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Polymorphisms of Aspirin-Metabolizing Enzymes *CYP2C9*, *NAT2* and *UGT1A6* in Aspirin-Intolerant Urticaria

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Acetyl salicylic acid (ASA) is metabolized by UDP-glucuronosyltransferase 1A6 (UGT1A6), cytochrome P4502C9 (CYP2C9), and N-acetyl transferase 2 (NAT2). Variations in the activities of these enzymes may modulate adverse ASA-related symptoms such as urticaria. We examined whether polymorphisms in the *UGT1A6*, *CYP2C9*, and *NAT2* genes are related to ASA-intolerant urticaria (AIU). The genotypes of 148 subjects with AIU (AIU group) and 260 normal healthy control subjects (NC group) were analyzed with respect to the following single nucleotide polymorphisms: *CYP2C9* –1188T>C and *CYP2C9**3A1075C; *UGT1A6* T181A A>G and *UGT1A6* R184S A>C; and *NAT2* 9796A>T, *NAT2* 197G>A, *NAT2* 286G>A, *NAT2* 9601A>G, and *NAT2* 9306A>G. There were significant differences in the allele frequencies for the *CYP2C9* polymorphisms between the two groups. The frequency of the minor allele *CYP2C9* –1188T>C was significantly higher in the AIU group than in the NC group (P=0.005). The frequency of the variant genotype CC was higher in the AIU group compared with the controls in both the co-dominant (P=0.007) and recessive models (P=0.012). The frequency of haplotype 2 [CA] was also significantly higher in the AIU group in both the co-dominant (P=0.006) and dominant models (P=0.012). There was no significant difference in genotype frequencies for any of the *UGT1A6* or *NAT2* polymorphisms between the two groups. Clinical parameters did not differ according to genotype. These results suggest that the C allele of *CYP2C9* –1188T>C may be associated with AIU.

Key Words: Aspirin; Cytochrome P4502C9; Metabolizing enzyme; Urticaria

Aspirin ingestion can induce a wide range of clinically recognized allergic reactions, including acetyl salicylic acid (ASA)-exacerbated respiratory disease (AERD), ASA-intolerant urticaria (AIU), chronic rhinitis, and anaphylaxis. Patients with AIU have high rates of atopy and increased total IgE levels,[1] but the pathogenic mechanism of AIU remains unclear. ASA is metabolized by UDP-glucuronosyltransferase 1A6 (UGT1A6), cytochrome P4502C9 (CYP2C9), and N-acetyl transferase 2 (NAT2). Inter-individual differences in the activities of these enzymes may be the underlying cause of adverse ASA-related symptoms such as urticaria.

The two polymorphic enzymes CYP2C9 and UGT1A6 are involved in hydroxylation and glucuronidation of ASA, respectively.[2,3] Two known variant alleles of *UGT1A6* result in amino acid changes at positions 181 and 184, and a 30–50% reduction in enzyme activity compared with wild type activity.[4] Similarly, two variant *CYP2C9* alleles, *CYP2C9**2 (R144C) and *CYP2C9**3 (I359L), produce slow metabolizing enzymes, with 5–30% of wild-type enzyme activity.[5] Variations in the enzymes that metabolize ASA may play a role in ASA-related diseases such as urticaria.

Leukotriene overproduction is a possible risk factor for ASA hypersensitivity, including AIU.[6] Cysteinyl leukotrienes are inactivated by acetyl coenzyme A-dependent N-acetyltransferase (NAT). Thus, functional alterations in the *NAT2* gene may contribute to the risk for ASA-intolerant symptoms such as asthma.[7] Genetically-determined rapid and slow acetylators produce variation in the elimination rates of xenobiotics, as well as in the levels of NAT2 in the liver and other tissues. Single nucleotide polymorphisms (SNPs) of the *NAT2* gene are markers of atopic asthma, high serum IgE levels, and high blood eosinophil counts.[8]

Based on the involvement of these enzymes in the metabolism of ASA, we investigated the relationship between polymorphisms in *UGT1A6*, *CYP2C9*, and *NAT2*, and the occurrence of ASA-intolerant urticaria in a Korean population. In total, 148 AIU patients (AIU group) and 260 normal healthy control sub-

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jects (NC) were enrolled at the Ajou University Hospital in Suwon, Korea. AIU was defined by a positive result on an ASA oral provocation test, which was performed using 500 mg of ASA (Rhonal; KunWha Pharmaceutical Co., Seoul, Korea).[5] A control group (NC) with no personal or family history of allergic diseases or past history of ASA hypersensitivity was recruited from the general population. Informed consent was obtained from all subjects prior to enrollment, and the study was approved by the Ajou University Hospital Institutional Review Board. Skin prick testing was performed with 55 common aero-allergens (Bencard Co., West Sussex, UK). Atopy was defined as one or more positive reactions to common inhalant allergens. Total IgE concentration was measured using a UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden), according to the manufacturer's instructions. SNPs in the promoter and/or exons of the *CYP2C9*, *UGT1A6*, and *NAT2* genes of 40 healthy volunteers were sequenced using an ABI Prism 3100 DNA analyzer (Applied Biosystems, Foster City, CA, USA). We identified two SNPs in *CYP2C9*, one in the promoter (*CYP2C9* –1188T>C) and one in an exon (*CYP2C9**3A1075C). For *UGT1A6*, we selected two exonic SNPs (*UGT1A6* T181A A>G and *UGT1A6* R184S A>C). We confirmed five SNPs for *NAT2* (*NAT2* 9796A>T, *NAT2* 197G>A, *NAT2* 286G>A, *NAT2* 9601A>G, and *NAT2* 9306A>G). These SNPs were genotyped using primer extension and a SNAPshot ddNTP primer extension kit (Applied Biosystems).

Differences in mean values of phenotypic characteristics among the patients were compared using a Student's *t*-test for continuous variables or the χ^2 test for categorical variables. Differences in genotype frequency between the two groups were examined using the χ^2 test. A logistic regression recessive analysis model was used after adjustment for age and gender covariates, followed by a multiple comparison test. All statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). In all analyses, $P<0.05$ was considered statistically significant.

The mean age was significantly higher in the AIU group than in the NC group (35.1±11.5 vs. 32.5±12.6 years, respectively; $P=0.041$). The AIU patients exhibited a significantly higher rate of atopy than the NC group (69.6 vs. 13.2%, respectively; $P<0.001$) and higher serum total IgE (log transformed; 5.1±1.2 vs. 3.6±1.3 IU/mL respectively; $P<0.001$).

The genotype frequencies for the two polymorphisms in each of *CYP2C9* and *UGT1A6* and the five polymorphisms in *NAT2* were analyzed and compared between AIU patients and NC subjects. The two polymorphisms in *CYP2C9* were in linkage disequilibrium (/D′/=1, $r^2=0.067$). The frequency of the minor C allele of *CYP2C9* –1188T>C was significantly higher in the AIU group than in the NC group ($P=0.005$), and remained significant after a multiple comparison ($P_{corr}=0.03$). The frequency of the variant genotype CC of *CYP2C9* –1188T>C was also higher in the AIU patients compared with the controls in both the co-dominant ($P=0.007$) and recessive models ($P=0.012$).

Table 1. Genotype and allele frequencies of CYP2C9, UGT1A6 and NAT2 gene polymorphisms

		AIU (n=148)	NC (n=260)	*P* value	P_{corr}
CYP2C9 -1188 T>C	TT	35 (23.6%)	96 (36.9%)	**0.007**	**0.042**
(Promoter)	TC	81 (54.7%)	126 (48.5%)	0.070	NS
(rs 4918758)	CC	32 (21.6%)	38 (14.6%)	**0.012**	NS
	q.	0.49	0.388	**0.005**	**0.030**
CYP2C9*3 A1075C	AA	136 (91.9%)	233 (89.6%)	0.385	NS
(Coding exon)	AC	12 (8.1%)	26 (10.0%)	1	NS
(rs 1057910)	CC	0 (0%)	1 (0.4%)	0.441	NS
	q.	0.41	0.54	0.397	NS
		AIU (n=146)	NC (n=258)	*P* value	P_{corr}
UGT1A6 T181A A>G	AA	85 (58.2%)	152 (58.9%)	0.762	NS
(Exon)	AG	54 (37%)	94 (36.4%)	0.988	NS
(rs 2070959)	GG	7 (4.8%)	12 (4.7%)	0.723	NS
	q.	0.233	0.229	0.892	NS
UGT1A6 R184S A>C	AA	79 (54.5%)	151 (58.5%)	0.251	NS
(Exon)	AC	55 (37.9%)	94 (36.4%)	0.355	NS
(rs 1105879)	CC	11 (7.6%)	13 (5.0%)	0.333	NS
	q.	0.266	0.233	0.296	NS
		AIU (n=148)	NC (n=260)	*P* value	P_{corr}
NAT2 -9796 T>A	TT	86 (58.1%)	157 (60.4%)	0.979	NS
(Promoter)	AT	58 (39.2%)	95 (36.5%)	0.821	NS
(rs 4646244)	AA	4 (2.7%)	8 (3.1%)	0.847	NS
	q.	0.223	0.213	0.249	NS
NAT2197 G>A	AA	85 (57.4%)	162 (62.3%)	0.509	NS
(Exon)	AG	59 (39.9%)	90 (34.6%)	0.979	NS
(rs1799930)	GG	4 (2.7%)	8 (3.1%)	0.452	NS
	q.	0.226	0.204	0.450	NS
NAT2 286 A>G	AA	118 (79.7%)	196 (75.4%)	0.336	NS
(Exon)	AG	28 (18.9%)	59 (22.7%)	0.669	NS
(rs1799931)	GG	2 (1.4%)	5 (1.9%)	0.346	NS
	q.	0.108	0.133	0.305	NS
		AIU (n=148)	NC (n=259)	*P* value	P_{corr}
NAT2 -9601 A>G	AA	42 (28.4%)	65 (25.1%)	0.47	NS
(Promoter)	AG	79 (53.4%)	139 (53.7%)	0.527	NS
(rs 4646267)	GG	27 (18.2%)	55 (21.2%)	0.402	NS
	q.	0.449	0.481	0.388	NS
NAT2 -9306 A>G	AA	102 (68.9%)	156 (60.2%)	0.176	NS
(Promoter)	AG	42 (28.4%)	96 (37.1%)	0.783	NS
(rs 4345600)	GG	4 (2.7%)	7 (2.7%)	0.11	NS
	q.	0.169	0.212	0.134	NS

P values were applied by binary logistic regression with sex and age. $P<0.05$ was considered to be significant. *P* value was further tested using multiple comparisons, as indicated, P_{corr} for AIU vs. NC.
AIU, aspirin intolerant urticaria; NC, normal control; CYP2C9, cytochrome P450C9; UGT1A6, UDP-glucuronosyltransferase 1A6; NAT2, N-acetyl transferase 2.

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In addition, the relationship between the *CYP2C9* –1188T>C polymorphism and AIU remained significant after a multiple comparison test (P_{corr}=0.042 [co-dominant model] for AIU vs. NC; Table 1). Using Power Analysis and Sample Size (PASS, 2008), the power of this genetic association study was calculated to be 74% for detecting an effect size (W) of 0.1430 using 2 degrees of freedom with the χ^2 test, at a significance level (alpha) of 0.05. The frequency of haplotype 2 [CA] was significantly higher in the AIU group compared with the NC group in both the co-dominant (P=0.006; P_{corr}=0.036) and dominant models (P=0.012) (Table 2).

Table 2. Haplotype frequencies of *CYP2C9*, *UGT1A6* and *NAT2* gene polymorphisms

CYP		AIU (n=148)	NC (n=259)	*P* value	P_{corr}
ht1 (TA)	+/+	35 (23.6%)	96 (37.1%)	**0.006**	**0.036**
	+/-	81 (54.7%)	126 (48.6%)	0.056	0.336
	-/-	32 (21.6%)	37 (14.3%)	**0.012**	0.072
ht2 (CA)	+/+	26 (17.6%)	29 (11.2%)	**0.006**	**0.036**
	+/-	87 (58.8%)	134 (51.7%)	**0.012**	0.072
	-/-	35 (23.6%)	96 (37.1%)	0.079	0.474
ht3 (CC)	+/+	NA	NA	0.522	1
	+/-	6 (4.1%)	8 (3.1%)	0.522	1
	-/-	142 (95.9%)	251 (96.9%)	NA	NA
UGT		**AIU (n=145)**	**NC (n=258)**	***P* value**	**P_{corr}**
ht1 (AA)	+/+	79 (54.5%)	151 (58.5%)	0.251	1
	+/-	55 (37.9%)	94 (36.4%)	0.355	1
	-/-	11 (7.6%)	13 (5.0%)	0.333	1
ht2 (GC)	+/+	7 (4.8%)	12 (4.7%)	0.887	1
	+/-	54 (37.2%)	94 (36.4%)	0.688	1
	-/-	84 (57.9%)	152 (58.9%)	0.977	1
ht3 (AC)	+/+	NA	NA	**0.01**	1
	+/-	9 (6.2%)	2 (0.8%)	**0.01**	1
	-/-	136 (93.8%)	256 (99.2%)	NA	1
NAT2		**AIU (n=148)**	**NC (n=258)**	***P* value**	**P_{corr}**
ht1 (TAAGG)	+/+	39 (26.4%)	65 (25.2%)	0.572	1
	+/-	82 (55.4%)	138 (53.5%)	0.459	1
	-/-	27 (18.2%)	55 (21.3%)	0.848	1
ht2 (AAAAG)	+/+	4 (2.7%)	8 (3.1%)	0.663	1
	+/-	57 (38.5%)	89 (34.5%)	0.614	1
	-/-	87 (58.8%)	161 (62.4%)	0.969	1
ht3 (TAGGA)	+/+	2 (1.4%)	4 (1.6%)	0.256	1
	+/-	25 (16.9%)	58 (22.5%)	0.225	1
	-/-	121 (81.8%)	196 (76.0%)	0.873	1
ht4 (TAGGG)	+/+	1 (0.7%)	1 (0.4%)	0.202	1
	+/-	17 (11.5%)	46 (17.8%)	0.154	1
	-/-	130 (87.8%)	211 (81.8%)	0.651	1
ht5 (TAAGG)	+/+	0 (0%)	2 (0.8%)	0.42	1
	+/-	20 (13.5%)	23 (8.9%)	0.273	1
	-/-	128 (86.5%)	233 (90.3%)	0.999	1

P values were applied by binary logistic regression with sex and age.

There was no significant difference in allele, genotype, or haplotype frequencies of the *UGT1A6* and *NAT2* genetic polymorphisms between the AIU and NC groups. Clinical parameters, including atopic status, serum total IgE levels, and autoantibody levels, did not differ between any of the genotypes for any of the polymorphisms.

We analyzed the genotype frequencies for two SNPs in both *CYP2C9* and *UGT1A6*, and five SNPs in *NAT2*, and compared them between AIU patients and normal control subjects in a Korean population. This is the first study to investigate the SNPs of *CYP2C9*, *UGT1A6*, and *NAT2* in AIU patients. Among the nine polymorphisms in the three metabolic enzyme genes analyzed, *CYP2C9* –1188T>C showed a significant association with the AIU phenotype. The frequencies of the minor C allele and the CC genotype of *CYP2C9* –1188T>C were significantly higher in the AIU group compared with the NC group. There was no significant difference in allele, genotype, or haplotype frequencies of the *UGT1A6* and *NAT2* polymorphisms between the AIU and NC groups.

CYP2C9 is involved in the metabolism of many therapeutic agents, including non-steroidal anti-inflammatory drugs, oral anticoagulants, and angiotensin receptor antagonists. The metabolism of non-steroidal anti-inflammatory drugs involves oxidation by CYP enzymes and conjugation, particularly glucuronidation, by phase II enzymes. Aspirin is deacetylated to salicylic acid and is further metabolized by glucuronidation, hydroxylation, and glycine conjugation, with CYP2C9 playing a major role in the metabolic process.[5] Genetic polymorphisms in *CYP2C9* have been associated with cutaneous adverse reactions induced by diphenylhydantoin.[9] The effects of inter-individual differences in *UGT1A6* and *CYP2C9* genotypes on ASA metabolism have been described in colon adenoma.[5] Recently, *CYP2C19* and *CYP2C9* genetic polymorphisms were shown to be significantly associated with an increased risk for the development of antituberculosis drug-induced maculopapular eruption in the Korean population.[10] Collectively, these findings suggest that the C allele may be involved in the pathogenesis of AIU; however, further studies of CYP2 enzyme activity are needed.

The *NAT2* gene variants and its subphenotypes have been linked to the development of asthma. Additionally, the NAT2 slow acetylator phenotype may be a determinant in a patient's susceptibility to asthma.[11] Another study indicated that SNPs in the *NAT2* gene are closely associated with asthma-related traits such as high serum IgE and blood eosinophil counts, and aspirin hypersensitivity in asthmatic subjects.[7,8] Significant associations between *UGT1A6* T181A gene polymorphisms and asthma susceptibility have also been reported.[12] However, the results of this study did not show a relationship between *NAT2* or *UGTA6* polymorphisms and AIU in a Korean population.

In summary, we identified a statistically significant associa-

tion between the C allele of *CYP2C9* –1188T>C and AIU, suggesting that this allele may modulate the risk for, and contribute to, the development of the AIU phenotype.

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