IL1RL1 single nucleotide polymorphisms are associated with asthma in the Iranian population

Maral Ranjbar[1], Mojdeh Matloubi[1], Shaghayegh Sadegh[1], Morteza Fallahpour[2,3], Leila Janani[4], Mohammad-Ali Assarehzadegan[1,5]

[1]Department of Immunology, School of Medicine, Iran University of Medical Science, Tehran; [2]Department of Allergy, Rasool-e-Akram Hospital, Iran University of Medical Sciences, Tehran; [3]Firoozabadi Hospital, Iran University of Medical Sciences, Tehran; [4]Department of Biostatistics, School of Public Health, Iran University of Medical Science, Tehran; [5]Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran

Correspondence: Mohammad-Ali Assarehzadegan, PhD, Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, and Department of Immunology, School of Medicine, Iran University of Medical Science, Hemmat Highway, 1449614535 Tehran, Iran.
Tel. +98.21.86703264.
E-mail: assarehma@gmail.com; assareh.ma@iums.ac.ir

Key words: Asthma; atopic; single nucleotide polymorphism; interleukin 1 receptor-like 1.

Contributions: MAM, MR, MM, SS, concept and design; MR, MM, MF, SS, data collection and/or processing; MR, MM, LJ, analysis and/or interpretation; MR, manuscript drafting. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: The authors declare no conflicts of interest.

Ethics approval and consent to participate: This study has been approved by the Ethics Review Committee of the Iran University of Medical Sciences (ID: IR.IUMS.REC1396.30759). Written informed consent was obtained from all participants.

Acknowledgments: The current study was supported by a grant (Grant Number: 96-01-138-30759) from Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Science, Tehran, Iran.

Received for publication: 24 November 2020.
Accepted for publication: 20 December 2021.

Licensee PAGEPress, Italy
Monaldi Archives for Chest Disease 2022; 92:1697
doi: 10.4081/monaldi.2021.1697

Abstract

Asthma is a chronic and multifactorial disease which is known to result from environmental and genetic factors. Interleukin 1 receptor-like 1 (IL1RL1) is a receptor, which promotes inflammatory responses after binding to its ligand IL-33. Several studies have shown that IL1RL1 gene polymorphisms are related to susceptibility or protection to asthma. The objective of this study was to evaluate the association between two IL1RL1 single nucleotide polymorphisms (rs10208293 and rs1041973) and the risk of asthma in the Iranian population. We performed genotyping of the IL1RL1 SNPs in 126 adult asthmatics and 300 healthy controls using TaqMan genotyping assay. Moreover, total serum IgE level, eosinophil count, and skin prick test were accomplished. The results indicated that the AA genotype of rs10208293 was positively associated with asthma susceptibility (p=0.028). We did not find any association between rs1041973 and asthma. Overall, our findings indicate that rs10208293 has a positive association with asthma in the Iranian population.

Introduction

Asthma is a chronic disease which is characterized by symptoms such as shortness of breath, chest tightness, and wheezing [1]. It is estimated that over 300 million people are suffering from this complex disease [2] and its prevalence is increasing among developing countries including Iran [3]. Asthma is a multifactorial disease and results from both environmental and genetic factors. It is shown that several gene polymorphisms have a direct impact on susceptibility to asthma [4].

Interleukin 1 receptor-like 1 (IL1RL1) also known as [ST2 (suppression of tumorigenicity 2)], is a receptor belonging to the IL-1 family. By binding to its ligand (IL-33), IL1RL1 plays an important role in development of inflammatory pathways and the production of type 2 cytokines, which consequently results in a variety of inflammatory associated diseases such as asthma [5, 6]. The IL1RL1 gene is located in the 2q12 chromosome. There are Four known isoforms of IL1RL1 in the human body, which are as a result of alternative splicing and post transcription process on IL1RL1 mRNA [7,8]. The two most important isoforms of IL1RL1 are [ST2 ligand (ST2L)] and [soluble ST2 (sST2)].

ST2L is a transmembrane form of IL1RL1 and is expressed on

various inflammatory cells including mast cells, [T helper 2 cells (Th2)], basophils, and eosinophils. While the bonding of ST2L and its ligand IL-33 promotes inflammatory responses, sST2 acts as a decoy receptor as it is a soluble isoform. Therefore, it downregulates the inflammation by binding to excess IL-33 and preventing it to bind to ST2L on cell surfaces [9-11].

[Single nucleotide polymorphisms (SNP)] are common variations in genes where one single nucleotide is replaced by another nucleotide which can happen in either coding or non-coding region of the genes and have remarkable impacts on the gene expression, protein production or even responses to different medications in patients [12]. To this date, several studies have shown the association of single nucleotide polymorphisms in the IL1RL1 gene and asthma in different populations [13-23].

The rs10208293 is an SNP located in the intron region of the IL1RL1 gene. In this SNP, the G allele is replaced by A (minor) allele [19]. Studies published on the relation of this SNP and asthma have demonstrated a negative association [21, 22]. The rs1041973 SNP is a missense variant located in the coding region of IL1RL1. The C allele is replaced by the A (minor) allele in this SNP [20]. As gene polymorphism studies strictly depend on racial or ethnic characteristics of a population [24], the evaluation of polymorphisms needs to be carried out in various parts of the world. In this study, we aimed to analyze the association of two commonly studied single nucleotide polymorphisms (rs10208293 and rs1041973) of the IL1RL1 gene and asthma in the Iranian population.

Materials and Methods

Subjects

In this case-control study, we recruited 126 confirmed asthmatic patients and 300 healthy subjects aged 18-64 years from Firoozabadi Hospital, Tehran, Iran from 2018 to 2019. All subjects were referred to Firoozabadi hospital from different cities of Iran. The two groups were matched based on sex and age. The inclusion and exclusion criteria for patients and controls are based on our previous paper [25]. The participants completed a demographic questionnaire designed based on the European community respiratory health survey and [Behavioral Risk Factor Surveillance System (BRFSS)], which is also used by our former paper [26]. Written informed consent was obtained from all participants. This study was approved by the Ethics Review Committee of the Iran University of Medical Sciences (ID: IR.IUMS.REC1396.30759).

Spirometry and allergic testing

Spirometry test was performed for patients based on American Thoracic Society guidelines [27]. Based on the data obtained from spirometry test and GINA guidelines [28], we categorized patients into three groups of mild (FEV_1 ³80), moderate (60<FEV_1<80), and severe (FEV_1 £60) asthmatics.

Aeroallergens common to Iran [29], including *Russian thistle*, tree mix, grass mix, weed mix, mite mix, cockroach, Mugwort, and mold mix were used for skin prick testing by European standards [30]. Patients were classified into atopic and non-atopic groups based on skin prick test results.

Total serum IgE levels were measured in both patients and the healthy control group by ELISA kit (EUROIMMUN AG, Lübeck, Germany). Moreover, patients were classified into childhood-onset (younger than 16 years old) and later-onset (16 years old and older) according to their age of asthma onset [15].

Genotyping

Five milliliters of whole blood was collected from all participants. Genomic DNA was extracted using the salting-out method [31]. The quality of DNA was confirmed by the NanoDrop 2000c spectrophotometer. We performed genotyping assay for determining two single nucleotide polymorphisms of the IL1RL1 gene (rs1041973 and rs10208293) using TaqMan genotyping assay kit (TaqMan® SNP genotyping assay kit, Applied Biosystems. Foster City, CA, USA).

Statistical analysis

Chi-square or fisher's exact test was used to analyze the association between categorical parameters. Independent *t*-test or its non-parametric counter-part, Mann-Whitney U test, was utilized for comparing numerical parameters between two subgroups of categorical parameters. Binary and multinomial logistic regressions were used to assess the adjusted correlation between IL1RL1 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). Two-tailed p-value <0.05 was considered statistically significant. The analysis was conducted using IBM SPSS advanced statistics (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, v. 24.0. IBM Corp , Armonk, NY, USA).

Results

Demographic characteristics

The mean (±SD) age of patients and healthy controls were 35.08 (±10.49) and 36.00 (±11.21), respectively (p=0.188). The frequency of males in patients and healthy controls were 25.4% and 22.0%, respectively (p=0.447). There was a significant association between risk of asthma and job status (p<0.001), smoking status (p<0.001), kindergarten attendance (p= 0.016), and types of elementary school (p=0.012). Moreover, serum IgE level and eosinophil count had an expectedly significant association with asthma (Table 1). we did not find any significant associations between other confounding factors, including age, sex, BMI, education level, place of birth, type of delivery, neonatal nutrition, exposure to pets, and risk of asthma (data not shown).

Association of IL1RL1 polymorphisms and risk of asthma

The results of the analysis of IL1RL1 gene polymorphisms and the risk of asthma are shown in Table 2. According to the table, the frequency of genotype (AA) of SNP rs10208293 in the case group was more pronounced than its frequency in the controls (23.8% *vs* 14.0%). There was a significant association between AA genotype of rs10208293 and asthma (OR=1.834, Cl=1.069-3.147, p=0.028). However, we could not find any significant correlation between the genotypes of rs1041973 and asthma.

Association of IL1RL1 polymorphisms with atopy, severity, and onset of asthma

We observed that the AA genotype of rs10208293 is more prevalent in non-atopic patients. Although not statistically significant, this genotype showed an inverse association with non-atopic asthma with OR <1.00. The frequency of the AA genotype of rs1041973 was slightly higher in the non-atopic group; however, no significant correlation was observed (Table 3).

There was no significant association between both SNPs and mild, moderate, and severe asthma (*data not shown*). Furthermore, we could not find any statistically significant association among childhood-onset asthma, later-onset asthma, and selected IL1RL1 SNPs (*data not shown*).

Discussion

In this study, we investigated the association between two commonly studied single nucleotide polymorphisms (rs10208293

Table 1. General characteristics of the study population.

Characteristics		Asthmatics n=126 (%)	Control subjects n=300 (%)	p-value
Age	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%)	
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%)	**0.000**[b]
	High risk	41 (32.5%)	3 (1.1%)	
Smoking status	Smoker	11 (8.7%)	7 (2.6%)	**0.000**[b]
	Expose to smoke	57 (45.2%)	42 (15.8%)	
	None	58 (46.0%)	216 (81.5%)	
Serum IgE	Mean	312.42	171.58	**0.000**[c]
EOS count	Mean ±SD	4.639±2.32	1.216±0.61	**0.000**[a]
FVC	Mean (min-max)	79.13 (36-156)	-	-
FEV_1	Mean (min-max)	72.55 (30-126)	-	-
FEV_1/FVC	Mean (min-max)	89.45 (56-124)	-	-
Skin prick test	Positive	64 (50.79)	-	-
	Negative	62 (49.20)	-	-

EOS, eosinophil; [a]independent *t*-test; [b]Pearson Chi-Square; [c]nonparametric tests (Mann-Whitney U Test); FVC, forced vitale capacity; FEV_1, forced expiratory volume in 1 second. Bold type indicates significant association.

Table 2. Relation between SNPs of IL1RL1 and asthma.

IL1RL1 SNPs	Genotype and alleles	Population		Logistic regression	
		Asthmatics n=126 (%)	Control subjects n=300 (%)	OR (95% CI)	p-value
rs10208293	GG1	74 (58.7%)	190 (63.3%)	1.00	-
	AG	22 (17.5%)	68 (22.7%)	0.831 (0.479-1.441)	0.509
	AA	30 (23.8%)	42 (14.0%)	**1.834 (1.069-3.147)**	**0.028**
rs1041973	CC*	51 (40.5%)	99 (33.0%)	1.00	-
	AC	58 (46.0%)	168 (56.0%)	0.670 (0.427-1.052)	0.082
	AA	17 (13.5%)	33 (11.0%)	1.00 (0.509-1.965)	1.000

*Reference; OR, odds ratio; CI, confidence interval; logistic regression analysis adjusted by job and smoking status, kindergarten, and elementary school. Bold type indicates statistically significant results.

Table 3. Relation between SNPs of IL1RL1 and atopic/non-atopic asthma.

IL1RL1 SNPs	Genotype	Population			Logistic regression			
		Control subjects n=300 (%)	Atopic n=64 (%)	Non-atopic n=62 (%)	Atopic asthma OR (95% CI)	p-value	Non-atopic asthma OR (95% CI)	p-value
rs10208293	GG1	190 (63.3%)	40 (62.5%)	34 (54.8%)	1.00	-	1.00	-
	AG	68 (22.7%)	12 (18.8%)	10 (16.1%)	1.265 (0.804-1.990)	0.310	1.685 (0.667-4.256)	0.269
	AA	42 (14.0%)	12 (18.8%)	18 (29.0%)	0.825 (0.525-1.297)	0.405	0.492 (0.228-1.060)	0.070
rs1041973	CC*	99 (33.0%)	28 (45.8%)	23 (37.1%)	1.00	-	1.00	-
	AC	168 (56.0%)	29 (45.3%)	29 (46.8%)	1.320 (0.923-1.888)	0.129	0.1.702 (0.851-3.406)	0.133
	AA	33 (11.0%)	7 (10.9%)	10 (16.1%)	1.358 (0.711-2.592)	0.354	0.938 (0.341-2.577)	0.901

*Reference; OR, odds ratio; CI, confidence interval; logistic regression analysis adjusted by job and smoking status, kindergarten, and elementary school.

and rs1041973) of IL1RL1 gene and asthma. The reason for choosing these two specific SNPs was to make comparisons between the results of other studies conducted in other part of the world and our data. We demonstrated that there is a significant association between rs10208293 and asthma. This association is in a positive manner. However, we did not find any significant association between rs1041973 and asthma despite its adequate frequency in both case and control groups. By designing a questionnaire and eliminating the confounding factors of asthma diseases, we also confirmed that there is a relationship between risk of asthma and job, smoking status, going to kindergarten, and type of elementary school.

Our study showed that the AA genotype of SNP rs10208293 has an association with the risk of asthma. In other words, individuals who carry this genotype are more susceptible to asthma compared to those who do not have this polymorphism. One possible explanation for this positive association is that the AA genotype of rs10208293 might increase the expression of ST2L on the surface of immune cells, which in turn leads to elevated production of inflammatory cytokines and asthma development. Further experiments are needed to confirm this hypothesis.

Among several studies that have investigated the association of IL1RL1 gene polymorphisms and asthma, we found only one study that has conducted research on the correlation of rs10208293 and asthma, the result of which is in contrast with ours. Savenije *et al*. has reported that rs10208293 has an inverse association with late-onset wheezing in childhood asthma [19]. The possible reason for these contradicting results may be the race and age differences between the studies. As it is proven that genetic variations have distinct impacts among ethnic groups [24], it is likely to assume that a single nucleotide polymorphism has a protective manner towards asthma in the Dutch population [19] whereas it is positively associated with asthma in the Iranian population.

The conflicting results between our study and studies carried out in other ethnic groups are also observed in the second SNP (rs1041973). In this study, we concluded that rs1041973 had no significant relation with the risk of asthma. In fact, the frequency of AA genotype was distributed almost equally between two groups of patients and healthy controls (13.5% and 11.0%, respectively). While we failed to find any significant association between rs1041973 and risk of asthma, Wu et al. demonstrated that this SNP is negatively associated with childhood asthma in Mexican population [20]. The same inverse fashion was also observed by Savenije *et al.* in the Dutch children [21]. Although not statistically significant, the AC genotype of rs1041973 showed a similar negative association with asthma. This negative fashion may be as a result of A allele, which decreases the possibility of developing asthma in those who are a carrier of this allele. Moreover, one study done in the Dutch population by Reijmerink *et al.* could not find any significant association between the mentioned SNP and asthma [22].

In this research, we investigated the association between IL1RL1 polymorphisms and atopic and non-atopic asthma. Although the AA genotype of rs10208293 was more prevalent in non-atopic patients, we could not detect any significant association between this genotype and non-atopic asthma. In general, around 55 percent of the genotype frequency belonged to the GG genotype of rs10208293 in both atopic and non-atopic groups. Furthermore, similar studies which have examined the relation of polymorphisms and atopy were also unable to detect any significant correlation [22,23]. We conducted a statistical analysis to search for any possible association between the studied polymorphisms and the severity as well as childhood/adulthood onset of asthma. However, due to similar frequency of genotypes in both case and healthy control group, we were not able to reach significance in our analysis (data not shown).

The findings of this study elucidated the undeniable impact of ethnicity on multifactorial diseases such as asthma. It may not be prudent to assume that a distinct polymorphism will have the same effects on the pathological and physiological aspects of a disease in different individuals. As it is shown in this study, the result of polymorphism studies is crucially dependent on the geographical area and the race of the study population. Although polymorphism studies are promising for developing personalized medicine, it should be kept in mind that the outcomes are mostly limited to the study population.

A rather small study population was a limitation to this study. Larger sample size would have allowed us to achieve statistical significance in our analysis. Moreover, gene expression and protein measurements of IL1RL1 and its ligand IL-33 would provide a comprehensive view of the function of the polymorphisms.

Conclusions

Our study demonstrated a positive association between IL1RL1 gene SNP rs10208293 and the risk of asthma in Iranian the population.

References

1. Mims JW. Asthma: definitions and pathophysiology. Int Forum Allergy Rhinol 2015;5:S2-6.
2. Masoli M, Fabian D, Holt S, et al. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004;59:469-78.
3. Fazlollahi MR, Najmi M, Fallahnezhad M, et al. The prevalence of asthma in Iranian adults: The first national survey and the most recent updates. Clin Respir J 2018;12:1872-81.
4. March ME, Sleiman PM, Hakonarson H. Genetic polymorphisms and associated susceptibility to asthma. Int J Gen Med 2013;6:253-65.
5. Murphy GE, Xu D, Liew FY, et al. Role of interleukin 33 in human immunopathology. Ann Rheum Dis 2010;69:i43-47.
6. Kumar S, Minnich MD, Young PR. ST2/T1 protein functionally binds to two secreted proteins from Balb/c 3T3 and human umbilical vein endothelial cells but does not bind interleukin 1. J Biol Chem 1995;270:27905-13.
7. Griesenauer B, Paczesny S. The ST2/IL-33 axis in immune cells during inflammatory diseases. Front Immunol 2017; 8:475.
8. Iwahana H, Yanagisawa K, Ito-Kosaka A, et al. Different promoter usage and multiple transcription initiation sites of the interleukin-1 receptor-related human ST2 gene in UT-7 and TM12 cells. Eur J Biochem 1999;264:397-406.
9. Ciccone MM,Cortese F, Gesualdo M, et al. A novel cardiac bio-marker: ST2: a review. Molecules 2013;18:15314-28.
10. Lingel A, Weiss TM, Niebuhr M, et al. Structure of IL-33 and its interaction with the ST2 and IL-1RAcP receptors--insight into heterotrimeric IL-1 signaling complexes. Structure 2009;17:1398-410.
11. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related pro-

tein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;23:479-90.
12. Marth GT, Korf I, Yandell MD, et al. A general approach to single-nucleotide polymorphism discovery. Nat Genet 1999;23:452-6.
13. Queiroz GA, Costa RS, Alcantara-Neves NM, et al. IL33 and IL1RL1 variants are associated with asthma and atopy in a Brazilian population. Int J Immunogenet 2017;44:51-61.
14. Akhabir L, Sandford A. Genetics of interleukin 1 receptor-like 1 in immune and inflammatory diseases. Curr Genomics 2010;11:591-606.
15. Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211-21.
16. Torgerson DG, Ampleford EJ, Chiu GY, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. Nat Genet 2011;43:887-92.
17. Grotenboer NS, Ketelaar ME, Koppelman GE, et al. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. J Allergy Clin Immunol 2013;131: 856-65.
18. Zhang G, Lu J, Yin X, et al. Association of IL-33, IL1RL1 gene polymorphisms with serum IL-33 levels and risk of asthma in adults and asthmatic bronchitis in children (Chinese). Biotechnol Biotechnol Equip 2018;32:1251-6.
19. Savenije OE, Mahachie John JM, Granell R, et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. J Allergy Clin Immunol 2014;134:170-7.
20. Wu H, Romieu I, Shi M, et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. J Allergy Clin Immunol 2010;125:321-7.e13.
21. Savenije OE, Kerkhof M, Reijmerink NE, et al. Interleukin-1 receptor-like 1 polymorphisms are associated with serum IL1RL1-a, eosinophils, and asthma in childhood. J Allergy Clin Immunol 2011;127:750-6.e1-5.
22. Reijmerink NE, Postma DS, Bruinenberg M, et al. Association of IL1RL1, IL18R1, and IL18RAP gene cluster polymorphisms with asthma and atopy. J Allergy Clin Immunol 2008;122:651-4.e8.
23. Chen J, Zhang J, Hu H, et al. Polymorphisms of RAD50, IL33 and IL1RL1 are associated with atopic asthma in Chinese population. Tissue Antigens 2015;86:443-7.
24. Weber WW. Populations and genetic polymorphisms. Mol Diagn 1999;4:299-307.
25. Ranjbar M, Matloubi M, Assarehzadegan MA, et al. Association between two single nucleotide polymorphisms of thymic stromal lymphopoietin (TSLP) gene and asthma in Iranian population. Iran J Allergy Asthma Immunol 2020;19:362-72.
26. Matloubi M, Ranjbar M, Assarehzadegan MA, et al. The impact of interleukin (IL)-33 gene polymorphisms and environmental factors on risk of asthma in the Iranian population. Lung 2020;198:105-12.
27. [No authors listed]. Standardization of spirometry, 1994 update. American Thoracic Society. Am J Respir Crit Care Med 1995;152:1107-36.
28. Bateman ED, Hurd SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008;31:143-78.
29. Assarehzadegan MA, Amini SA. Sensitization to common aeroallergens among asthmatic patients in a tropical region affected by dust storm. J Med Sci 2013;13:592-7.
30. Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test - European standards. Clin Transl Allergy 2013;3:3.
31. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.