

Genetic Polymorphisms in Aspirin-Intolerant Chronic Urticaria

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Background: Aspirin (acetylsalicylic acid, ASA)-intolerant urticaria with angioedema (AIU), a major type of aspirin-related hypersensitivity in ASA-intolerant asthma, can be classified into two groups, acute ASA-intolerant urticaria (AIAU) and chronic ASA-intolerant urticaria (AICU), according to the duration of the urticaria. The pathogenic mechanism of AICU is not well understood.

Methods/Data base: We present here a review of the recent findings from AICU molecular genetic studies.

Results: A previous study on human leukocyte antigen (HLA) genotypes suggested that the HLA alleles DRB1*1302 and DQB1*0609 may be genetic markers for ASA-induced urticaria/angioedema for both AIAU and AICU, and that they were significantly associated with the prevalence of serum-specific immunoglobulin E (IgE) to staphylococcal superantigens, particularly in AICU patients. A polymorphism study that examined four leukotriene-related genes, *ALOX5*, *ALOX5AP*, *PTGS2*, and *LTC4S*, found that two promoter polymorphisms of *ALOX5* at -1708A>G and *CysLTR1* at -634 C>T, but not *LTC4S* (-444A>C), were significantly different between the phenotypes of ASA-intolerant asthma and AICU, suggesting different contributions to the leukotriene pathway from two different pathogenic mechanisms. Another genetic polymorphism study in patients with aspirin-induced urticaria/angioedema did not find any significant associations with *HRH1*, *HRH2*, or *MS4A2*. However, the *FCER1A* promoter (-344 C>T) and the 3'UTR site of *HNMT* (939 A>G) gene polymorphisms were significantly associated with the AICU phenotype, and their functional abnormalities with the different genotypes were confirmed by *in vitro* functional studies.

Conclusions: The two HLA alleles DRB1*1302 and DOB1*0609 and the *ALOX5*, *FCER1A* promoter, and *HNMT* gene polymorphisms may contribute to the pathogenic mechanism of AICU. Further studies on the genetic mechanisms of AICU are needed to elucidate its pathogenic mechanism and aid in the development of new therapeutic strategies.

Keywords: aspirin-intolerant chronic urticaria, gene polymorphism, histamine, leukotriene-related genes

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Differences in the Clinical Features of ASA-Intolerant Chronic Urticaria

Aspirin (acetylsalicylic acid, ASA) ingestion can induce urticaria with angioedema (AIU) [1–4], which can be classified into two types, acute aspirin-intolerant urticaria (AIAU) and chronic aspirin-intolerant chronic urticaria (AICU). AIAU can be defined as urticaria with angioedema that develops only when patients are exposed to aspirin. In contrast, AICU is characterized by daily urticaria symptoms that have lasted for more than 6 weeks and are aggravated when the subject is exposed to aspirin. The phenotype of both types of AIU can be confirmed by an oral aspirin challenge test as described previously [5]. When the clinical features of AICU were compared with aspirin-tolerant chronic urticaria (ATCU) [5, 6], AICU patients were younger and had a higher atopy rate, higher total serum immunoglobulin E (IgE), and a greater association with a history of asthma (see Table 1). No significant differences were noted in the prevalence of thyroid autoantibodies and antinuclear antibodies. However, AICU patients required higher doses of antihistamines to control the urticaria, suggesting that AICU patients may show a more severe clinical course than ATCU patients. A recent study indicated that the prevalence of serum-specific IgE to staphylococcal superantigen, TSST-1, was significantly higher in AICU than in ATCU or normal control subjects, while the colonization rate of *Staphylococcus* was similar [7]. Patients with high levels of IgE to the superantigen showed a high atopy rate and had high serum total IgE levels [7]. These findings suggest a possible involvement of a T-helper cell type 2 (Th2) immune response to staphylococcal superantigens in the pathogenic mechanism of AICU.

TABLE 1
CLINICAL CHARACTERISTICS OF PATIENTS WITH ASPIRIN-INTOLERANT CHRONIC URTICARIA

	AICU [†] (n = 105)	ATCU [†] (n = 154)	NC [†] (n = 222)	AICU vs. ATCU	AICU vs. NC
Age (years)*	35.0 ± 11.5	39.3 ± 11.6	36.4 ± 15.2	0.003	0.362
Sex (male/total)	51/105 (48.6%)	74/154 (48.1%)	104/222 (46.8%)	0.935	0.771
Atopy (presence/total)	76/102 (74.5%)	73/150 (48.7%)	26/186 (14.0%)	<0.001	<0.001
Log (serum total IgE (IU/ml))*	2.2 ± 0.5	2.1 ± 0.5	1.7 ± 0.6	0.009	<0.001
Antithyroglobulin antibody (positive/total)	10/64 (15.6%)	22/115 (16.1%)	NA	0.938	NA
Antimicrosomal antibody (positive/total)	6/64 (9.4%)	17/137 (12.4%)	NA	0.529	NA
Antinuclear antibody (positive/total)	13/67 (19.4%)	22/129 (17.1%)	NA	0.684	NA

[†]AICU = ASA-intolerant chronic urticaria; ATCU = ASA-tolerant chronic urticaria; NC = normal healthy controls; NA = not applicable.

*Mean ± SD

Genetic mechanisms of aspirin-intolerant urticaria with angioedema

Although the genetic mechanisms of AIU and AICU are poorly understood, a few reports [2, 4] have suggested that an overproduction of cysteinyl leukotrienes may be involved. In this review, we summarize recent findings on the molecular genetic mechanisms of AIU and AICU.

HLA Alleles DRB1*1302 and DQB1*0609 Can Be Genetic Markers

We demonstrated an association between the human leukocyte antigen (HLA) genotype and AIU by using a high-resolution analysis technique, confirming a strong positive association between the HLA DRB1*1302 and HLA DQB1*0609 alleles common in both AIAU and AICU [8]; allelic frequencies of HLA DRB1*1302 and HLA DQB1*0609 were significantly higher in AIU patients than in AIA and normal healthy controls when clinical parameters were analyzed according to the presence of these two alleles. Patients carrying HLA DRB1*1302 or HLA DQB1*0609 (28.2 ± 7.0 and 29.0 ± 6.9 years old, respectively) were significantly younger (by 10 years) than those without either allele (38.0 ± 13.6 and 36.4 ± 13.4 years old, respectively), indicating that patients with these alleles develop AIU at an earlier age. No significant differences were found in the other clinical parameters, including atopy, total serum IgE, and circulating autoantibodies between the two groups.

Recent data also showed that the prevalence of serum-specific IgE to staphylococcal superantigens was significantly higher in AICU; IgE specific for TSST-1 was the most prevalent form in AICU patients (25.8%), and this prevalence was significantly higher compared to those for the normal control (6.5%) and ATCU (13.7%) patients. Significant associations were

noted between the prevalence of specific IgE to staphylococcal enterotoxin-A (SEA) and -B (SEB) and the HLA DQB1*0609 and DRB1*1302 alleles in the AICU group [7], suggesting that patients with these two HLA alleles may be more susceptible to acquiring a Th2 immune response of staphylococcal superantigens that develops into the AICU phenotype.

These findings suggest that the HLA alleles DRB1*1302 and DQB1*0609 may be strong HLA markers for determining the AICU phenotype. Further studies are needed to explore the genetic interactions between these HLA alleles and gene polymorphisms, as well as environmental interactions.

Genetic Polymorphisms of the Leukotriene Synthesis Pathway Gene and the AIU Phenotype

Leukotrienes have been suggested to participate in the pathogenesis of AIU as well as that of aspirin-intolerant asthma (AIA) [2–4]. Immunopharmacological studies have demonstrated that mast cells and basophils are activated to a greater extent in patients with AIU [9]. Mastalerz et al. and colleagues [10] demonstrated the overproduction of cysteinyl leukotrienes in association with a polymorphism of the leukotriene C4 synthase gene (*LTC4S*) at –444A>C in patients with AICU, and the frequency of subjects carrying the C allele of *LTC4S* –444A>C was significantly higher in AICU patients. Moreover, AIU was shown to be aggregated in families inheriting the *LTC4S* –444C allele [11], and the frequency of the –444C allele was significantly higher in patients with AICU than in those with ATCU. However, no associations were found in Spanish [12] or Korean [13] study populations.

We also investigated the genetic polymorphisms of candidate genes of the leukotriene synthesis pathway in a Korean population [13]. We examined 9 single-nucleotide polymorphisms (SNPs) of 4 leukotriene-related genes: arachidonate 5-lipoxygenase (*ALOX5*; –1708G>A, 270G>A, and 1728G>A), 5-lipoxyge-

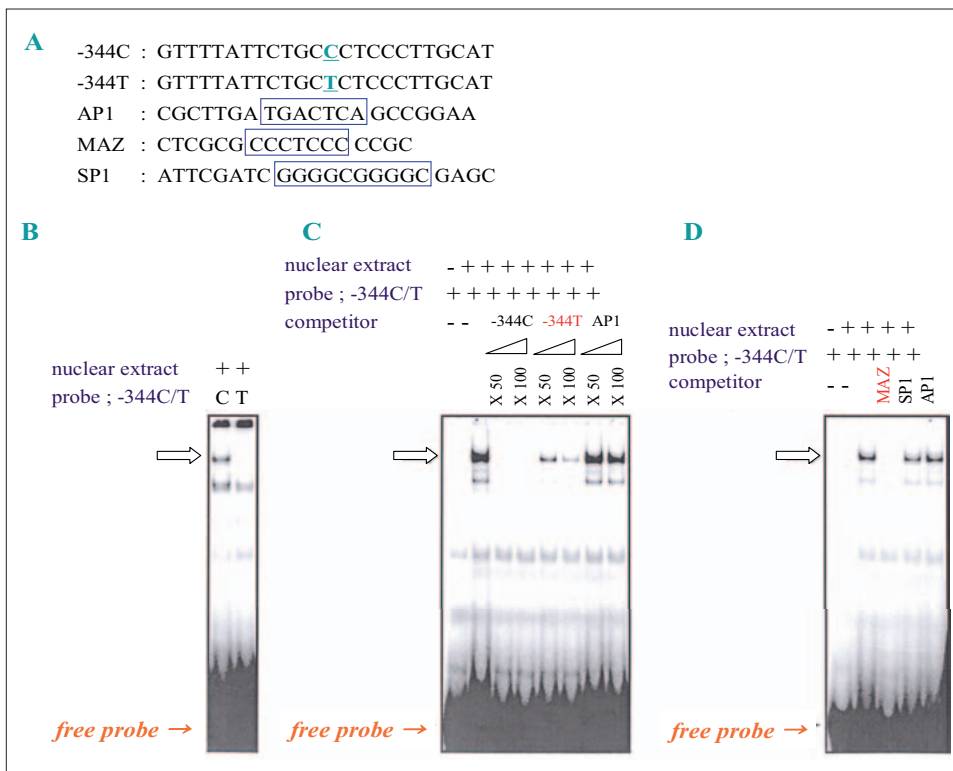


Figure 1. EMSA finding for the *FceRIα* -344 C>T polymorphism using nuclear extracts of KU 812 cells. A: nucleotide sequences used. B: Binding difference of a nuclear promoter to the -344 C/T allele. C: Different binding affinities with additions of competitors. D: Competition assay with the MAZ antibody.

nase-activating protein (*ALOX5AP*; 218A>G), cyclooxygenase-2 (*PTGS2*: -162C>G, 10T>G, and 228G>A), leukotriene C4 synthase (*LTC4S*) (-444A>C), and cysteinyl leukotriene receptor 1 (*CysLTR1*) (-634C>T) and found that the polymorphisms of *ALOX5* (-1708G>A) and *CysLTR1* (-634C>T) showed significant differences in genotype frequency between AICU and AIA patients [13]. The frequency of the *ALOX5* -1708A allele was significantly higher in the AIU group than in the normal control group [13], whereas the frequency of the *CysLTR1* -634T allele was significantly lower in the AICU group than in the AIA group [14]. These results suggest that *ALOX5* and *CysLTR1* make different contributions to two major aspirin-related conditions, AICU and AIA, in the Korean population.

Genetic Polymorphism of the High-Affinity IgE Receptor, Histamine Receptor Gene Polymorphisms, and the AICU Phenotype

Considering the pathogenic mechanism of AIU and AICU, we speculated that polymorphisms of candidate genes involved in the release and metabolism of histamine and of histamine receptor genes may be associated with the AICU phenotype.

We analyzed 11 known SNPs of the genes encoding high-affinity IgE receptor I (*MS4A2*: -109T>C, Rsa1_Int2, I181L(A>C), E237G(A>G) Rsa1_Ex7), histamine N-methyltransferase (*HNMT* T105I(C>T)), and histamine receptor type 1 (*HRH1*: -17C>T, D349N(G>A)) and type 2 (*HRH2*: 543G>A, 826C>T) and their haplotypes in AIU patients, and compared the frequencies with those in patients exhibiting other drug allergies and in normal controls [15]. No significant differences were observed in allele or genotype frequencies, or in the haplotype frequencies of the SNPs of the *MS4A2* gene, *HNMT*,

HRH1, and *HRH2*, among the three groups, suggesting that the polymorphisms of the *MS4A2* gene and the 3 histamine-related genes might not contribute to the development of the AIU phenotype in the Korean population [15].

Recently, we reported finding significant associations of 2 promoter polymorphisms (-334C>T and -95 T>C) of the *FCER1A* (encoding the alpha chain of the IgE high-affinity receptor) gene with AICU [6]. *FceRIα* is the first receptor to bind with IgE antibodies. The allelic frequency of the -344C>T polymorphism was significantly higher in AICU patients compared to the other subjects [6]. The *FCER1A* -344C>T polymorphism was also significantly associated with the total serum IgE level and a higher rate of atopy in the AICU patients. The *FCER1A* -344C>T polymorphism has been recently reported to predict higher levels of IgE in the sera of patients with other allergic diseases such as asthma or urticaria [16]. In a Korean study [6], the reporter plasmid that carried the -344T allele exhibited significantly higher promoter activity in a rat mast cell line (RBL-2H3) compared to the promoter activity of the -344C allele. It was also found that the transcription factor MAZ preferentially bound to the -344C>T polymorphism (see Figure 1). An *in vivo* functional study demonstrated that AICU patients with the T allele showed a higher histamine-releasing activity by IgE than those with the homozygous CC genotype (see Figure 2), while no significant differences were noted in the calcium ionophore-induced histamine-releasing activity. These findings suggest that the -344C>T polymorphism of the *FceRIα* promoter may be associated with an increased expression of *FceRIα* on mast cells and enhanced release of histamine, which ultimately contribute to the development of AICU. However, Sanak and colleagues recently reported the lack of an association of the *FCER1A* -344C>T polymorphism with aspirin hypersensitivity in a Caucasian Polish popula-

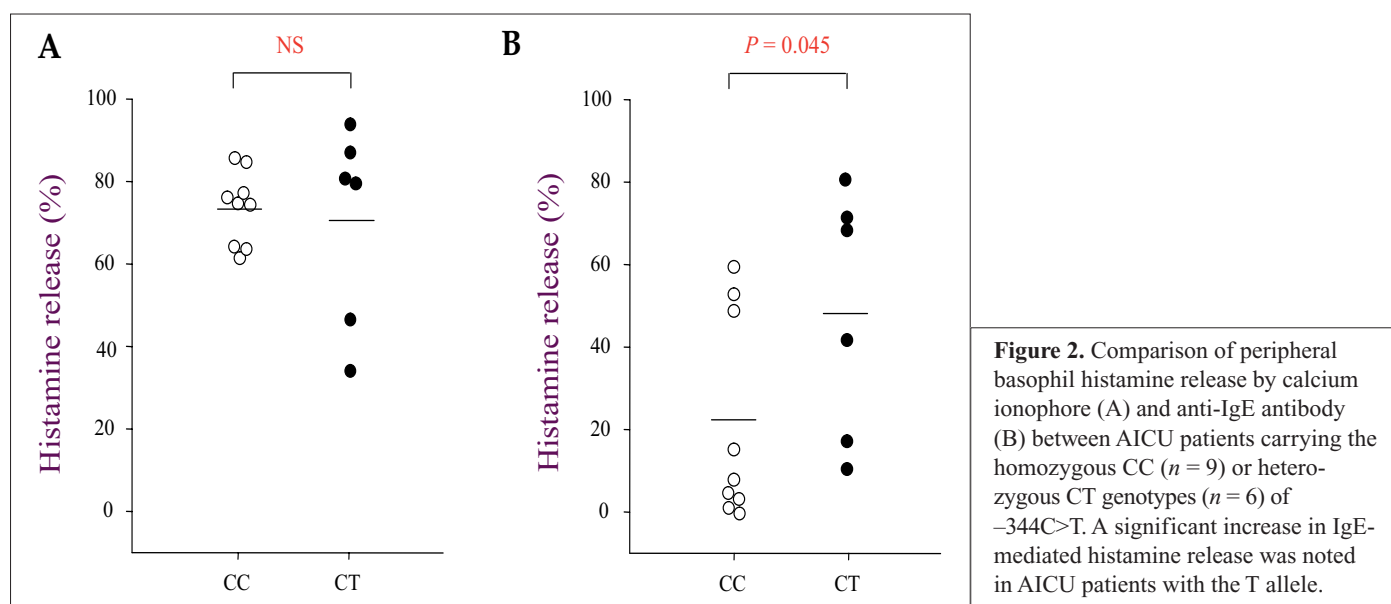


TABLE 2
SINGLE NUCLEOTIDE POLYMORPHISMS OF THE CANDIDATE GENES FOR ASA-INTOLERANT CHRONIC URTICARIA

Gene	Name	Loci	Single-nucleotide polymorphisms
<i>ALOX5</i>	arachidonate 5-lipoxygenase	10q11.2	-1708 G>A, 21 C>T, 270 G>A, 1728 A>G
<i>ALOX5AP</i>	arachidonate 5-lipoxygenase-activating protein	13q12.3	218 A>G
<i>CysLTR1</i>	cysteinyl leukotriene receptor 1	Xq13.2-21.1	-634 C>T, 927 T>C
<i>LTC4S</i>	leukotriene C4 synthase	5q35	-444 A>C
<i>PTGS2</i>	prostaglandin-endoperoxide synthase-2 (Cox2)	1q25.2-q25.3	-162 C>G, 10 T>G, 228 G>A
<i>FCER1A</i>	Fc fragment of IgE, high-affinity I, receptor for; alpha polypeptide	1q23	-344 C>T, -95 T>C
<i>MS4A2</i>	Fc fragment of IgE, high-affinity I, receptor for; beta polypeptide	11q12.1	-109 T>C, RsaI_in2 G>A, E237G, RsaI_ex7 T>C
<i>HNMT</i>	histamine N-methyltransferase	2q22.1	105 C>T, 939 A>G
<i>HRH1</i>	histamine receptor H1	3p25.3	-17 C>T
<i>HRH2</i>	histamine receptor H2	5q35.2	543 G>A

tion [17]. This inconsistent association may be explained by the different composition of patients with aspirin hypersensitivity because the Polish study included both patients with asthma and urticaria. Further investigations of the *FCER1A* gene in different populations need to be conducted to support its classification as a strong genetic marker for the AICU phenotype.

Genetic Polymorphism of the Histamine-Metabolizing Gene and the AICU Phenotype

Histamine is a biogenic amine chemical that plays an important role in the chronic skin inflammation of AICU, and the level of histamine is regulated by histamine N-methyl transferase (HNMT). We investigated the functional variability of the *HNMT* gene according to genetic polymorphisms in AICU patients [18] and found that the *HNMT* 939A>C polymorphism was significantly associated with the AICU phenotype. Moreover, an *in vitro* functional study demonstrated that the 939A>G conversion in the 3'-UTR increased mRNA stability and protein

expression, which suggests that the genetic variants of the *HNMT* 939A>C polymorphism may affect mRNA stability and protein expression, resulting in altered histamine metabolism and finally contributing to the development of the AICU phenotype. As the bioactive histamine level can also be regulated by the synthesizing enzyme histamine decarboxylase (HDC), further studies are needed to investigate the genetic contribution of HDC in association with HNMT.

Conclusion

The genetic polymorphisms of candidate genes investigated in AICU and the genetic associations are summarized in Tables 2 and 3. Further information on the genetic polymorphisms of candidate genes with supporting functional studies will make it pos-

TABLE 3
SUMMARY OF THE GENETIC STUDIES FOR ASPIRIN-INDUCED URTICARIA/ANGIOEDEMA

Gene	SNPs	Disease	Ethnicity	Phenotype [†]	Association [*]	Year	Reference
HLA	DRB1*1302	AIU vs. AIA (or NC)	Korean	R	Y	2005	[8]
	DQB1*0609	AIU vs. AIA (or NC)	Korean	R	Y	2005	[8]
ALOX5	-1708G>A	AIU vs. AIA	Korean	R	Y	2005	[13]
MS4A2	-109T>C	AIU vs. other drug allergy	Korean	–	N	2005	[15]
HNMT	105 C>T	AIU vs. other drug allergy	Korean	–	N	2005	[15]
	939 A>G	AICU vs. ATCU	Korean	P	Y	2007	[18]
HRH1	-17C>T	AIU vs. other drug allergy	Korean	–	N	2005	[15]
HRH2	543G>A	AIU vs. other drug allergy	Korean	–	N	2005	[15]
CysLTR1	-634 A>C	AICU vs. AIA	Korean	P	Y	2007	[14]
LTC4S	-444A>C	NSAID-induced periorbital angioedema vs. NC	Spanish	–	N	2001	[12]
		AICU vs. CIU	Polish	R	Y	2004	[10]
		AICU vs. ATCU	Korean	R	Y	2007	[5]
		patients with aspirin hypersensitivity (asthma + urticaria)	Polish	–	N	2007	[17]

[†]R = risk; P = protection. ^{*}Y = significant association; N = no association

sible to elucidate the molecular mechanism of AICU and identify the candidate genetic markers for differentiating AICU from other aspirin-related allergic conditions, which may lead to the development of new diagnostic markers and novel therapeutic strategies.

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