



Association of *TSLP* Gene's SNP Variants with Asthma Disease in Pakistan

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ABSTRACT

Asthma is the most common chronic inflammatory disease characterized by paroxysms of dyspnea, frequent wheezing, chest tightness, and cough that varies in intensity from person to person. Thymic stromal lymphopoietin (*TSLP*) is a cytokine residing within the cytokine gene cluster, on locus 5q22.1, that is involved in inflammation and thus plays main role in the pathogenesis of asthma and allergic diseases. The purpose of this study was to identify the potential asthma risk single nucleotide variants (SNVs) of the *TSLP* gene reported in genome-wide association studies (GWAS) and evaluate the most potential asthma risk SNP in the local Punjabi population of Lahore. GWAS catalog indicated eleven single nucleotide polymorphisms (SNPs) which are significantly associated ($p\text{-value} \geq 4 \times 10^{-6}$) with asthma disease. Out of 11 SNPs, rs1837253 (T/C) was considered the most potential asthma risk variant ($p\text{-value} \geq 4 \times 10^{-6}$) as it was reported in 44 GWAS. A total of 97 asthma patients and 53 healthy controls were recruited for the case-control study. The target region containing the selected SNP rs1837253 (T/C) was PCR amplified and capillary-based sequencing was performed on Genetic Analyzer (3130XL). The statistical analyses were performed using SHESis and SNPStat softwares. The statistical analysis predicted that rs1837253 SNP is significantly associated with asthma disease at the allelic level ($p\text{-value} 0.038$), however, "T" allele (Odds ratio 0.579) is a protective factor against asthma. The genotypic analysis also predicted a negatively significant association under the dominant inheritance model as the best-fit model (lowest AIC, $p\text{-value} 0.04$, Odds ratio 0.49), which showed that the persons with both homozygous "TT" and heterozygous "CT" genotypes are less susceptible to asthma disease in the studied population. This study reports the significant association of rs1837253 SNP of the *TSLP* gene with asthma, however, its allele "T" plays a protective role against asthma susceptibility in the Punjabi population of Lahore, Pakistan.

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Authors' Contribution

ZM and IK designed the study design and experiments. ZM wrote the manuscript. IB did clinical evaluation of patients and data analysis. MUG, MFS, MUK presented concept, supervised the study and edited the manuscript. HMR and MH evaluated the data. SA and AM conducted the experiments. IA, MS did data analysis, proofreading and editing.

Key words

Asthma, GWAS, Population study, Pakistan, *TSLP*

INTRODUCTION

Asthma is a complex, heterogeneous disorder categorized as bronchoconstriction and inflammation of the airways. There is an overproduction of mast cells, helper T-lymphocytes, and eosinophils. These cells release

inflammatory cytokines which cause; the constriction of airways, increased mucus production, and bronchial structural changes (Hamid and Tulic, 2009; Sabar *et al.*, 2018). It is estimated that asthma caused 455,000 deaths in 2019 and about 262 million people suffer from this disease worldwide with an increasing rate in underdeveloped countries (Kumari, 2022). Asthma affects more than six million individuals in Pakistan, with 32% of the asthmatic population being children (Dar *et al.*, 2017; Ghani *et al.*, 2017). Asthma is caused by a combination of hereditary and environmental factors. The illness is mostly caused by genetic factors that are activated by environmental events (Asher, 2011; MoF, 2010). There are more than 200 known genes that have a role in the development of asthma across the globe (Naeem *et al.*, 2020; Shahid *et al.*, 2015). The thymic stromal lymphopoietin (*TSLP*) gene is one of

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the potent asthma-susceptible genes located on 5q22.1. Elevated levels of *TSLP* are observed in the bronchi of asthma patients (Li *et al.*, 2018).

TSLP is a cytokine closely related to the IL-7 family but it has a discrete biological profile. *TSLP* was first isolated from a conditioned medium of thymic stromal cell lines and was shown to promote the maturation and proliferation of B-cells (Lee and Ziegler, 2007). The human orthologue of the *TSLP* gene was first identified using computational analysis techniques (Reche *et al.*, 2001) and is present on locus 5q22.1. It has two isoforms, long and short isoforms, made up of 159 amino acids and 60 amino acids respectively, which are produced as a result of alternative splicing in the promoter region (Landheer and Carla, 2015). Asthma phenotype is greatly influenced by the isoforms of *TSLP* in distinct ways. But majorly, the long pro-inflammatory isoform (*TSLP*-lf) (Afzal *et al.*, 2020) is studied by most researchers as compared to the lesser-known short isoform (*TSLP*-sf). *TSLP* is involved in both homeostasis and disease susceptibility (Friend *et al.*, 1994; Pandey *et al.*, 2000; Park *et al.*, 2000). It is largely expressed by epithelial cell lining in the skin, gastrointestinal tract, and lungs in the steady state; but, during inflammatory circumstances, numerous other types of cells, including airway smooth muscle cells and alveolar fibroblasts, also release *TSLP* (Rimoldi *et al.*, 2005; Soumelis *et al.*, 2002).

In inflammatory diseases such as asthma and atopic dermatitis, the synthesis of *TSLP* is dysregulated (Ferreira *et al.*, 2012). It has been demonstrated that the amount of *TSLP* expression in asthmatics correlates with bronchoconstriction and illness severity, and is believed that *TSLP* drives various elements of asthma pathophysiology like bronchoconstriction, airway hyper-responsiveness, and increased mucus production via its downstream, pro-inflammatory effects involving cytokines such as IL-4, IL-5, and IL-13 (Li *et al.*, 2018). Genome-wide association studies help us understand the role of *TSLP* single nucleotide polymorphism in different disorders. GWAS has also identified the link between single nucleotide polymorphism in the *TSLP* gene and asthma susceptibility (Gauvreau *et al.*, 2020).

rs1837253 is located in the promoter region of *TSLP*, 5.7 kb upstream of the transcription start site and has been identified as an important genetic variant in *TSLP* gene regulation by genome-wide association studies. There is a variable association of this genetic variant in different ethnic populations. It shows protective as well as increased risk association in different studies (Afzal *et al.*, 2020). SNP variations on genes provide protective benefits for some, whereas others are directly or indirectly implicated in the development of illness (Sabar *et al.*, 2016; Usman

Ghani *et al.*, 2021). The association of the genomic variant rs1837253 of the *TSLP* gene with asthma disease has been studied widely in different ethnic populations but it is rarely studied in Pakistan and especially no published research is available on the Punjabi population, which is the largest sub-ethnic population of Pakistan. This case-control study explains the association of the *TSLP* gene's SNP rs1837253 with asthma exacerbations in the Punjabi population of Lahore, Pakistan.

MATERIALS AND METHODS

In silico retrieval of SNVs

The single nucleotide variants (SNVs) of the *TSLP* gene which are significantly associated ($p\text{-value} \leq 4 \times 10^{-6}$) with asthma disease and reported in genome-wide association studies (GWAS) were retrieved from the GWAS catalog (<https://www.ebi.ac.uk/gwas/>). The GWAS catalog is an online tool that contains a catalog of human genome-wide association studies (Supplementary Table I).

SNP selection

Based on the findings of multiple GWAS, the rs1827253 SNP of the *TSLP* gene was considered a highly potent asthma-susceptible SNP variant and studied its association with asthma disease in local Punjabi population of Lahore.

Enrollment of study participants (cases/controls)

A total of 97 asthma patients and 53 controls were selected for this case-control study. The asthma patients as case subjects were enrolled from Children's Hospital Lahore, Pakistan. All the patients were clinically diagnosed with asthma by a pediatric physician. The healthy study participants as control subjects were randomly enrolled from the general Punjabi population of Lahore-Punjab, Pakistan. Written informed consent was obtained from all the study participants or their guardians before drawing blood from the subjects. A detailed questionnaire was also duly filled from study participants. The study was approved by the ethical review board of The Children's Hospital, Lahore. The case subjects were randomly selected irrespective of gender restrictions. Only those subjects were selected who had a reported asthma history or had been diagnosed with asthma by a physician. The following factors were considered for inclusion; (a) shortness of breath (b) chest tightness which requires a follow up (c) wheezing (d) difficulty in breathing and repeated respiratory inconsistency. Any subject with a history of bronchitis, tuberculosis, pneumonia, emphysema, or smokers were excluded from this study. Non-asthmatic controls sample were collected from the same ethnic group (Punjabi) and

no control was selected with any family history of asthma, allergies, HIV, and smoking. 2-3 ml blood samples of study subjects were drawn by the standard operating procedure. Each control and patient sample was given an id according to the protocol and samples were stored at -4 °C.

DNA extraction and sequencing

The genomic DNA was extracted from the blood samples through phenol/chloroform/Isoamyl alcohol as described previously (Sabar *et al.*, 2017). The SNP alleles were determined by modified DNA sequencing (capillary electrophoresis) assay (Ghani *et al.*, 2022). For DNA sequencing, the desired genomic region containing the

target SNP was PCR amplified by a primers pair in a 20 µl PCR reaction mix (Table I). The PCR amplicons were purified with magnetic beads (High Prep™ PCR kit) and purified PCR amplicons were used as a template DNA to perform DNA sequencing reaction as described in Table II. The Sequencing PCR reaction amplicons (product) were purified by ethanol precipitation method followed by analysis on automated Genetic Analyzer 3130XL (Applied Biosystems, USA). The sequencing results were interpreted using Sequencing Analysis software v.5.1 (Applied Biosystem, USA) to determine the alleles of SNPs.

Table I. Potential asthma susceptible SNVs of *TSLP* gene reported on GWAS catalog.

rs ID of SNVs	Chr. position	p-value range (as reported on GWAS catalog)	Type of the variant	Mapped gene	Associated disease (GWAS Report)
rs1837253	5:111066174	7×10^{-94} to 4×10^{-6} (Supplementary I)	intergenic variant	<i>TSLP</i>	Asthma (childhood onset)
rs10455025	5:111069301	9×10^{-26} to 2×10^{-21} (Supplementary I)	intergenic variant	<i>TSLP</i>	Eosinophil counts
rs1898671	5:111072304	1×10^{-38} to 8×10^{-10} (Supplementary I)	intron variant	<i>TSLP</i>	Asthma
rs3806933	5:111071044	3×10^{-26} 1×10^{-14} (Supplementary I)	5 prime UTR variant	<i>TSLP</i>	Asthma
rs252716	5:111089365	4×10^{-9} (Supplementary I)	intron variant	<i>I.1</i>	Asthma
rs58743367	5:111052064	2×10^{-13}	intergenic variant	<i>TSLP</i>	Atopic asthma
rs2289276	5:111071809	2×10^{-11}	5 prime UTR variant	<i>TSLP</i>	Atopic asthma
rs62375550	5:111051427	6×10^{-9} and 3×10^{-9}	intergenic variant	<i>TSLP</i>	Asthma
rs2289278	5:111073450	8×10^{-12} and 2×10^{-8}	5 prime UTR variant	<i>TSLP</i>	Asthma (childhood onset)
rs149096812	5:111082543	2×10^{-8}	Intron Variant	<i>TSLP</i>	Asthma
rs10061842	5:111067157	5×10^{-8}	intergenic variant	<i>TSLP</i>	Atopic asthma

Table II. Association of rs1837253 with asthma under allelic model.

SNP ID	Allele	Participants (Frequency)		p-Value	Odds ratio (CI 95%)
		Case	Control		
rs1837253	C	148(0.763)	69(0.663)	0.038	0.579620 (0.345070 ~ 0.973598)
	T	46(0.237)	37(0.349)		

Statistical analysis

An online version of SHEsis (<http://analysis.bio-x.cn/myAnalysis.php>) was used for allelic analysis (Yong and He, 2005), whereas, SNPStat: (<https://www.snptest.net/preproc.php>), an online tool for the analysis of single nucleotide polymorphism (Solé *et al.*, 2006), was used for genotypic analysis under different inheritance models. The p-value ≤ 0.05 was considered significant for all models and the odd ratio (OR) was determined under a confidence level of 95%.

RESULTS

SNVs selected using the GWAS catalog

GWAS catalog includes 11 SNVs from 83 different studies (Supplementary Table I). All of the 11 SNVs are from the *TSLP* gene and are associated with asthma disease (Table I). Out of the 11 reported SNVs, rs1837253 is the most studied SNP as it has been reported in 44 GWAS (Supplementary Table I). The p-value for

rs1837253 ranges from 7×10^{-94} to 4×10^{-6} (Supplementary Table I). rs10455025 is mentioned in four studies with p-value ranging from 9×10^{-26} to 2×10^{-21} . rs1898671 is reported in three GWAS studies mentioned in the GWAS catalog having a p-value ranging from 1×10^{-38} to 8×10^{-10} . Three SNVs rs3806933, rs2289278, and rs62375550 each are reported in two GWAS studies with a p-value ranging from 3×10^{-26} to 3×10^{-14} , 8×10^{-12} to 2×10^{-8} , and 6×10^{-9} to 3×10^{-9} , respectively. rs3806933, rs58743367, rs2289276, rs149096812, rs10061842, and rs149096812 are reported in a single GWAS study having p-value 4×10^{-9} , 2×10^{-13} , 2×10^{-11} , 2×10^{-8} , 5×10^{-8} , 2×10^{-8} , respectively (Supplementary Table I).

Allelic and genotypic associations

The major allele “C” has a frequency of 0.763 in the cases and 0.663 in the controls, whereas the minor allele “T” has a frequency of 0.237 in the cases and 0.337 in the control samples of the studied population. A p-value of 0.038 showed a statistically significant association of the “T” allele with asthma disease in the current case-control study. However, the odds ratio 0.579620 [0.345070~0.973598] indicated that the “T” allele of this SNP plays a protective role in asthma susceptibility in the study population (Table II).

Different inheritance models, including codominant, dominant, recessive and log additive models, were generated to check the association of rs1837253 SNP with asthma susceptibility at the genotypic level. The best-fit model was based on the lowest AIC value. The dominant model shows the lowest AIC value (194.6) and was considered the best-fit model for this study. In the dominant model, the heterozygous genotype “CT” and homozygous genotype “TT” predicted significant association (p-value = 0.04) with an odds ratio of 0.49 which indicated the protective roles of both the genotypes

in asthma susceptibility (Table III).

DISCUSSION

Asthma is a complex, heterogeneous respiratory disease that involves airflow obstruction, wheezing, inflammation, airway hyperresponsiveness, cough, and breathlessness. It is caused by genetic factors mostly influenced by environmental factors which play a significant role in the severity of asthma manifestation. More than 200 genes have been reportedly involved in the manifestation of asthma disease. The different studies, especially GWAS, have identified several genomic variants in asthma-susceptible genes which are associated with disease and regulate the expression of their corresponding genes (Naeem, 2022; Sabar *et al.*, 2018, 2020). Several genomic variants have been reported as highly significant in GWAS in large cohorts but lack similar association in replica studies on different ethnicities which are driving forces for researchers to find the true diagnostic and prognostic markers in different ethnic populations (Ghani *et al.*, 2017, 2019a).

TSLP is an important candidate gene for asthma pathogenesis and atopic diseases. Asthma disease is highly influenced by the single nucleotide polymorphisms in the genome (Ullah *et al.*, 2022). In the current study, the Genome-wide Association Studies (GWAS) catalog was explored (<https://www.ebi.ac.uk/gwas/>) which identified 11 SNVs in the *TSLP* gene that were highly significant (p-value $\leq 2 \times 10^{-8}$) to induce asthmatic complications in different populations (Supplementary Table I). The rs1837253 SNP was observed as the top risk asthma susceptible SNP of the *TSLP* as it was reported to be significantly associated with asthma disease in 44 different GWAS with the lowest p-value.

Table III. Association of rs1837253 with asthma under genotypic models.

Genotypic models	Genotype	Case participants (Percentage)	Control participants (Percentage)	p- value	Odds ratio (CI 95 %)	AIC*
Codominant	C/C	59 (60.8%)	23 (44.2%)	0.12	1.00	196.6
	C/T	30 (30.9%)	23 (44.2%)		0.51 (0.25-1.05)	
	T/T	8 (8.2%)	7 (13.2%)		0.45 (0.14-1.37)	
Dominant**	C/C	59 (60.8%)	23 (44.2%)	0.04	1.00	194.6
	C/T-T/T	38 (39.2%)	30 (56.6%)		0.49 (0.25-0.97)	
Recessive	C/C-C/T	89 (91.8%)	46 (88.5%)	0.34	1.00	197.9
	T/T	8 (8.2%)	7 (13.2%)		0.59 (0.20-1.73)	
Log-additive	---	---	---	0.052	0.61(0.37-1.01)	195.1

*, Akaike Information Criterion; **, best fit genotypic model on the basis of AIC.

97 asthma patients and 53 healthy controls were enrolled in a case-control study and their DNA samples were sequenced to determine the alleles (C/T) of rs1837253 SNP in the 234bp PCR amplicon of the *TSLP*. The statistical analysis predicted that the “T” allele of rs1837253 is a minor allele in the study population with allele frequency 0.237 in cases and 0.349 in controls, respectively, along p-value of 0.038 (Table II) which predicted a significant association of this allele against asthma susceptibility under the allelic model. However, the odds ratio 0.579620 [0.345070~0.973598] indicates that the “T” allele of this SNP plays a protective role in asthma susceptibility in the study population. Different inheritance models including codominant, dominant, recessive and log additive models were generated to check the association of rs1837253 SNP with asthma susceptibility at the genotypic level. The dominant model shows the lowest AIC value (194.6) and thus is considered to be the best-fit model for this study. In the dominant model, the heterozygous genotype, “CT” and homozygous genotype “TT” show an odds ratio of 0.49 with a p-value of 0.04 which indicates that the persons having any of genotype heterozygous “CT” or homozygous “TT” are less susceptibility to asthma. These results showed the protective role of the “T” allele of rs1837253 SNP against asthma disease in the Punjabi population of Lahore, Pakistan.

The protective role of the “T” allele of rs1837253 SNP is reported in several GWAS and case-control studies (Hunninghake *et al.*, 2010). The case-control association study on asthmatic children of Costa Rica reported the protective role of the “T” allele of rs1837253 SNP in the general population (men and women) of some of the sub-ethnic groups (Hunninghake *et al.*, 2010). The expression studies by Hui *et al.* show that the presence of allele “T” decreases the release of *TSLP* in nasal epithelial cells (NECs) by 1.8 folds in heterozygous genotype “CT” while the decrease is 2.5 folds in homozygous genotype “TT” as compared to the release of *TSLP* in NECs having homozygous genotype “CC” (Hui *et al.*, 2015), which supports the current study findings of significantly negative association (protective role) of “T” allele with asthma disease. In contrast to our findings, a significant association of rs1837253 with asthma has also been observed in different population studies (Afzal *et al.*, 2020; Nakayama *et al.*, 2020; Sun *et al.*, 2019). For example, the genome-wide study in the Guangxi Zhuang population of China reported that “T” allele of this SNP is significantly associated with (P=0.012) asthma disease increasing the risk of asthma (Sun *et al.*, 2019). Similarly, this SNP has been shown to increase asthma susceptibility in the Japanese population (Nakayama *et al.*, 2020). In Pakistani

Pashtun women, the “T” allele of rs1837253 is highly associated with asthma susceptibility (Afzal *et al.*, 2020). The varying results of this SNP in different populations suggest that the association is influenced by ethnicity and the specific genetic makeup of different global populations (Akram *et al.*, 2022; Ghani *et al.*, 2019b). To our knowledge, this is the first study conducted on the Punjabi population of Pakistan to find any association between the *TSLP* gene and asthma susceptibility.

CONCLUSION

We hereby conclude that *TSLP* SNP rs1837253 is significantly associated with asthma disease in both allelic and genotypic levels but has protective effects against asthma susceptibility in the Punjabi population of Lahore, Pakistan. However, there is a need to carry out more research on large-scale data for better understanding and to establish more precise results. Further, the expression studies for *TSLP* gene are suggested for further validation of the results.

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IRB approval

The study was approved by The Children's Hospital and The Institute of Child Health, Lahore, Pakistan

Ethics statement

It is certified that this research did not involve any hazardous experimental work. The study was accomplished following the standard protocols. All the standard ethical considerations were followed in case of the acquisition of data. The study did not have any obvious impact on the environment, human, animal, and plant life.

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20230218070210>

Statement of conflict of interest

The authors have declared no conflict of interest.

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