BMB
reports

Identifying the polymorphisms in the thymic stromal lymphopoietin receptor (*TSLPR*) and their association with asthma

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The present study aimed to investigate whether the polymorphisms in the *TSLPR* gene are associated with atopic and asthmatic disease in the Korean population. We identified eleven single nucleotide polymorphisms (SNPs) and two variation sites in the *TSLPR* gene, including the promoter region. The genotype and allele frequencies of g.33G>C of the *TSLPR* gene in asthma patients were significantly different from the respective frequencies of the control group (*P* = 0.006 and 0.003, respectively). Our additional analysis showed that the genotype and allele frequencies of the g.33G>C and g.19646A>G of the *TSLPR* gene were significantly associated in the atopic asthma patients rather than in the non-atopic asthma patients (genotype frequencies; *P* = 0.0001 and 0.0003 respectively, allele frequencies; *P* = 0.0005 and 0.0001 in that order). Our results suggest that the SNPs of the *TSLPR* gene could be associated with the susceptibility to atopic asthma in the Korean population. [BMB reports 2010; 43(7): 499-505]

INTRODUCTION

Allergic diseases such as asthma, allergic rhinitis and atopic dermatitis are usually due to an excessive immune response to certain antigens called allergens. Asthma is one of the most common multi-complex disorders and this is triggered by a genetic predisposition and environmental factors (1). This disorder is characterized by reversible airflow obstruction and airway inflammation, and persistent airway hyper-reactivity (AHR) and airway remodeling (2). Unlike other inflammatory diseases, the asthmatic inflammatory process is characterized by the predominant secretion of interleukin (IL)-4 and IL-5 by T-helper lymphocytes type 2 (Th2) and immunoglobulin (Ig)E synthesis. IgE mediates the early and late asthmatic responses that induce of lung eosinophil infiltration and Th2 cell cytokine production (3).

Thymic stromal lymphopoietin (TSLP) has been proposed to signal through a heterodimeric receptor complex, which is composed of the TSLP receptor (TSLPR; also known as CRLF2) and the IL-7R alpha chain (4, 5). Human TSLP is involved in dendritic cell maturation (6, 7) and it is produced by epithelial cells, stromal cells and mast cells. The single nucleotide polymorphisms (SNPs) of *TSLP* were associated with the susceptibility to allergic rhinitis (8). The human TSLPR located in the Xp22.3 and Yp11.3 regions has been identified from a human T lymphocyte and dendritic cell (DC) cDNA library (9, 10). TSLPR contains two fibronectin type III-like domains, four conserved cysteine residues and a WSXWS (Trp-Ser-X-Trp-Ser) box-like motif that is typical for type I cytokine receptors. The C-terminal intracellular region contains a membrane-proximal Box 1 motif, which also functions as a putative binding site for signal transduction molecules (9, 10). The TSLPR knockout mice have normal numbers of lymphocytes, but the TSLPR/γ_c double knockout mice display a greater lymphoid defect than the γ_c KO mice (11). Leonard and coworkers have also reported that these mice exhibit strong Th1 responses with high levels of IL-12, interferon-γ and IgG2a, but low levels of IL-4, IL-5, IL-10, IL-13 and IgE. *TSLPR* knockout mice fail to develop an inflammatory lung response to inhaled antigen unless they are supplemented with wild- type $CD4^+$ T cells (12). These results indicated that TSLPR plays an important role in the development of inflammatory and/or allergic responses. Shi and coworkers have recently suggested that the local application of anti-TSLPR prevented Th2- mediated airway inflammation by regulating the function of DCs, and this might be exploited to develop novel treatments for asthma (13).

Although *TSLPR* is one of the crucial candidate genes for the development of allergic responses and the differentiation of T cells, any correlation between the SNPs of *TSLPR* and the individual susceptibility to asthma has not been demonstrated. In an attempt to understand the genetic influences of *TSLPR* on asthma, we have identified the possible variation sites and SNPs through the eight exons and their boundary introns sequences of *TSLPR*, including the ~1.9 kb promoter regions.

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Received 30 March 2010, Accepted 30 June 2010

Keywords: Asthma, Atopic disease, *CRLF2*, Polymorphism, *TSLPR*

To determine whether these *TSLPR* SNPs are associated with the susceptibility to asthma, we analyzed the frequencies of the *TSLPR* SNPs on the genomic DNAs that were isolated from asthma patients and healthy controls. We further investigated the relationships between these polymorphisms and the total serum IgE levels, the peripheral blood eosinophil counts and the forced vital capacity (FVC) or forced expiratory volume in 1 second (FEV1) in asthma patients. Finally, we calculated the haplotype frequencies that were constructed by these SNPs in both groups.

RESULTS

The human *TSLPR* gene is located on chromosome Xp22.3 and Yp11.3, and it consists of seven (NM_001012288.1, isoform 2) or eight exons (NM_022148.2, isoform 1). We examined the expression pattern of *TSLPR* in 13 normal human tissues and peripheral leukocytes. The expression level of *TSLPR* mRNA was the highest in the ovary, pancreas and lung, yet that in brain and skeletal muscle was not detected (Fig. 1A).

To determine the possible variation sites in the entire coding regions and their boundary introns sequences of the *TSLPR* isoform 1, including ~1.9 kb promoter regions, we first scanned the genomic DNAs isolated from 24 unrelated asthma patients and 24 healthy controls with using a direct sequencing method. We identified eleven SNPs and two variation sites, g.-1446A>C (rs9780560) and g.-43T>C (novel, agtcatgagtgatctgttcg[t/c]tgtaggtccctgaggacctc) in the promoter region, g.33G>C (novel, gttctgctgtggggagctgc[g/c]gtctttctgctgggaggctg) in exon 1, g.214delT (novel, cttttctttttttttttttttttttt[t/]ccgggttca), g.10101G>T (novel, gggcggccgcgactcagggc[g/t]atggtggctgagcgtccccc), g.13732_13733(GT)19-24 (novel), g.16196C>A (novel, gccgcccgtgggtggagggga[c/a]ctgcttgcacaatgtctgtga) and g.16197C>T (novel, gccgcccgtgggtggagggac[c/t]tgcttgcacaatgtctgtga) in the introns, g.19646A>G (novel, ccgtctctatttcagagtga[a/g]gaagtttctcattcccagcg) in exon 7, g.21869T>C (rs36139698), g.21884G>A (rs36177645) and g.21995T>C (rs36133495) in exon 8 and g.22129T>C (rs36158404) in 3'-UTR (Fig. 1B). While the g.33G>C, g.21869T>C, g.21884G>A and g.21995T>C polymorphisms located in the coding region were synonymous SNPs (Ala11Ala, Pro308Pro, Val313Val and Gly350Gly, respectively) in isoform 1, the amino acids of g.19646A>G were substituted to Lys258Arg (K258R) in isoform 1. In the isoform 2 of TSLPR, the amino acid of g.19646A>G SNPs was substituted to Lys146Arg (K146R). The linkage disequilibrium (LD) coefficients (|D'|) between all the SNP pairs were calculated, and we found the absolute LD (|D'| = 1 and r^2 = 1) between g.16196C>A and g.16197C>T, and between g.21869T>C and g.22129T>C (data not shown). Among the identified polymorphisms, six SNPs (g.-43T>C, g.33G>C, g.19646A>G, g.21869T>C, g.21884G>A and g.21995T>C) were selected for large sample genotyping based on their locations and LD.

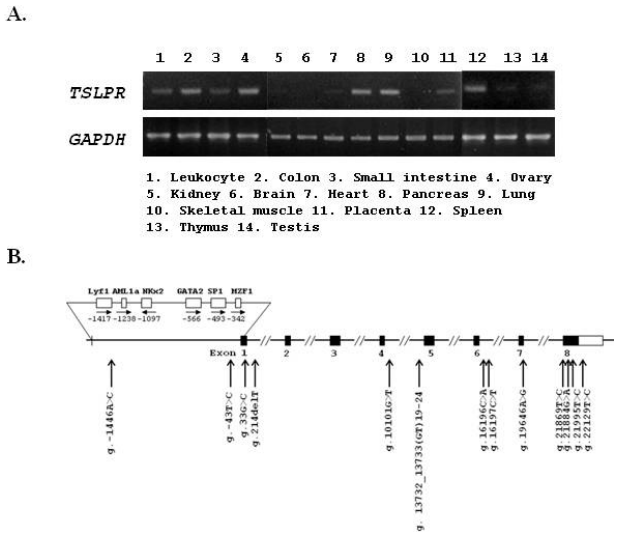

Fig. 1. Expression patterns of the *TSLPR* mRNA in the various human tissues and the peripheral blood cells by RT-PCR (A), and locations of each single nucleotide polymorphisms (SNPs) and variation sites in *TSLPR* (B). Coding exons are marked by black blocks and 3'-UTR by white blocks. The positions of SNPs were calculated from the translation start site. Putative transcription fac tor sites were searched at www.cbrc.jp/research/db/TFSEARCH.html. The reference sequence for *TSLPR* was based on the sequence of human chromosome X clone RP13-440N7 (CR936360).

To determine whether the *TSLPR* SNPs are associated with the susceptibility of asthma, the genotypes of the *TSLPR* SNPs were analyzed by the single-base extension (SBE), high resolution melting (HRM) or TaqMan probe method. The genotypes and allele frequencies were compared between the asthma patients and the healthy controls. All the genotype frequencies were in Hardy-Weinberg equilibrium (HWE), except for the g.-43T>C in the healthy controls and the g.19646A>G in the asthma patients (data not shown). The genotype and allele frequencies of g.-43T>C, g.19646A>G, g.21869T>C, g.21884G>A and g.21995T>C were not significantly different between the asthma patients and healthy controls. However, the genotype and allele frequencies of the g.33G>C of the *TSLPR* in the asthma patients were significantly different from the respective frequencies of the control group (P = 0.006 and 0.003, respectively, Table 1). We further analyzed the genotype and allele frequencies of the atopic asthma patients, the non-atopic asthma patients and the healthy controls (Table 1). In the atopic asthma patients rather than non-atopic asthma patients, the genotype and allele frequencies of the g.33G>C as well as those of the g.19646A>G were significantly different from the respective frequencies of the healthy controls group (genotype frequencies; P = 0.0001 and 0.0003 respectively, allele frequencies; P = 0.0005 and 0.0001 in that order). These results strongly suggest that the SNPs of the *TSLPR* gene could be associated with the susceptibility to atopic disease (Table 1).

We further investigated the relationships between the SNPs

Table 1. Genotype and allele analyses of the *TSLPR* gene polymorphisms in asthma patients and healthy controls

Position[a]	Genotype/ Allele	Control n (%)	Asthma n (%)	Odds ratio[b] (95% CI)	P[c]	Asthma n (%)		P[c]	
						Atopic	Non-atopic	Atopic	Non-atopic
g.-43T>C	TT	425 (76.6)	269 (74.5)	1.00	0.381	69 (67.0)	58 (73.4)	0.118	0.799
	TC	102 (18.4)	78 (21.6)	1.21 (0.87-1.68)		27 (26.2)	17 (21.5)		
	CC	28 (5.0)	14 (3.9)	0.79 (0.41-1.53)		7 (6.8)	4 (5.1)		
	T	952 (85.8)	616 (85.3)	1.00	0.786	165 (80.1)	133 (84.2)	0.044	0.628
	C	158 (14.2)	106 (14.7)	1.04 (0.80-1.35)		41 (19.9)	25 (15.8)		
g.33G>C	CC	520 (91.5)	341 (86.1)	1.00	0.006	100 (81.3)	78 (88.6)	0.0001	0.670
	CG	48 (8.5)	55 (13.9)	1.75 (1.16-2.63)		21 (17.1)	10 (11.4)		
	GG	0 (0.0)	2 (0.5)	-		2 (1.6)	0 (0.0)		
	C	1,088 (95.8)	737 (92.6)	1.00	0.003	221 (89.8)	166 (94.3)	0.0005	0.428
	G	48 (4.2)	59 (7.4)	1.82 (1.23-2.69)		25 (10.2)	10 (5.7)		
g.19646A>G	GG	250 (44.2)	159 (43.4)	1.00	0.243	33 (30.0)	40 (48.2)	0.0003	0.752
	GA	242 (42.7)	145 (39.6)	0.94 (0.71-1.25)		47 (42.7)	32 (38.6)		
	AA	74 (13.1)	62 (16.9)	1.32 (0.89-1.95)		30 (27.3)	11 (13.3)		
	G	742 (65.5)	463 (63.3)	1.00	0.321	113 (51.4)	112 (67.5)	0.0001	0.599
	A	390 (34.5)	269 (36.7)	1.11 (0.91-1.34)		107 (48.6)	54 (32.5)		
g.21869T>C (rs36139698)	CC	408 (72.1)	301 (76.6)	1.00	0.163	95 (78.5)	66 (75.9)	0.059	0.715
	CT	147 (26.0)	82 (20.9)	0.76 (0.56-1.03)		21 (17.4)	20 (23.0)		
	TT	11 (1.9)	10 (2.5)	1.23 (0.52-2.94)		5 (4.1)	1 (1.1)		
	C	963 (85.1)	684 (87.0)	1.00	0.231	211 (87.2)	152 (87.4)	0.366	0.489
	T	169 (14.9)	102 (13.0)	0.85 (0.65-1.11)		31 (12.8)	22 (12.6)		
g.21884G>A (rs36177645)	AA	249 (44.5)	182 (47.0)	1.00	0.724	60 (50.0)	42 (49.4)	0.249	0.641
	AG	260 (46.4)	170 (43.0)	0.90 (0.68-1.17)		46 (38.3)	37 (43.5)		
	GG	51 (9.1)	35 (9.0)	0.94 (0.59-1.50)		14 (11.7)	6 (7.1)		
	A	758 (67.7)	534 (69.0)	1.00	0.581	166 (69.2)	121 (71.2)	0.703	0.378
	G	362 (32.3)	240 (31.0)	0.94 (0.77-1.15)		74 (30.8)	49 (28.8)		
g.21995T>C (rs36133495)	TT	204 (36.4)	116 (31.4)	1.00	0.213	31 (27.7)	32 (40.0)	0.127	0.269
	TC	274 (48.8)	188 (50.8)	1.21 (0.90-1.62)		58 (51.8)	32 (40.0)		
	CC	83 (14.8)	66 (17.8)	1.40 (0.94-2.08)		23 (20.5)	16 (20.0)		
	T	682 (60.8)	420 (56.8)	1.00	0.092	120 (53.6)	96 (60.0)	0.052	0.863
	C	440 (39.2)	320 (43.2)	1.18 (0.98-1.43)		104 (46.4)	64 (40.0)		

[a]Calculated from the translation start site, [b]Logistic regression analyses were used for calculating OR (95% CI; confidence interval), [c]Value was determined by Fisher's exact test or χ^2 test from 2 × 2 contingency Table.

of *TSLPR* and the total serum IgE levels or the peripheral blood eosinophil counts in the asthma patients (Table 2). The polymorphisms of *TSLPR* in the asthma patients were shown to have no significant association with the levels of total serum IgE and the peripheral blood eosinophil counts. We also investigated the relationships between the SNPs of *TSLPR* and the FVC or FEV1 in asthma patients. However, those also had no significant association (Supplementary Table 3).

Finally, we estimated the haplotype frequencies of the block 1 (g.-43T>C and g.33G>C) and the block 2 (g.21869T>C and g.21884G>A) of the *TSLPR* gene between the healthy controls and the asthma patients (Table 3). Although the minor haplotype (TG) frequency was somewhat different in the block 1 of the asthma patients, as compared to that of the healthy controls (P = 0.001), the major haplotypes' (frequency > 0.05) distributions in block 1 and block 2 were not significant difference between the asthma patients and the healthy controls. These results suggest that the haplotypes of the *TSLPR* polymorphisms are not associated with asthma susceptibility.

DISCUSSION

Asthma is a multi-factorial inflammatory condition that's often associated with bronchial hyper-reactivity and atopy. Asthma is triggered by the genetic predisposition and environmental factors. In this study, we evaluated the associations between the *TSLPR* polymorphisms and the susceptibility to asthma.

The expression levels of *TSLPR* mRNA, as assessed by northern blot analysis, have been differently reported. Tonozuka *et al*. reported that *TSLPR* mRNA was expressed in liver, kidney,

Table 2. Analysis of serum total IgE levels and peripheral eosinophil counts among the genotypes of each SNP of the *TSLPR* in asthma patients

Position[a]	Genotype	IgE (IU/ml)			P[b]	Eosinophil[c]			P[b]
		n	Mean	SD		n	Mean	SD	
g.-43T>C	TT	247	247.6	300.0	0.940	242	4.16	5.02	0.510
	TC	76	239.2	337.0		75	4.10	4.28	
	CC	14	220.4	217.0		14	5.67	4.82	
g.33G>C	CC	318	238.8	238.8	0.850	313	4.24	5.87	0.890
	CG	53	247.0	247.0		51	4.12	4.11	
	GG	2	–	–		2	–	–	
g.19646A>G	GG	143	267.6	267.6	0.340	140	4.33	5.66	0.140
	GA	136	217.3	217.3		136	3.72	4.02	
	AA	62	267.6	220.7		59	5.49	8.72	
g.21869T>C (rs36139698)	CC	286	242.5	242.5	0.960	283	4.31	5.55	0.870
	CT	74	241.2	241.2		72	3.92	6.18	
	TT	9	214.1	214.1		7	4.34	4.49	
g.21884G>A (rs36177645)	AA	175	227.8	227.8	0.094	175	4.43	6.15	0.680
	AG	154	275.2	275.2		149	3.97	4.32	
	GG	34	162.3	162.3		32	4.75	8.09	
g.21995T>C (rs36133495)	TT	106	222.0	222.0	0.120	104	4.51	5.57	0.360
	TC	177	233.4	233.4		174	3.81	5.42	
	CC	62	315.4	315.4		62	4.80	4.70	

[a]Calculated from the translation start site, [b]Values were analyzed by ANOVA, [c]Values were determined by the eosinophil numbers (10^3/μl).

Table 3. The haplotype frequencies by *TSLPR* gene SNPs in asthma patients and healthy controls

Haplotype			Frequency[a]		Chi-square	P[b]
			Asthma	Control		
Block 1 (g.-43T>C, g.33G>C)	T	C	78.77	82.25	3.383	0.064
	C	C	13.92	13.50	0.065	0.808
	T	G	7.04	3.55	11.28	0.001
	C	G	0.27	0.7	1.565	0.273
Block 2 (g.21869T>C, g.21884G>A)	C	A	68.55	67.53	0.219	0.618
	C	G	18.58	17.48	0.372	0.563
	T	G	12.39	14.66	1.967	0.142
	T	A	0.48	0.33	0.242	0.628

[a]Values were constructed by EM algorithm with genotyped SNPs, [b]Values were analyzed by permutation P value.

heart and skeletal muscle (9), while Zhang *et al*. showed that it was restrictedly expressed in spleen and peripheral blood leukocytes, and it was undetectable in thymus, liver, kidney, heart, skeletal muscle, brain, small intestine, placenta, lung and colon tissue (10). In this study, we showed that the expressed levels of *TSLPR* mRNA by RT-PCR were highest in ovary, pancreas and lung, and they were only barely detected in kidney, heart, thymus and testis, while they were not detected in brain and skeletal muscle (Fig. 1A).

In this study, we identified eleven SNPs, including six novel SNPs and two novel variation sites in *TSLPR* (Fig. 1B), and we analyzed the genotype of these SNPs in asthma patients and healthy controls. The genotype and allele frequencies of the g.33G>C of *TSLPR* in asthma patients were significantly different from those in the controls group (Table 1). This result suggests that the g.33G>C polymorphism of *TSLPR* might be associated with the susceptibility to asthma. Our additional analysis of the genotype and allele frequencies between the atopic asthma or non-atopic asthma patients and the healthy controls showed that the genotype and allele frequencies of the g.33G>C and g.19646A>G were significantly associated in the atopic asthma patients rather than in the non-atopic asthma patients (Table 1). These results strongly suggest that the SNPs of *TSLPR* could be associated with the susceptibility

to atopic asthma. Interestingly, the amino acid of g.19646A>G was substituted to Lys258Arg and Lys146Arg in isoform 1 and 2, respectively, while the g.33G>C was the synonymous SNP (Ala11Ala). Generally, synonymous variations affect mRNA stability, mRNA secondary structure and synthesis of the receptor and it can have effects of potential pathophysiological importance (14, 15). These results and the LD block of *TSLPR* led to us think that the g.33G>C and g.19646A>G of *TSLPR* could be associated with the susceptibility to atopic disease with and without the change of amino acid, respectively.

Large numbers of eosinophils are accumulated in the lungs of asthmatic patients and they are believed to be essential in phagocytosis as well as in the allergic and inflammatory reactions of asthma. We previously reported that the SNPs or variations of the *Tim-1* and *IL-27* gene are associated with the susceptibility to asthma in a Korean population (16, 17) as well that the SNP of *Eotaxin-3* is associated with the total IgE level in the serum of asthma patients (18). However, our present study revealed that the SNPs of the *TSLPR* gene in asthma patients are not closely associated with the level of total serum IgE, the peripheral blood eosinophil counts (Table 2), the FVC and the FEV1 (Supplementary Table 3). Probably, the level of IgE and accumulation of eosinophil in chronic asthma patients come out of multiple physiological alterations that eventually leads to a late stage of asthmatic symptoms.

In conclusion, the results of this study suggest that *TSLPR* might be a candidate gene that is associated with the pathogenesis of atopic asthma, although we cannot rule out the possibility that multiple genetic alterations also lead to the aggravation of atopic disease, including asthma. Our results suggest that these polymorphisms are possibly correlated with the susceptibility to atopic or asthmatic disease.

MATERIALS AND METHODS

Patients and DNA samples

The DNA samples used in this study were provided by the Biobank of Wonkwang University Hospital, which is a member of the National Biobank of Korea and this Biobank is supported by the Ministry of Health, Welfare and Family Affairs. On the basis of approval and informed consent from the institutional review board, we obtained the genomic DNAs from 405 asthma patients and 570 healthy controls. The clinical parameters of the study subjects are summarized in Supplementary Table 1. Genomic DNA was extracted from the leukocytes of the peripheral blood by a standard phenol-chloroform method or by using a Genomic DNA Extraction kit (iNtRON Biotechnology, Korea) according to the manufacturer's directions. Asthma was diagnosed in reference to the criteria of the American Thoracic Society (19). The blood eosinophil counts and total serum IgE levels in the asthma patients were measured using a Coulter GenSTM Hematology Analyzer (Florida, USA) and a Roche COBAS-CORE II (Roche Diagnostics, Basal, Switzerland), respectively. All the subjects used in this study were Korean who lived in the same area.

Polymerase chain reaction (PCR) and sequencing analysis

The entire coding regions of the *TSLPR*, including the 1.9 kb promoter regions, were partially amplified with using eight primer pairs (Supplementary Table 2). Amplification was carried out in a PCR Thermal Cycler DICE Gradient (TaKaRa, Japan) at 95°C for 7 minutes to pre-denature the template DNA, followed by 31 cycles of denaturation at 95°C for 10 seconds, annealing at 65°C for 30 seconds and extension at 72°C for 2 or 2.5 minutes. The final extension was completed at 72°C for 10 minutes. The template DNA for sequencing analysis was prepared according to the previously described method (20). The same primers for PCR (Supplementary Table 2) were used as sequencing primers. An additional two primers were employed for the sequencing of the *TSLPR* promoter regions (Supplementary Table 2). The SNPs of *TSLPR* were detected by sequence analysis that was based on the reference sequence of human chromosome X clone RP13-440N7.

mRNA expression level

The expression assay for *TSLPR* mRNA was performed using the cDNAs obtained from various human tissues and peripheral blood cells (Clontech, USA). Approximately 1 μg of total RNA was used in the first-strand cDNA synthesis with using a sequence specific primer and M-MLV reverse transcriptase (Bioneer, Korea) for reverse transcription-polymerase chain reaction (RT-PCR). The PCR samples were prepared in a 20 μl reaction volume containing 50 ng cDNA, 10x buffer 2 μl, 10 mM dNTP 0.5 μl, EF-taq 0.2 U (Solgent, Korea) and 0.5 μM of each primer (Supplementary Table 2) under the following conditions: 10 minutes at 95°C for initial denaturation, and then 30 cycles of PCR that consisted of denaturation for 10 seconds at 98°C, annealing for 15 seconds at 55°C and extension for 30 seconds at 72°C and a final extension was done for 10 minutes at 72°C, and all this was done in a PCR Thermal Cycler DICE Gradient (TaKaRa, Japan).

HRM

Genotype analysis for g.-43T>C, g.19646A>G and g.21995T>C of the *TSLPR* gene was performed by HRM analysis. The 10 μl reaction mixture was made up using 1× QuantiTect Probe PCR Kit (Qiagen, Chatsworth, USA) and this consisted of 50 ng of genomic DNA, 100 nM of each primer (Supplementary Table 1) and 1× Evagreen solution (Biotium, Hayward, USA). The PCR cycling and HRM analysis were carried out using the Rotor-Gene thermal cycler RG6000 (Corbett Research, Sydney, Australia). The PCR cycling conditions were as follows; one cycle of 95°C for 15 minutes, 45 cycles of 95°C for 5 seconds (g.-43T>C) or 15 seconds (g.19646A>G and g.21995T>C), 58°C (g.21995T>C) or 60°C (g.-43T>C and g.19646A>G) for 10 seconds and 72°C for 15 seconds (g.-43T>C) or 30 seconds (g.19646A>G and g.21995T>C). The optical measurements in the green channel (excitation at 470 nm and detection at 519 nm) were recorded during the extension step.

The melting-curve data was generated by the rising the temperature from 75°C to 90°C (g.-43T>C) or from 75°C to 86°C (g.19646A>G) or from 77°C to 95°C (g.21995T>C) at 0.1°C per second. The HRM curve analysis was performed using the software Rotor-Gene 1.7.40 and the HRM algorithm that was provided.

SBE

The SBE method was used for genetic analysis of g.21884G>A in the *TSLPR*. PCR was performed with 50 ng each of genomic DNA and Taq DNA polymerase (EF Taq, Solgent, Korea) and 0.5 μM of each primer under the following conditions: 30 cycles of denaturation at 98°C for 10 seconds, annealing at 55°C for 10 seconds and extension at 72°C for 30 seconds. The final extension was completed at 72°C for 10 minutes in a thermocycler (PE Applied Biosystem, USA). The PCR products that were purified by a PCR purification kit (Millipore, USA) were used as the template DNA for the SBE primers (Supplementary Table 1). The SBE reaction mix was prepared according to the previously described method (21).

Genotype analysis by Taq-Man probe

The Taq-man assay probes for g.33G>C and g.21869T>C were designed by Integrated DNA Technologies (IDT, USA) and Operon Biotechnology (USA). FAM-fluorescence dye was attached to the TSLPR-1G, TSLPR-2T probe and the HEX-fluorescence dye was attached to the TSLPR-1C, TSLPR-2C probe (Supplementary Table 1). The PCR conditions were as follows: one cycle at 95°C for 15 minutes, 50 cycles at 95°C for 15 seconds and 64°C for 40 seconds. The PCR was performed in the Rotor-Gene thermal cycler RG6000 (Corbett Research, Sydney, Australia). The samples were read and analyzed using the software Rotor-Gene 1.7.40 (Corbett Research, Sydney, Australia).

Statistic analysis

The asthma patients and controls were compared using case-control association analysis. χ^2 tests were employed to estimate the HWE. Pair-wise comparison of the biallelic loci was employed for the analyses of LD. The haplotype frequencies of *TSLPR* for multiple loci were estimated using the expectation maximization (EM) algorithm with SNPAlyze software (DYNACOM, Yokohama, Japan). Logistic regression analyses (SPSS 11.5) were used to calculate the odds ratios (with the 95% confidence intervals). The ANOVA method was applied to define the IgE levels and the peripheral blood eosinophils counts of each genotype from the individual asthma patients. A P-value of less than 0.05 was considered to indicate statistical significance.

Acknowledgements

This research was supported by Wonkwang University in 2008.

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