Adverse Effects of Sulfasalazine in Patients with Rheumatoid Arthritis Are Associated with Diplotype Configuration at the N-Acetyltransferase 2 Gene

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ABSTRACT. ***Objective.*** *N*-acetyltransferase 2 (*NAT2*) is a key enzyme for the acetylation of sulfasalazine (SSZ). We examine whether there was a correlation between diplotype configurations (combinations of 2 haplotypes for a subject) at the *NAT2* gene and the adverse effects of SSZ used for the treatment of rheumatoid arthritis (RA).

Methods. The findings from 144 patients with RA who had been treated with SSZ were collected from our outpatient department and used for a retrospective study. Haplotype analysis was performed by the maximum-likelihood estimation based on the EM algorithm using the obtained polymorphism data.

Results. Sixteen patients (11.1%) had experienced adverse effects from SSZ, the most common being allergic reactions including rash and fever. The slow acetylators who had no *NAT2*4* haplotype had experienced adverse effects more frequently (62.5%) than the fast acetylators who had at least one *NAT2*4* haplotype (8.1%) ($p < 0.001$, OR 7.73, 95% CI 3.54–16.86). In 25% of the slow acetylators, the adverse effects were so severe that they were hospitalized.

Conclusion. Genotyping the *NAT2* gene followed by estimation of diplotype configuration before administration of SSZ is likely to reduce the frequency of adverse effects in Japanese patients with RA. (J Rheumatol 2002;29:2492–9)

Key Indexing Terms:

SULFASALAZINE | *N*-ACETYLTRANSFERASE 2 | POLYMORPHISM
RHEUMATOID ARTHRITIS | DIPLOTYPE CONFIGURATION | ADVERSE EFFECTS

Several prospective randomized trials have shown that sulfasalazine (SSZ) is an effective disease modifying antirheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA)[1,2]. SSZ is widely used as a second-line drug[3-7]. The outcome of SSZ treatment in RA has been considerably variable and unpredictable. Use of SSZ is sometimes limited because of the occurrence of the adverse effects. The frequency of the adverse effects with SSZ has been reported to be about 20–30% in patients with RA[8-10].

Acetylation of SSZ is mediated by *N*-acetyltransferase (*NAT*, EC 2.3.1.5) in the liver. Blum, *et al* cloned 3 human *NAT* genes from human leukocyte DNA using a rabbit cDNA for arylamine *NAT*[11]. Two of them, *NAT1* and *NAT2*, both with open reading frames of 870 bp and mapped to 8p21.3–23.1, were shown to be functional. The third appeared to be a pseudogene. The *NAT2* gene has several single-nucleotide polymorphisms (SNP) in the coding exon. SNP in the *NAT2* gene are known to result in variation of acetylation activity and have been associated with drug toxicity and/or various cancers[12]. Consensus nomenclature for *NAT2* genes has been published[13], and as of April 2001, 13 SNP sites have been identified within the *NAT2* coding region and a total of 29 *NAT2* alleles have been described in human populations at the *NAT2* nomenclature website[14,15] (www.louisville.edu/medschool/pharmacology/NAT.html).

The genetic state for each subject at the *NAT2* gene is most properly expressed as a combination of 2 haplotypes, i.e., a diplotype configuration[16,17]. The *NAT2* activity for a subject was related to the diplotype configuration. The representative 4 common alleles (haplotypes) that possess signature nucleotide substitutions at positions 341, 590, 857, and 191 are designated *NAT2*5*, *NAT2*6*, *NAT2*7*, and *NAT2*14* clusters, and several studies have shown that the members of those clusters are responsible for the slow acetylator phenotype. Most mutant alleles include some of the following 7 most frequent mutations — $G^{191}A$, $C^{282}T$, $T^{341}C$, $C^{481}T$, $G^{590}A$, $A^{803}G$, and $G^{857}A$ [11,16,18,19]. A schematic of part of the *NAT2* gene is shown in Figure 1. The subjects

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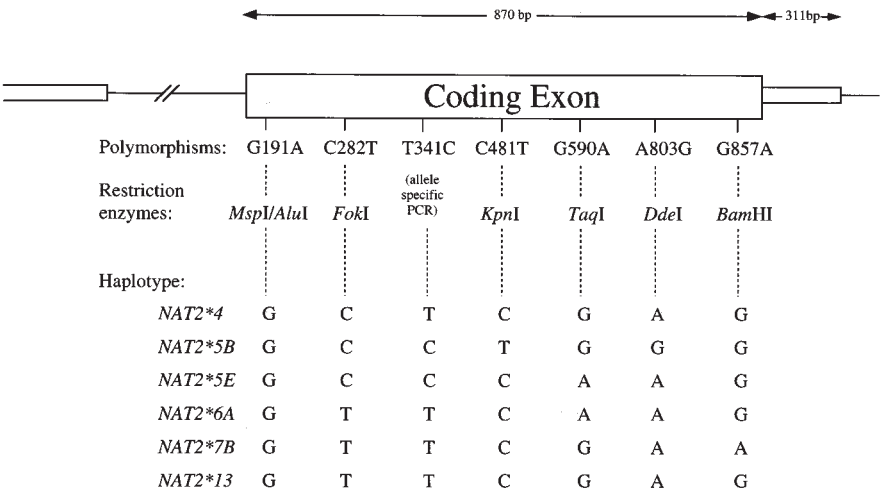

Figure 1. Schematic of part of the *N*-acetyltransferase 2 (*NAT2*) gene. Wide and narrow open boxes indicate coding and noncoding regions, respectively. Bar indicates the intron and 3′ flanking regions. The 5′ noncoding region of the cDNA was located separately from the coding region[18,36]. Seven common different single nucleotide polymorphism sites were observed within the exon 2 of *NAT2* gene. PCR products were digested by each restriction enzyme. The *NAT2*4* contained GCTCGAG at nucleotide positions 191, 282, 341, 481, 590, 803, and 857, respectively. Each haplotype observed in our population was characterized by specific patterns of the restriction sites, as shown.

with only these mutant haplotypes exhibit the slow acetylator phenotype, whereas the presence of at least one *NAT2*4* haplotype (wild-type allele) in a subject resulted in a rapid acetylator phenotype[16-18,20,21].

Some studies have examined the relationship between the effectiveness or adverse effects of SSZ and acetylator phenotypes of patients. Many studies have failed to find an association between the acetylator phenotype and either the effectiveness or the toxicity of SSZ[22-25]. However, some reports described that the frequency of particular adverse effects had occurred more frequently in slow than in rapid acetylators[22,23,26]. It is known that these acetylator types play an important role in the metabolism of drugs such as SSZ, isoniazid, phenelzine, hydralazine, procainamide, dapsone, and nitrazepam[27,28]. Timbrell, *et al* reported that hepatotoxicity with isoniazid was more frequent in the slow acetylators than the rapid acetylators[29].

There have been studies of correlation between the toxicity of SSZ and acetylator phenotypes in patients with RA[22,23,25]. They determined the phenotypes by measuring the ratios of the metabolites of SSZ, free sulfapyridine and acetylated sulfapyridine, in either serum or urine. Recently, Wadelius, *et al* reported that polymorphisms of *NAT2* were not associated with the risk of agranulocytosis caused by SSZ in patients with inflammatory diseases, although subjects in that study had diseases other than RA[30]. Therefore, there is no definitive report that found a correlation between the diplotype configurations at the *NAT2* gene and the occurrence of adverse effects of SSZ used for treatment of RA. We examined whether the diplotype configurations at the *NAT2* gene were associated with the adverse effects of SSZ in RA.

MATERIALS AND METHODS

Patients. The study was approved by the Genome Ethics Committee of Tokyo Women's Medical University (Approval Number 19). One hundred forty-four patients with RA (all Japanese nationals; 119 women, 25 men) who had been administered SSZ were randomly selected from the outpatient department of the Institute of Rheumatology, Tokyo Women's Medical University, between 1992 and 1999. All had been diagnosed with RA according to the 1987 classification criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) during their clinical course[31].

Genotype and haplotype determination. After informed consent, including approval for genetic analysis, was obtained between July 1999 and June 2000, a peripheral blood sample was taken from each patient, and genomic DNA was extracted from mononuclear cells using a Wizard Genomic DNA Purification Kit (#A1620, Promega, Madison, WI, USA) until August 2000. The genotypes at the 7 most frequent SNP sites were examined. The genotypes at 3 SNP sites ($C^{481}T$, $G^{590}A$, and $G^{857}A$) were determined by the single polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method[32]. A 815 bp fragment was amplified by PCR and subjected to *Kpn*I, *Taq*I, and *Bam*HI digestion for the mutations of $C^{481}T$, $G^{590}A$, and $G^{857}A$, respectively. Determination of the remaining genotypes at 4 SNP sites was performed according to Cascorbi, *et al*[21]. The $T^{341}C$ mutation was determined by allele-specific PCR and the other 3 mutations were identified by PCR-RFLP using a 1211 bp DNA fragment. The restriction enzymes *Msp*I/*Alu*I, *Fok*I, and *Dde*I were used for determination of the mutations of $G^{191}A$, $C^{282}T$, and $A^{803}G$, respectively. Each haplotype is characterized by specific patterns of the restriction sites, as shown in Figure 1. The haplotype and diplotype configuration (the combination of 2 haplotypes) of each subject was inferred based on these 7 genotypes. We applied the genotype findings to the LDSUPPORT program[33] to estimate the haplotype frequencies in the population and to calculate the probability of the diplotype distribution for each subject.

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Treatment with SSP. Treatment with SSZ was started at initial doses of 500 mg to 1000 mg per day. Thereafter, the SSZ dose was maintained or increased to 1500 mg per day according to each physician's decision without strict criteria. Usually the dosage was raised when the effect of SSZ was insufficient as judged from the clinical and laboratory findings. Clinical records were carefully examined to search for adverse effects of the drug.

Definition of adverse effects. The definition of an adverse effect was according to the criteria proposed by Karch and Lasagna[34]. Namely, the adverse effects were classified as "definite," "probable" "possible," "conditional," and "doubtful," according to their definition. A severe adverse effect was defined as a reaction that required hospitalization[35].

Statistical analysis. Statistical analyses to evaluate differences between groups were by Fisher's exact probability test or the chi-square test. Odds ratios and 95% confidence intervals were calculated when possible.

RESULTS

Polymorphisms at the NAT2 gene. Table 1 shows estimated numbers and frequencies of haplotypes and diplotype configurations at the *NAT2* gene in the 144 patients with RA. *NAT2*4* haplotype was the most frequent (72.2%). *NAT2*5B, NAT2*5E, NAT2*6A, NAT2*7B*, and *NAT2*13* haplotypes were present at frequencies of 0.3%, 0.3%, 19.1%, 7.6%, and 0.3%, respectively. The *NAT2* haplotype containing the $G^{191}A$ mutation (*NAT2*14* cluster) that has been reported only in Africans was not present in our subjects. The numbers of fast acetylators who had at least one *NAT2*4* haplotype and slow acetylators who had no *NAT2*4* haplotype were 136 and 8, respectively (Table 1). The number of subjects with each diplotype configuration did not indicate a departure from a Hardy-Weinberg equilibrium ($p > 0.05$, chi-square test). In the next step, the diplotype configuration for each subject was constructed by applying the same data to the LDSUPPORT program (maximum-likelihood method). When this method was used, the probability distribution of the diplotype configuration for each individual was concentrated on a single event in all the subjects.

Patient characteristics. The baseline features of the 144 RA patients treated with SSZ are shown in Table 2. The mean age was 49.9 ± 13.4 years, and the mean period between the onset of RA and the initiation of SSZ treatment was 51.1 ± 65.7 months. We aimed to detect any difference in the frequency of adverse effects of SSZ between individuals with RA with different diplotype configurations. Therefore, it was important to exclude the possibility that the baseline features were markedly different between the 2 groups. Table 2 indicates that there was no significant difference in baseline features between the 2 groups. However, the mean observation periods were significantly shorter for the slow acetylators than the fast acetylators because of the occurrence of adverse effects. During the treatment with SSZ, other DMARD were added in 41 patients (28.5%) because of the inefficacy of SSZ. By June 2000, when the last measurements were obtained, treatment with SSZ had been discontinued in 83 patients (57.6%) because of insufficiency of the treatment or adverse effects. Table 3 shows that there were no significant differences in the initial and the final amounts of SSZ used between the 2 groups.

Adverse effects of SSZ. All adverse effects noted in this study were "probable" adverse effects[34], because we did not perform a rechallenge with SSZ for ethical reasons. Adverse effects of SSZ had occurred in 16 patients (11.1%). Some patients had experienced multiple adverse effects. The frequencies of adverse effects of SSZ were as follows: rash in 12 patients (8.3%); fever, 9 (6.3%); increase in transaminases, 5 (3.5%); gastrointestinal (GI) symptoms, 3 (2.1%); myelosuppression, 3 (2.1%); stomatitis, 2 (1.4%); and edema, 1 (0.7%). The most common adverse effect was allergic reaction including rash and fever. Detailed clinical findings for the 16 patients with adverse effects and their diplotype configurations are given in Table 4. Most of the adverse effects occurred within 2 weeks after starting treatment with SSZ. Thirteen patients discontinued SSZ because of the occurrence of adverse effect.

Association between genotypes or diplotype configurations at the NAT2 gene and adverse effects of SSZ. When proportions of the haplotypes at the *NAT2* gene were compared between patients with and those without adverse effects, the proportion of the mutant haplotypes was significantly higher in the group with adverse effects than that of *NAT2*4* haplotype ($p < 0.05$, OR 2.02, 95% CI 1.06–3.87) (Table 5), and the proportion of *NAT2*6A* was significantly higher in the group with adverse effects than that without them ($p < 0.05$, chi-square test, OR 2.22, 95% CI 1.14–4.33). Patients

Table 1. Numbers and frequencies of haplotypes and diplotype configurations at the *NAT2* gene in 144 patients with RA.

Haplotype	Nucleotide Change (s)	Diplotype Configurations, n (%)						Numbers of Phenotypes, n (%)
		*NAT2*4*	*NAT2*5B*	*NAT2*5E*	*NAT2*6A*	*NAT2*7B*	*NAT2*13*	
*NAT2*4*	None	72 (50.0)	1 (0.7)	0	44 (30.5)	18 (12.5)	1 (0.7)	Fast acetylators: 136 (94.4)
*NAT2*5B*	$T^{341}C$, $C^{481}T$, $A^{803}G$	—	0	0	0	0	0	
*NAT2*5E*	$T^{341}C$, $G^{590}A$	—	—	0	1 (0.7)	0	0	
*NAT2*6A*	$C^{282}T$, $G^{590}A$	—	—	—	5 (3.5)	0	0	Slow acetylators: 8 (5.6)
*NAT2*7B*	$C^{282}T$, $G^{857}A$	—	—	—	—	2 (1.4)	0	
*NAT2*13*	$C^{282}T$	—	—	—	—	—	0	
Numbers of Haplotypes, n (%)		208 (72.2)	1 (0.3)	1 (0.3)	55 (19.1)	22 (7.6)	1 (0.3)	

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Table 2. Baseline features of patients with RA treated with SSZ.

	Total	Fast Acetylators	Slow Acetylators
No. of patients	144	136	8
Mean age ± SD, yrs*	49.9 ± 13.4	50.0 ± 13.3	48.0 ± 15.1
Female, n (%)	119 (82.6)	111 (81.6)	8 (100)
Mean disease duration ± SD, mo*	51.1 ± 65.7	46.6 ± 52.8	127.6 ± 165.4
Median disease duration, mo*	31	29.5	62.5
Mean observation period ± SD (range), mo	27.8 ± 26.8 (0.03–97)	26.3 ± 26.0 (0.1–97)	7.2 ± 9.9 (0.03–27)
Median observation period, mo	18	17	2.25
RF positivity (%)*	125 (86.8)	117 (86.0)	8 (100)
Mean ESR ± SD (range), mm/h*	51.0 ± 27.0 (3.2–142.0)	51.6 ± 27.4 (3.2–142.0)	37.2 ± 18.7 (3.8–63.8)
Mean CRP ± SD (range), mg/100 ml*	2.70 ± 2.98 (0.0–14.0)	2.70 ± 3.01 (0.0–14.0)	2.39 ± 2.27 (0.2–7.7)
Corticosteroids users, n (%)*	69 (47.9)	66 (48.5)	3 (37.5)
No. of other DMARD users, n (%)**	39 (27.1)	37 (27.2)	2 (25.0)

* Data were obtained when SSZ treatment was initiated. ** Number of patients who had continued taking other DMARD after administration of SSZ.

Table 3. The dose of SSZ in 144 patients.

	Total, n (%)	Fast Acetylators, n (%)	Slow Acetylators, n (%)
Initial SSZ doses, mg/day			
500	98 (68.1)	94 (69.1)	4 (50.0)
1000	46 (31.9)	42 (30.9)	4 (50.0)
Final SSZ doses, mg/day*			
500	39 (27.1)	37 (27.2)	2 (25.0)
1000	100 (69.4)	94 (69.1)	6 (75.0)
1500	5 (3.5)	5 (3.7)	0 (0)

* The doses of SSZ when findings were obtained at the latest occasion or those when SSZ was discontinued.

Table 4. Clinical characteristics and diplotype configurations at the *NAT2* gene in 16 patients with RA who had experienced adverse effects of SSZ.

Patient	Age, yrs sex	Duration 1, mo	Complication	Initial Dose, mg/day	Duration 2, weeks	Adverse Effects	Withdrawal of SSZ	Hospitalization	Diplotype Configurations	Acetylator Phenotype
1	59 F	8	—	500	2	R, S	+	–	*NAT2*4/*4*	Fast
2	46 F	130	—	500	1	F	+	–	*NAT2*4/*4*	Fast
3	44 F	3	—	1000	2	R	+	–	*NAT2*4/*4*	Fast
4	16 F	6	SLE, SS	500	2	F, R, T, M	+	+	*NAT2*4/*4*	Fast
5	50 F	2	SS	500	8	R	+	–	*NAT2*4/*4*	Fast
6	26 F	40	—	1000	2	F, R	+	–	*NAT2*4/*4*	Fast
7	55 F	1	—	1000	4	R	–	–	*NAT2*4/*4*	Fast
8	59 F	4	—	500	2	F, R	+	+	*NAT2*4/*6A*	Fast
9	61 F	78	—	500	120	S	–	–	*NAT2*4/*6A*	Fast
10	49 F	1	—	500	2	F, R, E	+	–	*NAT2*4/*6A*	Fast
11	65 F	87	—	1000	1	GI	+	–	*NAT2*4/*7B*	Fast
12	58 F	57	Hyperthyroidism	500	1	F, R, T, GI, M	+	+	*NAT2*6A/*6A*	Slow
13	31 F	0	SS, hypothyroidism	1000	2	F, R, T	+	+	*NAT2*6A/*6A*	Slow
14	35 F	113	—	500	1	F, R, GI	+	–	*NAT2*6A/*6A*	Slow
15	69 F	242	—	500	4	T	–	–	*NAT2*6A/*6A*	Slow
16	59 F	52	—	1000	2	F, R, T, M	+	–	*NAT2*7B/*7B*	Slow

Duration 1: period between onset of RA and initiation of SSZ treatment. Initial dose: initial dose of SSZ. Duration 2: period between initiation of SSZ treatment and onset of adverse effects. SLE: systemic lupus erythematosus, SS: Sjögren's syndrome, R: rash, F: fever, T: increase in transaminases, GI: gastrointestinal symptoms, S: stomatitis, M: myelosuppression, E: edema.

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Table 5. Correlation between genotypes at the *NAT2* gene and adverse effects of SSZ.

Haplotype	No. of Haplotypes	Without Adverse Effects of SSZ, n (%)	With Adverse Effects of SSZ, n (%)
*NAT2*4*	208	190* (91.3)	18* (8.7)
Mutant haplotype	80	66* (82.5)	14* (17.5)

* When the proportions of the haplotypes at the *NAT2* gene were compared between patients with and those without adverse effects, the proportion of the mutant haplotypes, including *NAT2*5B, NAT2*5E, NAT2*6A, NAT2*7B,* and *NAT2*13* haplotypes, was significantly higher in the group with adverse effects than that of *NAT2*4* haplotype ($p < 0.05$, OR 2.02, 95% CI 1.06–3.87).

without the *NAT2*4* haplotype had developed adverse effects more frequently than those with homozygous *NAT2*4* haplotypes ($p < 0.01$, Fisher's exact probability test) and more frequently than those heterozygous with both *NAT2*4* and a mutant haplotype ($p < 0.01$, Fisher's exact probability test). The frequency of adverse effects was not different between the patients with homozygous *NAT2*4* haplotypes and those heterozygous with both *NAT2*4* and a mutant haplotype ($p = 0.357$, Fisher's exact probability test). The frequency of the patients with adverse effects was 5 of 8 patients (62.5%) in the slow acetylators, while it was 11 of 136 patients (8.1%) in the fast acetylators ($p < 0.001$, Fisher's exact probability test, OR 7.73, 95% CI 3.54–16.86) (Table 6). The adverse effects in the 4 patients were so severe that they were hospitalized and treated with corticosteroid (Table 4). The percentage of patients who had the severe adverse effects with SSZ was very small (1.5%) in the fast acetylators, while it was relatively high (25.0%) in the slow acetylators. The difference was significant ($p < 0.05$, Fisher's exact probability test, OR 17.0, 95% CI 2.74–105.5) (Table 6).

DISCUSSION

We examined whether there was any difference in the frequency of the adverse effects of SSZ between RA patients with different diplotype configurations at the *NAT2* gene. The slow acetylators showed a higher frequency (62.5%) of adverse effects than the fast acetylators (8.1%). Surprisingly, 25.0% of the slow acetylators who took SSZ had to be hospitalized and treated with corticosteroid, while adverse effects with hospitalization occurred in only 1.5% of the fast acetylators. The physicians would not have administered SSZ to the patients without *NAT2*4* haplotype if they had known the high frequency of adverse effects in such subjects. It is suggested that genotyping for *NAT2* gene to determine the patient's diplotype configuration before the administration of SSZ is quite important.

Table 6. Correlation between diplotype configurations at the *NAT2* gene and adverse effects of SSZ.

	Fast Acetylators, n = 136	Slow Acetylators, n = 8
With:without adverse effects	11:125*	5:3*
With:without hospitalization	2:134**	2:6**

* The slow acetylators developed adverse effects more frequently than the fast acetylators ($p < 0.001$, OR 7.73, 95% CI 3.54–16.86). ** The slow acetylators had been hospitalized because of severe adverse effects of SSZ at a higher frequency than the fast acetylators ($p < 0.005$, OR 17.0, 95% CI 2.74–105.5).

This study is interpreted as a cohort study rather than a case-control study because the patients with and those without adverse effects were not separately selected. In addition, the use of SSZ was not influenced by any genetic information, i.e., physicians were not aware of the genetic information for patients when they administered SSZ. Therefore, both the subjects with and those without adverse effects should be considered as belonging to a single group at the beginning.

The frequencies of the haplotypes at the *NAT2* gene determined in this study in patients with RA were similar to those determined in Japanese controls[32,36-39]. These frequencies are different from those of other ethnic groups. Thus, the *NAT2*14* cluster observed only in subjects of African origin[16] was not found in our Japanese population. The diplotype configuration of each subject in our population was inferred based on the genotypes at 7 loci. Gross, *et al* described that the genotype data alone provided efficient and accurate information as to acetylator status[40]. In addition, Grant, *et al* described that the genotype determination at nucleotide positions 191, 341, 590, and 857 can efficiently provide a high degree of phenotype predictability[12]. Lin, *et al* showed that slow acetylators could be detected as mutations at positions 481, 590, and 857 in the *NAT2* gene[41]. Base substitutions at positions 341 and 803 were almost always associated with the 481 mutation, and mutation at position 282 was strongly associated with the 590 mutation. The 481, 590, and 857 mutations accounted for virtually all of the slow acetylator alleles in Asian and white populations[41]. Other studies have also observed high correlation between genotype and phenotype in Japanese, Chinese, Caucasians, and Australian Aborigines. The prediction rate has been reported to be between 88% and 100%[17,18,42-45]. In our population, *NAT2*5B*, *NAT2*5E*, *NAT2*6A*, *NAT2*7B*, and *NAT2*13* mutant haplotypes were present. Among these alleles, *NAT2*5B*, *NAT2*6A*, and *NAT2*7B* have been asso-

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ciated with reduced *N*-acetylation activities in *in vivo* and/or *in vitro* studies[17,19,36,41,46,47].

Many studies have examined the difference in the rate of the adverse effects of SSZ between slow and rapid acetylator phenotypes in patients with RA[22-26]. Although most concluded that there was no association, some showed that the slow acetylators had a higher rate of particular adverse effects than the rapid acetylators[22,23,26]. The present findings are in agreement with some previous observations. Pullar, *et al* showed that upper GI adverse effects were more common in slow acetylators, while serious toxicity was not different between the slow and the rapid acetylators[22]. Kitas, *et al* showed that significant increases in aspartate transaminase were noted mainly in slow acetylators[23]. Laversuch, *et al* showed that 3 of 4 patients with RA who developed cutaneous vasculitis and lupus-like disease after intake of SSZ had the slow acetylator phenotype[26]. Patients with inflammatory bowel disease with the slow acetylator phenotype had a higher frequency of adverse effects, including GI symptoms, cyanosis, rashes, and fever, than the rapid acetylators[48-50]. In most studies, the acetylator phenotypes were determined by measuring either the serum or urinary ratios of acetylated sulfapyridine to free sulfapyridine. However, in this study, an extensive analysis of the association between the adverse effects of SSZ and the genotypes and/or diplotype configurations at the *NAT2* gene in patients with RA was performed for the first time. Although we observed that patients with the mutant haplotype had developed adverse effects more frequently than those with the *NAT2*4* haplotype, the relationship to adverse effects became more evident through analysis of the diplotype configuration (Tables 5 and 6).

The majority of reported adverse effects were GI symptoms in Caucasians[8-10], while allergic reactions such as rashes and fever were the most frequent adverse effects in our study. Most of the patients with RA in this study were administered nonsteroidal antiinflammatory drugs (NSAID) or corticosteroids, and GI symptoms are one of the common adverse effects of both NSAID and corticosteroids. Since it was often difficult to discriminate between GI symptoms caused by SSZ and those caused by NSAID or corticosteroids, GI symptoms that definitely followed a reasonable time sequence from administration of SSZ or that were confirmed by improvement on stopping SSZ were recorded as adverse effects of SSZ. It might be possible that applying this procedure underestimated the frequency of GI symptoms caused by SSZ. However, a prospective longterm study of 1 g of SSZ in Japanese patients with RA revealed that the frequencies of allergic reactions and GI symptoms were 38.2% and 27.3%, respectively[51]. A retrospective study of SSZ in Japanese patients with inflammatory bowel diseases also showed that allergic reactions were more frequent than GI symptoms (61 vs 22 cases, respectively)[52]. Thus the frequency of GI symptoms and allergic reactions caused by SSZ in Japanese might be different from that of a Caucasian population. For these reasons, allergic reactions such as rash, etc., are the most frequent adverse effects in this report. Rashes and fever were generally considered to be allergic reactions. In a double blind prospective study in Japanese patients with RA, those patients who were administered 1 g and 2 g SSZ per day developed allergic adverse reactions such as rashes and fever at frequencies of 7.3% and 41.8%, respectively[53]. That report was compatible with findings in the present study that the frequency of allergic reactions was much higher in patients with slow acetylator diplotype configurations than in patients with rapid acetylator diplotype configurations. Further, Sabbagh, *et al* examined the *NAT2* genotype in patients with chronic discoid lupus erythematosus and found that the slow acetylator genotype seemed to be more prone to adverse effects such as rash caused by SSZ[54]. That report together with our results suggested that a pharmacogenetic study might be able to predict not only dose dependent adverse effects but also the allergic adverse effects. Interestingly, there is a case report that describes delayed hypersensitivity to 5-fluorouracil associated with reduced dihydropyrimidine dehydrogenase (DPD) activity[55].

In this study, the patients with *NAT2*4* haplotype also developed adverse effects, although the frequency was much lower than that of patients without *NAT2*4* haplotype. SSZ is split by the action of bacterial azoreductases into sulfapyridine and 5-aminosalicylic acid (5-ASA). Sulfapyridine is almost completely absorbed from the colon and is acetylated in the liver, although hydroxylation and glucuronation also occur[56]. Therefore, although *NAT2* gene polymorphism seems to be one of the most important factors, some factors such as enzymes other than *NAT2* might be associated with the adverse effects of SSZ. Further studies will be needed to clarify these factors.

If the metabolism of SSZ or sulfapyridine is different between subjects with different diplotype configurations at the *NAT2* gene, the same difference is likely to cause a difference in the efficacy of the drug. However, the high frequency of adverse effects in the subjects without *NAT2*4* haplotype obscured the beneficial effects of the drug. Since more than half of such subjects experienced adverse effects and many had to stop taking the drug, it was not possible to compare the effect of the treatment with SSZ between the subjects with different diplotype configurations without any bias.

In summary, the Japanese RA patients without the *NAT2*4* haplotype showed a higher rate (62.5%) of adverse effects than those with at least one *NAT2*4* haplotype (8.1%). These findings indicate that Japanese patients with RA should be examined with regard to diplotype configuration at the *NAT2* gene before they are treated with SSZ.

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