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Thymic Stromal Lymphopoietin (*TSLP*) gene variant rs1837253 is significantly associated with Asthma prevalence in Pakistani Pashtun women

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Abstract: Asthma is a chronic inflammatory disease of the airways characterized by airway hyperresponsiveness and remodeling. Thymic stromal lymphopoietin (*TSLP*), a member of the interleukin-2 family of cytokines, is produced by activated lung and intestinal epithelial cells, mast, and other immune cells. Population-based studies identified associations between SNPs in the *TSLP* promoter region and asthma pathogenesis. In this study, we analyzed the genotypic association of *TSLP* rs1837253 with asthma predisposition in the Pashtun population of Khyber Pakhtunkhwa, Pakistan. Target DNA sequence of 250 asthmatics and an equal number of healthy individuals was PCR amplified, and allelic determination was performed by Sanger sequencing. Statistical analysis was conducted using chi-square tests and logistic regression analysis. Homozygous T/T genotype was frequent in the asthmatic subjects with a statistically significant level ($P<0.05$). Genetic models, including recessive, dominant, co-dominant, over-dominant, and additive were tested while adjusting allele frequencies with covariates (gender and age). Combined C/T and T/T individuals had higher odds ratios of 3.00, 1.91, and 1.73 in co-dominant, dominant, and additive models with statistically significant P-values of 0.029*, 0.022*, and 0.02*, respectively. T allele of rs1837253 was associated with increased susceptibility to asthma among Pashtuns, particularly in females, and we corroborate rs1837253 as a SNP of interest with a potential functional role.

Keywords: Asthma, *TSLP*, single nucleotide polymorphism, rs1837253, association analysis, Pakistan

INTRODUCTION

Thymic stromal lymphopoietin (*TSLP*), an interleukin (IL)-7 like pleiotropic cytokine, has been linked to the pathogenesis of asthma and allergic diseases (Liu, YJ *et al*. 2007). TSLP exerts its genetic effects by binding to a high-affinity heterodimeric TSLP receptor (TSLPR), composed of TSLP chain (TSLPRα) and the IL-7 receptor α (IL7Rα) (Hofmeister *et al*. 1999). *TSLP* gene is located on chromosome 5q22.1, and alternative splicing of *TSLP* transcript due to different promoter regions results in the production of two TSLP isoforms; long isoform TSLP-Lf, which encodes a 159 amino acid protein (18.1 kDa) and a short TSLP-Sf isoform, encoding a sequence that is identical in C-terminal portion consisting of 63 amino acids (7 kDa) (Fornasa *et al*. 2015). Both the TSLP isoforms can be upregulated and secreted by many cell types, e.g., epithelial, keratinocytes, airway smooth muscle cells, fibroblasts, dendritic cells (DCs) and mast cells; and tissues including lung parenchyma, skin, intestine, ocular tissues, and spinal cord (Takai 2012; Wallmeyer *et al*. 2017). Several cellular targets for TSLP have been identified, including immune (DCs, ILC2, T and B cells, NKT and Treg cells, eosinophils, basophils, neutrophils, monocytes, macrophages, and mast cells) and non-immune cells (sensory neurons and platelets). The immune responses promoted by TSLP isoforms can be protective as well as detrimental to the host. TSLP generated from the olfactory epithelium serves as a tight epithelial protective barrier between neighboring cells through tight junctions and desmosomes while preserving tissue homeostasis (Kamekura *et al*. 2009). Excessive secretion of TSLP was observed in the airway epithelium of diseased mice and patients having asthma, and this factor is correlated with asthma severity (Al-Shami *et al*. 2005; Zhou *et al*. 2005; Ying *et al*. 2008). TSLP produced

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by regulatory dendritic cells (DC) initiates and controls differentiation of Th17 and natural Treg cells in thymus and intestine, nurtures the development of regulatory T-cells, and guards against colitis (Iliev *et al*., 2009; Spadoni *et al*. 2012). TSLP activated DCs, also control the inflammatory Th2 cells that secrete large amounts of cytokines such as IL-4, IL-5, IL-13, and tumor necrosis factor (TNF)-α (Ito *et al*., 2005).

Both TSLP isoforms play distinct roles in the asthma phenotype. The majority of the studies have focused on the pro-inflammatory long isoform TSLP-Lf than the lesser-known anti-inflammatory short isoform TSLP-Sf. TSLP-Lf was highly induced in healthy human bronchial epithelial cells after exposure to polyinosinic-polycytidylic acid (poly I:C) (Harada *et al*., 2009). Gene expression of TSLP-Lf is predominantly upregulated in primary human keratinocytes by TNF-α, toll-like receptor TLR ligands, and Th2-type cytokines (IL-4 and IL-13) (Xie *et al*., 2012). The region of the TSLP containing the C-terminal, chiefly mediates vigorous inhibitory activity against the disease-causing microbes (Sonesson *et al*. 2011). TSLP-Sf is the predominant form of TSLP and did not activate signal transducer and activator of transcription 5 (STAT5) signaling in $CD1c^+$ DCs, nor it interferes with STAT5 activation by TSLP-Lf. TSLP-Sf, therefore, act as having a potent antimicrobial activity and is constitutively expressed in saliva and skin keratinocytes to create a defense barrier to control both commensal and pathogenic microbes (Bjerkan *et al*., 2015). TSLP-Lf is expressed and upregulated during inflammation, whereas TSLP-Sf is expressed under steady-state circumstances, downregulated with inflammation, inhibits cytokine secretion of DCs, and plays a homeostatic role (Fornasa *et al*., 2015).

Three major mitogen-activated protein kinases (MAPKs): c-JUN N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK), have been known as cell cycle regulators that transform extracellular stimuli into a variety of cellular responses (Cargnello & Roux 2011). IL-33-induced TSLP expression is mediated by MAPK signaling via JNK, p38, and ERK activation. Additionally, IL-25 and TSLP expression were inhibited by ERK, JNK, and p38-specific MAPK inhibitors. The allergen-induced IL-25 and TSLP production were regulated via MAP kinase pathways (Yu *et al*., 2010; Omori-Miyake & Ziegler 2012; Ryu *et al*. 2015). Bronchial epithelial cells express TSLPR, which interacts with TSLP to encourage cell proliferation and injury repair facilitated by STAT5 phosphorylation (Semlali *et al*., 2010).

TSLP and its downstream target molecules involved in signaling pathways could provide a basis for possible treatment modalities to control asthma inflammation (Cianferoni & Spergel 2014). Genome-wide association studies have identified associations of a single nucleotide polymorphism (SNP) rs1837253, located 5.7 kb upstream of the *TSLP* transcription start site in its promoter region (UCSC hg19; chr5:110401872), to have a relevance to its gene expression. This genetic variant has shown a protective association with asthma, other allergic disorders, and related traits by altering the gene expression (He *et al*., 2009; Hunninghake *et al*., 2010; Hui *et al*. 2015; Moorehead *et al*. 2020). In some studies, rs1837253 in *TSLP's* promoter region had been significantly associated with a lower risk for asthma and hay fever (Ferreira *et al*. 2014). Reports in the literature provided variable associations of this particular *TSLP* variant with the asthma risk across different ethnic groups, including the Chinese population (Jiang *et al*., 2017; Sun *et al*., 2019). However, studies in the Pakistani population are largely missing, thereby validating studies are required to demonstrate the reproducibility of the previous findings in our population. Therefore, in this present study, we analyze the genotypes of the *TSLP* promotor SNP rs1837253 to identify its possible association with asthma predisposition in the Pakistani Pashtun population of Khyber Pakhtunkhwa (KPK) province.

MATERIALS AND METHODS

Patients and subjects

A total of 250 asthmatic non-familial cases from the Pashtun population of KPK were enrolled during the two-year study period (2017-2018). Recruited asthma patients were selected after detailed interviews, and all the patients were examined by pulmonologists at Khyber Medical University, Peshawar, Pakistan. A standardized questionnaire was used to gather information regarding the age of onset, family history, risks or environmental factors, and related atopic disorders. The patients with any indication or history of other respiratory lung diseases were not included in the study. Eligibility of the selected asthma cases was based on the following criteria: (a) history of at least one year of asthma diagnosed by a physician, and (b) Pakistani nationals were recruited only. Atopic asthmatics were identified by total serum IgE levels (>100 IU/ml), spirometry, and skin prick tests (Hunninghake *et al*., 2010). 250 ethnically matched, unrelated healthy controls without a previous history of any respiratory, dermatological, immunological, cardiac or renal disorder were enrolled in the study. Non-asthmatics control subjects were not related to the recruited asthmatic patients, and subjects having a positive family history of asthma and allergies were also excluded. In addition, all the recruited control subjects were screened by spirometry. 3-5 ml peripheral blood samples were collected from equally selected asthmatic cases and non-asthmatic control subjects (total of 500 participants).

Selection of SNP and genotyping

TSLP SNP, rs1837253 investigated in our study cohort

was selected due to its reported association with asthma susceptibility in many populations (Hunninghake *et al*., 2010; Liu, W *et al*., 2012; Birben *et al*., 2014). Genomic DNA was extracted from the blood samples using the QIAamp DNA Blood Midi kit (Qiagen, Germantown, MD), following the manufacturer's guidelines. SNP genotyping was performed by using Veriti Thermal Cycler (Thermo Fisher Scientific). Briefly, PCR amplification was done in a total volume of 25µl reaction mix, containing 20ng DNA, dNTPs, recommended amounts of specific primers for rs1837253: 5'-CTG AGA AGA GTG GGA CTC ACA A-3' (forward) and 5'-CAG GAA ACC GTG GCT CTT AAT G-3' (reverse) (Promega, USA), and HotStar Taq DNA Polymerase (Qiagen, Germantown, MD). PCR amplicons were sequenced using the Big Dye Terminator sequencing kit and ABI PRISM 3730 XL automated sequencer (Applied Biosystems, Foster City, CA). Sequence analysis was performed using the SeqMan 6.1 module of the Lasergene (DNA Star Inc. WI, USA) software package.

Ethical approval

The study was approved by the ethical review committee of the Imperial College of Business Studies, Lahore, Pakistan. Informed written consents were obtained from all the individuals. Please insert ethical approval statement with Ref. No.ERB/ICBS/35 dated 18 December 2018.

STATISTICAL ANALYSIS

Incidences of allelic and genotypic frequencies, C/T for rs1837253 were calculated separately. Calculations of Hardy-Weinberg equilibrium were performed among the cases and control subjects using the chi-square test. The relationship of rs1837253 with asthma was calculated using a web-based tool, SNPStats, to perform logistic regression analysis, modified for age and sex (Solé *et al*. 2006). For each genetic model, genotypic percentages, the crude odds ratios, 95% confidence intervals, and the P-values were calculated, with the reference category being the homozygote of the major allele 'C'. Akaike information criterion (AIC) and Bayesian information criterion (BIC) for each genetic model were calculated, where a lower AIC or BIC value indicates a better fit. The P-value <0.05 was considered statistically significant (Elston 2000; Iniesta, Guino & Moreno 2005).

Functional Studies

Physical and functional interactions between TSLP and other proteins were determined by employing the STRING database version 11.0 at EMBL (https://string-db.org/). The STRING database accumulates, scores, and incorporates curated protein-protein interactions' data and align them with the computational predictions. Primary sources to derive the data for interactions in STRING include genomic context predictions, high-throughput lab experiments, co-expression relationships, or physical interactions extracted from pathway databases such as KEGG, Biocarta, BioCyc, Gene Ontology, and Reactome (Szklarczyk *et al*., 2019). Functional and regulatory features for rs1837253 were interpreted using rSNPBase Softwares (Guo & Wang 2018) and RegulomeDB (Dong & Boyle 2019).

RESULTS

Clinical and demographics data

The asthma cases included 126 (50.4%) males and 124 (49.6%) females; the mean age was 33.964±1.733 SEM years. The controls included 127 males (50.8%) males and 123 (49.2%) females with a mean age of 34.194 ±1.744 SEM years. For the asthmatic phenotype, 54% of the patients were mild, 37% were moderate, and 9% were severe asthmatics. 60% of the asthmatic participants were from urban areas, whereas 40% belonged to rural areas of KPK.

Allelic and genotypic associations

TSLP rs1837253 is confirmed to be distributed in Hardy-Weinberg equilibrium in both the asthmatic and healthy groups with an overall HWE P-value of 0.00057 (Table 1). The global mean frequency (GMF) of the minor allele T was 40% (44% in asthmatics, 37% in healthy subjects). The allele and genotype frequencies are presented in table 1. Higher values of Pearson's chi-square (X^2) tests of goodness of fit revealed considerable differences in occurrences of allele frequencies among the asthmatic and healthy groups. The rate of risk allele 'C' was lower (0.56) in asthmatics as compared with controls (0.63) with significant results (P-value<0.05*). The relative frequencies for the minor allele, 'T' were slightly higher (0.44) in asthmatics compared to the control (0.37) group, with significant differences (Yates' corrected Chi-squared tests (X^2=4.52, P=0.034*). Taking into account genotype frequencies, homozygous T/T was slightly more frequent in asthmatic subjects (0.14) compared to healthy controls (0.11), with statistically significant differences (Yates' corrected tests (X^2=6.35, P=0.042*).

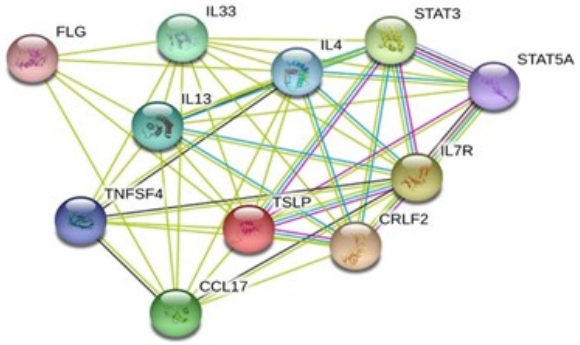

Fig. 1: Protein-protein interactions of *TSLP*, including physical and functional associations utilizing STRING database.

Table 1: Exact test for Hardy-Weinberg Equilibrium (N=500) and Allelic/Genotypic frequencies of SNP rs1837253.

	C/C	C/T	T/T	C	T	HWE P-value	
All Subjects	158	279	63	595	405	0.00057*	
Controls	92	130	28	314	186	0.1	
Asthmatics	66	149	35	281	219	0.0013*	
Allele or Genotype	All Subjects (Proportion)	Asthmatics # & (%)	Healthy # & (%)	Percent Deviation		Pearson's Chi-Square	P-value*
				Asthma	Healthy		
C	595 (0.6)	281 (0.56)	314 (0.63)	+4%	-3%	4.52	0.034*
T	405 (0.4)	219 (0.44)	186 (0.37)	-4%	+3%		
C/C	158 (0.32)	66 (0.26)	92 (0.37)	+6%	-5%	6.35	0.042*
C/T	279 (0.56)	149(0.60)	130 (0.52)	-4%	+4%		
T/T	63 (0.13)	35 (0.14)	28 (0.11)	-2%	+1%		

Percentage deviation is calculated as: [(observed-expected/expected) x 100]. *Significance is set at P-value < 0.05.

Table 2: Genotype frequency distribution of SNP rs1837253 and its association with response to disease status (adjusted by sex).

Model	Genotype	Asthma (%)	Healthy (%)	OR (95% CI)	P-value*	AIC	BIC
Co-dominant	C/C	66 (26.4%)	92 (36.8%)	1.00 (Reference)	0.042*	694.8	711.6
	C/T	149 (59.6%)	130 (52%)	1.60 (1.08-2.37)			
	T/T	35 (14%)	28 (11.2%)	1.74 (0.97-3.14)			
Dominant	C/C	66 (26.4%)	92 (36.8%)	1.00 (Reference)	0.012*	692.9	705.5
	C/T-T/T	184 (73.6%)	158 (63.2%)	1.62 (1.11-2.38)			
Recessive	C/C-C/T	215 (86%)	222 (88.8%)	1.00 (Reference)	0.35	698.2	710.9
	T/T	35 (14%)	28 (11.2%)	1.29 (0.76-2.19)			
Overdominant	C/C- T/T	101 (40.4%)	120 (48%)	1.00 (Reference)	0.087	696.2	708.9
	C/T	149 (59.6%)	130 (52%)	1.36 (0.96-1.94)			
Additive	---	---	---	1.39 (1.05-1.83)	0.02*	693.8	706.4

Analysis of allele frequencies adjusted by covariate gender, showing odds ratio (OR) and 95% confidence interval (CI) for five genetic models of SNP association with asthma, using the most frequent homozygous genotype as reference. *Significance is set at P-value<0.05.

Table 3: Analysis of interaction between gender, genotypes and with asthma.

Gender	Genotype	Asthma	Healthy	OR (95% CI)	Chi-Square	P-value*
Males	C/C	38	48	1 (Reference)	2.51	0.285
	C/T	74	62	1.51 (0.88-2.60)		
	T/T	14	17	1.04 (0.46-2.37)		
Females	C/C	28	44	1 (Reference)	7.02	0.029*
	C/T	75	68	1.73 (0.97-3.08)		
	T/T	21	11	3.00 (1.26-7.16)		

Analysis of interaction between gender, genotypes and with asthma showing odds ratios (OR) and 95% confidence intervals. The C/C genotype is taken as a reference. *Yates P-values; significance is set at two-tailed P <0.05.

We further performed a logistic regression analysis of the association of genotypes with asthma. Five genetic models (co-dominant, dominant, recessive, over-dominant, and additive), with frequencies adjusted for covariates age and gender, were tested (Table 2). Genotype frequency analysis was performed using subjects with the C/C genotype of rs1837253 as a reference group, after adjustment for age and gender. The combination of C/T and T/T genotypes had a significantly increased risk of developing the diseased state (asthma) under the co-dominant, dominant, and additive models.

The relative frequency for the heterozygous allele, C/T in the SNP, was higher (59.6%) in asthmatics, comparatively to the control (52%) group in the co-dominant model, with statistically significant differences (OR=1.60, 95% CI= 1.08-2.37, P-value=0.042*). The odds ratios for the dominant model also proved statistically significant results (OR=1.62, 95% CI= 1.11-2.38, P-value=0.012*). No such significant association was found in the recessive or over-dominant or genetic models. An analysis of the interaction of asthma and genotypes is shown in Table 3, with a statistically significant odds ratio for T/T genotype

in female asthmatics (OR=3.00, 95% CI=1.26-7.16, X^2=7.02, P-value=0.029*). The crude analysis was also performed to study the frequency distribution of genotypes of rs1837253 in female subjects and its association with asthma (Table 4). The co-dominant (OR=3.00, 95% CI=1.26-7.16, P-value=0.029*) and dominant (OR=1.91, 95% CI= 1.09-3.34, P-value=0.022*) genetic models proved statistically significant (P<0.05) association with asthma.

DISCUSSION

Asthma is a chronic inflammatory disorder of the airways, which is characterized by bronchial hyperresponsiveness, resulting in recurrent cough, dyspnea, discomfort, wheezing, anxiety, and airway obstruction, and an underlying inflammation of the bronchial mucosa (Cockcroft & Davis 2006). The global burden and morbidity of asthma have been increasing for several decades. By the year 2025, the number of people with asthma is expected to be over 400 million worldwide, but essential predictors of asthma are not well known (Masoli *et al*. 2004; To *et al*. 2012). More than six million people affected by asthma exist in Pakistan. Heritability estimates indicate that the genetic variation among individuals' accounts for one-half of the risk of asthma (Ober 2016). Single-gene association studies, family-based genome-wide linkage analyses, and genome-wide association studies (GWASs) are the most prominent genetic methodologies for identifying asthma genes (Hernandez-Pacheco, Pino-Yanes & Flores 2019). Single nucleotide polymorphisms (SNPs) have been identified as independent risk factors for asthma and elucidate molecular pathways within and outside the cells that lead to asthma pathogenesis (Hui *et al*. 2015; Jiang *et al*. 2017; Sun *et al*. 2019; Moorehead *et al*. 2020). This feature of asthma has implications for the diagnosis, management, and potential prevention of the disease.

TSLP is like a pleiotropic cytokine and is overexpressed in the airways of severe asthmatics. TSLP is implicated in various allergic diseases, chronic inflammatory, and autoimmune disorders (Varricchi *et al*., 2018). The broad pathophysiological role of TSLP has proposed therapeutic targeting to this cytokine. Tezepelumab is a potential IgG2 monoclonal antibody that binds to TSLP and inhibits its interaction with the TSLP receptor complex on effector cells (Marone *et al*., 2019). Several clinical trials have evaluated its safety, tolerability, and efficacy alone or as an add-on-therapy with allergen immunotherapy to patients with severe uncontrolled asthma and other inflammatory disorders. *TSLP* polymorphisms appear to be associated with a higher risk of allergic disorders.

Our study is the first to report genetic associations between *TSLP* SNP rs1837253 and asthma predisposition among the Pashtun population in KPK, Pakistan. Our results indicated that the rs1837253 is significantly (P-value <0.05) associated with asthma patients and showed significant differences between the patients and controls, not only in the frequencies of genotypes and alleles but also in the co-dominant, dominant and additive genetic models. Similar results had been observed in a study that stated an association of rs1837253 with increased asthma risk and susceptibility in Guangxi's Zhuang people of China (Sun *et al*., 2019). The frequency of 'C/T' alleles in our studied population for rs1837253 SNP (0.595/0.405) closely matched to its frequency in the population of Gujarati Indians in Houston, Texas, and Mexican ancestry in Los Angeles, California, i.e., 0.62/0.38 and 0.59/0.41 respectively (Flicek *et al*., 2014).

In our study samples, the stratified analysis showed that the combined C/T and T/T genotypes of rs1837253 were significantly associated with an increased risk of asthma exclusively in Pashtun females of Pakistan. The minor T allele of rs1837253 was found to have higher odds ratios

Table 4: Genotype frequency distribution of SNP rs1837253 and its association with response to disease status in females only (crude analysis)

Model	Genotype	Asthma (%)	Healthy (%)	OR (95% CI)	P-value*	AIC	BIC
Co-dominant	C/C	28 (22.6%)	44 (35.8%)	1.00 (Reference)	0.029*	341.3	351.8
	C/T	75 (60.5%)	68 (55.3%)	1.73 (0.97-3.08)			
	T/T	21 (16.9%)	11 (8.9%)	3.00 (1.26-7.16)			
Dominant	C/C	28 (22.6%)	44 (35.8%)	1.00 (Reference)	0.022*	341.2	348.2
	C/T-T/T	96 (77.4%)	79 (64.2%)	1.91 (1.09-3.34)			
Recessive	C/C-C/T	103(83.1%)	112 (91.1%)	1.00 (Reference)	0.06	342.9	349.9
	T/T	21 (16.9%)	11 (8.9%)	2.08 (0.95-4.52)			
Overdominant	C/C-T/T	49 (39.5%)	55 (44.7%)	1.00 (Reference)	0.41	345.7	352.7
	C/T	75 (60.5%)	68 (55.3%)	1.24 (0.75-2.05)			
Additive	---	---	---	1.73 (1.15-2.61)	0.02*	693.8	706.4

Analysis of allele/genotype frequencies of female subjects and their association with disease status, showing odds ratio (OR) and 95% confidence interval (CI) for five genetic models of SNP association with asthma, using the most frequent homozygous genotype as reference. *Significance is set at P-value<0.05.

of (3.00, 1.91, and 1.73) in three of the five tested genetic models (co-dominant, dominant, and additive) with statistically significant P-values of 0.029*, 0.022*, and 0.02* respectively. Our findings were supported by the study of Miyake *et al*. (2015), which described the combined effect of T/C and C/C genotypes of rs1837253 to be significantly associated with an increased risk of eczema, while T/T allele is associated with a reduced risk of eczema in Japanese females (Miyake *et al*., 2015).

Several studies had identified significant genetic associations in distinctive ethnicities between rs1837253 and asthma. The most common SNPs in the *TSLP* gene promoter region had been associated with asthma in Asian, European and American communities in several gene-specific associations and GWAS studies at a genome-wide level (He *et al*. 2009; Hunninghake *et al*., 2010; Bunyavanich *et al*., 2011; Miyake *et al*., 2015; Hui *et al*., 2015; Jiang *et al*., 2017; Varricchi *et al*., 2018; Johansson *et al*. 2019; Kristjansson *et al*., 2019; Sun *et al*., 2019; Moorehead *et al*. 2020). The *TSLP* gene showed a significant correlation of the Japanese population with adult asthma (P=0.023) for rs1837253 (Hirota *et al*. 2011). The T allele of rs1837253 was associated with asthma protection and airway hyper-responsiveness among the Canadian and Australian populations (He *et al*., 2009). In contrast, the T allele of rs1837253 was inversely associated with allergic rhinitis in Costa Rican boys with asthma (Bunyavanich *et al*. 2011). Interestingly, another study in Costa Rica showed that the T alleles of rs1837253 and rs2289276 of the *TSLP* gene were associated with a reduced risk of asthma in men and women, respectively (Hunninghake *et al*., 2010). Our finding observed that the T allele of rs1837253 was associated with increased susceptibility to asthma among Pashtuns, particularly in females. The subjects in our study cohort were mostly adults, compared to other case-control studies across the globe. Results from this study clarify the dissimilarities and highlight the specific genetic architecture among different populations worldwide.

TSLP isoforms, TSLP-Sf, and TSLP-Lf, are known for their different biological properties, associated with immune pathologies (Fornasa *et al*., 2015; Dong *et al*., 2016). This dichotomous role of TSLP in immunoregulatory pathways and its underlying mechanisms makes it very complicated to understand the gene-disease relationship data in a given population. TSLP-Sf may also act as a novel therapeutic agent for individual personalized asthma care. However, many questions remain unanswered, including the reproducibility of SNPs functional studies and their impact on each of the TSLP-Lf and TSLP-Sf isoforms' expression and activity. The interaction network of TSLP and different proteins must be carefully considered for the complete understanding of the biological phenomena. The analyzed protein interaction network in this study was constructed by the STRING database. The curated interactions included both the direct (physical) and indirect (functional) associations between TSLP and different proteins, as shown in fig. 1.

SNP rs1837253 of *TSLP* was further analyzed for gene expression and functional parameters using RegulomeDB (Dong & Boyle 2019). RegulomeDB ranks the SNP rs1837253, located in the transcription binding site of the target gene *TSLP*, as 2b (TF binding/DNase peak) with a score of 0.2664. This SNP in the *TSLP* promoter region is suggested to disrupt a transcription factor binding site for the transcription factor activating protein (AP)-1 and lead to *TSLP*'s downstream effects. It also acts as an enhancer for different types of cell lines (e.g., skeletal muscle satellite cells, mammary epithelial cells, IMR-90 cell line, and foreskin keratinocyte) derived from different body organs such as the musculature of the body, epithelium, mammary gland, lung, the skin of the body, and epithelium. Analysis by the SNP base, a database of regulatory SNPs, further revealed that the rs1837253 is involved in both proximal and distal transcription regulation of the *TSLP* (Guo & Wang 2018). TSLP is predicted to be expressed in choroid plexus epithelial cells, fibroblasts of lung and heart, aortic smooth muscle cells, and fibroblasts of aortic adventitia (brain, epithelium, vasculature, arterial blood vessel, blood vessel, connective tissue, and body fluids).

Our finding provides important insights into the differential regulation and function of TSLP isoforms, including the role of *TSLP* rs1837253 polymorphism in asthma. Besides, we corroborate rs1837253 as a SNP of interest with a potential functional role. This study's limitation includes a lack of evidence for any interactions between rs1837253 and asthma-influencing lung function tests. Further evidence from the genetic association and functional studies as well are needed to strengthen the epidemiological evidence. Additional replicative studies are warranted from different regions of KPK and other ethnic groups of Pakistan with a higher number of adult and pediatric asthmatic cases, and control subjects to characterize the molecular basis of asthma pathogenesis further and enhance our understanding regarding the role of *TSLP* SNPs in asthma susceptibility in our population.

CONCLUSION

Our study describes *TSLP* rs1837253 association among the Pashtun population and suggests the combination of TC and TT genotypes of *TSLP* rs1837253 are significantly associated with an increased risk of asthma, exclusively in the female asthmatics. GWAS and candidate-gene studies in independent, diverse populations will help to understand the molecular pathways, predict disease prognosis, provide an insight in

pharmacogenomics of asthma therapies for better treatment response, and minimize adverse effects by personalized and precision medicine.

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