



Polymorphisms in thymic stromal lymphopoietin gene demonstrate a gender and nasal polyposis-dependent association with chronic rhinosinusitis [☆]

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ABSTRACT

Background: Recent studies have suggested that thymic stromal lymphopoietin (TSLP), a key cytokine involved in the dendritic cell-mediated activation of Th2-mediated inflammatory responses, is significantly increased in nasal polyps from atopic individuals. Our objective was therefore to explore firstly any associations between single nucleotide polymorphisms (SNPs) in and around the TSLP gene and development of chronic rhinosinusitis (CRS; with (CRSwNP) or without (CRSsNP) nasal polyps and, and secondly the influence of nasal polyposis and gender.

Methods: A population-based case-control association analysis was performed in a Han Chinese cohort. DNA extracted from peripheral blood leukocytes from 638 subjects with CRS (306 CRSwNP and 332 CRSsNP) and 325 healthy controls was assessed for 11 SNPs in and around TSLP gene, selected from the Chinese HapMap genotyping dataset. Genetic association tests were performed using the Haploview and STATA software package.

Results: Single-locus analysis of CRS risk, showed no significant differences in allele or genotype frequencies between CRS subjects and controls. Stratified analyses of association between the selected SNPs and CRS adjusted for gender demonstrated that rs13156068 (CC genotype: $P = 0.010$, OR = 0.289) and rs764917 (CC genotype: $P = 0.040$, OR = 0.509) were significantly protective against CRS, whereas rs6886755 (GT genotype: $P = 0.040$, OR = 0.509) presented a risk among females. In contrast, rs764917 (CA genotype: $P = 0.033$, OR = 1.553) presented risk for CRS in males. Furthermore, SNPs rs252706 (AA genotype: $P = 0.012$, OR = 0.552) and rs764917 (CA genotype: $P = 0.001$, OR = 0.182) displayed protective roles among CRSwNP, but not CRSsNP, subjects.

Conclusions: This study suggests that SNPs in TSLP gene may exert a gender and/or nasal polyposis-dependent risk for development of CRS in Chinese subjects.

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1. Introduction

Chronic rhinosinusitis (CRS) is a common inflammatory disorder of the sinus and paranasal sinus mucosa. Based on the absence or presence of nasal polyps (NPs), CRS is classified into two pheno-

types, which include CRS without NPs (CRSsNP) and CRS with NPs (CRSwNP) [1]. Although CRS has a highly heterogeneous pathogenesis, the development of this disease is believed to be the result of interactions between the genetic background of the affected subject and environmental factors [2,3].

Evidence suggests that the epithelial cell-derived thymic stromal lymphopoietin (TSLP); which has been shown to profoundly influence the polarization of dendritic cells to drive T helper (Th) 2-inflammatory response as well as Th2 cytokine production [4,5]; may play a role in the aetiology of CRS with or without NPs; particularly as these may be characterized by different cytokine profiles. Thus, while Th2 cytokines and eosinophilic infiltration are more specific of CRSwNP, neutrophils appear to play a major role in CRSsNP [6,7]. Indeed, emerging evidence from more recent studies has suggested that TSLP may possibly play a role also in the aetiology of CRS [8,9]. A recent study has reported that the expression of TSLP mRNA was markedly higher in NPs,

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compared to the allergic nasal mucosa, and strongly correlated with the number of eosinophils and the levels of IgE in NPs [8]. Similarly, another study demonstrated that expression of the TSLP receptor was significantly higher in the inflammatory infiltrate and epithelial cells of both CRSwNP and CRSsNP patients, compared to controls [9].

Consequently, we hypothesized that genetic variations in TSLP gene are associated with the risk of developing CRS risk and may potentially be a critical mechanism underlying the etiology of CRS. The objective of this study was therefore to investigate the associations between tagging single nucleotide polymorphisms (SNPs) in/around the TSLP gene and susceptibility to develop CRS with or without nasal polyposis.

2. Materials and methods

A population-based case-control association study design was used to assess the risk of CRS conferred by specific SNPs in TSLP gene regions.

2.1. Study subjects

Six hundred and thirty-eight adult individuals fulfilling the criteria of CRS according to the current European EAACI Position Paper on Rhinosinusitis and Nasal Polyps [10] and American guidelines [1] were recruited from the Rhinology ward of the Otolaryngology, Head and Neck Surgery Department of Beijing Tongren Hospital, from February 2010 to November 2010. The diagnosis of CRS was made by trained rhinologists; based on history, clinical examination, nasal endoscopy, and computed tomographic scanning of the sinuses. Patients with CRS were additionally required to have failed to respond to medical therapy and subsequently undergone endoscopic sinus surgery. All eligible CRS subjects were divided into two subgroups based on the absence or presence sinonasal polyposis; i.e. CRSsNP and CRSwNP, respectively.

Individuals with a diagnosis of CRS were excluded from the study if they suffered from (1) allergic rhinitis, eczema, asthma, or any other allergic disease, (2) hypertension, diabetes or any other chronic disease, or (3) tumor in the nasal cavity and any other inflammatory nasal disease. The diagnosis of asthma was confirmed by a chest physician according to Global Initiative for Asthma 2006 guidelines based on symptoms and pulmonary function tests [11].

A total of 325 adult healthy control subjects; presenting no clinical nasal features, local nasal cavity signs, history of allergic disease, or any other nasal disease were also recruited from an ethnically similar local population to determine background population allele frequencies.

All subjects were ethnic Han Chinese from the Beijing region of China, and provided written informed consent, prior to recruitment. The study protocol was approved by the Ethics Committee of Beijing Tongren Hospital and performed in accordance with the guidelines of the World Medical Association's Declaration of Helsinki.

2.2. Selection of polymorphisms in the human TSLP gene

The International Haplotype Mapping (HapMap) (www.hapmap.org) SNP databases were used to select tSNPs in the TSLP gene region, and the screened region was extended 10 kilobases upstream of the annotated transcription start site and downstream at the end of the last exon in each gene. Twenty five tSNPs were selected in the region using the CHB genotyping data from the HapMap database (HapMap data rel 27 Phase II+III, Feb2009) [12], and the dataset for these tSNPs loaded in the Haploview software version 4.2 ([http://www.broad.](http://www.broad.mit.edu/haploview/)

[haploview/](http://www.broad.mit.edu/haploview/) haploview-downloads) [13]. Further selection of the eventual tSNPs to be investigated was then made using a pair wise tagging algorithm [13]; setting the Hardy–Weinberg p value, minor allele frequency (MAF), and r^2 thresholds at 0.01, 0.05 and 0.8, respectively. The linkage disequilibrium (LD) pattern of the TSLP gene in the CHB population exhibited strong LD in several groups of tSNPs ($r^2 \geq 0.8$), indicating that most common SNPs can be captured by a subset of tagging SNPs [14]. Consequently, 11 SNPs for TSLP (including rs1545169, rs764917, rs12653736, rs1837253, rs12654933, rs10455025, rs11466741, rs13156086, rs6886755, rs252706 and rs2416259) were selected for further assessment, representing the entire 25 loci for genotyping.

2.3. Single nucleotide polymorphism genotyping

DNA was isolated from peripheral blood leukocytes, using the DNA Isolation Kit for Mammalian Blood (Roche, Indianapolis, USA). Genotyping of candidate SNPs was performed by a contract service-based specialist company (BGI Company, <http://en.genomics.cn/navigation/index.action>), using the Sequenom MassARRAY technology platform with the iPLEX GOLD chemistry (Sequenom, San Diego, CA) [15]. Briefly, polymerase chain reaction (PCR) was carried out in standard 384-well plates, using extension primers designed using MassARRAY Assay Design 4.0 software (Supporting information, Table 1) according to the supplier's instructions. The final product was transferred to a 384-well Spectro-CHIP (Sequenom, San Diego, CA), and analyzed in a MassARRAY Analyzer Compact MALDI-TOF mass spectrometer (Sequenom, San Diego, CA), using the MassARRAY Typer 4.0 Software. The PCR assay was arrayed with ten no-template controls and ten duplicated samples in each 384-well format as quality controls. All genotyping results were generated and checked by an investigator blinded to the clinical status of the subject from whom the sample was derived.

2.4. Statistical analyses

Quality tests were initially performed using the Haploview version 4.1 software to filter and assess the suitability of the data for the further statistical analyses. Hardy–Weinberg equilibrium (HWE) for each SNP was assessed in control samples only, and a threshold $P < 0.001$ was regarded to indicate deviation from HWE. The minor allele frequency (MAF), non-missing genotype percentage, and other criteria in the CRS cases as well as controls were additionally assessed to filter the data. Among them, MAF and non-missing genotype percentage thresholds were set at <0.001 and $<75\%$ respectively.

Differences in frequencies of the alleles and genotypes between the CRS patients and controls were evaluated using the χ^2 -test. HWE in the control group was tested using the chi-square test for goodness of fit according to the Web-based program <http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>; with a P -value of 0.05 being considered significant. Akaike's information criteria (AIC) were used to select the most parsimonious genetic model for each SNP. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis, adjusted for age and gender. Stratification analyses were also performed by variables of interest, such as CRS item and sex. These analyses were conducted with STATA statistical package (version 11.0; Stata Corp LP, College Station, TX, USA). All P values are two tailed.

Additionally, pair-wise linkage disequilibrium (LD) among the SNPs was examined using Lewontin's standardized coefficient D' and LD coefficient r^2 [16], and haplotype blocks were defined according to the method of Gabriel et al. [17] in Haploview 4.2 with default settings. The HAPLO.STATS package (www.mayo.edu/hsr/Sfunc.html) in software language R developed by Schaid et. al. [18] was used for the haplotype analysis. PHASE 2.1 Bayesian algo-

Table 1

Demographic characteristics of the study population.

	Age (Mean $\pm$ SE (Range); years)	Male/Female (Number (%))	Total IgE, kU/l
CRS (n = 638)	40.66 $\pm$ 0.62 (18–77)	370 (57.99)/268 (42.01)	116.0 $\pm$ 247.375
CRSsNP (n = 332)	38.87 $\pm$ 15.36	190 (57.23)/142 (42.77)	112.1 $\pm$ 277.1
CRSwNP (n = 306)	42.59 $\pm$ 15.80	180 (58.82)/126 (41.18)	120.2 $\pm$ 211.4
Control (n = 325)	36.22 $\pm$ 0.84 (18–78)	174 (53.54)/151 (46.46)	57.4 $\pm$ 111.9

CRS, Chronic rhinosinusitis; CRSsNP, Chronic rhinosinusitis without sinonasal polyposis; CRSwNP, Chronic rhinosinusitis with sinonasal polyposis

Table 2

SNPs genotyped for TSLP gene.

Gene: locus and OMIM No. ^a	No.	SNP_ID	Chromosome Position ^b	Intermarker distances (bp)	Genic location	Base Change	MAF ^c			P ^f	P value for HWE ^g test	Call rate(%)
							NCBI ^d	Case ^e	Control			
TSLP: 5q22.1	1	rs1545169	110427275		5' near gene	T/G	0.476	0.293	0.278	0.4175	<0.0000	97.5
	2	rs764917	110428406	1131	5' near gene	A/C	0.205	0.283	0.289	0.9646	0.0004	95
	3	rs12653736	110428526	120	5' near gene	G/T	0.062	0.101	0.113	0.2152	0.6270	99
	4	rs1837253	110429771	1245	5' near gene	T/C	0.389	0.460	0.460	0.7107	0.7337	98.7
	5	rs12654933	110430654	883	5' near gene	C/A	0.244	0.155	0.144	0.4099	0.8902	99.8
	6	rs10455025	110432898	2244	5' near gene	A/C	0.067	0.050	0.048	0.7126	0.3650	99.6
	7	rs11466741	110436604	3706	Intron 2	C/T	0.211	0.309	0.329	0.2771	0.2428	80.7
	8	rs13156086	110443368	6764	3' near gene	A/C	0.144	0.233	0.251	0.2909	0.7148	93.2
	9	rs6886755	110443500	132	3' near gene	G/T	0.089	0.079	0.073	0.4408	0.5559	99.7
	10	rs252706	110444759	1259	3' near gene	G/A	0.337	0.325	0.337	0.3088	0.0635	99.5
	11	rs2416259	110447641	2882	3' near gene	T/C	0.411	0.370	0.366	0.9903	0.1416	97.5

^a OMIM, Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/Omim>).^b SNP position in Chromosome 5 in the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>).^c MAF, minor allele frequency.^d MAF for Chinese in the NCBI dbSNPs database.^e Chronic rhinosinusitis subjects.^f P value for difference in allele distributions between chronic rhinosinusitis and control subjects.^g HWE, Hardy–Weinberg equilibrium.**Table 3**

Genotype frequencies of tag SNPs among CRS and control subjects and their associations with CRS risk.

SNP ID	Genotype	CRS subjects		Control subjects		P (2 df) ^a	Logistic regression	
		No.	Frequency (%)	No.	Frequency (%)		OR (95%CI)	P ^b
rs12653736	GG	517	81.80	255	78.95	0.386	1.00 (referent)	
	GT	110	17.41	63	19.50		0.863 (0.609–1.222)	0.405
	TT	5	0.79	5	1.55		0.475 (0.135–1.675)	0.247
rs1837253	TT	180	28.35	90	28.66	0.693	1.00 (referent)	
	TC	337	53.07	159	50.64		0.901 (0.604–1.342)	0.607
	CC	118	18.58	65	20.70		1.068 (0.776–1.470)	0.686
rs12654933	CC	451	70.69	237	73.37	0.683	1.00 (referent)	
	CA	172	26.96	79	24.46		1.140 (0.834–1.558)	0.413
	AA	15	2.35	7	2.17		1.181 (0.471–2.959)	0.723
rs10455025	AA	570	89.62	292	90.40	0.705	1.00 (referent)	
	AC	66	10.38	31	9.60		1.114 (0.707–1.755)	0.643
	CC	0	0.00	0	0.00		NA ^c	NA ^c
rs11466741	CC	263	48.08	112	46.67	0.198	1.00 (referent)	
	CT	238	43.51	98	40.83		1.047 (0.756–1.450)	0.782
	TT	46	8.41	30	12.50		0.670 (0.401–1.120)	0.127
rs13156086	AA	353	58.64	169	56.52	0.308	1.00 (referent)	
	AC	223	37.04	110	36.79		0.988 (0.735–1.329)	0.936
	CC	26	4.32	20	6.69		0.623 (0.336–1.155)	0.133
rs6886755	GG	533	83.54	276	85.71	0.574	1.00 (referent)	
	GT	104	16.30	45	13.98		1.226 (0.837–1.798)	0.296
	TT	1	0.16	1	0.31		0.550 (0.034–8.904)	0.674
rs252706	GG	296	46.47	149	46.27	0.093	1.00 (referent)	
	GA	282	44.27	129	40.06		1.100 (0.823–1.470)	0.518
	AA	59	9.26	44	13.66		0.648 (0.416–1.009)	0.055
rs2416259	TT	254	40.06	126	42.14	0.433	1.00 (referent)	
	TC	296	46.69	127	42.47		1.139 (0.843–1.540)	0.396
	CC	84	13.25	46	15.38		0.895 (0.586–1.365)	0.606

CRS, Chronic rhinosinusitis

^a Global P values [2 degrees of freedom (df)], genotype frequencies in cases and controls were compared using a χ^2 test with 2 df.^b P values from unconditional logistic regression analyses, adjusted for age and gender.^c NA, not available because of the rarity of genotype.

rithm [19] was used to estimate the haplotype frequencies and haplotypes with a frequency of less than 0.03 were pooled into a combined group. Empirical *P*-values, based on 100,000 simulations, were computed for the global score test and each of the haplotype-specific score tests. Diplotype (haplotype dosage, an estimate of the number of copies of the haplotype) was the most probable haplotype pair for each individual. Unconditional logistic regression analyses, adjusted for age and gender, were conducted to estimate ORs and 95% CIs for participants carrying one to two copies versus zero copy of each common haplotype for the dichotomized diplotypes.

The statistical power for the present study was calculated using G*Power 2 software (<http://www.psych.uni-duesseldorf.de/aap/projects/gpower/>).

3. Results

3.1. Demographic characteristics of the study population

Table 1 demonstrates the demographic characteristics of the present study population. Both the CRS and control groups were well matched with respect to age and male to female ratios; although the mean age of CRS subjects (41 years old) was found to be slightly higher than for controls (36 years old). The Pearson Chi-Square test of the ratios for male/female between CRS (370/268, M/F) and control (174/151, M/F) groups showed that these were not significantly different (*P* = 0.138). The serum total IgE measurements for CRS as a whole, CRSsNP, CRSwNP and control groups were 116.0 ± 247.375 , 112.1 ± 277.1 , 120.2 ± 211.4

and 57.4 ± 111.9 KU/l respectively. Meanwhile, the level of serum total IgE between CRSsNP and controls (*P* = 0.0183) as well as CRSsNP vs controls (*P* = 0.0006) were significantly different. Among the CRS subjects, 332 patients (52.04%) presented without nasal polyps (i.e. CRSsNP) 306 patients (47.96%) with nasal polyps (i.e. CRSwNP). The characteristics of the subgroups including CRSsNP and CRSwNP were also exhibited in Table 1.

3.2. Individual SNP association analysis

Table 2 shows the tag SNPs assessed for associations with risk of CRS. The initial quality tests for the SNPs in the TSLP gene selected for genotyping demonstrated that two SNPs (rs1545169) and (rs764917) were not suitable for study, based on their HWE values (*P* < 0.001), and data for these two loci were therefore excluded of further whole population analyses. In the single-locus analyses of CRS risk the allele frequencies of all the nine passed tag SNPs were not significantly different between the CRS and control subjects (*P* > 0.05).

The genotype distributions of the nine selected SNPs in CRS and control subjects are summarized in Table 3. None of the genotypes was found to be associated with CRS susceptibility. Moreover, age and gender adjusted logistic regression analyses further revealed that in the codominant-effect model, as assessed by the Akaike's information criteria (AIC), no significant protective or risk effects against CRS were associated with SNPs in TSLP gene, compared with wild-type carriers (Table 3).

Stratified analyses of CRS associations on gender (Table 4), however, demonstrated that rs13156068 (CC genotype: *P* = 0.010,

Table 4
Stratified analyses of CRS associations on gender.

SNP ID	Genotype	Male			Female		
		No.	OR (95%CI)	P	No.	OR (95%CI)	P
rs1545169	TT	144/73	1.000 (referent)		112/69	1.000 (referent)	
	GT	218/96	1.194 (0.819–1.740)	0.357	152/78	1.216 (0.808–1.831)	0.349
	GG	0/1	N.A	N.A	0/0	N.A	N.A
rs764917	AA	183/100	1.000 (referent)		123/70	1.000 (referent)	
	CA	146/52	1.553 (1.036–2.327)	0.033	113/50	1.322 (0.845–2.068)	0.222
	CC	24/14	0.905 (0.443–1.846)	0.784	23/25	0.509 (0.268–0.969)	0.040
rs12653736	GG	303/136	1.000 (referent)		214/119	1.000 (referent)	
	GT	62/37	0.731 (0.461–1.159)	0.182	48/26	1.058 (0.622–1.800)	0.836
	TT	1/1	0.420 (0.026–6.784)	0.541	4/4	0.514 (0.125–2.108)	0.355
rs1837253	TT	107/49	1.000 (referent)		73/41	1.000 (referent)	
	CC	201/84	0.779 (0.456–1.331)	0.360	136/75	1.083 (0.596–1.970)	0.794
	TC	62/37	1.099 (0.716–1.688)	0.665	56/28	1.030 (0.638–1.662)	0.905
rs12654933	CC	265/127	1.000 (referent)		186/110	1.000 (referent)	
	AA	7/5	0.694 (0.214–2.248)	0.542	8/2	2.487 (0.514–12.029)	0.257
	CA	98/41	1.156 (0.754–1.772)	0.506	74/38	1.128 (0.712–1.787)	0.607
rs10455025	AA	334/151	1.000 (referent)		236/141	1.000 (referent)	
	CA	36/22	0.727 (0.410–1.289)	0.275	30/9	2.136 (0.979–4.662)	0.057
	CC	0/0	N.A	N.A	0/0	N.A	N.A
rs11466741	CC	155/56	1.000 (referent)		108/56	1.000 (referent)	
	CT	136/49	1.022 (0.651–1.603)	0.924	102/49	1.077 (0.672–1.726)	0.758
	TT	26/16	0.634 (0.315–1.278)	0.203	20/14	0.721 (0.337–1.539)	0.397
rs13156086	AA	204/100	1.000 (referent)		149/69	1.000 (referent)	
	CA	128/52	1.235 (0.822–1.854)	0.309	95/58	0.757 (0.489–1.172)	0.212
	CC	18/8	1.177 (0.490–2.832)	0.715	8/12	0.289 (0.112–0.746)	0.010
rs6886755	GG	316/138	1.000 (referent)		217/138	1.000 (referent)	
	GT	53/33	0.763 (0.469–1.241)	0.276	51/12	2.617 (1.343–5.098)	0.005
	TT	1/1	0.514 (0.032–8.351)	0.640	0/0	N.A	N.A
rs252706	GG	161/81	1.000 (referent)		135/68	1.000 (referent)	
	AA	173/65	0.574 (0.321–1.027)	0.061	109/64	0.751 (0.376–1.502)	0.418
	AG	35/27	1.315 (0.885–1.955)	0.175	24/17	0.878 (0.573–1.347)	0.552
rs2416259	TT	137/70	1.000 (referent)		117/56	1.000 (referent)	
	CC	51/26	0.934 (0.533–1.639)	0.812	33/20	0.829 (0.435–1.580)	0.568
	TC	180/66	1.290 (0.856–1.944)	0.223	116/61	0.953 (0.608–1.493)	0.832

^a Numbers of CRS subjects/ control subjects.

^b OR, odds ratio; CI, confidence interval, adjusted for age.

^c NA, not available because of the rarity of genotype.

Table 5

Item stratification associations between genotypes and CRS risk.

SNP ID	Genotype	CRSsNP			CRSwNP		
		No.	OR (95%CI)	P	No.	OR (95%CI)	P
rs1545169	TT	133/142	1.000 (referent)		123/142	1.000 (referent)	
	GT	191/174	1.210 (0.881–1.662)	0.239	179/174	1.166 (0.840–1.618)	0.358
	GG	0/1	NA.	NA.	0/1	NA.	NA.
rs764917	AA	166/170	1.000 (referent)		140/170	1.000 (referent)	
	CA	116/102	1.193 (0.845–1.685)	0.315	143/102	1.812 (1.279–2.568)	0.001
	CC	24/39	0.633 (0.363–1.104)	0.107	23/39	0.749 (0.420–1.333)	0.326
rs12653736	GG	274/255	1.000 (referent)		243/255	1.000 (referent)	
	GT	53/63	0.774 (0.516–1.162)	0.217	57/63	0.973 (0.646–1.464)	0.894
	TT	2/5	0.366 (0.070–1.923)	0.235	3/5	0.664 (0.155–2.852)	0.582
rs1837253	TT	96/90	1.000 (referent)		84/90	1.000 (referent)	
	TC	179/159	1.040 (0.725–1.491)	0.831	158/159	1.119 (0.766–1.637)	0.56
	CC	55/65	0.781 (0.491–1.240)	0.294	63/65	1.068 (0.670–1.704)	0.782
rs12654933	CC	234/237	1.000 (referent)		217/237	1.000 (referent)	
	CA	89/79	1.117 (0.783–1.592)	0.542	83/79	1.153 (0.798–1.666)	0.447
	AA	9/7	1.322 (0.483–3.620)	0.587	6/7	1.128 (0.364–3.499)	0.835
rs10455025	AA	298/292	1.000 (referent)		272/292	1.000 (referent)	
	CA	34/31	1.079 (0.644–1.807)	0.774	32/31	1.140 (0.666–1.953)	0.633
	CC	0/0	NA.	NA.	0/0	NA.	NA.
rs11466741	CC	153/112	1.000 (referent)		110/112	1.000 (referent)	
	CT	136/98	1.026 (0.717–1.468)	0.888	102/98	1.099 (0.743–1.625)	0.637
	TT	26/30	0.637 (0.356–1.140)	0.129	20/30	0.745 (0.395–1.406)	0.364
rs13156086	AA	190/169	1.000 (referent)		163/169	1.000 (referent)	
	CA	103/110	0.849 (0.603–1.194)	0.346	120/110	1.192 (0.843–1.684)	0.32
	CC	11/20	0.496 (0.230–1.069)	0.073	15/20	0.835 (0.408–1.710)	0.622
rs6886755	GG	276/276	1.000 (referent)		257/276	1.000 (referent)	
	GT	55/45	1.243 (0.808–1.911)	0.322	49/45	1.230 (0.784–1.930)	0.368
	TT	1/1	1.010 (0.062–16.334)	0.995	0/1	NA.	NA.
rs252706	GG	150/149	1.000 (referent)		146/149	1.000 (referent)	
	AG	150/129	1.162 (0.837–1.614)	0.371	132/129	1.016 (0.722–1.430)	0.928
	AA	32/44	0.706 (0.423–1.179)	0.184	27/44	0.552 (0.319–0.953)	0.033
rs2416259	TT	126/126	1.000 (referent)		128/126	1.000 (referent)	
	TC	153/127	1.203 (0.854–1.695)	0.291	143/127	1.025 (0.721–1.459)	0.889
	CC	50/46	1.093 (0.681–1.753)	0.713	34/46	0.667 (0.397–1.123)	0.128

CRSsNP, Chronic rhinosinusitis without sinonasal polyposis

CRSwNP, Chronic rhinosinusitis with sinonasal polyposis.

^a Numbers of chronic rhinosinusitis /control subjects.^b OR, odds ratio; CI, confidence interval, adjusted for age and gender.^c NA, not available because of the rarity of genotype.

OR = 0.289, 95% CI = 0.112–0.746) and rs764917 (CC genotype: $P = 0.040$, OR = 0.509, 95% CI = 0.268–0.969) were significantly associated with protective effects against CRS development; while rs6886755 (GT genotype: $P = 0.005$, OR = 2.617, 95% CI = 1.343–5.098) presented a risk role among female group. In contrast, only rs764917 exhibited risk role for CA genotype ($P = 0.033$, OR = 1.553, 95% CI = 1.036–2.327) in male CRS subjects.

Stratified analysis of CRS risk on category further revealed that rs252706 (AA genotype: $P = 0.012$, OR = 0.552, 95% CI = 0.319–0.953) and rs764917 (CA genotype: $P = 0.001$, OR = 0.182, 95% CI = 1.279–2.568) played protective roles among only CRSwNP group, whereas none of the SNPs showed any significant associations in CRSsNP subjects (Table 5).

3.3. LD analysis and haplotype block structure

Fig. 1 shows plots of the pair wise LD (r^2 and D') values for the tag SNPs and LD structures in selected chromosome 5 region (Fig. 1A). The LD plot indicates that, for selected region in chromosome 5, only on block were identified in CRS vs. control analysis and block partial transcribed TSLP gene and 3' region of TSLP gene (Fig. 1B).

3.4. Haplotype Analysis

Table 6 summarizes the associations between frequencies of the haplotypes in block rs10455025–rs11466741–rs13156086–

rs6886755–rs252706–rs2416259 and the risk of CRS. None of the haplotypes investigated was found to be associated with increased risk of CRS in the cohort studied. Likewise, there was no association between the diplotypes evaluated in the same block and risk of CRS (Table 7).

4. Discussion

Here we aimed to explore the contribution of genetic variations in TSLP gene region against CRS susceptibility in a Chinese cohort. In the single-locus analysis of CRS risk, no significant differences in allele and genotype frequencies were found between CRS patients and the controls. Further logistic regression analyses adjusted by age and gender also failed to reveal significant associations between CRS and the selected SNPs. However, stratified analyses of CRS associations on gender, demonstrated rs13156086 (CC) and rs764917 (CC) to be significantly associated with protective effects against CRS while rs6886755 (GT) presented a risk role among female group. In contrast, only rs764917 exhibited risk role for CA genotype regarding to CRS in male subjects. Furthermore, stratified analysis of CRS risk on category revealed that rs252706 (AA) and rs764917 (CA) played protective roles only among CRSwNP group while none of the SNPs showed significant association in CRSsNP subjects. These findings suggest that SNPs in TSLP gene are likely to modify the risk for development of CRS in Chinese subjects and exert a gender as well as comorbid nasal polyposis-dependent association pattern.

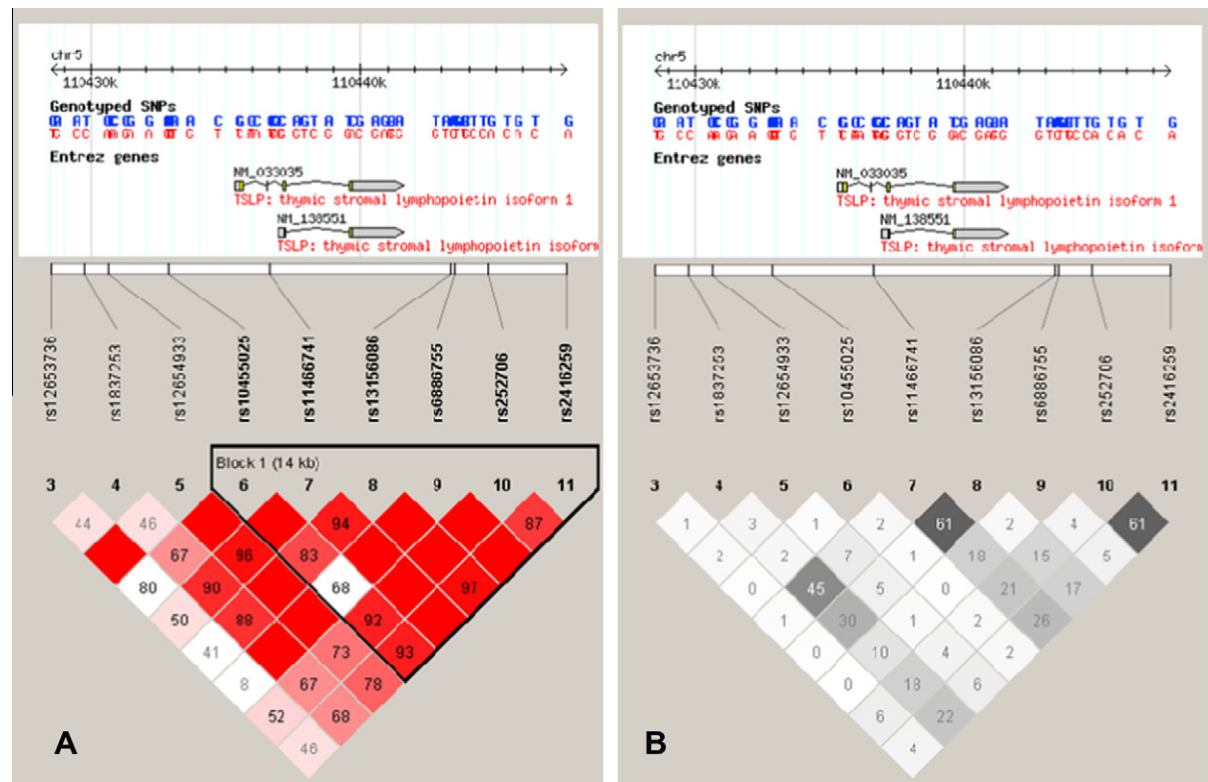


Fig. 1. Graphical representation of the SNP locations and LD structure of TSLP gene. Each figure was composed of chromosome scale (the top line with even division), the transcription string (the thick bars represent exon (yellow) or UTR (blue), the thin lines represent intron), SNP scale (the hollow bar with scales representing SNPs location), and block definition (flattened) or graphic of LD (black-and-white). A: The measure of LD (D') among all possible pairs of SNPs is shown graphically according to the shade of color, where white represents very low D' and scarlet represents very high D' . The numbers in squares are D' values ($D' \times 100$). B: The measure of LD (r^2) among all possible pairs of SNPs is shown graphically according to the shade of color, where white represents very low r^2 and black represents very high r^2 . The numbers in squares are r^2 values ($r^2 \times 100$).

Table 6
Associations between common haplotypes near TSLP gene and CRS risk.

Block	CRS subjects		Control subjects		P ^a	P _{sim} ^b	Hap. Score ^c	Logistic Regression		Global score test ^f
	No.	Frequency	No.	Frequency				OR (95% CI)	p ^d	
Block1: rs10455025–rs11466741–rs13156086–rs6886755–rs252706–rs2416259										
ACAGAC	371	29.08	203	31.23	0.25268	0.25268	1.14386	1.00 (referent)		global–stat = 22.33835, df = 6, p–val = 0.0010, P_{sim}^b = 0.00083
ATCGGT	292	22.88	167	25.69	0.54458	0.54665	0.60591	0.976 (0.753–1.264)	0.852	
ACAGGT	307	24.06	143	22.00	0.02064	0.02118	2.31455	1.176 (0.902–1.533)	0.231	
ATATGT	104	8.15	48	7.38	0.17303	0.17203	1.36254	1.220 (0.829–1.796)	0.312	
ACAGGC	92	7.21	37	5.69	0.04288	0.0421	2.02484	1.367 (0.896–2.085)	0.147	
CCAGGT	63	4.94	31	4.77	0.27754	0.2777	1.08586	1.135 (0.710–1.813)	0.597	
Others	47	3.68	21	3.23	NA ^e	NA ^e	NA ^e	NA ^e	NA ^e	

CRS, Chronic rhinosinusitis

^a P value for difference in haplotype frequency between CRS and control subjects.

^b Generated by permutation test with 100,000 times simulation.

^c A positive (or negative) score for a particular haplotype would have suggested that the haplotype was associated with increased (or decreased) risk of CRS

^d P values from unconditional logistic regression analyses

^e NA, not available because of the rarity of genotype.

^f df, degrees of freedom.

The TSLP gene is located on human chromosome 5q22 which near the gene cluster encoding Th2 cytokines [20,21], and plays a critical role in Th2 cell differentiation [5,22,23]. There is strong evidence that the Th2 skewing properties of TSLP play an important role in the pathogenesis of allergic disorders such as asthma, atopic dermatitis and allergic rhinitis [4]. Moreover, association studies on polymorphisms in the TSLP gene and allergy related phenotypes have verified the important effects of TSLP towards allergy in different populations [24–30]. With regard to the possible role of

the TSLP gene in CRS development, to our knowledge there are only two studies to date. In one study Kimura and colleagues [8] compared the expression of TSLP in nasal polyps and nasal mucosa from atopics and non-atopics. The authors reported that the mRNA expression of TSLP was markedly high in NPs compared to the allergic nasal mucosa. Furthermore, the number of TSLP+ cells in nasal polyps from atopics was significantly greater than that in the allergic nasal mucosa and in non-atopics, and strongly correlated with the number of eosinophils and the levels of IgE in nasal

Table 7

Diplotype analysis of common haplotypes near TSLP gene with CRS risk.

Block	0-copy		1-copy Logistic Regression			2-copy Logistic Regression			P (2 df) ^b
	CRS/C	OR (95%CI)	CRS/C	P ^a	OR (95%CI)	CRS/C	P ^a	OR (95%CI)	
Block: rs10455025–rs11466741–rs13156086–rs6886755–rs252706–rs2416259									
ACAGGT	380/204	1.000 (referent)	225/105	0.374	1.141 (0.853–1.527)	33/16	0.784	1.092 (0.584–2.041)	0.624
ACAGGC	558/291	1.000 (referent)	73/31	0.358	1.234 (0.788–1.931)	7/3	0.860	1.131 (0.287–4.464)	0.641
ACAGAC	336/167	1.000 (referent)	252/123	0.894	1.020 (0.765–1.360)	50/35	0.113	0.680 (0.422–1.096)	0.314
ATATGT	544/280	1.000 (referent)	93/44	0.631	1.100 (0.745–1.626)	1/1	0.670	0.546 (0.034–8.841)	0.811
ATCGGT	386/191	1.000 (referent)	230/114	0.879	1.023 (0.767–1.363)	22/20	0.065	0.549 (0.290–1.037)	0.151
BCAGGT	575/294	1.000 (referent)	63/31	0.828	1.052 (0.666–1.664)	0/0	NA ^c	NA ^c	0.868

CRS, Chronic rhinosinusitis subjects; C, Control subjects

^a P values from unconditional logistic regression analyses, adjusted for age and gender.^b Global P values [2 degrees of freedom (df)], diplotype frequencies in cases and controls were compared using a χ^2 test with 2 df.

polyps. The authors concluded that their findings suggested a potential role for TSLP in the pathogenesis of nasal polyps by regulating the Th2 type and eosinophilic inflammation [8]. In another study Boita and colleagues [9] investigated the expression of TSLP Receptor (TSLP R) in surgical specimens from CRSwNP and CRSsNP patients and demonstrated that expression of TSLP R was equally and significantly higher in both the inflammatory infiltrate and epithelial cells of both CRSwNP and CRSsNP patients, compared to controls. To our knowledge, the present study provides the first data and evidence for a genetic association between the TSLP gene and development of CRS, excluding an allergy factor.

Studies in Caucasians have shown that CRSwNP and CRSsNP display distinct features on the basis of histomorphology and the expression of inflammatory and remodeling mediators; with CRSwNP characterized by a Th2-skewed eosinophilic inflammation, whereas CRSsNP represents a predominant Th1 milieu [6,31,32]. Recently, however, several studies in Asian subjects suggested that CRSwNP presented different immunopathologic features from those in white patients, indicating distinct mechanisms underlying the pathogenesis of nasal polyps between the two ethnic cohorts [7,33–35]. Moreover, Cao and colleagues [35] reported that the Th2-dominated reactions can only be found in subjects eosinophilic CRSwNP instead of all CRSwNP. Here we only detected a significant association between TSLP and CRSwNP but not CRSsNP, implying the potential role of TSLP in nasal polyp development at the genetic level. However, in the present study we did not aim to evaluate either the immunopathologic characteristic of each subject or any potential relationship between inflammatory category (eosinophils or neutrophils dominated), Th subtype and TSLP genetic variations. Thus, it would be interesting to assess whether or not the associations between SNPs in the TSLP gene and CRS are also dependent on nasal inflammatory milieu in future studies.

Evidence from studies in both animal models and humans has indicated that gender may modify the role of TSLP in asthma. Transgenic expression of *TSLP* in mice leads to perivascular leukocytic infiltration with prominent eosinophilia, with increased severity noted in female mice compared to male mice [36]. In human studies, Hunninghake and colleagues [37] have reported a sex-specific association between a polymorphism, rs2289276, and serum total IgE in girls in two independent populations. More recently these authors showed that TSLP polymorphisms were also associated with asthma in a sex-specific fashion [38]. In particular, the T allele of rs1837253 was significantly associated with a reduced risk of asthma in males whereas the T allele of rs2289276 was significantly associated with a reduced risk of asthma in females [38]. Likewise, our study has demonstrated that there was a gender-dependent pattern between TSLP genetic variations and CRS; i.e. rs13156068, rs764917 and rs6886755 were associated with CRS in females whereas only rs764917 exhibited an association

in male subjects. It is worth mentioning that rs764917_CC presented as a protective role and rs764917_CA as a risk factor.

Last but not least, all the identified positive SNP sites in present study (rs764917, rs6886755, rs252706 and rs13156068) in present study are at the transcriptional regulation site of TSLP gene but not at the coding site, which means they can only influence the transcriptional expression but not the sequence or structure of this gene, let alone the TSLP receptor. Accordingly, more attention on SNPs in the coding region of TSLP gene needs to be paid in the future.

Taken together, we provide the first data for the genetic association between TSLP gene and CRS etiology. These findings suggest that SNPs in TSLP gene modify the risk for development of CRS in Chinese subjects and exert a gender as well as comorbid nasal polyposisdependent association pattern. Further studies are needed to combine the histopathology characteristics, CRS category and TSLP variations and thus explore in-depth the potential mechanisms involved in the etiology of CRS.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.humimm.2012.11.004>.

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SUPPORTING INFORMATION:**Table 1.** Details of the primers used in the screening of SNPs by MassArray.

SNP	Alleles	Position	Function	Primers	Extension Primers
1545169	T/G	110427275	Unknown	ACGTTGGATGCTTACAGTTCTGAATGCTGG ACGTTGGATGGCAAACTAAGGCCATCTGC	CTGAATGCTGGAAGTCC
764917	A/C	110428406	Unknown	ACGTTGGATGGAACAGTTATTCCAAAATGC ACGTTGGATGTCCAGTTTTGTAAAATCGC	AAAAATGCATTTGAAAAGAAGA
12653736	G/T	110428526	Unknown	ACGTTGGATGGCTCTTGTTCTCAACATTTG ACGTTGGATGAAGACTTGGGAACCTAGGAG	CTCATAACTCATATAGAGTACCT
1837253	T/C	110429771	Unknown	ACGTTGGATGACCTACTGGACTCTATTGTG ACGTTGGATGCCCTTGACTCACATAATGG	TGTAATTTGCTTCATAGTTTAGACAC
12654933	C/A	110430654	Unknown	ACGTTGGATGGCTTTATGCCTTGAAGCAG ACGTTGGATGAGGTGAACTCAACCAGAAGC	GGAAGCAGACCCATAAG
10455025	A/C	110432898	Unknown	ACGTTGGATGAGGAGACCACTTTTGGAGAC ACGTTGGATGCTCTGAGCTGTTTTCTTCCC	CAAATAAGATGAACATGGCA
11466741	C/T	110436604	nearGene-5	ACGTTGGATGCATTTTGGAGAGGGAGTATC ACGTTGGATGAGCCCAGATGTCCCCTTTC	GGAGAGGGAGTATCCTGCTA
13156086	A/C	110443368	Unknown	ACGTTGGATGCTATTGCCACAAAGCAGTCC ACGTTGGATGGGAATAAAGACTGTTACAC	AGACAGAAAACCTAAAAATAAAGTTAC
6886755	G/T	110443500	Unknown	ACGTTGGATGGCACACAGTTATTGACAGCC ACGTTGGATGTTCTCTGTGCACCTCTTACC	TAGCAGAGTTCATAAGACA
252706	G/A	110444759	Unknown	ACGTTGGATGGATTTCTACCTGTTAGCTTC ACGTTGGATGTCAAGAATAGTGGAGAATGG	CTGTTTAAGTAGGAGGGT
2416259	T/C	110447641	Unknown	ACGTTGGATGGTCATCCTTGGACACACAAC ACGTTGGATGGCCACAGGTTACAAAAGGAC	gTTGGAAACCTTAACATCATATT