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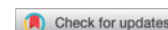
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Association of IL-13 rs20541 and rs1295686 variants with symptomatic asthma in a Saudi Arabian population

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ABSTRACT

Objective: Interleukin 13 (IL-13) plays a critical pro-inflammatory role in asthma. Several single nucleotide polymorphisms (SNPs) are associated with asthma susceptibility in specific populations; however, further replicative studies in other ethnic groups are mandatory. **Methods:** The association between IL-13 SNPs rs762534, rs20541, rs1295686, and rs1800925 (risk alleles A, A, T, and A, respectively) and asthma predisposition in a Saudi Arabian cohort was examined via a case-control cross-sectional study. **Results:** The frequencies of alleles between asthmatics and control populations were significantly different for rs20541 and rs1295686 SNPs ($p < 0.001$), whereas the frequencies of genotypes between asthmatics and controls were significantly different only for rs20541. The association of the risk (minor) alleles with asthma was examined using the dominant genetic model. Individuals with at least one copy of the risk alleles A (for rs20541) and T (for rs1295686) had significantly greater odds of being asthmatic (OR = 2.13, 95% CI = 1.39–3.26, $p < 0.0001$; OR = 1.69, 95% CI = 1.12–2.54, $p = 0.008$) relative to their most common homozygous genotypes. On the other hand, the minor A alleles for rs762534 and rs1800925 were not significantly associated with asthma risk. Regarding haplotype association analysis, individuals with at least one copy of the minor “risk” allele for both rs20541 and rs1295686 (CATG and CATA, respectively) had greater odds of being asthmatic relative to CGCG haplotype; however, this trend was not statistically significant ($p > 0.3$). **Conclusions:** IL-13 minor T and A alleles for rs1295686 and rs20541, respectively, were associated with significantly higher risk of asthma in the Saudi Arabian population.

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Abbreviations

SNPs	single nucleotide polymorphisms
OR	odds ratio
CSF2	colony stimulating factor 2
CI	confidence interval
STAT6	signal transducer and activator of transcription 6
RANTES	Regulated on Activation Normal T Cell Expressed and Secreted
GATA-3	GATA binding protein 3
ERK	extracellular signal-regulated kinase
LD	linkage disequilibrium

Introduction

Interleukin 13 (IL-13), since its discovery in 1993 by differential screening of a subtracted cDNA library from activated peripheral blood mononuclear cells (1), has

attracted considerable interest as a therapeutic target (2). It is encoded by a 4.8 kbp gene that maps within chromosome 5q31, clustered with several Th-2 cytokine genes in proximity, including interleukin 5 (IL-5), granulocyte-macrophage colony-stimulating factor (CSF2) and its closest neighbor, and interleukin 4 (IL-4), with which it shares significant similarity in structural topology and functional activity (3). Consensus on the molecular size of IL-3 is lacking with some studies documenting IL-13 to be composed of 112 amino acids (aa) and a mass of 10 kDa (4) or about 13 kDa (2), or as a 146 aa protein with a molecular weight of 15.8 kDa (5); the differences may reflect distinct analytical techniques employed.

Both IL-4 and IL-13 cytokines transduce signals to signal transducer and activator of transcription 6 (STAT6) via their cognate plasma membrane receptors. The common gamma chain receptor (Interleukin-2 Receptor Gamma subunit, IL-2R γ) can associate either with the IL-4 receptor alpha subunit (IL-4R α), or with the

IL-13R α 1/IL-13R α 2 subunits. Binding of IL-4 or IL-13 to their receptors results in a number of cell-specific cytokine responses, including differentiation of Th2 cells, lymphocyte proliferation, and IgE production (6,7). IL-13 is primarily expressed in activated Th2 cells; however, it can be induced in other effector cells by several factors involved in either Th1 or Th2 responses. These factors include IL-6 cytokine in mast cells, via extracellular signal-regulated kinase (ERK) and Akt cell signaling pathways (8); IL-18 in CD4⁺ T cells and in natural killer (NK) cells (9); IL-4/Fc(epsilon)RI crosslink in mast cells, which can be either STAT-6- or GATA-3-dependent (10,11); IL-33 in eosinophils after exposure to schistosome adult worm antigen (12); the chemokine “Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES)” via Akt cell signaling pathway in mast cells (13); lipopolysaccharide (LPS) in dendritic cells (14); and by enteric parasitic nematode infection (15).

Although IL-13 is a key beneficial mediator in protective immune responses against pathogenic parasites, such as in the control of intestinal nematode infection (16), and perhaps also contributing in protection against types 1/2 diabetes (17), it is also a major health detrimental agent in several auto-immune diseases, such as rheumatoid arthritis (18); in microbial infections, including tuberculosis (TB) (19); and also in allergy and asthma pathophysiology (20). Persistent induction of IL-13 in pulmonary tissues of asthmatics has been implicated in inflammation and tissue remodeling. This is believed to be a consequence of IL-13-induced pro-inflammatory cell infiltration in the airway tissues (21), release of mucus (22), collagen, and metalloproteinases (23,24) along with airway structural cells metaplasia (25) and hyperplasia (26).

Despite the existence of contradictory evidence, a number of studies have reported several common IL-13 gene single-nucleotide variants (SNPs) in association with allergy and asthma risk in different ethnic populations (27–32). These variants, either coding or non-coding, are found scattered in IL-13 gene in the promoter region and throughout the exons and introns in strong linkage disequilibrium (LD) (29). Among these variants, data regarding non-coding variant rs1295686 (+1923C/T) have been contradictory, with some studies showing no association with asthma (33,34), while others observing a significant association with asthma risk in both Asians and Caucasians (35,36). Another variant located in the promoter region, rs1800925 (–1112C/T), has also been associated with an increased risk of asthma in Caucasians with adult-onset asthma (37). However, among Caucasian children, this SNP was not associated with asthma exacerbations, except in a subgroup of children receiving inhaled corticosteroid therapy (38). Similarly, two variants in strong LD, rs848 and rs20541 (R130Q), have been linked with asthma phenotypes, such as increased risk of wheezing

(39) among children with no significance among adult asthma patients (31), except when adult asthmatics were presented with allergic comorbidities such as subject-reported allergic rhinitis (AR) and/or allergic conjunctivitis (AC) (40). Therefore, to help elucidate the apparent discrepancies, further replicative studies in different ethnic groups are needed.

Objective

In this view, we aimed to investigate the association of four single-nucleotide variants (rs20541/R130Q; rs1295686/+1923C/T; rs1800925/–1112C/T; rs762534/C/T) within/near the IL-13 gene, with risk of asthma in a Saudi cohort of asthmatic patients; to that end, we tested the model of dominant inheritance, in which at least one copy of the minor (risk) allele of each SNP genotyped could be associated with an increased risk of asthma.

Methods

Patients and subjects

We recruited 472 consenting subjects, including healthy controls and asthmatics, comprising 41% females and 59% males, at two major hospitals (King Khalid University Hospital and King Fahad National Guard Hospital), in Riyadh, Saudi Arabia. The inclusion criteria for the patients ($n = 235$) were: (a) having physician diagnosed asthma for at least 1 year; (b) age >3 years; (c) only autochthonous Saudi Arabian citizens (citizens of Saudi Arabia, born from Saudi parents, and are not foreigners). Principal clinical characteristics of the recruited asthmatics are shown in Table 1; additional demographic and clinical information of this cohort has been published elsewhere (41). Briefly, of these patients, 15.7% were intermittent, 43.4% mild, 22.3% moderate, and 18.6% severe asthmatics (41,42); the majority (67.3% of men; 56.1% of women) reported having one or more allergy types, with AR (mean 57.7%) being the most frequent followed by eczema (mean 25.3%) and allergy to some foods (mean 21.4%), antibiotics (2.9%), and aspirin (1.4%). Total serum IgE level analyses (>100 IU/ml (43)) and/or skin prick tests were used to identify atopic asthmatics. Patients were excluded if they were found to have cardiac and renal dysfunction, uncontrolled hypertension, acute respiratory tract infection, or if they were tobacco smokers. Non-asthmatic control subjects ($n = 237$) were recruited based on the following criteria: (a) of age ≥ 3 years old; (b) non-smoker; (c) no history of asthma or allergies; (d) not taking any medications at the time of recruitment; (e) be of Saudi ethnicity/nationality; (f) must not have any respiratory, cardiac, or renal dysfunction, or acute respiratory infections at the time

Table 1. Principal clinical characteristics of asthmatic patients.*

Variables	Levels	Males	Females
Age (in years)		13.2 ± 18.8	19.1 ± 21.2
Age of asthma diagnosis (in years)		6.0 ± 12.0	9.2 ± 16.9
Asthma severity	Intermittent	16.2%	14.9%
	Mild persistent	42.6%	44.7%
	Moderate persistent	23.6%	20.2%
	Severe persistent	17.6%	20.2%
Major symptoms	Cough	94.0%	91.8%
	Wheezing	86.7%	78.6%
	Shortness of breath	73.3%	51.0%
	Chest pain	52.7%	48.0%
Allergies?	Yes	67.3%	56.1%
Allergic to aspirin?	Yes	1.2%	1.8%
Allergic to some medicines?	Yes	1.3%	5.3%
Allergic to some food?	Yes	22.8%	19.3%
Eczema?	Yes	31.6%	15.8%
Allergic rhinitis?	Yes	63.3%	49.1%
Parental history of allergic rhinitis?	Yes	51.8%	40.0%
Parental history of eczema?	Yes	18.5%	14.3%

*See (41) for additional information about the clinical and demographic characteristics of the recruited asthmatics.

of recruitment. All individuals or the parents signed a written consent form; this study was approved by the Ethical Committee of the King Khalid University Hospital, approval no. E-10-122.

Genotyping

A minimal sample of recruited patients ($n = 200$) was calculated using Cochran's formula: $n = Z^2pq/d^2$, where Z is the standard deviation at 95% CI (1.96; two-tailed) and p is asthma's prevalence (effect size) in Saudi Arabia: estimated at 8–23% (44,45). Briefly, peripheral blood was drawn and DNA was purified using a QIAamp DNA Blood Midi Kit (QIAGEN) in accordance with the manufacturer's instructions. Genotyping of four SNPs (rs20541, rs1295686, rs1800925, and rs762534) was performed by PCR amplification of the DNA segments (about 150–250 bp) encompassing each variant, using specific primers (rs20541: F: 5'-GTAAAACGACGGCCAGTCTGGCGTTCTACTCATG TGCT-3', R: 5'-CAGGAAACAGCTATGACCGAGGTCG GCTAGGCTGAAGA-3'; rs1295686: F: 5'-GTAAAACG ACGGCCAGTGACTTCCGTGAGGACTGAATG-3', R: 5'-CAGGAAACAGCTATGACCTCCTGTCTCTGCAA ATAATGATG-3'; rs1800925: F: 5'-GTAAAACGACGG CCAGTAGCCTGAGCAGGCAGAGAG-3', R: 5'-CAGG AAACAGCTATGACCGCAGAATGAGTGCTGTGGAG -3'; rs762534: F: 5'-GTAAAACGACGGCCAGTTCATTT CAGCAACTCAAGATAGGA-3', R: 5'-CAGGAAACAG CTATGACCGCATGTGTCATCCAGAAAGG-3') and Taq Polymerase (Quick-Load Taq 2X Master Mix, New England BioLabs). The conditions for PCR were the following: 10 min at 95°C for initial denaturation, followed by 35 cycles of 30 s at 95°C for denaturation, 30 s at 60°C for annealing, 1 min at 72°C for extension, and 7 min at 72°C for the final extension. After

Sephadex column purification, the PCR segments were then Sanger-sequenced using the BigDye Terminator kit and resolved by capillary electrophoresis (Sequencer model 3730XL, Applied Biosystems). In the case of rs762534 and rs1295686, genotyping was possible for only 181 and 204 patients, respectively, due to limitations in DNA sample. Genotyping analysis and base calling were performed with the Sequencing Analysis software version 5.2 (Applied Biosystems).

Statistical analysis

Both allele and genotype frequencies were analyzed as described previously (41). Yates corrected Chi-square tests and Yates p values were calculated. Two-tailed $p < 0.05$ was considered statistically significant. A Bonferroni correction was applied, specifying the new alpha thresholds of significance, where needed. Hardy-Weinberg (H-W) equilibrium tests were done using SNPStats computer program (46). Logistic regressions for each SNP were performed to test the interaction of genotypes with gender on asthma, by using the computer program IBM SPSS Statistics v.19. Calculations of odds ratios and confidence intervals were done using an interactive calculation tool (47).

Results

Hardy-Weinberg equilibrium and linkage disequilibrium

All the four SNPs genotyped had genetic distributions in H-W equilibrium among the control groups ($p > 0.05$); this was also observed among the asthma groups, except for rs1800925 SNP, which was significantly out of H-W equilibrium ($p = 0.045$). All SNPs were in LD among each

Table 2. Frequencies of alleles and genotypes in asthmatics and healthy controls for IL-13 SNPs.

SNP	Alleles or genotypes	Asthmatics <i>N</i> (%)	Healthy <i>N</i> (%)	Percentage deviations [§]		Yates Chi-square [‡]	<i>p</i> values*
				Asthmatics	Healthy		
rs762534	C	339 (0.94)	433 (0.95)	−0.4%	+0.3%	0.15	0.69
	A	23 (0.06)	25 (0.05)	+6.2%	−4.9%		
	C/C	158 (0.87)	204 (0.89)	−1.1%	+0.9%	0.31	0.85
	C/A	23 (0.13)	25 (0.11)	+8.5%	−6.8%		
rs20541	A/A	0	0				
	G	361 (0.78)	397 (0.87)	−5.4%	+5.5%	12.96	0.0003*
	A	103 (0.22)	59 (0.13)	+25.5%	−25.9%		
	G/G	141 (0.61)	175 (0.77)	−11.5%	+11.7%	13.75	0.001*
rs1295686	G/A	79 (0.34)	47 (0.21)	+24.3%	−24.7%		
	A/A	12 (0.05)	6 (0.03)	+32.2%	−32.7%		
	C	279 (0.68)	335 (0.77)	−5.8%	+5.5%	7.18	0.0074*
	T	129 (0.32)	101 (0.23)	+15.6%	−14.6%		
rs1800925	C/C	99 (0.49)	134 (0.61)	−12.1%	+11.3%	7.32	0.0257
	C/T	81 (0.4)	67 (0.31)	+13.2%	−12.4%		
	T/T	24 (0.12)	17 (0.08)	+21.1%	−19.7%		
	G	348 (0.76)	354 (0.77)	−0.7%	+0.7%	0.15	0.6985
	A	110 (0.24)	104 (0.23)	+2.3%	−2.3%		
	G/G	138 (0.6)	141 (0.62)	−1.1%	+1.1%	0.29	0.865
	G/A	72 (0.031)	72 (0.31)	0%	0%		
	A/A	19 (0.08)	16 (0.07)	+8.6%	−8.6%		

[§]Percentage deviations were calculated as: [(observed − expected/expected) × 100].

*Statistical significance threshold was set at the Bonferroni corrected *p* value < 0.0250.

[‡]Yates corrected Chi Square test values.

other: the highest LD was observed between rs762534 and rs1295686 ($D' = 0.65$; $p < 0.001$), and between rs762534 and rs1800925 ($D' = 0.60$; $p < 0.001$), or about 60% of the theoretical maximum; the lowest LD was observed between rs1800925 and rs20541 ($D' = 0.12$; $p < 0.001$).

Frequencies of alleles and genotypes and asthma susceptibility in a Saudi Arabian population

Alleles and genotype frequencies of the four SNPs are shown in Table 2. The minor (risk) alleles for three SNPs, namely A, T, and A, for rs20541, rs1295686, and rs1800925, respectively, were quite common in this cohort, representing between 22% and 32% of the total; this contrasted with rs762534 SNP, for which the minor A allele was only 6% of the total, with no homozygous A/A individuals found in this cohort. The frequencies of alleles between asthmatics and control populations were significantly different only for rs20541 and rs1295686 SNPs ($p < 0.001$), whereas the frequencies of genotypes between asthmatics and controls were significantly different only for rs20541 (Table 2).

Table 3 shows the analysis of association between genotypes and the risk of asthma, when the dominant genetic model (one copy of the minor allele is enough to produce an effect) was tested. The minor A alleles of both rs762534 and rs1800925 SNPs were not significantly associated with asthma, whereas those of rs20541(A allele) and rs1295686 (T allele) SNPs were significantly associated ($p < 0.05$); those individuals who inherited at least one copy of the minor risk allele, G/A+A/A or T/A+T/T, had greater odds (OR = 2.13, 95% CI = 1.39–3.26 and OR = 1.69, 95% CI = 1.12–2.54, for rs20541 and rs1295686, respectively) of being asthmatics.

A logistic regression analysis of the effect of interaction of gender with the two SNPs shown to be associated with asthma (Gender × rs20541 and Gender × rs1295686) failed to find significant differences between males and females (Table 4).

Last, an analysis of association of haplotypes with asthma risk was performed; three major haplotypes accounted for the majority (76%) of the subjects, with

Table 3. Analysis of association of genotypes with asthma when the dominant model of inheritance is tested, adjusted by gender.

SNP	Genotypes	Asthma <i>N</i> (%)	Healthy <i>N</i> (%)	Odds ratio	95% confidence interval	<i>p</i> value*
rs762534	C/C	158 (87.3%)	204 (89.1%)	1.00		
	C/A + A/A	23 (12.7%)	25 (10.9%)	1.19	0.62–2.26	0.64
rs20541	G/G	141 (60.8%)	175 (76.8%)	1.00		
	G/A + A/A	91 (39.2%)	53 (23.2%)	2.13	1.39–3.26	<0.0001*
rs1295686	C/C	99 (48.5%)	134 (61.5%)	1.00		
	C/T + T/T	105 (51.5%)	84 (38.5%)	1.69	1.12–2.54	0.008*
rs1800925	G/G	138 (60.3%)	141 (61.6%)	1.00		
	G/A + A/A	91 (39.7%)	88 (38.4%)	1.06	0.7–1.6	0.77

*Statistical significance was considered at *p* value < 0.05.

Table 4. A logistic regression model showing odds ratios and 95% confidence intervals for gender and its interaction with IL-13 SNPs on asthma.

Predictors	Estimate	<i>p</i> value*	OR	95% CI	
				Upper	Lower
Gender male (base = female)	− 0.370	0.611	0.7	0.2	2.9
Gender × rs20541		0.464			
Gender × A/A	− 0.973	0.584	0.4	0.1	12.3
Gender × G/A	0.839	0.446	2.3	0.3	20.1
Gender × G/G	0.633	0.185	1.9	0.7	4.8
Gender × rs1295686		0.279			
Gender × C/C	2.227	0.089	9.3	0.7	120.9
Gender × C/T	0.297	0.694	1.3	0.3	5.9
Gender × T/T	− 0.012	0.988	1.0	0.2	4.4
Intercept	0.196	0.65	1.2		

*Wald Chi-square statistic was considered significant at 0.05 level.

Note. Correlation coefficients for this model: Cox and Snell's $r^2 = 0.07$; Nagelkerke's $r^2 = 0.09$.

CGCG (rs762534, rs20541, rs1295686, and rs1800925, in that order) being the most common (55%). Individuals who inherited the CGTG haplotype (10.4%) had significantly lower odds of being asthmatics (OR = 0.24 (0.13–0.44); $p < 0.0001$), in relation to the common CGCG haplotype (Table 5). In contrast, individuals who inherited CATG and CATA haplotypes showed greater odds of being asthmatics (OR = 1.29 and 1.98, respectively), although this trend was not significant. It should be noted that those haplotypes containing both minor “risk” alleles, “A” and “T,” for rs20541 and rs1295686, respectively (shown to be significantly associated with asthma in the SNP analysis) were found at very low frequencies (some of them absent) in our sample population; we speculate that this could be one reason for the observed a lack of significance in the haplotype analysis of association.

Discussion

Given the prominent role of IL-13 in Th-2 mediated asthma pathogenesis, it is not surprising that several specific therapeutic strategies have been developed as potential alternatives for a better control of asthma symptoms (20,22,26,48,49). Accordingly, many studies have reported that single-nucleotide mutations in

IL-13 gene are associated with asthma susceptibility and/or other allergic disorders, albeit not without discrepancies (27–29, 31, 33, 36–38,50). In the backdrop of these observations, functional studies have sought to understand the mechanisms by which SNPs in many genes including IL-13 (either coding or non-coding) modify gene expression and/or protein function/activity, thus influencing the severity of asthma symptoms and/or the patient's response to medications (31,51–53).

In this cross-sectional study of asthmatic patients from Saudi Arabia, we analyzed the possible link between asthma susceptibility and four single-nucleotide variants located within or in near proximity to IL-13 gene, previously reported to be associated with allergic diseases and/or helminthic infections. rs20541/R130Q was found in strong association with elevated serum IgE levels, atopic dermatitis, allergy, airway hyperresponsiveness, and asthma severity symptoms in several populations (29,31,43,54–56). This variant is a coding variant located at codon 130, where the G→A substitution results in changing arginine for glutamine, possibly affecting IL-13 ligand–receptor interaction (57). Although some conflicting results have been reported (58), our analysis indicated that when the genetic dominant model is considered, the minor “A” allele is significantly associated with asthma susceptibility. In fact, individuals with at least one copy

Table 5. Analysis of association of haplotypes with asthma.

	rs762534	rs20541	rs1295686	rs1800925	Freq.	OR (95% CI)	<i>p</i> value
1	C	G	C	G	0.549	1.00	–
2	C	G	C	A	0.104	1.11 (0.61–2.01)	0.74
3	C	G	T	G	0.104	0.23 (0.12–0.44)	<0.0001*
4	C	G	T	A	0.046	0.43 (0.19–0.94)	0.03
5	C	A	C	G	0.041	0.00	1
6	C	A	T	G	0.061	1.29 (0.54–3.05)	0.56
7	C	A	T	A	0.029	1.98 (0.50–7.88)	0.34
8	A	A	T	A	0.027	0.60 (0.21–1.73)	0.34
9	A	G	T	A	0.014	0.04 (0.00–1.49)	0.08

Note. Haplotypes were compared to the most common CGCG haplotype.

*Statistical significance threshold was set at the Bonferroni corrected p value < 0.006.

of “A” variant have odds over twice of being asthmatics in relation to those having the common C/C genotype. Another variant, rs1295686/+1923C/T, is an intronic mutation that has been linked with atopic dermatitis (54) and was associated with asthma susceptibility in Mauritanian Indians, but not in Han Chinese populations (28,59); here, we confirm a significant association of the minor (risk) “T” allele with asthma in Saudi Arabians. We also genotyped rs1800925/−1112C/T, a variant located 5′ upstream in the promoter region of IL-13, for which the minor “T” allele was linked with elevated IL-13 expression in fibrotic liver tissues from patients infected with *Schistosoma japonicum*, and driving higher reporter luciferase activity, comparatively to C allele (60); however, non-significant association results with parasitic susceptibility have also been published (61). Our results indicate that this variant is not associated with asthma susceptibility in the Saudi Arabian cohort, and is compatible with other previous reports involving different ethnic groups; hence, a possible risk association with asthma remains ambiguous. For instance, in a meta-analysis study, the minor “T” allele was globally significantly associated with asthma risk according to the dominant model. However, when stratifying by race differences were uncovered, Caucasians were distinguished with a higher risk of asthma susceptibility, whereas a lack of significant association was observed in Asians and African Americans (37,62). Furthermore, in another study involving Han Chinese children, this variant was not significantly different to healthy controls (59). In fact, a potentially protective role was suggested in a functional analysis study, where the homozygous T/T genotype was associated with improved FEV₁/FVC and increased methylation at a specific CpG site in IL-13 promoter, comparatively to T/C and C/C genotypes (63). The fourth variant analyzed, rs762534/C/T, is located in a sequence-tagged region (STS) 3′ downstream of IL-13 and about 4.9 kbp 5′ upstream from the transcription initiation site of IL-4 gene; whether this SNP is part of the IL-4 promoter or is within 3′ regulatory sequences of IL-13 gene remains unclear. We observed a lack of significant association with asthma risk in the Saudi Arabian cohort. Although no studies involving asthmatics have been published so far, our results are in agreement with two reports describing non-significant association results of this variant with schistosoma infection and diabetes (61,64). We observed that the minor risk “A” allele was the least frequent in the Saudi Arabian cohort (5% and 6% for control and asthmatics, respectively), among the four SNPs analyzed, so much so that no homozygous A/A individual was found; this could be interpreted a priori as bias sampling, yet our recruitment was planned to avoid it, which is supported by the fact that the alleles were in

the H-W equilibrium. Therefore, the lower frequency is a characteristic of this population, and would require to increase considerably the number of controls and asthmatics to get a more representative cohort. Hence, further replicative studies are needed with larger populations and in other ethnic groups, before a more confident conclusion can be reached.

Our study highlights the possible implication of rs20541/R130Q and rs1295686/+1923C/T as factors significantly influencing asthma susceptibility in the Saudi Arabian ethnic group. This was determined by testing the dominant model of inheritance, in which individuals inheriting at least one copy of the minor risk alleles for both SNPs had greater odds of being asthmatics.

The mechanisms by which the minor variants influence IL-13 gene expression and/or activity remain obscure. Regarding rs20541, evidence suggests that the arginine-to-glutamine substitution increases its biological activity at the ligand–receptor interface (29). In the case of rs1295686 SNP, no functional studies of the risk allele have been performed, to the best of our knowledge, and therefore its biological role remains unknown. However, accumulating evidence has indicated that rs1295686/+1923C/T polymorphism plays a critical role in the regulation of IgE level, with IgE-dependent mechanisms being crucial in the pathophysiology of airway inflammation during asthma (36,65). Interestingly, rs1295686/+1923C/T and rs20541/R130Q SNP were reported in complete LD and associated with a risk of pediatric food allergy (66); our findings go in parallel with those findings, as we also observed that both variants were in high LD and associated with asthma risk; this may suggest that both SNPs could act in concert, enhancing the susceptibility to IgE-mediated inflammatory-allergic disorders. On the other hand, given that asthmatics usually have elevated IL-13 cytokine levels (21,22,26), it could be speculated that the risk T allele of rs1295686 influences the rate of IL-13 transcription, as it has been reported for some intronic variants acting as enhancers of transcription (67). Further studies are needed to demonstrate the functional significance of this SNP.

Limitations of the study

Although we obtained valuable information and our main goal was achieved, our study has some potential limitations. Principally, we were expecting to analyze the association of SNP alleles with different asthma severity levels; however, stratification by disease severity was not feasible as it would require a lot more patients recruited to obtain groups of larger size; hence, we confounded all asthmatics in one group. Another limitation is that our study was

comprised of both children and adults, who were grouped together; since age is known to influence asthma susceptibility, confounding by age could have influenced the results. Therefore, future studies are needed to address these issues.

Conclusion

IL-13 cytokine has been singled-out, along with IL-4, as a prominent factor in promoting allergic diseases and asthma pathophysiology; it is also a part of the Th2 response against helminth infections and has been targeted specifically with antibodies aiming to control inflammation (20,26,48,49). We have confirmed two variants within this gene, rs20541/R130Q and rs1295686/+1923C/T, in significant association with asthma risk in a cohort of Saudi Arabians, as reported in other populations. These results could be useful in the future, with the development of therapeutic programs tailored for identified susceptible individuals who would benefit from a more specialized and effective medical treatment.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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