood eosinophil count. Since TSLP has a critical role in the activation of eosinophils,[30] the presence of rs2280276 in women's *TSLP* gene might reduce the total count of eosinophil cells with a consequent reduction in the pathological damages generated by these cells.[6]

The presence of nearly 10 million SNPs justifies the importance of studying the potential effects of SNPs on gene function and human health. Determining the frequency of effective SNPs in each population can be important in the management of corresponding diseases. As the first study in the Iranian population, we have found that the frequency of the GG genotype of SNPrs2289278 was extremely low (0% in the case and 0.3% in the control group). However, the frequency of this genotype has been reported to be 5.3% and 4.5% in the Chinese[13] and the Japanese [11] populations, respectively. This inconsistency can be explained by racial diversity and/or interactions between genetic and environmental factors. It seems that this SNP is rarely impressive in the Iranian population. However, further studies with larger sample sizes are required to confirm it.

Although the population size of the current study was acceptable to find significant correlations between SNP rs2289276 and asthma, we could not demonstrate any associations for SNPrs2289278. This could be due to a relatively low sample size of our study for this SNP. This underlines the need for studies with a larger sample size on the Iranian population to investigate the potential associations of this SNP. Moreover, measuring serum levels of type II cytokines is complementary to the study which is suggested to be assessed in further studies. Lack of evidence on the functional role of rs2289276 and rs2289278 SNPs in gene expression, protein level, and protein activity, justifies the requirement to elucidate these effects in

future studies. Furthermore, investigating the association between *TSLP* polymorphisms and the patient's response to therapies in asthma and other inflammatory lung diseases in future studies could pave the way towards personalized medicine for the management of such diseases.

In conclusion, our study has shown a significant inverse association between rs2289276 SNP of the *TSLP* gene and the risk of asthma among the Iranian population. The protective phenotype of this SNP was more pronounced in the female population.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Mukherjee AB, Zhang Z. Allergic asthma: influence of genetic and environmental factors. J Biol Chem. 2011;286(38):32883-9.
2. Braman, SS. The global burden of asthma. Chest. 2006;130(1 Suppl):4s-12s.
3. Fazlollahi MR, Najmi M, Fallahnezhad M, Sabetkish N, Kazemnejad A, Bidad K, et al. The prevalence of asthma in Iranian adults: The first national survey and the most recent updates. Clin Respir J. 2018;12(5):1872-81.
4. Liu YJ. TSLP in epithelial cell and dendritic cell cross talk. Adv Immunol. 2009;101:1-25.
5. Tsilingiri K, Fornasa G, Rescigno M. Thymic Stromal Lymphopoietin: To Cut a Long Story Short. Cell Mol Gastroenterol Hepatol. 2017;3(2):174-82.
6. Wong CK, Hu S, Cheung PF, Lam CW. Thymic stromal lymphopoietin induces chemotactic and prosurvival effects in eosinophils: implications in allergic inflammation. Am J Respir Cell Mol Biol. 2010;43(3):305-15.
7. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol. 2002;3(7):673-80.
8. Birben E, Sahiner UM, Karaaslan C, Yavuz TS, Cosgun E, Kalayci O, et al. The Genetic Variants of Thymic Stromal Lymphopoietin Protein in Children with Asthma and Allergic Rhinitis. Int Arch Allergy Immunol. 2014;163(3):185-92.
9. Elmaraghy MA, Hodieb MM, Khattab RAER, Abdelgalel MN. Association between TSLP gene polymorphism and bronchial asthma in children in Beni Suef Governorate in Egypt. Comp Clin Path. 2018;27(3):565-70.
10. Hunninghake GM, Soto-Quiros ME, Avila L, Kim HP, Lasky-Su J, Rafaels N, et al. TSLP polymorphisms are associated with asthma in a sex-specific fashion. Allergy. 2010;65(12):1566-75.
11. Harada M, Hirota T, Jodo AI, Hitomi Y, Sakashita M, Tsunoda T, et al. Thymic stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. Am J Respir Cell Mol Biol. 2011;44(6):787-93.
12. Liu M, Rogers L, Cheng Q, Shao Y, Fernandez-Beros ME, Hirschhorn JN, et al. Genetic variants of TSLP and asthma in an admixed urban population. PLoS ONE. 2011;6(9):e25099.
13. Liu W, Xu LS, Liu QJ, Dong FZ, Qiu RF, Wen MC, et al. Two single nucleotide polymorphisms in TSLP gene are associated with asthma susceptibility in Chinese Han population. Exp Lung Res. 2012;38(8):375-82.
14. Saba N, Raja GK, Yusuf O, Rehman S, Munir S, Mansoor A. Impacts of different cytokine and chemokine polymorphisms in Pakistani asthmatics a case control study. COPD Research and Practice. 2017;3(1):8.
15. Weber WW. Populations and genetic polymorphisms. Mol Diagn. 1999;4(4):299-307.
16. Mete F, Ozkaya E, Aras S, Koksal V, Etlik O, Baris I. Association between gene polymorphisms in TIM1, TSLP, IL18R1 and childhood asthma in Turkish population. Int J Clin Exp Med. 2014;7(4):1071-7.
17. Heinrich J, Richter K, Frye C, Meyer I, Wölke G, Wjst M, et al. European Community Respiratory Health Survey (ECRHS). Pneumologie. 2002;56(5):297-303.
18. Matloubi M, Ranjbar M, Assarehzadegan MA, Fallahpour M, Sadeghi F, Soleiman-Jahi S, et al. The Impact of Interleukin (IL)-33 Gene Polymorphisms and Environmental Factors on Risk of Asthma in the Iranian Population. Lung. 2020;198(1):105-112.
19. Le Moual N, Zock J-P, Dumas O, Lytras T, Andersson E, Lillienberg L, et al. Update of an occupational asthma-specific job exposure matrix to assess exposure to 30 specific agents. Occup Environ Med. 2018;75(7):507-14.
20. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A Large-Scale, Consortium-Based

Genomewide Association Study of Asthma. N Engl J Med. 2010;363(13):1211-21.
21. Heinzerling L, Mari A, Bergmann K-C, Bresciani M, Burbach G, Darsow U, et al. The skin prick test – European standards. Clin Transl Allergy. 2013;3(1):3.
22. Assarehzadegan MA SH, Amini A. Sensitization to Common Aeroallergens among Asthmatic Patients in a Tropical Region Affected by Dust Storm. J Med Sci. 2013;13(7):592-7.
23. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36.
24. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J. 2008;31(1):143-78.
25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.
26. Harada M, Hirota T, Jodo A, Doi S, Kameda M, Fujita K, et al. Functional analysis of the thymic stromal lymphopoietin variants in human bronchial epithelial cells. Am J Respir Cell Mol Biol. 2009;40(3):368-74.
27. Williams T, Admon A, Luscher B, Tjian R. Cloning and expression of AP-2, a cell-type-specific transcription factor that activates inducible enhancer elements. Genes Dev. 1988;2(12a):1557-69.
28. Keselman, A, Heller N. Estrogen Signaling Modulates Allergic Inflammation and Contributes to Sex Differences in Asthma. Front Immunol. 2015;6:568.
29. Yung JA, Fuseini H, Newcomb DC. Hormones, sex, and asthma. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2018;120(5):488-94.
30. Cook EB, Stahl JL, Schwantes EA, Fox KE, Mathur SK. IL-3 and TNFα increase Thymic Stromal Lymphopoietin Receptor (TSLPR) expression on eosinophils and enhance TSLP-stimulated degranulation. Clin Mol Allergy. 2012;10(1):8.