Respiratory Medicine (2008) **102**, 1132–1139

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Association of three sets of high-affinity IgE receptor (FcepsilonR1) polymorphisms with aspirin-intolerant asthma☆

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Received 30 July 2007; accepted 14 March 2008
Available online 1 July 2008

KEYWORDS
Aspirin-intolerant asthma;
FCER1A;
FCER1G;
MS4A2;
Serum total IgE;
Specific IgE to *Staphylococcal* superantigen

Summary
Background and objective: The high-affinity IgE receptor comprises a tetramer of the ligand-binding alpha chain, a signal-augmenting beta chain, and a signal-transducing gamma chain dimer on mast cells. We hypothesized that the three subsets of the *FCER1* gene may play a role in the development of the aspirin-intolerant asthma (AIA) phenotype and analyzed these genetic polymorphisms in association with clinical parameters in AIA patients.
Subjects and methods: Six polymorphisms of *FCER1* (*FCERIA*-344C>T, *FCER1A*-95T>C, *MS4A2*-109T>C, *MS4A2* E237G, *FCER1G*-237A>G, *FCER1G*-54G>T) were genotyped in 126 AIA patients compared to 177 patients with aspirin-tolerant asthma (ATA) and 222 normal health controls (NC).
Results: A significant difference in the genotype frequencies of *FCER1G*-237A>G was detected between AIA and ATA patients ($p < 0.05$) both in co-dominant and recessive analysis models, whereas no significant relationships were identified between the frequencies of the other five single-nucleotide polymorphisms and AIA, ATA, and NC subjects. In addition, AIA patients carrying the homozygous AA genotype of *FCER1G*-237A>G exhibited significantly higher total serum IgE levels than did those with the GG/AG genotype ($p = 0.012$). AIA patients expressing the CT/TT genotype at *FCERIA*-344C>T showed a higher prevalence of serum IgE specific to *Staphylococcal* enterotoxin A than did those with the CC genotype ($p = 0.008$).
Conclusion: The *FCER1G*-237A>G and *FCERIA*-344C>T polymorphisms may contribute to the development of AIA in a Korean population.
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☆ This study was supported by the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (03-PJ10-PhoG13-GD01-0002).
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doi:10.1016/j.rmed.2008.03.017

Introduction

Aspirin-intolerant asthma (AIA) is a clinically distinct syndrome characterized by the onset of asthma attacks following the ingestion of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Symptoms of AIA include aspirin sensitivity, asthma, and rhino sinusitis. AIA is observed in 5–15% of adult asthmatic patients and is most commonly observed in middle-aged females whose clinical symptoms are more severe than those of aspirin-insensitive asthma patients.[1–3]

IgE is thought to play a key role in the pathogenesis of asthma and other allergic diseases because elevated serum IgE levels have been closely associated with atopy and allergic asthma.[4] AIA patients, however, have been shown to exhibit increased levels of serum total and specific IgE to *Staphylococcal* superantigens independent of atopic status. AIA patients having high serum specific IgE antibody also showed increased airway hyper responsiveness to methacholine.[5] The other study showed that IgE antibodies specific for *Staphylococcal* enterotoxin A (SEA) and *Staphylococcal* enterotoxin B (SEB) were present in approximately one half of polyp tissues from patients with nasal polyposis and the presence of enterotoxin-specific IgE correlated with the presence of polyclonal IgE antibodies specific for inhaled allergens. Furthermore, SEA and SEB positive samples were found to have eosinophils, IL-5, eotaxin and cys-leukotrienes and they were more likely to be associated with asthma or aspirin sensitivity.[6] We have also reported that the levels of SEA and SEB specific IgE antibodies in nasal polyps were higher in AIA than those with ATA and the levels of enterotoxin-specific IgE correlated with the degree of eosinophilic inflammation.[7] During the IgE-mediated response, IgE binds with a high-affinity IgE receptor, which consists of a tetramer of a ligand-binding α chain, a signal-augmenting β, and a signal-transducing γ chain dimer. The receptor is abundantly expressed on the surface of mast cells and basophils and is thought to be involved in allergic inflammation of the asthmatic airway.[8]

The *FCER1G* gene is located on chromosome 1q23 in humans. *FcεR1γ* plays an essential role in the induction of mast cell degranulation and survival.[9–11] To date, the only study suggesting an association of *FcεR1γ* with asthma and allergic diseases reported a statistically insignificant association of an *FCER1G* gene polymorphism with systemic lupus erythematosus.[12] The *FCER1A* gene is located on chromosome 1q23 and known as the first IgE-binding site to induce mast cell activation. This initiates receptor stabilization, which is critical to the IgE-mediated allergic response.[13] Although some studies have suggested a possible role of this genetic polymorphism in allergic asthma and atopic dermatitis,[14,15] no published data support any association with AIA patients.

The *MS4A2* gene, the β chain of the high-affinity receptor for immunoglobulin (IgE), is located on chromosome 11q13. The β chain of *FcεR1* is an amplifier for cell surface expression in association with the alpha chain, as well as an enhancer for signal-transduction via an immunoreceptor tyrosine activation motif (ITAM) of the *FcεRI* gamma chain.[16] The *MS4A2* gene polymorphism is associated with atopy, asthma, and serum total IgE in asthmatic patients.[17,18] One study reported an association of *MS4A2* gene polymorphism with the prevalence of serum IgE specific to *Staphylococcal* superantigens in AIA patients.[19]

We examined whether genetic polymorphisms of *FCER1* in mast cells play an important role in AIA pathogenesis by analyzing the genotypes and haplotypes of three subsets of *FCER1* genes in association with various clinical parameters. We also investigated the possible genetic/environmental component of AIA development by examining the prevalence of serum IgE specific for three *Staphylococcal* superantigens: SEA, SEB, and toxic shock syndrome toxin 1 (TSST-1).

Materials and methods

Study subjects

The three study groups of 126 patients with AIA, 177 patients exhibiting aspirin-tolerant asthma (ATA), and 222 normal health controls (NC) were recruited from Ajou University Hospital, Suwon, Korea. The diagnosis of AIA was based on a positive response to a lysine-acetyl salicylic acid (ASA) bronchoprovocative test, which was performed on all study subjects according to a previously described method.[20] Change of forced expiratory volume (FEV_1) in 1 s was followed up for 5 h following the final dose of the aspirin challenge. The ASA-induced change (%) in FEV_1 was calculated as the percentage of post-challenge FEV_1 relative to pre-challenge FEV_1.

Methacholine bronchial challenge tests were performed as described previously.[21] NC individuals were chosen from the general population using a screening questionnaire in which they indicated no history of respiratory symptoms or aspirin hypersensitivity. All NC subjects also exhibited an FEV_1 greater than 80% of predicted, provocation concentration (PC20) methacholine greater than 25 mg/ml, and normal findings on simple chest radiograms. Atopy was defined as one or more positive reactions in a skin prick test using 12 common aeroallergens (Bencard, Brendford, UK), with histamine and saline controls. To measure specific IgE, venous blood was collected from the antecubital vein, allowed to clot for 1–3 h at 4 °C and centrifuged at 1500 × *g* for 10 min at 4 °C. The serum was aspirated, separated and stored in aliquots at −20 °C until analysis. Serum total IgE, SEA, SEB, and TSST-1 were measured using the UniCAP system (Pharmacia Diagnostics, Valinge, Sweden), according to the manufacturer's instructions. The threshold cut off value for specific IgE level is 0.35 KU/L as measured by UniCAP.

The presence of rhinosinusitis and nasal polyps was evaluated using a paranasal sinus X-ray and rhinoscopy. Informed consent was obtained from all subjects, and the institutional review board of Ajou University Hospital (Suwon, Korea) approved the study.

SNP identification and genotyping

The single-nucleotide polymorphisms (SNPs) *FCERIA*-344C>T, *FCERIA*-95T>C, *MS4A2*-109T>C, and *MS4A2* E237G were described previously for a Korean

Table 1 Amplifying and extension primers of the candidate genes for SNP genotyping

Gene	SNP	Position		Primers
FCER1A	-344C>T	Promoter	Forward	tggcatatgtttggtattcagt
			Reverse	aatctgtcaatctgtgtacaactatttag
			Extension	cttagaaaagtgggatgcaagggag
	-95T>C	Promoter	Forward	agaaagaagcaaaaccaggc
			Reverse	aatataggcttaaaccaaaaagca
			Extension	caaaaagcagxaggaaatgttttctgt
MS4A2	-109T>C	Promoter	Forward	aaaattatgctccaggagtctca
			Reverse	ataagtttcttggctgattaagatca
			Extension	tttacttgtgatgaatagaaaaatt
	E237G	Exon	Forward	agaggatcgtgtttatgaagaattaaac
			Reverse	gaatcagagtgttctggacacgt
			Extension	cttacagtgagttggaagacxcagggg
FCER1G	-237A>G	Promoter	Forward	gtggagtggaaaatggca
			Reverse	actgctggaatcatcttgg
			Extension	tgtagacagcctttcctgagcgtga
	-54G>T	Promoter	Forward	aaagcatgggggaaggcg
			Reverse	actgctggaatcatcttgg
			Extension	aggggactctgtggtcagggaact

population.[19,24] Briefly, genomic DNA was obtained from 40 healthy Korean volunteers. Promoter regions were sequenced to examine SNPs by using the ABI Prism 3100 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing analysis was based on GenBank sequences. Sequence variants were verified using chromatograms.

SNPs in the promoter region of *FCERIG* were screened by SNP-IT™ assays. Information regarding SNPs in the promoter region of FcεRIG was obtained from the NCBI SNP database and two SNPs were selected.

SNP genotyping was performed using SNP-IT™ assays with the SNPstream 25K™ system (Orchid Biosciences, Princeton, NJ, USA). The region of genomic DNA spanning the polymorphic site was amplified using PCR with one phosphothiolated primer and one regular PCR primer. The sequences of the amplifying and extension primers for *FCERIA*-344C>T (rs 2427827), *FCERIA*-95T>C (rs 2251746), *MS4A2*-109T>C (rs 1441586) *MS4A2* E237G (rs 569108), *FCERIG*-237A>G (rs 11587213), and *FCERIG*-54G>T (rs 2070901) were shown (Table 1). BIONEER (http://www.bioneer.com) programme was used for primer composition and further verified by NCBI BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Statistical analysis

Significant departures of genotype frequency from Hardy–Weinberg equilibrium at each polymorphic site were tested using chi-square analyses. Haplotypes of the FcεRI gene were analyzed using Haploview v2.0 based on the

Table 2 Clinical characteristics of the study subjects

	AIA ($N = 126$)	ATA ($N = 177$)	NC ($N = 222$)	p-value		
				AIA vs. ATA	AIA vs. NC	ATA vs. NC
Age (year)[a]	44.65 ± 12.95	39.95 ± 13.89	35.83 ± 15.05	**0.003**	**<0.001**	**<0.001**
Sex (male/total)	48/126 (38.1%)	98/177 (44.6%)	102/222 (45.9%)	0.288	0.177	0.840
Atopy (presence/total)	66/119 (55.7%)	118/172 (68.6%)	28/189 (14.8%)	**0.026**	**<0.001**	**<0.001**
FEV_1 (%)[a]	78.09 ± 25.75	87.73 ± 22.48	NA	**0.001**	NA	NA
Fall_FEV_1[a]	23.48 ± 10.07	7.15 ± 3.83	NA	**0.001**	NA	NA
Log [PC20 methacholine (mg/mL)][a]	0.06 ± 0.66	0.48 ± 0.72	NA	**<0.001**	NA	NA
Serum total IgE (IU/mL)[a]	358.70 ± 436.59	423.96 ± 698.08	NA	0.379	NA	NA
Log [serum total IgE (IU/mL)][a]	2.24 ± 0.57	2.24 ± 0.66	1.71 ± 0.58	0.987	**<0.001**	**<0.001**
Rhinosinusitis (presence/total)	83/101 (82.2%)	130/171 (76.0%)	NA	0.287	NA	NA

[a] This value was presented as means ± SD. Values in bold indicate significant p value. AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NC, normal control; FEV_1%, forced expiratory volume in 1 s; IgE, immunoglobulin E. N = number of patients, NA = not applicable.

Table 3 Genotype and allele frequencies of *FCER1*

Loci	Genotype	AIA	ATA	NC	*p*-value		
					AIA vs. ATA	AIA vs. NC	ATA vs. NC
FCERIA-344C>T	CC	86/125 (68.80%)	113/177 (63.80%)	148/222 (66.70%)	0.296	0.904	0.390
	CT	34/125 (27.20%)	57/177 (32.20%)	70/222 (31.50%)	0.906	0.204	0.197
	TT	5/125 (4.00%)	7/177 (4.00%)	4/222 (1.80%)	0.231	0.557	0.607
	*q	0.176	0.201	0.176	0.287	0.905	0.401
	Total	125	177	222			
FCERIA-95T>C	TT	120/126 (95.20%)	164/177 (92.70%)	200/222 (90.10%)	0.468	0.109	0.319
	CT	6/126 (4.80%)	13/177 (7.30%)	22/222 (9.90%)	NA	NA	NA
	CC				0.468	0.109	0.319
	*q	0.024	0.037	0.050	0.475	0.117	0.330
	Total	126	177	222			
MS4A2-109T>C	TT	63/126 (50.00%)	91/177 (51.40%)	99/222 (44.60%)	0.901	0.517	0.243
	CT	56/126 (44.40%)	74/177 (41.80%)	104/222 (46.80%)	0.717	0.369	0.631
	CC	7/126 (5.60%)	12/177 (6.80%)	19/222 (8.60%)	0.742	0.739	0.226
	*q	0.278	0.277	0.320	0.905	0.534	0.258
	Total	126	177	222			
MS4A2 E237G	AA	96/126 (76.20%)	134/177 (75.70%)	151/222 (68.00%)	0.926	0.252	0.093
	AG	27/126 (21.40%)	40/177 (22.60%)	65/222 (29.30%)	0.542	0.962	0.569
	GG	3/126 (2.40%)	3/177 (1.70%)	6/222 (2.70%)	0.926	0.187	0.088
	*q	0.131	0.130	0.173	0.925	0.252	0.094
	Total	126	177	222			
FCER1G-237A>G	AA	111/126 (88.10%)	136/177 (76.80%)	183/222 (82.40%)	**0.035**	0.114	0.213
	AG	15/126 (11.90%)	40/177 (22.60%)	35/222 (15.80%)	1.000	0.999	0.316
	GG	0/126 (0.00%)	1/177 (0.60%)	4/222 (1.80%)	**0.039**	0.184	0.097
	*q	0.060	0.119	0.097	**0.043**	0.102	0.210
	Total	126	177	222			
FCER1G-54G>T	GG	35/126 (27.80%)	39/168 (23.20%)	62/221 (28.10%)	0.689	0.721	0.701
	GT	54/126 (42.90%)	85/168 (50.60%)	101/221 (45.70%)	0.740	0.548	0.777
	TT	37/126 (29.40%)	44/168 (26.20%)	58/221 (26.20%)	0.315	0.998	0.361
	*q	0.508	0.515	0.491	0.681	0.706	0.695
	Total	126	168	221			

Values in bold indicate significant p value. *q = minor allele frequency.

expectation maximization (EM) algorithm.[22] Pairwise linkage disequilibrium (LD) between SNP loci was measured using the absolute value of Lewontin's D' and r^2.[23] Differences in the mean values of phenotypic characteristics among AIA patients were compared using analysis of t-tests. Statistical analyses were performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) with a significance level of $p < 0.05$.

Results

Clinical characteristics of the study subjects

The mean ages of both AIA and ATA patients were significantly higher than that of the NC group ($p < 0.001$; Table 2). The atopy rate was significantly lower for AIA patients compared to those with ATA ($p = 0.026$). The percent fall in forced expiratory volume (FEV_1) after the lysine aspirin inhalation was significantly lower in the AIA patients compared to the ATA patients ($p = 0.001$). The AIA patients also exhibited significantly lower baseline PC20 methacholine compared to the ATA patients ($p < 0.001$). Significant differences in serum total IgE were noted for both the AIA and ATA patients compared to the NCs; however, there was no significant difference in serum total IgE between AIA and ATA patients.

Allele, genotype, and haplotype frequencies of the FCER1 gene with the AIA phenotype

Genetic associations were examined among the six genetic polymorphisms of *FCERIA*-344C>T, *FCERI*A-95T>C, *MS4A2*-109T>C, *MS4A2* E237G, *FCERIG*-237A>G, and *FCERIG*-54G>T and the three study groups of AIA, ATA, and NC. The genotype distribution of six polymorphisms did not depart significantly from Hardy–Weinberg equilibrium ($p>0.05$). The genotypic frequency of *FCERIG*-237A>G was significantly different between AIA and ATA patients (Table 3). Specifically, AIA patients showed a significantly higher frequency of the homozygous AA genotype of *FCERIG*-237A>G compared to ATA patients ($p < 0.05$) both in co-dominant and recessive analysis models. However, there were no significant differences among the test groups with respect to the allele and genotype frequencies of the other *FCERI* SNPs, including *FCERIA*-344C>T, *FCERIA*-95T>C, *MS4A2*-109T>C, *MS4A2* E237G, and *FCERIG*-54G>T ($p>0.05$) (Table 3). Two polymorphisms in the *FCER1A* gene were not in linkage disequilibrium ($|D'| = 0.74$ and $r^2 = 0.01$). Two polymorphisms in the *MS4A2* and *FCER1G* gene were in linkage disequilibrium ($|D'| = 0.95$) and ($|D'| = 0.97$) respectively and three common haplotypes, ht1[TA], ht[CA], ht3[CG] and ht1[AT], ht[AG], ht3[GG] were constructed utilizing the EM algorithm. No significant differences in haplotypes frequencies were observed among the three groups ($p>0.05$) (data not shown). In addition, there were no significant associations between clinical parameters such as atopy, FEV_1, log PC20 methacholine, and rhinosinusitis and any polymorphism within the AIA patients.

FCERIG-237A > G and total serum IgE levels

Of the six target SNPs of the FcεRI gene, a significant association was detected between the *FCERIG*-237A>G polymorphism and total serum IgE level in AIA patients. In addition, AIA patients carrying the homozygous AA genotype of *FCERIG*-237A>G exhibited significantly higher levels of total serum IgE than did those with the AG/GG genotype ($p = 0.012$) (Table 4). This association was not found in ATA patients ($p>0.05$, data not shown). Furthermore, no significant associations were found between the total serum IgE level and the other five SNPs, including *FCERIG*-54G>T, *FCERIA*-344C>T, *FCERIA*-95T>C, *MS4A2*-109T>C, *MS4A2* E237G, and *FCERIG*-54G>T (Tables 4–6).

Table 4 Comparison of the clinical characteristics according to the genotype of *FCERIG* in AIA

	-237A > G		*p*-value	-54G > T		*p*-value
	AA	AG or GG		GG	GT or TT	
Sex (male/total)	43/111 (38.7%)	5/15 (33.3%)	0.783	13/35 (37.14%)	35/91 (38.46%)	1.000
Atopy (presence/total)	58/105 (55.2%)	8/14 (57.1%)	1.000	21/33 (63.64%)	45/86 (52.33%)	0.307
Asthma duration (year)[a]	6.24 ± 5.29/89	9.58 ± 9.7/12	0.070	7.69 ± 6.68/28	6.23 ± 5.73/73	0.279
FEV_1 (%)[a]	77.31 ± 25.39/108	83.75 ± 28.52/15	0.366	77.84 ± 28.36/35	78.2 ± 24.82/88	0.944
Fall_FEV_1[a]	23.33 ± 9.52/69	24.4 ± 13.56/11	0.747	25.02 ± 10.53/20	22.97 ± 9.95/60	0.433
Log [PC20 methacholine (mg/mL)][a]	0.02 ± 0.65/76	0.41 ± 0.68/11	0.066	0.25 ± 0.74/20	0.02 ± 0.64/67	0.179
Log [serum total IgE (IU/mL)][a]	2.29 ± 0.54/98	1.89 ± 0.68/14	**0.012**	2.21 ± 0.68/32	2.26 ± 0.52/80	0.705
Serum total IgE (IU/mL)[a]	384.10 ± 454.65/98	180.88 ± 214.99/14	**0.009**	436.05 ± 589.26/32	327.76 ± 358.00/80	0.338
Rhinosinustis (presence/total)	72/90 (80.0%)	11/11 (100.0%)	0.206	24/29 (82.76%)	59/72 (81.94%)	1.000
Specific IgE to SEA (positive/total)	7/36 (19.4%)	1/4 (25.0%)	1.000	2/12 (16.67%)	6/28 (21.43%)	1.000
Specific IgE to SEB (positive/total)	10/36 (27.8%)	1/4 (25.0%)	1.000	3/12 (25%)	8/28 (28.57%)	1.000
Specific IgE to TSST-1 (positive/total)	7/36 (19.4%)	1/4 (25.0%)	1.000	3/12 (25%)	5/28 (17.86%)	0.677

[a] This value was presented as means ± SD. Values in bold indicate significant *p* value. FEV_1%, forced expiratory volume in 1 s; IgE, immunoglobulin E; SEA, *Staphylococcal* enterotoxin A; SEB, *Staphylococcal* enterotoxin B; TSST-1, toxic shock syndrome toxin 1.

Association between the genetic polymorphism of FCERI with serum IgE specific for *Staphylococcal* superantigens

Asthma-related phenotypes such as atopy, total serum IgE level, FEV_1, and PC20 methacholine, as well as IgE specific for SEA, SEB, and TSST-1, were examined for associations with six polymorphisms of the FcεRI gene (Tables 4–6). Significant associations were noted between two SNPs, i.e., *FCERIA*-344C>T and *FCERIA*-95T>C, and the prevalence of serum IgE specific to SEA. Specifically, AIA patients carrying the CT/TT genotype at *FCERIA*-344C>T had a significantly higher prevalence of SEA-specific IgE than did those with the CC genotype ($p = 0.008$). In addition, AIA patients with the CT/CC genotype at *FCERIA*-95T>C had a higher prevalence of SEA-specific IgE than did those with the TT genotype ($p = 0.035$). The *FCERIA*-95T>C genetic polymorphism was also significantly associated with the prevalence of IgE specific for TSST-1, whereby patients carrying the CT/CC genotype exhibited a significantly higher prevalence than those with the homozygous TT genotype ($p = 0.036$; Table 5). However, no such associations were found in case of ATA patients ($p>0.05$; data not shown). AIA patients expressing the TT genotype of the *MS4A2*-109T>C genetic polymorphism showed a significantly higher prevalence of IgE specific for SEB than did those with CT/CC genotype ($p = 0.012$). There was no association between the *MS4A2* E237G polymorphism and IgE specific for SEA, SEB, or TSST-1 (Table 6).

Discussion

We examined six genetic polymorphisms of *FCER1A*, *MS4A2*, and *FCER1G* for associations with the AIA phenotype in a Korean population. The *FCER1G*-237A>G was significantly associated with the AIA phenotype, as well as total serum IgE levels, within AIA patients. FcεR1γ is essential for initiating the signal-transduction pathway necessary to induce cell degranulation, although its intensity is less than that of endogenous tetrameric FcεR1.[10] The promotion of mast cell survival by IgE without antigens is mediated by signals through the ITAM. Therefore, FcεR1γ-mediated signals differentially upregulate the receptor expression, activation, and survival of mast cells.[10] No study has examined the genetic association of this polymorphism with the pathogenic mechanism of AIA, however, recent data suggest that mRNA levels of FcεR1γ in peripheral blood mononuclear cells are upregulated by aspirin treatment.[25] Even though serum total IgE may be more important for asthma rather than AIA, our present statistical significant association of *FCER1G*-237A>G and serum total IgE in AIA group cannot be precluded. Therefore, functional role of this SNP might be beneficial for AIA pathogenesis. Given our results, we propose that the *FCER1*G-237A>G polymorphism may be a risk factor for AIA susceptibility.

We previously demonstrated a significant association of *FCER1A* with aspirin-intolerant chronic urticaria,[24] in which an *FCER1A* promoter polymorphism was associated with increased expression of FcεR1α on mast cells via the enhanced release of histamine. Similarly, the study also indicated that the *FCER1A*-344C>T is associated with total serum IgE levels in patients with allergic diseases.[26] However, we did not find any significant association between allelic frequencies of *FCER1A*-344C>T with the total serum IgE level within the AIA patients, but a significant association was detected between *FCER1A*-344C>T and the prevalence of SEA-specific serum IgE. Functional role of *FCER1A*-344C>T implicated that the reporter plasmid

Table 5 Comparison of the clinical characteristics according to the genotype of *FCERIA* in AIA

	-344C > T		*p*-value	-95T > C		*p*-value
	CC	CT or TT		TT	CT or CC	
Sex (male/total)	34/86 (39.53%)	14/39 (35.9%)	0.843	46/120 (38.33%)	2/6 (33.33%)	1.000
Atopy (presence/total)	44/81 (54.32%)	22/37 (59.46%)	0.691	65/115 (56.52%)	1/4 (25%)	0.322
Asthma duration (year)[a]	7.1 ± 6.61/72	5.54 ± 4.09/28	0.246	6.7 ± 6.12/97	5 ± 1.41/4	0.581
FEV_1 (%)[a]	78.96 ± 24.63/85	76.33 ± 28.73/37	0.607	79.06 ± 24.48/117	59.29 ± 42.81/6	0.311
Fall_FEV_1[a]	22.35 ± 8.99/52	25.76 ± 11.89/27	0.158	23.09 ± 9.62/78	38.85 ± 20.01/2	0.028
Log [PC20 methacholine (mg/mL)][a]	0.08 ± 0.74/58	0.04 ± 0.47/29	0.773	0.07 ± 0.66/82	0.11 ± 0.78/5	0.890
Log [serum total IgE (IU/mL)][a]	2.19 ± 0.6/77	2.38 ± 0.49/34	0.107	2.25 ± 0.57/108	2.07 ± 0.48/4	0.543
Serum total IgE (IU/mL)[a]	323.84 ± 381.46/77	443.76 ± 542.61/34	0.249	365.09 ± 442.07/108	186.20 ± 202.42	0.423
Rhinosinustis (presence/total)	59/71 (83.1%)	23/29 (79.31%)	0.775	81/98 (82.65%)	2/3 (66.67%)	0.449
Specific IgE to SEA (positive/total)	2/27 (7.41%)	6/13 (46.15%)	**0.008**	6/38 (15.79%)	2/2 (100%)	**0.035**
Specific IgE to SEB (positive/total)	6/27 (22.22%)	5/13 (38.46%)	0.451	10/38 (26.32%)	1/2 (50%)	0.479
Specific IgE to TSST-1 (positive/total)	5/27 (18.52%)	3/13 (23.08%)	1.000	6/38(15.79%)	2/2(100%)	**0.036**

[a] This value was presented as means ± SD. Values in bold indicate significant *p* value. FEV_1%, forced expiratory volume in 1 s; IgE, immunoglobulin E; SEA, *Staphylococcal* enterotoxin A; SEB, *Staphylococcal* enterotoxin B; TSST-1, toxic shock syndrome toxin 1.

Table 6 Comparison of the clinical characteristics according to the genotype of *MS4A2* in AIA

	-109T > C		*p*-value	E237G		*p*-value
	TT	CT or CC		AA	AG or GG	
Sex (male/total)	23/63 (36.51%)	25/63 (39.68%)	0.855	38/96 (39.58%)	10/30 (33.33%)	0.668
Atopy (presence/total)	33/59 (55.93%)	33/60 (55%)	1.000	47/91 (51.65%)	19/28 (67.86%)	0.192
Asthma duration (year)[a]	7.46 ± 6.42/52	5.76 ± 5.48/49	0.156	6.89 ± 5.99/78	5.79 ± 6.14/23	0.444
FEV_1 (%)[a]	77.3 ± 23.9/61	78.88 ± 27.63/62	0.734	76.48 ± 25.43/94	83.34 ± 26.56/29	0.211
Fall_FEV_1[a]	22.79 ± 8.97/39	24.13 ± 11.09/41	0.555	23.4 ± 9.85/62	23.77 ± 11.09/18	0.892
Log [PC20 methacholine (mg/mL)][a]	0 ± 0.71/43	0.13 ± 0.62/44	0.365	0.08 ± 0.68/68	0.04 ± 0.64/19	0.836
Log [serum total IgE (IU/mL)][a]	2.34 ± 0.58/56	2.15 ± 0.55/56	0.072	2.25 ± 0.57/86	2.21 ± 0.57/26	0.739
Serum total IgE (IU/mL)[a]	438.98 ± 508.52/56	278.42 ± 336.10/56	0.052	364.39 ± 448.37/86	339.89 ± 402.85/26	0.803
Rhinosinustis (presence/total)	43/50 (86%)	40/51 (78.43%)	0.437	66/79 (83.54%)	17/22 (77.27%)	0.534
Specific IgE to SEA (positive/total)	6/19 (31.58%)	2/21 (9.52%)	0.120	8/28 (28.57%)	0/12 (0%)	0.079
Specific IgE to SEB (positive/total)	9/19 (47.37%)	2/21 (9.52%)	0.012	10/28 (35.71%)	1/12 (8.33%)	0.124
Specific IgE to TSST-1 (positive/total)	6/19 (31.58%)	2/21 (9.52%)	0.120	7/28 (25%)	1/12 (8.33%)	0.396

[a] This value was presented as means ± SD. FEV_1%, forced expiratory volume in 1 s; IgE, immunoglobulin E; SEA, *Staphylococcal* enterotoxin A; SEB, *Staphylococcal* enterotoxin B; TSST-1, toxic shock syndrome toxin 1.

carrying -344T allele exhibited significantly higher promoter activity in a rat mast cell line (RBL-2H3) compared to -344C allele. The association of transcription factor Myc-associated zinc finger protein bound the -344C promoter and also showed that heterozygous CT genotype of the -344C>T exhibited greater anti-IgE-mediated histamine release compared to CC genotype.[24]

Although, we identified significant associations between the *FCER1A*-95T>C polymorphism and serum IgE specific for SEA and TSST-1, further studies using a larger sample size are necessary to confirm these associations.

Previous population studies have reported significant associations between the *MS4A2* polymorphism and total serum IgE levels in asthmatic patients.[27,28] However, our results suggest that this is not the case for AIA patients.

Kim et al.[19] suggested a synergistic effect between the *MS4A2*-109T>C polymorphism and serum IgE specific to SEB in AIA patients. These results were supported by our current study population. It has been proposed that this genetic/environmental interaction occurs via the binding of SEB-specific IgE to its high-affinity receptor FcεR1 on the mast cell surface, resulting in mast cell activation and the enhanced release of inflammatory mediators and cytokines, which could ultimately contribute to AIA development. *In vitro* functional study of *MS4A2*-109T>C by luciferase reporter assays showed that FcεR1β-109T allele was associated with higher promoter activity in both RBL-2H3 and A549 cell lines.[19] Therefore, increased expression of FcεR1β-109T on mast cells may enhance the inflammatory mediators and Th2 cytokines and development of AIA.

With respect to the role of *Staphylococcal* superantigens in AIA pathogenesis, it is possible that AIA patients with high levels of IgE specific for the three *Staphylococcal* superantigens exhibit more severe airway hyperresponsiveness.[8] Specifically, the *Staphylococcal* superantigens may stimulate mast cell activation and polyclonal activation of T cells and antigen-specific CD4 + T cells with the release of Th2 cytokines and other proinflammatory mediators. This would promote the activation of IgE-producing B cells, which could contribute to the development of airway inflammation in AIA patients.[29,30] Given our data, we suggest that two genetic polymorphisms, *FCER1A*-344C>T and *MS4A2*-109T>C, may interact with the specific IgE response to *Staphylococcus aureus*, which enhances the progression of airway inflammation in AIA patients.

In conclusion, we examined three sets of *FCER1A*, *MS4A2* and *FCER1G* genetic polymorphism in AIA patients comparing with ATA and NC. Among them, *FCERIG*-237A>G may be more susceptible in AIA in a Korean population in association with serum total IgE and it is also speculated that *FCER1A*-344C>T and *MS4A2*-109T>C may increase the severity of AIA in association with specific IgE responses to *Staphylococcal* superantigens.

Conflict of interest statement

There are no conflicts of interest.

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