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Sequence variants of the secreted phosphoprotein 1 gene are associated with total serum immunoglobulin E levels in a Japanese population

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Summary

Background Secreted phosphoprotein 1 (SPP1) is a cytokine with pleiotrophic immunological activities, including activation of macrophage chemotaxis and T-helper type 1 (Th1) immune responses. SPP1 gene polymorphisms have been shown to be associated with several immune inflammatory diseases including multiple sclerosis (MS), which is characterized by fewer allergic symptoms and lower numbers of allergen sensitizations.

Objective The present study examined whether SPP1 gene polymorphisms are associated with total serum IgE levels, atopy and asthma in a Japanese population.

Methods This case-control association analysis examined 611 subjects, including 268 subjects with asthma. We genotyped three promoter and two exon polymorphisms at SPP1: –1687A/G; –381T/C; –94 deletion/G; 5891C/T; and 7052T/C.

Results Association analyses of SPP1 polymorphisms showed that homozygosities for the 5891T allele ($P=0.009$) and 7052C allele ($P=0.001$) were significantly associated with increased levels of total IgE in non-asthmatic subjects. However, these variants were not associated with asthma and atopy. Interestingly, individuals carrying the 5891C allele, which is more prevalent in patients with MS in Japanese populations, displayed significantly lower levels of total serum IgE. Individuals homozygous for the 7052C allele, which is associated with development of systemic lupus erythematosus, displayed significantly higher total serum IgE levels.

Conclusion These findings suggest that genetic polymorphisms in SPP1 may play a role in controlling basal levels of total serum IgE, independent of atopy.

Keywords asthma, atopy, polymorphism, SPP1, total serum IgE

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Introduction

Recent findings from linkage studies for complex diseases in both rodent models and human genetic studies have suggested the probable co-localization of susceptibility genes for several immune inflammatory diseases, including allergic diseases [1, 2]. Various complex immune inflammatory diseases may be affected by several susceptibility alleles, either individually or in combination. Alternatively, the same susceptibility alleles may influence various immune inflammatory diseases. Genes involved in a more generalized pathway of the immune response or inflammatory network may thus ultimately play central roles in the overall pathophysiological factors

of allergic disease, whereas other genes may be critical to specific clinical phenotypes.

Secreted phosphoprotein 1 (SPP1, also known as osteopontin, early T lymphocyte activation protein 1 or bone sialoprotein I) is a phosphorylated glycoprotein secreted by activated macrophages, leucocytes and activated T lymphocytes [3]. SPP1 may be involved in the pathophysiology of several immune-mediated inflammatory diseases with pleiotrophic immunological activities, including activation of macrophage chemotaxis, T-helper type 1 (Th1) immune responses at sites of inflammation and activation of B cells [3–5]. In addition, an *in vitro* study suggested that high SPP1 levels may be involved in apoptosis defects [6]. Konno *et al.* [7, 8] recently identified

SPP1 as a promising candidate biomarker associated with successful venom immunotherapy, suggesting that the known Th1-inducing activity of SPP1 may potentially play a role in modulating allergic responses.

The human SPP1 gene is located on chromosome 4q22.1 (<http://www.ncbi.nlm.nih.gov/>), and single-nucleotide polymorphisms (SNPs) of the gene are significantly associated with the development and/or disease activity of several inflammatory diseases, including multiple sclerosis (MS) [9, 10], systemic lupus erythematosus (SLE) [11] and type C hepatitis [12]. Haplotypes of SPP1 gene polymorphisms are also associated with higher production of SPP1 and the development of autoimmune lymphoproliferative disease (ALD) [6], although the functional consequences of these haplotypes remain unclear.

No studies appear to have investigated genetic associations of SPP1 polymorphisms with asthma, atopy or total serum IgE. The presumed biological function of SPP1 involves the regulation of Th1/Th2 balance and apoptosis. This and the recent finding that SPP1 polymorphisms are associated with several immune-mediated inflammatory diseases prompted the present examination of whether susceptibility alleles in the SPP1 gene exert influences on the development of asthma, atopy or increased levels of total serum IgE in a case-control study of 611 unrelated Japanese subjects.

Materials and methods

Study population

A total of 611 unrelated Japanese subjects were enrolled, mostly from the Sapporo area in Hokkaido, Japan, including patients with bronchial asthma ($n = 268$) recruited from the pulmonary clinic in the First Department of Medicine at Hokkaido University Hospital. Patients with asthma had experienced recurrent episodes of ≥ 2 of 3 symptoms (cough, wheeze and dyspnoea) associated with demonstrable reversible airflow limitation (15% variability in forced expiratory volume in 1 s (FEV₁) or peak expiratory flow rate, either spontaneously or with inhaled short-acting β_2 -agonist), increased airway responsiveness to methacholine or both, as described previously [13]. Healthy controls ($n = 343$) who had requested annual physical examinations, were included in the study only if they had no history of chronic pulmonary diseases, including bronchial asthma.

Total serum IgE levels and specific IgE responses were determined for 10 commonly inhaled allergens, including *Dermatophagoides farinae*, grass pollens, animal dander and moulds. Atopy was defined as a positive response to ≥ 1 of these 10 allergens. An increase in specific IgE antibody levels (IgE CAP RAST > 0.35 UA/mL, or Mast > 1.0 lumicount) was considered indicative of a positive response [14].

All participants were Japanese, and each provided written informed consent for enrollment in the study and all associated procedures. Study protocols were approved by the Ethics Committee of the School of Medicine at Hokkaido University.

Identification of polymorphisms

Genotypes of subjects were determined for three promoter and two exon polymorphisms in SPP1: –1687A/G (rs2728127, JST171775); –381T/C; –94deletion/G (JST171776); 5891C/T (rs4754); and 7052T/C (rs1126616). Nucleotide numbering starts from the first nucleotide of the transcription start site. In Japanese, 17 SNPs have been described in the SPP1 gene, including four promoter, three exon and 10 intron SNPs [9, 15]. As none of these SNPs have been shown to be functional, we selected five common SNPs based on location (three in the promoter region, two in the coding region), and also because these five SNPs have previously been associated with several immune-mediated diseases such as hepatitis C virus, MS and SLE [9, 11, 12].

Alleles were identified using an assay combining kinetic (real-time quantitative) polymerase chain reaction (PCR) with allele-specific amplification, as described elsewhere [16]. Primers were designed using Primer Express software (PE Applied Biosystems, Foster City, CA, USA) to specifically amplify each SNP allele in separate PCRs. PCR products were detected using an ABI 7700 Sequencer (PE Applied Biosystems). Primer sequences for each polymorphism are shown in Table 1. Real-time PCR was conducted using Syber Green I Master Mix (PE Applied Biosystems) and an ABI PRISMTM 7700 Sequence Detection System (PE Applied Biosystems). In addition, 24 samples were directly sequenced to confirm the accuracy of genotyping using allele-specific PCR.

Statistical analysis

Statistical analyses were performed using SYSTAT software (SPSS, Chicago, IL, USA). A χ^2 goodness-of-fit test was used to assess deviation from Hardy-Weinberg equilibrium for genotype and allele frequencies at each locus. A χ^2 test was also used to compare qualitative risk factors (sex, smoking status and atopic status) among four groups (asthmatics (atopic and non-atopic) and non-asthmatic controls (atopic and non-atopic)), and to compare genotype frequencies between asthmatics and controls or between atopic subjects and non-atopic subjects for each SPP1 polymorphism. The statistical power of the current study was calculated for asthma using the Genetic Power Calculator (<http://statgen.iop.kcl.ac.uk/gpc/>) [17]. One-way analysis of variance was used to compare quantitative risk factors (age and serum IgE levels) among these four groups.

Table 1. Primers for genotyping

Polymorphism*	Methods	Primer	Sequence 5'–3'
– 1687A/G	Direct sequence	S F	tggcattccaagaaatggag
		S R	ggtttcatgaacatttgtaggg
	Allele-specific PCR	P F1	ggaaattttgtgttttagaatttcA
		P F2	ggaaattttgtgttttagaatttcG
– 381T/C	Direct sequence	P R	gcataagcgtcatgttgca
		S F	acaggcaagagtgggtgcag
	Allele-specific PCR	S R	cagctgaatgcacaaccag
		P F1	ttgtgtgtgtgcgttttgGt
– 94 deletion/G	Direct sequence	P F2	ttgtgtgtgtgcgttttgTt
		P R	cccctctggttttgggttaaa
	Allele-specific PCR	S F	gggaacaaggataggtaggc
		S R	ggcattcagcatccagggaag
5891C/T	Direct sequence	P F1	ttgagtagtaaggacagaggcaagtT
		P F2	ttgagtagtaaggacagaggcaagtC
	Allele-specific PCR	P R	tgttcaagcctgcaaggagtt
		S F	atttcccggccatctaat
7052T/C	Direct sequence	S R	aggtccgtgggaaatcagt
		P F	aagccaacgaaagccatga
	Allele-specific PCR	P R1	ctggctgtccacatggtcA
		P R2	ctggctgtccacatggtcG
7052T/C	Direct sequence	S F	ccgtgggaaggacagttatg
		S R	ttaattgacctcagaagatgcac
	Allele-specific PCR	P F	cagtccagattatataagcggaagcT
		P R1	cagtccagattatataagcggaagcC
		P R2	ctacaaccagcatatcttcatggc

*Nucleotide numbering starts from the first nucleotide of the transcription start site.

SF, forward primer for direct sequence; SR, reverse primer for direct sequence; PF, forward primer for allele-specific PCR; PR, reverse primer for allele-specific PCR; PCR, polymerase chain reaction.

Total serum IgE levels were log-transformed (log10) to approximate a normal distribution. Total serum IgE levels were correlated with genotypes of the SPP1 polymorphisms (separate comparisons for patients with asthma and control subjects) using analysis of variance models including sex, age, smoking status and/or the presence of atopy as possible confounding variables.

Using genotype data of 611 unrelated Japanese individuals, pair-wise linkage disequilibrium (LD) was calculated between each polymorphism by estimating the coefficient of LD (D'), as described elsewhere [18]. In non-asthmatic controls, statistical associations between SPP1 gene haplotypes and total serum IgE levels were also tested using Haplo.score software that adjusts for covariates and calculates simulation P -values for each haplotype [19].

Results

The characteristics of patients with asthma and controls are summarized in Table 2. The mean age was highest for subjects with non-atopic asthma, and females predominated in this group. Atopic individuals and patients with atopic asthma displayed higher total serum IgE levels than

non-atopic counterparts. Table 3 shows genotype frequencies in SPP1 gene polymorphisms for all 611 subjects. Genotype distributions for each polymorphism were in Hardy–Weinberg equilibrium for all subjects. Neither asthma nor atopy was associated with any of the SPP1 polymorphisms. The current study with 611 subjects (268 asthmatics and 343 controls) would provide > 80% power to detect an effect of the 7052T/C polymorphism on asthma at $P = 0.05$, with a relative risk of 1.7 for the CC genotype. Our study may thus have failed to detect smaller genetic contributions of SPP1 polymorphisms to asthma and atopy.

In non-asthmatic subjects, 5891C/T and 7052T/C polymorphisms were significantly associated with levels of total serum IgE (Table 4). Individuals homozygous for the 5891T allele ($P = 0.009$) or the 7052C allele ($P = 0.001$) had higher levels of total IgE. The – 1687A/G, – 381T/C and – 94 deletion/G polymorphisms displayed no association with total serum IgE levels. Associations between these SPP1 polymorphisms and total serum IgE levels remained significant even after adjusting for the presence of atopy ($P = 0.012$ for 5891C/T; $P = 0.002$ for 7052 T/C).

Analyses of D' indicated a strong LD between each of the five polymorphisms in the SPP1 gene (Table 5). In

Table 2. Characteristics of the study population

Characteristics	Healthy controls		Patients with asthma		<i>P</i> value*
	Non-atopic	Atopic	Non-atopic	Atopic	
No. of subjects	148	195	71	197	
Sex (M/F)	85/63	136/59	22/49	102/95	< 0.0001*
Age (year, mean (SD))	40.6 (13.4)	33.5 (13.29)	55.2 (10.9)	40.1 (16.5)	< 0.0001*
Smoking (never/ex/current)	101/4/43	140/8/47	47/12/12	110/41/46	< 0.0001*
Log (total IgE) (IU/mL)	1.552 (0.577)	2.178 (0.539)	1.902 (0.487)	2.622 (0.555)	< 0.0001*

* χ^2 or one-way analysis of variance was used when appropriate.

Table 3. Genotype frequencies of polymorphisms in the secreted phosphoprotein 1 gene

OPN polymorphisms		Healthy controls (n, %)		Patients with asthma (n, %)		<i>P</i> value*	
		Non-atopic	Atopic	Non-atopic	Atopic	Between atopics and non-atopics	Between asthmatics and non-asthmatics
– 1687 (promoter)	A/A	78 (52.7)	120 (61.5)	42 (59.2)	112 (56.9)	0.5	0.54
	A/G	56 (37.8)	62 (31.8)	26 (36.6)	73 (37.0)		
	G/G	14 (9.5)	13 (6.7)	3 (4.2)	12 (6.1)		
– 381 (promoter)	T/T	54 (36.5)	63 (32.3)	18 (25.4)	67 (34.0)	0.48	0.83
	T/C	69 (46.6)	88 (45.1)	39 (54.9)	97 (49.2)		
	C/C	25 (16.9)	44 (22.6)	14 (19.7)	33 (16.8)		
– 94 (promoter)	Deletion/deletion	89 (60.1)	123 (63.1)	51 (71.8)	119 (60.4)	0.88	0.61
	Deletion/G	49 (33.1)	60 (30.8)	15 (21.1)	68 (34.5)		
	G/G	10 (6.8)	12 (6.1)	5 (7.0)	10 (5.1)		
5891 (exon 6)	C/C	55 (37.1)	83 (42.6)	32 (45.1)	82 (41.6)	0.85	0.8
	C/T	71 (48.0)	85 (43.6)	28 (39.4)	89 (45.2)		
	T/T	22 (14.9)	27 (13.8)	11 (15.5)	26 (13.2)		
7052 (exon 7)	T/T	75 (50.7)	89 (45.6)	32 (45.1)	82 (41.6)	0.41	0.42
	T/C	56 (37.8)	77 (39.5)	28 (39.4)	84 (42.6)		
	C/C	17 (11.5)	29 (14.9)	11 (15.5)	31 (15.7)		

*Calculated through use of χ^2 test (2 × 3).

haplotype analysis of total serum IgE, a common haplotype comprising the – 1687A/– 381T/– 94 deletion/5891T/7052C showed the highest haplotype-specific score of 1.64, although this result was not statistically significant (haplotype frequency was 0.21, $P=0.088$) (Table 6).

We initially conducted 20 statistical comparisons (five markers × four phenotypes (asthma, atopy, serum IgE levels in both controls and patients with asthma)). Although these statistical tests were not independent because of a strong LD between the five polymorphisms, an association between total serum IgE levels and the 7052T/C polymorphism in control subjects remained significant at the level of $P < 0.05$ even after correction for multiple comparisons by multiplying significance levels by 20.

Discussion

Overlapping susceptibility loci among different immune-mediated diseases have been reported [1, 2], and the hypothesis has been put forward that clinically distinct immune-mediated inflammatory diseases are controlled

by a common set of susceptibility alleles. The current study therefore focused on the genetic associations of asthma and asthma-related phenotypes with SPP1 gene polymorphisms that have been linked to several inflammatory immune diseases such as MS [9, 10], SLE [11], type C hepatitis [12] and ALD [6]. The failure to find significant associations between asthma and atopy and these SPP1 gene polymorphisms suggests that, at least in this study population, common allelic variations in the SPP1 gene do not contribute significantly to asthma or atopy.

Two polymorphisms of the SPP1 gene, however, were associated with total serum IgE levels in non-asthmatic subjects. Individuals carrying the C allele at position 5891 in exon 6, which is more prevalent in patients with MS in Japanese populations [9], displayed significantly lower levels of total serum IgE compared with non-carriers of this allele. Individuals homozygous for the C allele at position 7052 in exon 7, which is associated with development of SLE [11], exhibited significantly higher total serum IgE levels compared with carriers of the 7052T allele. Interestingly, patients with MS, a Th1 immune

Table 4. Association of total serum IgE levels with secreted phosphoprotein 1 polymorphisms

Polymorphism	Genotype	Healthy controls	<i>P</i> value*	Patients with asthma	<i>P</i> value*
- 1687	A/A	1.916 (0.045)	0.95	2.415 (0.047)	0.63
	A/G	1.900 (0.058)		2.436 (0.059)	
	G/G	1.883 (0.121)		2.569 (0.154)	
- 381	T/T	1.907 (0.058)	0.99	2.459 (0.064)	0.87
	T/C	1.905 (0.050)		2.422 (0.050)	
	C/C	1.916 (0.076)		2.414 (0.086)	
- 94	Deletion/deletion	1.924 (0.043)	0.82	2.421 (0.041)	0.92
	Deletion/G	1.878 (0.060)		2.444 (0.059)	
	G/G	1.897 (0.134)		2.472 (0.137)	
5891	C/C	1.955 (0.053)	0.009	2.407 (0.055)	0.74
	C/T	1.806 (0.050)		2.462 (0.054)	
	T/T	2.099 (0.089)		2.405 (0.097)	
7052	T/T	1.891 (0.048)	0.001	2.415 (0.055)	0.9
	T/C	1.820 (0.054)		2.450 (0.055)	
	C/C	2.222 (0.092)		2.424 (0.091)	

Mean log[total IgE] (SD) was shown for each genotype at 5 polymorphisms.

*Calculated through use of ANOVA adjusted for sex, age and smoking status.

Table 5. Pairwise linkage disequilibrium coefficients among polymorphisms in secreted phosphoprotein 1SPP1 gene

Polymorphism	- 1687	- 381	- 94	5891	7052
- 1687	1	0.71	0.86	0.68	0.52
- 381		1	0.93	0.7	0.59
- 94			1	0.88	0.7
5891				1	0.82
7052					1

Pairwise linkage disequilibrium (LD) between each polymorphism was calculated by estimating the coefficient of LD.

disease, display significantly fewer allergic symptoms, a lower number of allergen sensitizations [20] and reduced risk of asthma [21] compared with individuals without MS. Conversely, SLE is characterized by Th2 immune responses, and some reports have shown increased IgE production in SLE and have associated SLE with allergy [22]. Certain SPP1 polymorphisms may thus up-regulate some immune states and down-regulate others, providing genetic mechanisms underlying epidemiological associations between immune-mediated inflammatory diseases including MS and SLE, and allergic phenotypes including elevated levels of total serum IgE.

At least two different genetic mechanisms controlling the IgE regulation have been suggested: one involved in specific IgE responses, and the other in non-specific basal responses. Genetic regulation of basal IgE production has been suggested to be independent of specific responses to allergens [23]. In the current study, atopy defined by the presence of antigen-specific IgE responsiveness was not associated with SPP1 gene polymorphisms. Furthermore, significant associations between total serum IgE levels and SPP1 gene polymorphisms were evident only in healthy individuals, and these associations were indepen-

dent of the presence of atopy. Accordingly, SPP1 gene polymorphisms may play some role in regulating basal levels of total serum IgE rather than in regulating antigen-specific IgE responses. Genetic effects of SPP1 gene polymorphisms on levels of total serum IgE were not evident in patients with asthma. Development of asthma is determined by interactions between several genetic factors and various environmental exposures [24, 25]. The genetic effects of SPP1 polymorphisms may thus be overcome or masked by the effects of several additional genetic and environmental factors related to asthma that exert much stronger influences on levels of total serum IgE, such as genes encoding the β -chain of the high-affinity receptor for IgE [26], G protein-coupled receptor for asthma susceptibility [27] and plant homeodomain finger protein 11 [28].

Neither the 5891C/T nor the 7052T/C polymorphism involves any alteration in amino acid sequence. The National Center for Biotechnology Information dbSNP database contains an SNP that causes an amino acid exchange at position 7204 (rs4660) in exon 7, but no variations were found at this locus in our population after direct sequencing of 50 subjects (100 chromosomes). In addition, five polymorphisms fell within an extensive block of LD across the SPP1 gene, and no single haplotype was strongly associated with total serum IgE levels. These findings raise the possibility that an unidentified true susceptibility allele within or near the SPP1 gene is responsible for increased levels of total serum IgE, or alternatively, that complex interactions among different SPP1 gene haplotypes may provide an overall risk factor for increased levels of total serum IgE. These possibilities may also underlie the finding that heterozygotes at the 7052C/T or 5891C/T polymorphisms displayed the lowest levels of total serum IgE in the current study. Further

Table 6. Association analysis of haplotypes in secreted phosphoprotein 1 gene with total serum IgE levels

– 1687	– 381	– 94	5891	7052	Haplotype frequency	Haplotype score	Empirical <i>P</i> value
A	T	Deletion	T	T	0.07	– 1.57	0.11
G	T	G	C	T	0.177	– 1.21	0.24
A	C	Deletion	T	C	0.034	– 0.85	0.38
A	C	Deletion	T	T	0.024	– 0.62	0.54
A	C	Deletion	C	T	0.305	– 0.6	0.53
A	T	G	T	C	0.01	0.1	0.92
A	T	Deletion	C	T	0.057	0.5	0.65
G	T	G	C	C	0.019	0.68	0.52
A	C	Deletion	C	C	0.036	0.83	0.43
G	T	Deletion	T	C	0.015	1	0.31
G	C	Deletion	C	T	0.02	1.28	0.19
A	T	Deletion	T	C	0.21	1.64	0.088

studies are needed to elucidate the functional consequences of SPP1 gene variants.

In conclusion, as SPP1 is classified as a Th1 cytokine, up-regulating IFN- α and IL-12 production, the identification of significant associations between total serum IgE and SPP1 gene polymorphisms in non-asthmatic individuals suggests that the SPP1 gene may represent a genetic factor that plays a role in basal IgE production in a non-antigen-specific (non-cognate) fashion.

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