INTRODUCTION

Environmental pollutants, such as tobacco smoke, support an immune milieu that promotes allergic asthma (Spann, Snape, Baturcam, & Fantino, 2016). Individuals with long-term cigarette consumption have substantially increased risk of developing asthma, chronic obstructive pulmonary disease (COPD), and oral and lung cancers (Centers for Disease Control & Prevention, 2010). Cigarette smoking (CS) is a major public health concern that causes a global increase in mortality rates and vulnerability to certain diseases (Baig et al., 2016). It was estimated that globally, there are currently 1.2 billion smokers over the age of 15 years WHO (2018).
with around 6 million deaths per year worldwide. More than 5 million of these are caused by direct tobacco use, and over 600,000 are due to exposure to secondhand smoke. In the Kingdom of Saudi Arabia (KSA), the incidence of CS in certain regions is greater than 50% (Bassioni, 2009). Based on the WHO analyses, 3 million KSA residents were smokers in 2010; however, this number is predicted to increase to around 6 million by 2025. The above findings have prompted government agencies to increase public awareness on the health risks of tobacco use. A wide variety of diseases are attributed to smoking (Qiu et al., 2017). In developed countries, CS is responsible for ~30% of all cancer mortalities and morbidities, most of which are attributed to lung cancer (ACS Inc, 2014; Gutierrez, Suh, Abtin, Genshaft, & Brown, 2013) and diseases affecting the cardiovascular system (Menotti, Puddu, Maiani, & Catasta, 2015). Previous reports by Alamri et al. (2015) have also emphasized the role of tobacco in causing damage to gingival cells. In particular, CS deregulates multiple cell functions, including growth (Alamri et al., 2015), adhesion, and migration (Semlali, Chakir, Goulet, Chmielewski, & Rouabha, 2011), which have been observed in fibroblasts and human gingival epithelial cells (Semlali, Chakir, Goulet, et al., 2011; Semlali, Chakir, & Rouabha, 2011). In addition, CS has been reported to promote apoptosis in epithelial cells and impair the cell repair process (Semlali, Chakir, & Rouabha, 2011). Multiple chemical and biological studies have also revealed the harmful effects of many tobacco components, which have been particularly demonstrated to influence mutagenesis and DNA methylation (Steenaard et al., 2015) and induce genetic alterations in pro-oncogenes and tumor suppressor genes, as well as p53 (Husgafvel-Pursiainen & Kannio, 1996; Pfeifer et al., 2002; Taghavi et al., 2010) and innate immunity genes (Kohailan et al., 2017, 2016).

Previous studies have clearly demonstrated that CS induces chronic inflammation in the conducting airways through multiple mechanisms. Direct activation of immune cells induces the secretion of proinflammatory factors, as well as IL-6, TNF-α, and TSLP (thymic stromal lymphopoietin).

TSLP is an interleukin 7 (IL-7)-like cytokine secreted primarily by human bronchial epithelial cells (Liu et al., 2011). TSLP has been recognized as a primary instigator of allergic inflammation at the dendritic and epithelial cell interface (Liu et al., 2007) and has been shown to play an important role in innate immune response by inducing the differentiation of T-helper type 2 (Th2) effector cells in asthma patients. Various protease allergens, respiratory viruses, and inflammatory cytokines are known to induce TLSP upregulation in airway epithelial cells (Tsilingiri, Fornasa, & Rescigno, 2017; Ziegler & Artis, 2010). The human TSLP is located on chromosome 5q22.1 and is adjacent to the gene cluster that encodes Th2 cytokines (Quentmeier et al., 2001). TSLP comprises the TSLP receptor (TSLPR) and interleukin 7 receptor (IL7R) alpha chain (Pandey et al., 2000; Park et al., 2000). TSLPR is a novel receptor subunit that forms the receptor for TSLP in conjunction as a heterodimeric complex with the IL7R alpha chain (Pandey et al., 2000). Like all cytokine receptors, the TSLP subunit has a conserved WSXWS (Trp-Ser-X-Trp-Ser) motif in the extracellular domain; however, its role is not precisely understood (Hilton, Watowich, Katz, & Lodish, 1996; Tonozuka et al., 2001; Zhang et al., 2001). Knockout experiments in mice have demonstrated that TSLPR plays a crucial role in the lung inflammatory response and/or allergic responses (Al-Shami, Spolski, Kelly, Keane-Myers, & Leonard, 2005). Recently, Shi et al. suggested that local inhibition of TSLPR alleviated allergic responses by regulating the function of dendritic cells (DCs) (Shi et al., 2008). Furthermore, a recent study indicated TSLP as a strong susceptibility gene for asthma among adult Japanese populations (Harada et al., 2011). TSLP is strongly expressed in the submucosa and bronchial epithelia of clinically stable asthmatic patients and is also correlated with airway obstruction (Ying et al., 2005). Recently, it was proved that cigarette smoke induces TSLP expression, leading to T(H)2-type immune responses and airway inflammation.

Recent studies provided evidence that CS induces further genetic alterations, such as single nucleotide polymorphisms (SNPs), in innate immunity genes (Kohailan et al., 2016) that can in turn lead to a range of diseases (Steenaard et al., 2015) or induce transitions or transversions (Acevedo, Brodsky, & Andino, 2014; Farrell et al., 2014). One study found significant correlations between genetic variants of TSLP and asthma (Liu et al., 2011). Another study showed that the rs1837253 SNP, which is located 5.7 kb upstream of the TSLP transcription start site, was linked to asthma in a Canadian population (He et al., 2009). Furthermore, significant differences in the genotypes and allele frequencies of TSLPR were found between asthmatic patients and healthy controls in a Korean population (Semlali, Parine, et al., 2017). We hypothesized that the development of smoking-induced respiratory and cancer diseases is mediated by genetic changes in the genes encoding TSLP and TSLP receptors (TSLPR and IL7R). Interestingly, no previous studies have investigated the relationship between smoking and the SNPs in these three genes. Thus, the present study aimed to determine whether genetic variants in TSLP (rs3806933, rs2289276, and rs10043985), TSLPR (rs36133495, rs36177645, and rs36139698), and IL7R (rs1053496 and rs12516866) are associated with cigarette smoking in Saudi Arabians. The SNPs studied were selected based on their known involvement in various diseases, which could be explained by their ability to alter gene function and to ultimately influence the pathogenesis of other unstudied diseases.
2 | MATERIALS AND METHODS

2.1 | Ethics statement and sample collection

All methods were carried out in accordance with relevant guidelines and regulations and all experimental protocols were approved by a Research Ethics Committee of the College of Applied Medical Sciences at King Saud University (KSU) in Riyadh, Saudi Arabia (Approval Number: CAMS 13/3536). In this sense, written ethical consent for this study was reviewed by and obtained from this Research Ethics Committee of the College of Applied Medical Sciences at King Saud University (KSU). Participants who smoked cigarettes were termed smokers, whereas individuals who did not consume any kind of tobacco product were referred to as nonsmokers. Smokers were divided into two groups based on cigarette consumption, namely, those who smoked ≥20 cigarettes/day and those who smoked <20 cigarettes/day. All volunteer smokers and nonsmokers signed a written informed consent. Clinical data on smoking history, allergic symptoms and diseases, number of cigarettes smoked daily, and body mass index (BMI) were obtained through a self-completed questionnaire. Saliva samples were collected from a group of 177 cigarette smokers (smokers) and a group of 126 healthy controls (nonsmokers) recruited from academic staff and only male students at KSU between January 2015 and April 2015. Participating volunteers were not suffering from any diseases or disorders. Detailed clinical characteristics of the participants are summarized in Table 1.

2.2 | DNA extraction

DNA extraction was performed as previously described (Kohailan et al., 2017, 2016; Semlali, Jalouli, et al., 2017; Semlali, Parine, et al., 2017; Semlali, Parine, et al., 2016). Briefly, saliva samples were diluted twice in phosphate-buffered saline, and DNA was isolated using the PureLink® Genomic DNA Mini Kit (Catalog No K1820-01; Invitrogen™, Carlsbad, CA) according to the manufacturer’s instructions. DNA concentration was quantitated using a NanoDrop 8000 (Thermo Fisher Scientific, Waltham, MA) instrument, and DNA purity was determined by calculating the A_{260 nm}/A_{280 nm} and A_{260 nm}/A_{230 nm} ratios.

2.3 | Candidate SNP selection and TaqMan genotyping assay

10 ng/μl of each genomic DNA collected from saliva was used for genotyping. Eight tagged SNPs in TSLP and TSLPR were used in this study. Three SNPs in TSLP (rs3806933 [1350T/C, Ser450Ser], rs2289276 [1350T/C, Ser450Ser], and rs10043985 [597T/C, Asn199Asn]), three SNPs in TSLPR (rs36133495 [1350T/C, Ser450Ser], rs36177645 [1350T/C, Ser450Ser], and rs36139698 [597T/C, Asn199Asn]), and two SNPs in IL7R (rs10534946 [979 G/A, Val327Met] and rs12516866 [745T/C, Ser249Pro]) were selected based on their locations in the gene regulatory regions. All SNPs were located either in the promoter regions, 5'‐untranslated regions (5'-UTR), or exons (Table 2). These SNPs were also selected based on literature reviews of SNP associations with various diseases in diverse ethnic groups. Each genotyping reaction contained 0.2 μl of 40× TaqMan® Genotyping SNP Assay (Applied Biosystems), 5.6 μl of TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA), and 20 ng of DNA. Reactions were run on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems) with an end point reading of the genotypes (Semlali, Jalouli, et al., 2017; Semlali, Parine, et al., 2017).

2.4 | Data analysis

As described in our previous work (Semlali, Jalouli, et al., 2017; Semlali, Parine, et al., 2017), the calculated genotypic and allelic frequencies of each SNP were checked for the Hardy-Weinberg equilibrium deviation. Genetic comparisons were performed using the χ² test and calculation of allelic odds ratios (ORs). In addition, 95% confidence intervals (CIs) were determined using Fisher's exact test (two-tailed). All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 16.0 statistical software (SPSS, Chicago, USA). p < 0.05 was considered statistically significant.

Homology modeling of the 3D structure of the human TSLPR was performed on the SWISS-MODEL server using the X-ray structure of the mouse TSLPR included in the TSLPN123Q-TSLPRN53Q-IL7Rα complex (Protein Data Bank entry 4NN7) (Verstraete et al., 2014), with which it shares 35% sequence identity, as a model.
The resulting homology model of the human TLSPR was used to estimate the impact of the selected mutations on protein structure. Changes in thermal protein stability for the rs36139698 mutant was predicted using the CUPSAT stability prediction server (Parthiban, Gromiha, & Schomburg, 2006), which evaluates the changes in free energy during the protein folding-unfolding process ($\Delta \Delta G$) as a result of the mutation. A positive or negative $\Delta \Delta G$ value indicates that the mutation is thermodynamically stabilizing or destabilizing, respectively, while the magnitude of $\Delta \Delta G$ is a measure of the extent of the alteration.

3 | RESULTS

3.1 | General clinical patient characteristics

A total of 177 smoker patients and 126 nonsmoker controls from the Saudi Arabian population were included in the present study. The clinical and the demographic characteristics of the study population are described in Table 1. Our analysis revealed no significant differences in BMI and age between smoking and nonsmoking individuals (Table 1). The average ages for both groups were 20 ± 21 years for nonsmokers and 24 ± 27 years for smokers; 17% of nonsmokers and 20% of smokers were suffering from obesity. The smoker group was divided into two subgroups based on duration of smoking, namely, individuals who smoked for > 5 years, which comprised 63% of all smokers, and individuals who had smoked for ≤ 5 years, which comprised 37% of all smokers. The smoker subgroups were further classified into two categories according to the number of cigarettes smoked daily, namely, smokers who consumed ≥ 20 cigarettes (one pack of cigarettes) daily and those who consumed < 20 cigarettes daily (Table 1).

3.2 | Genotypic patterns of TSLP, TSLPR, and IL7R SNPs among smokers and nonsmokers

In this study, we collected a total of 177 samples from smokers and 126 samples from nonsmokers and studied the association of genetic variants in TSLP, TSLPR, and IL7R with smoking behavior. A general comparison between the genotype distribution and allele frequencies between smokers and controls for the eight tested SNPs are described in Table 3. Only rs10043985 and rs3806933 showed statistically significant correlations with smoking behavior. For rs10043985, the genotypic distribution was 84% AA, 7% AC, and 9% CC in nonsmokers and 79% AA, 21% AC, and 0.65% CC in smokers ($p < 0.05$). In particular, “AC” heterozygous allele showed around one third higher correlation with smoking than the homozygous “AA” allele (OR = 3.44; CI = 1.278–9.248; $p = 0.0103$). The homozygous “CC” allele was found to be significantly correlated with smoking (OR = 0.08; CI = 0.009–0.637; $p < 0.005$), and the allele distribution was similar between smokers and controls ($p = 0.679$). For rs3806933, smoker groups and control groups showed significant differences in genotype frequencies of “CT”, “TT”, and “CT + TT” ($p < 0.005$) when compared to the wild-type “CC” genotype. In addition, the “T” allele showed a significant phenotypic correlation with smoking individuals when compared to the “C” reference allele. The phenotypic distribution was 33% C and 67% T in normal controls and 45% C and 55% T in smokers ($p = 0.0075$). By contrast, rs2289276 showed similar genotype and allele frequencies between smokers and controls (Table 3).

The observed genotype frequency distribution for TSLPR revealed that out of the three SNPs tested, only rs36139698 exhibited significant differences between smoker and nonsmokers, with OR = 4.43 and $p = 0.04$. However, the genotype distributions were 11% CC, 20% CT, and 69% TT in nonsmokers and 6% CC, 50% CT, and 44% TT in smokers. The “CT” heterozygous allele showed around 25% higher correlation with smoking than the “CC” homozygous allele (OR = 4.43; CI = 1.287–15.260; $p = 0.0127$). Notably, an association was found between the “T” allele in rs36139698 and smoking when compared to the “C” allele (OR = 0.59; CI = 0.348–1.003; $p = 0.0497$) (Table 3). In addition, the genotype and allele frequencies for rs36177645 and rs36133495 in TSLPR did not appear to be influenced by cigarette smoking (Table 3).
| Gene | SNP       | Alleles | Controls |  | Smokers |  | OR |  | 95% CI |  | χ² |  | p value |
|------|-----------|---------|----------|----------|---------|----------|----|----|--------|----|----|----|--------|
|      |           | N       | Percent  | N         | Percent  |        |    |    |        |    |    |    |        |
| TSLP | rs10043985 |         |          |           |          |        |    |    |        |    |    |    |        |
|      | total     | 77      | 100      | 154       | 100      |        |    |    |        |    |    |    |        |
|      | AA        | 65      | 84       | 121       | 79       | Ref    |    |    | 1.2781–9.2484 | 6.5824 | 0.0103² |
|      | AC        | 5       | 7        | 32        | 21       | 4.44   |    |    | 0.0092–0.6374 | 9.0765 | <0.005² |
|      | CC        | 7       | 9        | 1         | 0        | 0.08   |    |    | 0.7146–3.0539 | 1.1177 | 0.2904 |
|      | AC+CC     | 12      | 16       | 33        | 21       | 1.48   |    |    | 0.4848–1.6034 | 0.1705 | 0.6797 |
|      | A         | 135     | 88       | 274       | 89       | Ref    |    |    |        |    |    |    |        |
|      | C         | 19      | 12       | 34        | 11       | 0.88   |    |    | 0.4848–1.6034 | 0.1705 | 0.6797 |
|      | CC        | 45      | 40       | 62        | 37       | Ref    |    |    |        |    |    |    |        |
|      | CT        | 56      | 49       | 82        | 49       | 1.06   |    |    | 0.6365–1.7745 | 0.0542 | 0.8159 |
|      | TT        | 12      | 11       | 23        | 14       | 1.39   |    |    | 0.6272–3.0853 | 0.6627 | 0.4156 |
|      | CT+TT     | 68      | 60       | 105       | 63       | 1.12   |    |    | 0.6864–1.8300 | 0.2077 | 0.6486 |
|      | C         | 146     | 65       | 206       | 62       | Ref    |    |    |        |    |    |    |        |
|      | T         | 80      | 35       | 128       | 38       | 1.13   |    |    | 0.7985–1.6103 | 0.4940 | 0.4821 |
| TSLPR | rs2289276 |         |          |           |          |        |    |    |        |    |    |    |        |
|      | total     | 113     | 100      | 167       | 100      |        |    |    |        |    |    |    |        |
|      | CC        | 45      | 40       | 62        | 37       | Ref    |    |    |        |    |    |    |        |
|      | CT        | 56      | 49       | 82        | 49       | 1.06   |    |    | 0.6365–1.7745 | 0.0542 | 0.8159 |
|      | TT        | 12      | 11       | 23        | 14       | 1.39   |    |    | 0.6272–3.0853 | 0.6627 | 0.4156 |
|      | CT+TT     | 68      | 60       | 105       | 63       | 1.12   |    |    | 0.6864–1.8300 | 0.2077 | 0.6486 |
|      | C         | 146     | 65       | 206       | 62       | Ref    |    |    |        |    |    |    |        |
|      | T         | 80      | 35       | 128       | 38       | 1.13   |    |    | 0.7985–1.6103 | 0.4940 | 0.4821 |
| TSLPR | rs3806933 |         |          |           |          |        |    |    |        |    |    |    |        |
|      | total     | 98      | 100      | 124       | 100      |        |    |    |        |    |    |    |        |
|      | CC        | 45      | 40       | 62        | 37       | Ref    |    |    |        |    |    |    |        |
|      | CT        | 56      | 49       | 82        | 49       | 1.06   |    |    | 0.6365–1.7745 | 0.0542 | 0.8159 |
|      | TT        | 12      | 11       | 23        | 14       | 1.39   |    |    | 0.6272–3.0853 | 0.6627 | 0.4156 |
|      | CT+TT     | 68      | 60       | 105       | 63       | 1.12   |    |    | 0.6864–1.8300 | 0.2077 | 0.6486 |
|      | C         | 146     | 65       | 206       | 62       | Ref    |    |    |        |    |    |    |        |
|      | T         | 80      | 35       | 128       | 38       | 1.13   |    |    | 0.7985–1.6103 | 0.4940 | 0.4821 |
| TSLPR | rs36139698|         |          |           |          |        |    |    |        |    |    |    |        |
|      | total     | 55      | 100      | 131       | 100      |        |    |    |        |    |    |    |        |
|      | CC        | 6       | 6        | 48        | 39       | Ref    |    |    |        |    |    |    |        |
|      | CT        | 52      | 53       | 16        | 13       | 0.04   |    |    | 0.0139–0.1063 | 51.5551 | <0.005² |
|      | TT        | 40      | 41       | 60        | 48       | 0.19   |    |    | 0.0734–0.4792 | 13.9699 | <0.005² |
|      | CT+TT     | 92      | 94       | 76        | 61       | 0.10   |    |    | 0.0419–0.2543 | 31.5786 | <0.005² |
|      | C         | 64      | 33       | 112       | 45       | Ref    |    |    |        |    |    |    |        |
|      | T         | 132     | 67       | 136       | 56       | 0.59   |    |    | 0.3988–0.8691 | 7.1587 | 0.0075² |
| TSLPR | rs36177645|         |          |           |          |        |    |    |        |    |    |    |        |
|      | total     | 119     | 100      | 125       | 100      |        |    |    |        |    |    |    |        |
|      | CC        | 24      | 20       | 32        | 20       | Ref    |    |    |        |    |    |    |        |
|      | CT        | 61      | 51       | 79        | 50       | 0.97   |    |    | 0.5194–1.8162 | 0.0083 | 0.9274 |
|      | TT        | 34      | 29       | 46        | 30       | 1.01   |    |    | 0.5088–2.0238 | 0.0017 | 0.9669 |
|      | CT+TT     | 95      | 80       | 125       | 80       | 0.99   |    |    | 0.5456–1.7851 | 0.0019 | 0.9651 |
|      | C         | 109     | 46       | 143       | 46       | Ref    |    |    |        |    |    |    |        |
|      | T         | 129     | 54       | 171       | 54       | 1.01   |    |    | 0.7205–1.4170 | 0.0036 | 0.9521 |

(Continues)
Finally, our results showed no statistically significant correlations between smoking and the IL7R SNPs rs12516866 and rs1053496. For IL7R, the genotype frequencies for rs12516866 were 40% GG, 50% GT, and 10% TT in nonsmokers and 48% GG, 44% GT, and 8% TT in smokers. On the other hand, the genotype frequencies in the IL7R rs1053496 SNP were 12% CC, 22% CT, and 66% TT in nonsmokers and 12% CC, 27% CT, and 61% TT in smokers (Table 3).

### 3.3 Association of gene polymorphisms of TSLP, TSLPR, and IL7R with duration of smoking

As mentioned earlier, patients in the present study were classified into the following two categories based on smoking duration: long-term smokers, which included individuals who had been smoking for >5 years, and short-term smokers, which included individuals who had smoked for ≤5 years. Table 4 shows the statistical analyses and genotype distributions for the TSLP, TSLPR, and IL7R variants for each subgroup when compared with the nonsmoking individuals. Analysis of the genotype distributions and allele frequencies for TSLP showed that rs10043985 results in a fourfold higher risk for developing cigarette-associated diseases in long-term smokers but not in short-term smokers and compared to nonsmokers. In addition, the genotype frequency of “AC” was 7% in controls and 24% in long-term smokers (p < 0.005). However, “AC” genotype frequencies were not statistically significant between nonsmokers and short-term smokers (Table 4). Conversely, the TSLP rs2289176 variant was clearly more highly associated with short-term smokers compared to control subjects by approximately 3.75 times but was not associated with long-term smokers. The genotype and allele frequencies for rs2289176 were 22%, 9%, and 11% for short-term smokers, long-term smokers, and nonsmoker subjects, respectively, for the homozygote genotype TT and 50%, 32%, and 35% for the “T” allele. For TSLP rs3806933, the “TT” genotype displayed a significant association with smoking in the two smoker subgroups relative to nonsmoker patients. In addition, “CT,” “TT,” and combined “CT+TT” genotypes appeared to exhibit significant associations relative to the “CC” homozygous reference allele in both long-term (OR = 0.04, CI = 0.012–0.124, p = 0.005; OR = 0.22, CI = 0.081–0.592, p = 0.005; and OR = 0.12, CI = 0.045–0.306, p = 0.005, respectively) and short-term smokers (OR = 0.04, CI = 0.012–0.143, p = 0.005; OR = 0.17, CI = 0.057–0.493, p = 0.005; and OR = 0.10, CI = 0.034–0.269, p = 0.005, respectively). However, the “T” allele showed a significant association with smoking only in short-term smokers (p = 0.0172) but not in long-term smokers relative to the “C” allele (Table 4). For TSLP rs36139693, the “TT” genotype displayed a significant association with smoking in the two smoker subgroups relative to nonsmoker patients. In addition, “CT,” “TT,” and combined “CT+TT” genotypes appeared to exhibit significant associations relative to the “CC” homozygous reference allele in both long-term (OR = 0.04, CI = 0.012–0.124, p = 0.005; OR = 0.22, CI = 0.081–0.592, p = 0.005; and OR = 0.12, CI = 0.045–0.306, p = 0.005, respectively) and short-term smokers (OR = 0.04, CI = 0.012–0.143, p = 0.005; OR = 0.17, CI = 0.057–0.493, p = 0.005; and OR = 0.10, CI = 0.034–0.269, p = 0.005, respectively). However, the “T” allele showed a significant association with smoking only in short-term smokers (p = 0.0172) but not in long-term smokers relative to the “C” allele (Table 4). For TSLP rs36139693, the “TT” genotype displayed a significant association with smoking in the two smoker subgroups relative to nonsmoker patients. 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| Gene  | SNP                 | Allele | Controls | >5 years | OR      | 95% CI  | χ²     | p value |
|-------|---------------------|--------|----------|----------|---------|---------|--------|---------|
|       |                     |        | N    | Percent | N    | Percent |        |         |
|       |                     |        |      |         |      |         |        |         |
|       |                     |        |      |         |      |         |        |         |
|Patients smoking for >5 years| | | | | | | | |
|       |                     |        |      |         |      |         |        |         |
|       |                     |        |      |         |      |         |        |         |
|       |                     |        |      |         |      |         |        |         |
| TSLP  | rs10043985 total    |        | 77   | 100     | 88   | 100     |        |         |
|       |                     | AA     | 65   | 84      | 67   | 76      | Ref    |         |
|       |                     | AC     | 5    | 7       | 21   | 24      | 4.07   | 1.4499–11.4509 | 7.9287 | <0.005* |
|       |                     | CC     | 7    | 9       | 0    | 0       | —      | 6.8593 | 0.0088* |
|       |                     | AC+CC  | 12   | 16      | 21   | 24      | 1.70   | 0.7728–3.7300 | 1.7593 | 0.1847 |
|       |                     | A      | 135  | 88      | 155  | 88      | Ref    |         |
|       |                     | C      | 19   | 12      | 21   | 24      | 1.70   | 0.7728–3.7300 | 1.7593 | 0.1847 |
|       |                     | rs2289276 total | 113  | 100     | 97   | 100     |        |         |
|       |                     | CC     | 45   | 40      | 43   | 44      | Ref    |         |
|       |                     | CT     | 56   | 49      | 45   | 47      | 0.84   | 0.4740–1.4919 | 0.3510 | 0.5536 |
|       |                     | TT     | 12   | 11      | 9    | 9       | 0.78   | 0.3005–2.0500 | 0.2452 | 0.6205 |
|       |                     | CT+TT  | 68   | 60      | 54   | 56      | 0.83   | 0.4795–1.4402 | 0.4355 | 0.5093 |
|       |                     | C      | 146  | 65      | 131  | 68      | Ref    |         |
|       |                     | T      | 80   | 35      | 63   | 32      | 0.88   | 0.5849–1.3169 | 0.3975 | 0.5928 |
|       |                     | rs3806933 total | 98   | 100     | 73   | 100     |        |         |
|       |                     | CC     | 6    | 6       | 26   | 36      | Ref    |         |
|       |                     | CT     | 52   | 53      | 9    | 12      | 0.04   | 0.0128–0.1243 | 39.5418 | <0.005* |
|       |                     | TT     | 40   | 41      | 38   | 52      | 0.22   | 0.0813–0.5915 | 9.8701 | <0.005* |
|       |                     | CT+TT  | 92   | 94      | 47   | 64      | 0.12   | 0.0454–0.3063 | 23.9247 | <0.005* |
|       |                     | C      | 64   | 33      | 61   | 42      | Ref    |         |
|       |                     | T      | 132  | 67      | 85   | 58      | 0.68   | 0.4333–1.0534 | 3.0600 | 0.0830 |
| TSLPR | rs36139698 total    | 55    | 100   | 84     | 100   |        |        |         |
|       |                     | CC     | 6    | 11      | 4    | 5       | Ref    |         |
|       |                     | CT     | 11   | 20      | 46   | 55      | 6.27   | 1.5072–26.1067 | 7.4432 | 0.0064* |
|       |                     | TT     | 38   | 69      | 34   | 40      | 1.34   | 0.3489–5.1622 | 0.1842 | 0.6678 |
|       |                     | CT+TT  | 49   | 89      | 80   | 95      | 2.45   | 0.6580–9.1144 | 1.8811 | 0.1702 |
|       |                     | C      | 23   | 21      | 54   | 32      | Ref    |         |
|       |                     | T      | 87   | 79      | 114  | 68      | 0.56   | 0.3181–0.9792 | 4.1890 | 0.0407* |
|       |                     | rs36177645 total | 119  | 100     | 47   | 100     |        |         |
|       |                     | AA     | 13   | 11      | 3    | 6       | Ref    |         |
|       |                     | AG     | 39   | 33      | 18   | 38      | 2.00   | 0.5062–7.9025 | 1.0034 | 0.3165 |
|       |                     | GG     | 67   | 56      | 26   | 56      | 1.68   | 0.4427–6.3874 | 0.5926 | 0.4414 |
|       |                     | AG+GG  | 106  | 89      | 44   | 94      | 1.80   | 0.4884–6.6245 | 0.7978 | 0.3717 |
|       |                     | A      | 65   | 27      | 24   | 26      | Ref    |         |
|       |                     | G      | 173  | 73      | 70   | 74      | 1.10   | 0.6359–1.8886 | 0.1087 | 0.7416 |
|       |                     | rs36133495 total | 119  | 100     | 90   | 100     |        |         |
|       |                     | CC     | 24   | 20      | 16   | 18      | Ref    |         |
|       |                     | CT     | 61   | 51      | 43   | 48      | 1.06   | 0.5028–2.2235 | 0.0216 | 0.8830 |
|       |                     | TT     | 34   | 29      | 31   | 34      | 1.37   | 0.6156–3.0382 | 0.5926 | 0.4414 |
|       |                     | CT+TT  | 95   | 80      | 74   | 82      | 1.17   | 0.5792–2.3571 | 0.1892 | 0.6636 |
| Gene      | SNP        | Allele | Controls | >5 years |
|-----------|------------|--------|----------|----------|
|           |            |        | N        | Percent  | N        | Percent | OR       | 95% CI    | χ²  | p value |
| IL-7R     | rs12516866 | C      | 109      | 46       | 75       | 42      | Ref      |           |     |         |
|           | rs1053496  | T      | 129      | 54       | 105      | 58      | 1.18     | 0.8002–1.7488 | 0.7100 | 0.3995  |
| IL-7R     | rs1053496  | T      | 87       | 35       | 28       | 30      | 0.80     | 0.4776–1.3386 | 0.7253 | 0.3944  |
| IL-7       | rs12516866 | GG     | 49       | 40       | 22       | 48      | Ref      |           |     |         |
| IL-7       | rs12516866 | GT     | 61       | 50       | 20       | 43      | 0.73     | 0.3580–1.4895 | 0.7497 | 0.3866  |
| IL-7       | rs12516866 | TT     | 13       | 10       | 4        | 9       | 0.69     | 0.2006–2.3408 | 0.3663 | 0.5450  |
| IL-7       | rs12516866 | GT+TT  | 74       | 60       | 24       | 52      | 0.72     | 0.3653–1.4286 | 0.8770 | 0.3490  |
| IL-7       | rs12516866 | G      | 159      | 65       | 64       | 70      | Ref      |           |     |         |
| IL-7       | rs1053496  | G      | 159      | 65       | 64       | 70      | Ref      |           |     |         |
| Patients   | smoking    |        |          |          |          |          |          |           |     |         |
| for ≤5     | years:     |        |          |          |          |          |          |           |     |         |
| TSLP       | rs10043985 | AA     | 65       | 84       | 45       | 82      | Ref      |           |     |         |
| TSLP       | rs10043985 | AC     | 7        | 9        | 1        | 2       | 0.21     | 0.0245–1.7356 | 2.5304 | 0.1117  |
| TSLP       | rs10043985 | CC     | 12       | 16       | 10       | 18      | 1.20     | 0.4791–3.0243 | 0.1558 | 0.6930  |
| TSLP       | rs10043985 | AC+CC  | 12       | 16       | 10       | 18      | 1.20     | 0.4791–3.0243 | 0.1558 | 0.6930  |
| TSLP       | rs10043985 | A      | 135      | 88       | 99       | 90      | Ref      |           |     |         |
| TSLP       | rs10043985 | C      | 19       | 12       | 11       | 10      | 0.79     | 0.3595–1.7336 | 0.3481 | 0.5552  |
| TSLP       | rs2289276  | CC     | 11       | 12       | 3        | 11      | Ref      |           |     |         |
| TSLP       | rs2289276  | CT     | 19       | 22       | 8        | 28      | 1.54     | 0.3375–7.0630 | 0.3159 | 0.5741  |
| TSLP       | rs2289276  | TT     | 59       | 66       | 17       | 61      | 1.06     | 0.2642–4.2245 | 0.0060 | 0.9380  |
| TSLP       | rs2289276  | CT+TT  | 78       | 88       | 25       | 89      | 1.18     | 0.3035–4.5504 | 0.0547 | 0.8150  |
| TSLP       | rs2289276  | C      | 41       | 23       | 14       | 25      | Ref      |           |     |         |
| TSLP       | rs2289276  | T      | 137      | 77       | 42       | 75      | 0.90     | 0.4466–1.8049 | 0.0916 | 0.7622  |
| TSLP       | rs3806933  | CC     | 45       | 40       | 13       | 22      | Ref      |           |     |         |
| TSLP       | rs3806933  | CT     | 56       | 49       | 32       | 56      | 1.98     | 0.9300–4.2071 | 3.1906 | 0.0741  |
| TSLP       | rs3806933  | TT     | 12       | 11       | 13       | 22      | 3.75     | 1.3820–10.1758 | 7.1085 | 0.0077a |
| TSLP       | rs3806933  | CT+TT  | 68       | 60       | 45       | 78      | 2.29     | 1.1117–4.7203 | 5.1827 | 0.0228a |
| TSLP       | rs3806933  | C      | 146      | 65       | 58       | 50      | Ref      |           |     |         |
| TSLP       | rs3806933  | T      | 80       | 35       | 58       | 50      | 1.83     | 1.1582–2.8758 | 6.7904 | 0.0092a |
| TSLP       | rs3613998  | CC     | 6        | 6        | 17       | 41      | Ref      |           |     |         |
| TSLP       | rs3613998  | CT     | 52       | 53       | 6        | 14      | 0.04     | 0.0116–0.1432 | 32.7314 | <0.005a |
| TSLP       | rs3613998  | TT     | 40       | 41       | 19       | 45      | 0.17     | 0.0570–0.4932 | 11.6898 | <0.005a |
| TSLP       | rs3613998  | CT+TT  | 92       | 94       | 25       | 59      | 0.10     | 0.0342–0.2687 | 25.2719 | <0.005a |
| TSLP       | rs3613998  | C      | 64       | 33       | 40       | 48      | Ref      |           |     |         |
| TSLP       | rs3613998  | T      | 132      | 67       | 44       | 52      | 0.53     | 0.3164–0.8989 | 5.6410 | 0.0175a |
| TSLP       | rs3613998  |        |          |          |          |          |          |           |     |         |

(Continues)
phenotypic and genotypic levels when compared to those of the “CC” genotype and “C” phenotype references (OR = 6.27, CI = 1.507–26.107, \( p = 0.0064 \) and OR = 0.56, CI = 0.318–0.979, \( p = 0.0407 \), respectively). However, rs36139698 showed no association with short-term smokers and controls. The genotype distribution of rs36139698 was 11% CC, 20% CT, and 69% TT in nonsmokers and 5% CC, 55% CT, and 40% TT in long-term smokers. The “T” allele frequency distribution was 79%, 68%, and 71% in nonsmokers, long-term smokers, and short-term smokers, respectively (Table 4). An association was observed between the TSLPR SNPs rs36177645 and rs36133495 with both short-term and long-term smokers (Table 4). TSLPR rs36177645 had the following genotype frequency distributions: 11% AA, 33% AG, and 56% GG in nonsmokers; 6% AA, 38% AG, and 56% GG in long-term smokers; and 16% AA, 43% AG, and 41% GG in short-term smokers. Subjects carrying the TSLPR rs36177645 variant showed more similar phenotypes within the smoker subgroups than those in nonsmoker controls (Table 4). In addition, TSLPR rs36133495 had the following genotype frequencies: 20% CC, 51% CT, and 29% TT in nonsmokers; 18% CC, 51% CT, and 29% TT in long-term smokers.

| Gene  | SNP   | Allele | Controls | >5 years | OR   | 95% CI    | \( \chi^2 \) | \( p \) value |
|-------|-------|--------|----------|----------|------|-----------|-------------|-------------|
|       |       |        | \( N \)  | \%      | \( N \) | \%        |             |             |
|       |       | CT+TT  | 49       | 89       | 35   | 90        | 1.07        | 0.2813–4.0815 | 0.0102 | 0.9195 |
|       |       | C      | 23       | 21       | 23   | 29        | Ref         |             |         |
|       |       | T      | 87       | 79       | 55   | 71        | 0.63        | 0.3237–1.2347 | 1.8171 | 0.1777 |
| total |       |        | 119      | 100      | 37   | 100       |             |             |         |
|       | rs36177645 | AA   | 13       | 11       | 6    | 16        | Ref         |             |         |
|       |       | AG    | 39       | 33       | 16   | 43        | 0.89        | 0.2875–2.7486 | 0.0418 | 0.8379 |
|       |       | GG    | 67       | 56       | 15   | 41        | 0.49        | 0.1586–1.4832 | 1.6534 | 0.1985 |
|       |       | AG+GG | 106      | 89       | 31   | 84        | 0.63        | 0.2224–1.8051 | 0.7389 | 0.3900 |
|       |       | A     | 65       | 27       | 28   | 38        | Ref         |             |         |
|       |       | G     | 173      | 73       | 46   | 62        | 0.62        | 0.3563–1.0694 | 2.9898 | 0.0838 |
| total | rs36133495 | CC   | 24       | 20       | 13   | 23        | Ref         |             |         |
|       |       | CT    | 61       | 51       | 31   | 54        | 0.94        | 0.4209–2.0913 | 0.0243 | 0.8761 |
|       |       | TT    | 34       | 29       | 13   | 23        | 0.71        | 0.2786–1.7883 | 0.5413 | 0.4619 |
|       |       | CT+TT | 95       | 80       | 44   | 77        | 0.86        | 0.3984–1.8352 | 0.1617 | 0.6876 |
|       |       | C     | 109      | 46       | 57   | 50        | Ref         |             |         |
|       |       | T     | 129      | 54       | 57   | 50        | 0.84        | 0.5404–1.3212 | 0.5461 | 0.4599 |
|       | IL‐7R | rs12516866 | total | 123     | 100  | 16   | 100       |             |         |
|       |       | GG    | 49       | 40       | 7    | 44        | Ref         |             |         |
|       |       | GT    | 61       | 50       | 8    | 50        | 0.92        | 0.3112–2.7083 | 0.0240 | 0.8768 |
|       |       | TT    | 13       | 10       | 1    | 6         | 0.54        | 0.0607–4.7764 | 0.3175 | 0.5731 |
|       |       | GT+TT | 74       | 60       | 9    | 56        | 0.85        | 0.2974–2.4369 | 0.0901 | 0.7641 |
|       |       | G     | 159      | 65       | 22   | 69        | Ref         |             |         |
|       |       | T     | 87       | 35       | 10   | 31        | 0.83        | 0.3763–1.8339 | 0.2112 | 0.6459 |
| total | rs1053496 | CC   | 11       | 12       | 4    | 17        | Ref         |             |         |
|       |       | CT    | 19       | 22       | 7    | 31        | 1.01        | 0.2411–4.2570 | 0.0003 | 0.9858 |
|       |       | TT    | 59       | 66       | 12   | 52        | 0.56        | 0.1521–2.0562 | 0.7798 | 0.3772 |
|       |       | CT+TT | 78       | 88       | 19   | 83        | 0.67        | 0.1920–2.3368 | 0.3989 | 0.5276 |
|       |       | C     | 41       | 23       | 15   | 33        | Ref         |             |         |
|       |       | T     | 137      | 77       | 31   | 67        | 0.62        | 0.3046–1.2559 | 1.7873 | 0.1813 |

*\( p < 0.05 \), Ref = Reference allele.
| Gene      | SNP          | Allele | Controls N | Controls Percent | ≥20 Cig. N | ≥20 Cig. Percent | OR     | 95% CI         | \( \chi^2 \) | p value |
|-----------|--------------|--------|------------|------------------|------------|-----------------|--------|----------------|------------|---------|
| TSLP      | rs10043985   | total  | 77         | 100              | 85         | 100              |        |                |            |         |
|           |              | AA     | 65         | 84               | 70         | 82               | Ref    |                |            |         |
|           |              | AC     | 5          | 7                | 15         | 18               | 2.79   | 0.9584–8.0968  | 3.7689    | 0.0522  |
|           |              | CC     | 7          | 9                | 0          | 0                | —      | —              | 7.1584    | 0.0075a |
|           |              | AC+CC  | 12         | 16               | 15         | 18               | 1.16   | 0.5057–2.6640  | 0.1238    | 0.7250  |
|           |              | A      | 135        | 88               | 155        | 91               | Ref    |                |            |         |
|           |              | C      | 19         | 12               | 15         | 9                | 0.69   | 0.3363–1.4059  | 1.0624    | 0.3027  |
|           | rs2289276    | total  | 113        | 100              | 92         | 100              | Ref    |                |            |         |
|           |              | CC     | 45         | 40               | 36         | 39               | Ref    |                |            |         |
|           |              | CT     | 56         | 49               | 44         | 48               | 0.98   | 0.5445–1.7716  | 0.0036    | 0.9523  |
|           |              | TT     | 12         | 11               | 12         | 13               | 1.25   | 0.5020–3.1126  | 0.2303    | 0.6313  |
|           |              | CT+TT  | 68         | 60               | 56         | 61               | 1.03   | 0.5861–1.8079  | 0.0102    | 0.9196  |
|           |              | C      | 146        | 65               | 116        | 63               | Ref    |                |            |         |
|           |              | T      | 80         | 35               | 68         | 37               | 1.07   | 0.7136–1.6038  | 0.1068    | 0.7439  |
|           | rs3806933    | total  | 98         | 100              | 68         | 100              |        |                |            |         |
|           |              | CC     | 6          | 6                | 27         | 40               | Ref    |                |            |         |
|           |              | CT     | 52         | 53               | 11         | 16               | 0.05   | 0.0157–0.1409  | 37.5074   | <0.005a |
|           |              | TT     | 40         | 41               | 30         | 44               | 0.17   | 0.0611–0.4546  | 13.7746   | <0.005a |
|           |              | CT+TT  | 92         | 94               | 41         | 60               | 0.10   | 0.0380–0.2582  | 28.4268   | <0.005a |
|           |              | C      | 64         | 33               | 65         | 48               | Ref    |                |            |         |
|           |              | T      | 132        | 67               | 71         | 52               | 0.53   | 0.3378–0.8304  | 7.7475    | 0.0054a |
| TSLPR     | rs36139698   | total  | 55         | 100              | 75         | 100              |        |                |            |         |
|           |              | CC     | 6          | 11               | 5          | 6                | Ref    |                |            |         |
|           |              | CT     | 11         | 20               | 35         | 47               | 3.82   | 0.9735–14.9748 | 3.9800    | 0.0460a |
|           |              | TT     | 38         | 69               | 35         | 47               | 1.11   | 0.3096–3.9458  | 0.0238    | 0.8775  |
|           |              | CT+TT  | 49         | 89               | 70         | 94               | 1.71   | 0.4952–5.9341  | 0.7373    | 0.3905  |
|           |              | C      | 23         | 21               | 45         | 30               | Ref    |                |            |         |
|           |              | T      | 87         | 79               | 105        | 70               | 0.62   | 0.3464–1.0986  | 2.7156    | 0.0994  |
|           | rs36177645   | total  | 119        | 100              | 54         | 100              |        |                |            |         |
|           |              | AA     | 13         | 11               | 4          | 7                | Ref    |                |            |         |
|           |              | AG     | 39         | 33               | 20         | 37               | 1.67   | 0.4806–5.7800  | 0.6567    | 0.4177  |
|           |              | GG     | 67         | 56               | 30         | 56               | 1.46   | 0.4381–4.8341  | 0.3783    | 0.5385  |
|           |              | AG+GG  | 106        | 89               | 50         | 93               | 1.53   | 0.4758–4.9395  | 0.5185    | 0.4715  |
|           |              | A      | 65         | 27               | 28         | 26               | Ref    |                |            |         |
|           |              | G      | 173        | 73               | 80         | 74               | 1.07   | 0.6406–1.7989  | 0.0725    | 0.7877  |
|           | rs36133495   | total  | 119        | 100              | 86         | 100              |        |                |            |         |
|           |              | CC     | 24         | 20               | 19         | 22               | Ref    |                |            |         |
|           |              | CT     | 61         | 51               | 43         | 50               | 0.89   | 0.4346–1.8244  | 0.1006    | 0.7511  |
|           |              | TT     | 34         | 29               | 24         | 28               | 0.89   | 0.4018–1.9786  | 0.0796    | 0.7779  |
|           |              | CT+TT  | 95         | 80               | 67         | 78               | 0.89   | 0.4521–1.7554  | 0.1116    | 0.7383  |
|           |              | C      | 109        | 46               | 81         | 47               | Ref    |                |            |         |

(Continues)
| Gene     | SNP          | Allele | Controls | ≥20 Cig. | OR    | 95% CI            | χ²       | p value |
|----------|--------------|--------|----------|----------|-------|-------------------|----------|---------|
|          |              | N      | Percent  | N        | Percent | ≥20 Cig.          |          |         |
|          |              |        |          |          |        |                   |          |         |
| T        | 129          | 54     | 91       | 53       | 0.95   | 0.6406–1.4067     | 0.0673   | 0.7953  |
| IL-7R    | rs12516866   |        |          |          |        |                   |          |         |
|          | total        | 123    | 100      | 33       | 100    |                   |          |         |
|          | GG           | 49     | 40       | 16       | 49     | Ref               |          |         |
|          | GT           | 61     | 50       | 14       | 42     | 0.70              | 0.3127–1.5798 | 0.7319   | 0.3923  |
|          | TT           | 13     | 10       | 3        | 9      | 0.71              | 0.1784–2.7992 | 0.2460   | 0.6199  |
|          | GT+TT        | 74     | 60       | 17       | 51     | 0.70              | 0.3250–1.5229 | 0.8005   | 0.3709  |
|          | G            | 159    | 65       | 46       | 70     | Ref               |          |         |
|          | T            | 87     | 35       | 20       | 30     | 0.79              | 0.4420–1.4284 | 0.5919   | 0.4417  |
| IL-7R    | rs1053496    |        |          |          |        |                   |          |         |
|          | total        | 89     | 100      | 37       | 100    |                   |          |         |
|          | CC           | 11     | 12       | 5        | 14     | Ref               |          |         |
|          | CT           | 19     | 22       | 9        | 24     | 1.04              | 0.2779–3.9072 | 0.0037   | 0.9512  |
|          | TT           | 59     | 66       | 23       | 62     | 0.86              | 0.2684–2.7406 | 0.0672   | 0.7954  |
|          | CT+TT        | 78     | 88       | 32       | 86     | 0.90              | 0.2903–2.8063 | 0.0314   | 0.8594  |
|          | C            | 41     | 23       | 19       | 26     | Ref               |          |         |
|          | T            | 137    | 77       | 55       | 74     | 0.87              | 0.4625–1.6226 | 0.2011   | 0.6538  |
| TSLP     | rs10043985   |        |          |          |        |                   |          |         |
|          | total        | 77     | 100      | 53       | 100    |                   |          |         |
|          | AA           | 65     | 84       | 40       | 76     | Ref               |          |         |
|          | AC           | 5      | 7        | 12       | 22     | 3.90              | 1.2787–11.8953 | 6.3165   | 0.0120  |
|          | CC           | 7      | 9        | 1        | 2      | 0.23              | 0.0275–1.9574 | 2.1065   | 0.1467  |
|          | AC+CC        | 12     | 16       | 13       | 24     | 1.76              | 0.7317–4.2355 | 1.6167   | 0.2036  |
|          | A            | 135    | 88       | 92       | 87     | Ref               |          |         |
|          | C            | 19     | 12       | 14       | 13     | 1.08              | 0.5162–2.2650 | 0.0429   | 0.8360  |
|          | T            | 113    | 100      | 57       | 100    |                   |          |         |
|          | rs2289276    |        |          |          |        |                   |          |         |
|          | total        | 113    | 100      | 57       | 100    |                   |          |         |
|          | CC           | 45     | 40       | 18       | 32     | Ref               |          |         |
|          | CT           | 56     | 49       | 30       | 52     | 1.34              | 0.6625–2.7075 | 0.6635   | 0.4153  |
|          | TT           | 12     | 11       | 9        | 16     | 1.88              | 0.6743–5.2134 | 1.4737   | 0.2248  |
|          | CT+TT        | 68     | 60       | 39       | 68     | 1.43              | 0.7310–2.8122 | 1.1040   | 0.2934  |
|          | C            | 146    | 65       | 66       | 58     | Ref               |          |         |
|          | T            | 80     | 35       | 48       | 42     | 1.33              | 0.8370–2.1047 | 1.4521   | 0.2282  |
|          | rs3806933    |        |          |          |        |                   |          |         |
|          | total        | 98     | 100      | 43       | 100    |                   |          |         |
|          | CC           | 6      | 6        | 16       | 37     | Ref               |          |         |
|          | CT           | 52     | 53       | 4        | 9      | 0.03              | 0.0072–0.1151 | 35.6327  | <0.005* |
|          | TT           | 40     | 41       | 23       | 54     | 0.22              | 0.0740–0.6282 | 8.6147   | <0.005* |
|          | CT+TT        | 92     | 94       | 27       | 63     | 0.11              | 0.0392–0.3088 | 21.9330  | <0.005* |
|          | C            | 64     | 33       | 36       | 42     | Ref               |          |         |
|          | T            | 132    | 67       | 50       | 58     | 0.67              | 0.3995–1.1351 | 2.2141   | 0.1368  |
| TSLPR    | rs36139698   |        |          |          |        |                   |          |         |
|          | total        | 55     | 100      | 42       | 100    |                   |          |         |
|          | CC           | 6      | 11       | 3        | 7      | Ref               |          |         |
|          | CT           | 11     | 20       | 24       | 57     | 4.36              | 0.9180–20.7425 | 3.7495   | 0.0528  |
|          | TT           | 38     | 69       | 15       | 36     | 0.79              | 0.1745–3.5713 | 0.0945   | 0.7585  |

(Continues)
smokers; and 23% CC, 54% CT, and 23% TT in short-term smokers. The rs36133495 phenotype distribution was more similar among the different smoker subgroups when compared to nonsmoker controls (Table 4).

Finally, we investigated the potential association between IL7R SNPs and cigarette smoking based on duration of smoking. We observed no significant correlations with smoking behavior for both rs12516866 and rs1053496. IL7R rs12516866 showed the following genotype distribution: 40% GG, 50% GT, and 10% TT in nonsmokers; 48% GG, 43% GT, and 9% TT in long-term smokers; and 44%, 50%, and 6% in short-term smoker. However, the phenotype distribution was 65% G and 35% T in long-term smokers and 70% G and 30% T in nonsmokers. Phenotype B for this SNP showed a genotype distribution of 40% GG, 50% GT, and 10% TT in nonsmokers and 44% GG, 50% GT, and 6% TT in smokers. Phenotype G was observed in 65%, 70%, and 69% of non-smokers, long-term smokers, and short-term smokers, respectively, while the mutant phenotype T was observed in 35%, 30%, and 31%, respectively (Table 4). By contrast, the respective genotype distributions of the IL7R rs1053496 SNP for nonsmokers, long-term smokers, and short-term smokers were 12%, 11%, and 17% for the “CC” genotype, 22%, 28%, and 31% for “CT,” and 66%, 61%, and 52% for “TT”

**Table 5 (Continued)**

| Gene | SNP | Allele | Controls | OR | 95% CI | χ² | p value |
|------|-----|--------|----------|----|--------|----|---------|
|      |     |        | ≥20 Cig. |    |        |    |         |

|                        | N  | Percent | N  | Percent |     |       |
|------------------------|----|---------|----|---------|-----|--------|
| **CT+TT**              | 49 | 89      | 39 | 93      | 1.59| 0.3740–6.7750 | 0.4013 | 0.5264 |
| **C**                  | 23 | 21      | 30 | 36      | Ref |        |
| **T**                  | 87 | 79      | 54 | 64      | 0.48| 0.2508–0.9030 | 5.2578 | 0.0218 |
| **total**              | 119| 100     | 28 | 100     |     |        |
| **rs36177645**         |    |         |    |         |     |        |
| **AA**                 | 13 | 11      | 5  | 18      | Ref |        |
| **AG**                 | 39 | 33      | 13 | 46      | 0.87| 0.2591–2.8988 | 0.0540 | 0.8162 |
| **GG**                 | 67 | 56      | 10 | 36      | 0.39| 0.1138–1.3236 | 2.4004 | 0.1213 |
| **AG+GG**              | 106| 89      | 23 | 82      | 0.56| 0.1830–1.7388 | 1.0139 | 0.3140 |
| **A**                  | 65 | 27      | 23 | 41      | Ref |        |
| **G**                  | 173| 73      | 33 | 59      | 0.54| 0.2947–0.9861 | 4.0929 | 0.0431 |
| **total**              | 119| 100     | 55 | 100     |     |        |
| **rs36133495**         |    |         |    |         |     |        |
| **CC**                 | 24 | 20      | 9  | 16      | Ref |        |
| **CT**                 | 61 | 51      | 29 | 53      | 1.27| 0.5235–3.0703 | 0.2771 | 0.5986 |
| **TT**                 | 34 | 29      | 17 | 31      | 1.33| 0.5094–3.4900 | 0.3443 | 0.5573 |
| **CT+TT**              | 95 | 80      | 46 | 84      | 1.29| 0.5557–3.0003 | 0.3542 | 0.5517 |
| **C**                  | 109| 46      | 47 | 43      | Ref |        |
| **T**                  | 129| 54      | 63 | 57      | 1.13| 0.7180–1.7866 | 0.2869 | 0.5922 |
| **IL‐7R rs12516866**   |    |         |    |         |     |        |
| **GG**                 | 49 | 40      | 12 | 46      | Ref |        |
| **GT**                 | 61 | 50      | 13 | 50      | 0.87| 0.3646–2.0773 | 0.0981 | 0.7541 |
| **TT**                 | 13 | 10      | 1  | 4       | 0.31| 0.0373–2.6424 | 1.2475 | 0.2640 |
| **GT+TT**              | 74 | 60      | 14 | 54      | 0.77| 0.3297–1.8099 | 0.3542 | 0.5518 |
| **G**                  | 159| 65      | 37 | 71      | Ref |        |
| **T**                  | 87 | 35      | 15 | 29      | 0.74| 0.3851–1.4255 | 0.8105 | 0.3680 |
| **total**              | 123| 100     | 26 | 100     |     |        |
| **rs1053496**          |    |         |    |         |     |        |
| **CC**                 | 11 | 12      | 1  | 8       | Ref |        |
| **CT**                 | 19 | 22      | 5  | 38      | 2.89| 0.2985–28.0715 | 0.9000 | 0.3428 |
| **TT**                 | 59 | 66      | 7  | 54      | 1.31| 0.1458–11.6842 | 0.0570 | 0.8113 |
| **CT+TT**              | 78 | 88      | 12 | 92      | 1.69| 0.2000–14.3186 | 0.2380 | 0.6256 |
| **C**                  | 41 | 23      | 7  | 27      | Ref |        |
| **T**                  | 137| 77      | 19 | 73      | 0.81| 0.3192–2.0675 | 0.1907 | 0.6623 |

*p < 0.05, Ref = Reference allele.
In addition, the respective phenotype distributions for nonsmokers, long-term smokers, and short-term smokers were 23%, 25%, and 33% for the “C” reference allele and 77%, 75%, and 67% for the “T” mutant allele (Table 4).

### 3.4 | Association between TSLP, TSLPR, and IL7R SNPs and daily cigarette consumption

To investigate the association between daily cigarette consumption and genetic variations in TSLP and its receptors, smokers were categorized into the following two subgroups according to smoking frequency: heavy smokers, who consumed ≥20 cigarettes per day (about one pack; termed group A) and moderate smokers, who smoked <20 cigarettes daily (termed group B). Table 5 displays the genotypic distributions of the selected SNPs in either group A or group B relative to the entire control group. Two of the three TSLP SNPs analyzed showed statistically significant associations with smoking in both smokers subgroup (categories A and B) relative to non-smokers. The first TSLP SNP, rs10043985, had the following respective genotype distributions for nonsmokers, group A and B: 84%, 82%, and 76% for the “AA” reference allele; 7%, 18%, and 22% for heterozygous “AC”; and 9%, 0%, and 2% for double mutant “CC.” Notably, the double mutant “CC” genotype showed a clear association with group A smokers (p = 0.0075), whereas the heterozygous “AC” genotype showed more than fourfold higher correlation with group B smokers when compared to the “CC” homozygous reference genotype (OR = 3.90; CI = 1.279–11.895; p = 0.0120). The second SNPs is rs3806933, which showed a strong association with smoking in group A and B smokers relative to nonsmoker subjects (p < 0.005). The “T” allele was highly associated with group A smokers relative to controls (p = 0.0054) but did not appear to be associated with group B smokers (p = 0.1368) (Table 5). However, there were no significant associations between TSLP rs2289276 and both smoking groups. rs2289276 showed the following genotype distributions: 40% CC, 49% CT, and 11% TT in nonsmokers; 39% CC, 48% CT, and 13% TT in group A smokers; and 32% CC, 52% CT, and 16% TT in group B smokers (Table 5).

To evaluate the association between TSLPR SNPs and smoking based on daily cigarette consumption, we examined the genotype distributions and allele frequencies for the three TSLPR SNPs. Results of the analysis are summarized in Table 5. Only rs36139698 was found to be associated with group A smokers relative to control subjects. We observed that the “CT” genotype had a fourfold higher association with smoking (OR = 3.82; CI = 0.974–14.975; p = 0.0460) in group A smokers compared to controls. In addition, rs36139698 showed no association with smoking at the phenotypic level; however, there was a protective association between allele T and smoking in group B smokers (OR = 0.48; CI = 0.251–0.903; p = 0.0218). For TSLPR SNP rs36177645, our analysis showed no significant differences between nonsmokers and group A smokers at both the genotype and phenotype levels; however, the “G” allele was strongly associated with smoking in the second category compared to control subjects (p = 0.0431). Additionally, TSLPR rs36133495 did not show any correlation with smoking in either group A or group B smokers (Table 5).

Finally, the two IL7R SNPs, namely, rs12516866 and rs1053496, showed no significant correlations with either group A or group B smokers (Table 5).

### 3.5 | Structural and functional analysis of the P195L mutation in rs36139698

We examined the effects of the polymorphisms on the structure and function of TSLP and TSLPR. The TSLP SNPs selected in the current study were located in the promoter and 5′-UTR regions and can influence TSLP expression in smokers by increasing promoter activity and enhancing transcription. However, TSLPR SNPs were located in the exon region and thus potentially affected TSLPR function. Only rs36139698 appeared to be associated with smoking in the Saudi population. Structural analysis showed that rs36139698 results in a proline 195 to leucine mutation. This residue is located on the surface of the extracellular domain of TSLPR close to a WS motif located between residues 200 and 204.

Sequence alignment of several TSLPRs (Figure 1) indicated that this proline residue is partially conserved and is replaced by a leucine in the mouse, similar to the rs36139698 variant. The P195L mutation is located on the surface and is accessible for hydrophobic interactions with TSLP, as observed in the mouse TSLPR structure.

From the X-ray structure of the mouse TSLP-TSLPR-IL7α complex, this leucine is located in a loop at the interface and participates in hydrophobic interactions with TSLP. Substitution of proline by a leucine in the TSLPR human variant facilitates additional hydrophobic interactions that can further strengthen the binding with TSLP. No similar human protein structures are available. The stability of the P195L variant was assessed using CUPSAT stability prediction server. The variant has a predicted ΔΔG increase of 2.15 kcal/mol, thereby increasing the stability of the protein structure. This increased stability could increase the half-life of the receptor and make it available for stronger interactions with TSLP, which in turn prolongs inflammation.

### 4 | DISCUSSION

For a long period of time, scientific studies have not investigated the harmful effects of cigarette smoking on the oral cavity, lungs, and respiratory system. However, tobacco
smoke has been later demonstrated to disrupt the lung and gingival epithelial barrier function (Semlali, Witoled, Alanazi, & Rouabhia, 2012), impair the innate immune system, and damage tissues by activating a variety of inflammatory immune cells. Semlali et al. provided substantial evidence that cigarette smoking (CS) promotes inflammation in the oral cavity and contributes to the development of gingival and periodontal disease by promoting the secretion of inflammatory cytokines (Rouabhia et al., 2017; Semlali, Chakir, Goulet, et al., 2011; Semlali, Chakir, & Rouabhia, 2011; Semlali et al., 2012). Genetic variants in the genes encoding these cytokines may contribute to susceptibility to smoking-related diseases. Identifying the specific role of CS in acute inflammation is an important step towards elucidating the mechanisms underlying tobacco-induced disease and can be used to develop novel therapeutic approaches for the management of diseases that afflict smokers. To our knowledge, the current study is the first to describe the association between variations in genes encoding TSLP and its receptors (TSLPR and IL7R) in smokers in Saudi Arabia, which has relatively high rates of smoking. The Saudi population has a considerably high incidence of respiratory diseases like asthma, COPD, periodontal diseases, oral cancers, and other tobacco-related diseases. Thus, we analyzed and compared the frequencies of the TSLP and TSLPR polymorphisms from DNA isolated from smokers and healthy controls. Our findings highlight significant associations of TSLP and TSLPR SNPs, but not IL7R SNPs, with smoking behavior among Saudi smokers. Two TSLP SNPs, namely, rs10043985 and rs3806933, showed the strongest associations with smoking (p = 0.01 and p < 0.005, respectively). Furthermore, the SNPs rs3806933 and 10,043,985 were predicted to be implicated in proximal transcriptional regulation of TSLP. These polymorphisms are located in the promoter region of TSLP and could thus influence TSLP expression in smokers by increasing promoter activity and enhancing the binding of the transcription factor activating protein AP-1 to the regulatory element of TSLP (Harada et al., 2009, 2011). This site is known to bind major transcription factors that regulate the expression of multiple inflammatory cytokines that play crucial roles in the pathogenesis of various airway diseases. Conversely, alterations in TSLP gene expression can directly affect the pathways involved in the development of inflammatory diseases.

Although the 5’-UTR rs2289276 polymorphism was reported to be associated with higher risk of respiratory disease, such as asthma (Harada et al., 2011), it was not found to be associated with smoking in the population studied. Previous genome-wide association studies have documented an association between the TSLP SNPs and risk for allergy diseases, such as asthma and airway hyperresponsiveness (Ferreira et al., 2014; Hirota et al., 2011; Torgerson et al., 2011). The principal role of the polymorphisms selected in the current study in diseases related to smoking still unclear. Thus, the functional role of the TSLP polymorphism requires further investigation. Accumulating evidence has also supported the role of TSLP in promoting inflammation in the pathogenesis of infectious and autoimmune diseases, including oral cancer and asthma. We (Semlali, Jacques, Koussih, Gounni, & Chakir, 2010) and other authors (Hui et al., 2014; Lee et al., 2012) have previously demonstrated that TSLP expression is upregulated in asthma patients relative to healthy controls.

TSLPR and IL7R are the core subunits of the TSLP receptor and play crucial roles in TSLP signaling during inflammatory response. All three TSLPR SNPs studied herein are located in the exon region, and we hypothesized that the mutant TSLPR exhibits higher stability than the wild-type TSLPR. In turn, this increased stability can prolong TSLP-induced signal transduction and induce constitutive activation of the principal pathway of TSLP (Jak-STAT pathway), causing inflammatory diseases as suggested.

FIGURE 1 (a) Homology modeling of human TSLP receptor with P195L mutation. (b) Sequence alignment of TSLPR from different species near Proline 195. TSLPR rs 36139698 is located in the exon region and results in a proline 195 to leucine mutation.
recently by Mullighan et al (Ferreira et al., 2014). The results appear to support our hypothesis that the rs36139698 polymorphism, which corresponds to substitution of proline 195 into leucine and produces a TSLPR variant with a predicted ∆ΔG increase of 2.15 kcal/mol, making the variant more stable than its wild-type counterpart. This increased stability might increase the half-life of the receptor making it available for interaction with TSLP maintaining the inflammation. P195L mutation located in the extracellular protein domain is able to bind to TSLP and is close to a WS motif, located between residues 200 and 204 involved in receptor activation. Changes in the structural rigidity of this segment introduced by the P195L mutation may affect the function of the WS domain.

Consistent with previous studies, TSLPR gene polymorphisms were found to be correlated with increased susceptibility to atopic asthma in the Korean population (Yu et al., 2010) and with systemic lupus erythematosus (Yu, Chun, Yun, Moon, & Chae, 2012). However, although several SNPs in IL7R have been associated with a wide range of diseases like liver disease in HIV/HCV infected patients (Guzmán-Fulgencio et al., 2015) and sclerosis risk (Wu et al., 2016), finally, our analysis demonstrated that smoking duration and consumption are correlated with the genotype frequencies of TSLP and TSLPR variants.

5 | CONCLUSIONS

Although TSLP and TSLPR play crucial roles in inflammatory responses, the results of our study demonstrated a correlation between the TSLP and TSLPR variants and smoking behavior. Overall, our findings suggested that these genes can be utilized as diagnostic markers for all cigarette-related diseases.

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CONFLICTS OF INTEREST

All authors declare no conflict of interest and all authors approved the manuscript.

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REFERENCES

Acevedo, A., Brodsky, L., & Andino, R. (2014). Mutational and fitness landscapes of an RNA virus revealed through population sequencing. Nature, 505(7485), 686–690. https://doi.org/10.1038/nature12861

ACS Inc. (2014). *Inc A: Cancer Facts & Figures 2014*. Atlanta: ACS Inc.

Alamri, A., Semlali, A., Jacques, E., Alanazi, M., Zakrzewski, A., Chmielewski, W., & Rouabhia, M. (2015). Long-term exposure of human gingival fibroblasts to cigarette smoke condensate reduces cell growth by modulating Bax, caspase-3 and p53 expression. *Journal of Periodontal Research*, 50(4), 423–433. https://doi.org/10.1111/jre.12223

Al-Shami, A., Spolski, R., Kelly, J., Keane-Myers, A., & Leonard, W. J. (2005). A role for TSLP in the development of inflammation in an asthma model. *Journal of Experimental Medicine*, 202(6), 829–839. https://doi.org/10.1084/jem.20050199

Baig, M., Bakarmar, M. A., Gazzaz, Z. J., Khabal, M. N., Ahmed, T. J., Qureshi, I. A., … Alshehi, F. M. (2016). Reasons and motivations for cigarette smoking and barriers against quitting among a sample of young people in Jeddah, Saudi Arabia. *Asian Pacific Journal of Cancer Prevention*, 17(7), 3483–3487.

Bassiony, M. M. (2009). Smoking in Saudi Arabia. *Saudi Medical Journal*, 30(7), 876–881.

Centers for Disease Control and Prevention. (2010). *In: How tobacco smoke causes disease: the biology and behavioral basis for smoking-attributable disease: A Report of the Surgeon General*. Atlanta, GA.

Darrell, A., Coleman, B. I., Benenati, B., Brown, K. M., Blader, I. J., March, G. T., & Gubbels, M. J. (2014). Whole genome profiling of spontaneous and chemically induced mutations in *Toxoplasma gondii*. *BMC Genomics*, 15, 354. https://doi.org/10.1186/1471-2164-15-354

Ferreira, M. A., Matheson, M. C., Tang, C. S., Granell, R., Ang, W., Hui, J., … Hopper, J. L. (2014). Genome-wide association analysis identifies 11 risk variants associated with the asthma with hay fever phenotype. *The Journal of Allergy and Clinical Immunology*, 133(6), 1564–1571.

Gutierrez, A., Suh, R., Abtin, F., Genshaft, S., & Brown, K. (2013). Lung cancer screening. *Seminars in Interventional Radiology*, 30(2), 114–120. https://doi.org/10.1055/s-0033-1342951

Guzmán-Fulgencio, M., Berenguer, J., Jiménez-Sousa, M. A., Pineda-Tenor, D., Aldamiz-Echevarría, T., Garcia-Broncano, P., … Resino, S. (2015). Association between IL7R polymorphisms and severe liver disease in HIV/HCV coinfected patients: A cross-sectional study. *Journal of Translational Medicine*, 13. https://doi.org/10.1186/s12967-015-0577-y

Harada, M., Hirota, T., Jodo, A. I., Doi, S., Kameda, M., Fujita, K., … Tamarai, M. (2009). Functional analysis of the thymic stromal lymphopoietin variants in human bronchial epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, 40(3), 368–374. https://doi.org/10.1165/rcmb.2008-0041oc

Harada, M., Hirota, T., Jodo, A. I., Hitomi, Y., Sakashita, M., Tsunoda, T., … Tamarai, M. (2011). Thymic stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. *American Journal of Respiratory Cell and Molecular Biology*, 44(6), 787–793. https://doi.org/10.1165/rcmb.2009-0418oc

He, J. Q., Hallstrand, T. S., Knight, D., Chan-Yeung, M., Sandford, A., Tripp, B., … Daley, D. (2009). A thymic stromal lymphopoietin gene variant is associated with asthma and airway hyperresponsiveness. *Journal of Allergy and Clinical Immunology*, 124(2), 222–229. https://doi.org/10.1016/j.jaci.2009.04.018

Hilton, D. J., Watowich, S. S., Katz, L., & Lodish, H. F. (1996). Saturation mutagenesis of the WSXWS motif of the erythropoietin receptor. *The Journal of Biological Chemistry*, 271(9), 4699–4708. https://doi.org/10.1074/jbc.271.9.4699
Hirota, T., Takahashi, A., Kubo, M., Tsunoda, T., Tomita, K., Doi, S., … Tamarai, M. (2011). Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. *Nature Genetics, 43*(9), 893–896.

Hui, C. C., Murphy, D. M., Neighbour, H., Al-Sayegh, M., O’Byrne, S., Thong, B., … Larche, M. (2014). T cell-mediated induction of thymic stromal lymphopoeitin in differentiated human primary bronchial epithelial cells. *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology, 44*(7), 953–964.

Husgafvel-Pursiainen, K., & Kannio, A. (1996). Cigarette smoking and p53 mutations in cancer and bladder cancer. *Environmental Health Perspectives, 104*, 553–556. https://doi.org/10.1289/ehp.96104s3553

Kohailan, M., Alazani, M., Rouabha, M., Al Amri, A., Parine, N. R., & Semlali, A. (2017). Two SNPs in the promoter region of Toll-like receptor 4 gene are not associated with smoking in Saudi Arabia. *OncoTargets and Therapy, 10*, 745–752. https://doi.org/10.2147/ott.s11971

Kohailan, M., Alazani, M., Rouabha, M., Alamri, A., Parine, N. R., Alhadheq, A., … Semlali, B. (2016). Effect of smoking on the genetic makeup of toll-like receptors 2 and 6. *Onco Targets and Therapy, 9*, 7187–7198.

Lee, H. C., Headley, M. B., Loo, Y. M., Berlin, A., Gale, M. Jr, Debley, J. S., … Ziegler, S. F. (2012). Thymic stromal lymphopoietin is induced by respiratory syncytial virus-infected airway epithelial cells and promotes a type 2 response to infection. *The Journal of Allergy and Clinical Immunology, 130*(5), 1187–1196.e1185.

Liu, M., Rogers, L., Cheng, Q., Shao, Y., Fernandez-Beros, M. E., Hirschhorn, J. N., … Reibman, J. (2011). Genetic variants of TSLP and asthma in an admixed urban population. *PLoS ONE, 6*(9), e25099. https://doi.org/10.1371/journal.pone.0025099

Liu, Y. J., Soumelis, V., Watanabe, N., Ito, T., Wang, Y. H., Malefyt, R. D., … Ziegler, S. F. (2007). TSLP: An epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annual Review of Immunology, 25*, 193–219. https://doi.org/10.1146/annurev.immunol.25.022106.141718

Menotti, A., Puddu, P. E., Maiani, G., & Catasta, G. (2015). Lifestyle behavior and lifetime incidence of heart diseases. *International Journal of Cardiology, 201*, 293–299. https://doi.org/10.1016/j.ijcard.2015.08.050

Pandey, A., Ozaki, K., Baumann, H., Levin, S. D., Puel, A., Farr, A. G., … Lodish, H. F. (2000). Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. *Nature Immunology, 1*(1), 59–64.

Park, L. S., Martin, U., Garka, K., Gliniak, B., Di Santo, J. P., Muller, W., … Sims, J. E. (2000). Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: Formation of a functional heteromer complex requires interleukin 7 receptor. *The Journal of Experimental Medicine, 192*(5), 659–670.

Parthiban, V., Gromiha, M. M., & Schomburg, D. (2006). CUPSAT: Prediction of protein stability upon point mutations. *Nucleic Acids Research, 34*, W239–W242.

Pfeifer, G. P., Demissaenko, M. F., Olivier, M., Tretyakova, N., Hecht, S. S., & Hainaut, P. (2002). Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene, 21*(48), 7435–7451. https://doi.org/10.1038/sj.onc.1205809

Qiu, F., Fan, P., Nie, G. D., Liu, H., Liang, C. L., Yu, W., & Dai, Z. (2017). Effects of cigarette smoking on transplant survival: Extending or shortening it? *Frontiers in Immunology, 8*, 127. https://doi.org/10.3389/fimmu.2017.00127

Quentmeier, H., Drexler, H. G., Fleckenstein, D., Zaborski, M., Armstrong, A., Sims, J. E., & Lyman, S. D. (2001). Cloning of human thymic stromal lymphopoetin (TSLP) and signaling mechanisms leading to proliferation. *Leukemia, 15*(8), 1286–1292.

Rouabha, M., Park, H. J., Semlali, A., Zakrzewski, A., Chmielewski, W., … Chakir, J. (2017). E-cigarette vapor induces an apoptotic response in human gingival epithelial cells through the caspase-3 pathway. *Journal of Cellular Physiology, 232*(6), 1539–1547.

Semlali, A., Chakir, J., Goulet, J. P., Chmielewski, W., & Rouabha, M. (2011). Whole cigarette smoke promotes human gingival epithelial cell apoptosis and inhibits cell repair processes. *Journal of Periodontal Research, 46*(5), 533–541. https://doi.org/10.1111/j.1600-0765.2011.01370.x

Semlali, A., Chakir, J., & Rouabha, M. (2011). Effects of whole cigarette smoke on human gingival fibroblast adhesion, growth, and migration. *Journal of Toxicology and Environmental Health Part A, 74*(13), 848–862. https://doi.org/10.1080/15287394.2011.570230

Semlali, A., Jacques, E., Koushish, L., Gounni, A. S., & Chakir, J. (2010). Thymic stromal lymphopoietin-induced human asthma airway epithelial cell proliferation through an IL-13-dependent pathway. *The Journal of Allergy and Clinical Immunology, 125*(4), 844–850.

Semlali, A., Jalouli, M., Parine, N. R., Al Amri, A., Arafah, M., Al Naeem, A., … Alazani, M. S. (2017). Toll-like receptor 4 as a predictor of clinical outcomes of estrogen receptor-negative breast cancer in Saudi women. *Onco Targets and Therapy, 10*, 1207–1216. https://doi.org/10.2147/ott.s112165

Semlali, A., Parine, N. R., Al Amri, A., Azzi, A., Arafah, M., Kohailan, M., … Alazani, M. S. (2017). Association between TLR-9 polymorphisms and colon cancer susceptibility in Saudi Arabian female patients. *Onco Targets and Therapy, 10*, 1–11.

Semlali, A., Reddy Parine, N., Arafah, M., Mansour, L., Azzi, A., Al Shahrani, O., … Alazani, M. S. (2016). Expression and polymorphism of toll-like receptor 4 and effect on NF-kappaB mediated inflammation in colon cancer patients. *PLoS ONE, 11*(1), e0146333.

Semlali, A., Witoled, C., Alazani, M., & Rouabha, M. (2012). Whole cigarette smoke increased the expression of TLRs, HBDS, and proinflammatory cytokines by human gingival epithelial cells through different signaling pathways. *PLoS ONE, 7*(12), e52614.

Shi, L. Y., Leu, S. W., Xu, F., Zhou, X. L., Yin, H. P., Cai, L. F., & Zhang, L. H. (2008). Local blockade of TSLP receptor alleviated allergic disease by regulating airway dendritic cells. *Clinical Immunology, 129*(2), 202–210.

Spann, K., Snape, N., Baturcam, E., & Fantino, E. (2016). The impact of early-life exposure to air-borne environmental insults on the function of the airway epithelium in asthma. *Annals of Global Health, 82*(1), 28–40.

Steenard, R. V., Ligthart, S., Stolk, L., Peters, M. J., van Meurs, J. B., Uitterlinden, A. G., … Dehghan, A. (2015). Tobacco smoking is associated with methylation of genes related to coronary artery disease. *Clinical Epigenetics, 7*(1), https://doi.org/10.1007/s13148-015-0088-y

Taghavi, N., Birmajimal, F., Sotoudeh, M., Moaven, O., Khademii, H., Abbaszadegan, M. R., & Malekzadeh, R. (2010). Association of p53/p21 expression with cigarette smoking and prognosis in esophageal squamous cell carcinoma patients. *World Journal of Gastroenterology, 16*(39), 4958–4967. https://doi.org/10.3748/wjg.v16.i39.4958

Tonoizuka, Y., Fujiyo, K., Sugiymata, T., Nosaka, T., Hirai, M., & Kitamura, T. (2001). Molecular cloning of a human novel type I
cytokine receptor related to delta1/TSLPR. *Cytogenetics and Cell Genetics, 93*(1–2), 23–25.

Torgerson, D. G., Ampleford, E. J., Chiu, G. Y., Gauderman, W. J., Gignoux, C. R., Graves, P. E., … Hancock, D. B., et al. (2011). Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nature Genetics, 43*(9), 887–U103.

Tsilingiri, K., Fornasa, G., & Rescigno, M. (2017). Thymic stromal lymphopoietin: To cut a long story short. *Cellular and Molecular Gastroenterology and Hepatology, 3*(2), 174–182.

Verstraete, K., van Schie, L., Vyncke, L., Bloch, Y., Tavernier, J., Pauwels, E., … Savvides, S. N. (2014). Structural basis of the proinflammatory signaling complex mediated by TSLP. *Nature Structural & Molecular Biology, 21*(4), 375–382.

WHO. (2018). Organization WH: Description of the global burden of NCDs, their risk factors and determinants. Global Status Report on Non-Communicable Diseases 2018.

Wu, S., Liu, Q., Zhu, J. M., Wang, M. R., Li, J., & Sun, M. G. (2016). Association between the IL7R T244I polymorphism and multiple sclerosis risk: A meta analysis. *Neurological Sciences, 37*(9), 1467–1474. https://doi.org/10.1007/s10072-016-2608-8

Ying, S., O’Connor, B., Ratoff, J., Meng, Q., Mallett, K., Cousins, D., … Corrigan, C. (2005). Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of TH2-attracting chemokines and disease severity. *Journal of Immunology, 174*(12), 8183–8190. https://doi.org/10.4049/jimmunol.174.12.8183

Yu, J. I., Chun, S. W., Yun, K. J., Moon, H. B., & Chae, S. C. (2012). TSLPR gene polymorphism is associated with systemic lupus erythematosus in the Korean population. *Genes & Genomics, 34*(1), 77–82. https://doi.org/10.1007/s13258-011-0148-4

Yu, J. I., Kang, I. H., Chun, S. W., Yun, K. J., Moon, H. B., & Chae, S. C. (2010). Identifying the polymorphisms in the thymic stromal lymphopoietin receptor (TSLPR) and their association with asthma. *BMB Reports, 43*(7), 499–505. https://doi.org/10.5483/bmbrep.2010.43.7.499

Zhang, W., Wang, J., Wang, Q., Chen, G., Zhang, J., Chen, T., … Cao, X. (2001). Identification of a novel type I cytokine receptor CRL2 preferentially expressed by human dendritic cells and activated monocytes. *Biochemical and Biophysical Research Communications, 281*(4), 878–883. https://doi.org/10.1006/ bbrc.2001.4432

Ziegler, S. F., & Artis, D. (2010). Sensing the outside world: TSLP regulates barrier immunity. *Nature Immunology, 11*(4), 289–293. https://doi.org/10.1038/ni.1852

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