N-acetyltransferase 2 slow acetylator genotype associated with adverse effects of sulfasalazine in the treatment of inflammatory bowel disease

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AIM: *N*-acetyltransferase 2 (NAT2) is an important enzyme catalyzing *N*-acetylation of sulfasalazine (SASP). The aim of the present study was to investigate associations of the genotypes of *NAT2* with inflammatory bowel disease (IBD), and with adverse effects of SASP, which is used as the first-line treatment of IBD.

PATIENTS AND METHODS: The wildtype allele (*NAT2*4*) and three variant alleles (*NAT2*5B*, *NAT2*6A* and *NAT*7B*) of the *NAT2* gene were determined in 101 patients with IBD (84 patients with ulcerative colitis and 17 patients with Crohn's disease) and 109 healthy controls by the polymerase chain reaction-restriction fragment length polymorphism method. Sixty-eight patients with IBD treated with SASP were followed, and their adverse reactions were recorded.

RESULTS: Eleven patients (16%) experienced adverse effects from SASP, including nine cases of sulfapyridine (SP) dose-related adverse effects and two cases of hypersensitivity (skin rash). Patients with the slow acetylator genotypes without the *NAT2*4* allele experienced adverse effects more frequently (36%) than those with the fast acetylator genotypes with at least one *NAT2*4* allele (11%), but the results were not significantly different (OR of 0.26, 95% CI 0.065 to 1.004; P=0.051). However, those with the slow acetylator genotypes experienced more SP dose-related adverse effects than those with the fast acetylator genotypes (36% versus 8%, OR of 0.17, 95% CI 0.039 to 0.749; P=0.019).

CONCLUSIONS: The *NAT2* gene polymorphism was not associated with susceptibility to IBD in Chinese populations, but the *NAT2* slow acetylator genotypes were significantly associated with SP dose-related adverse effects of SASP in the treatment of IBD.

Key Words: *Adverse effects; Genetic polymorphism; Inflammatory bowel disease; N-acetyltransferase 2; Sulfasalazine*

Génotype acétylateur lent de la *N*-acétyltransférase 2 associé aux effets indésirables de la sulfasalazine dans le traitement des maladies inflammatoires de l'intestin

OBJECTIF : La *N*-acétyltransférase 2 (NAT2) est une importante enzyme qui catalyse la *N*-acétylation de la sulfasalazine. Le but de la présente étude est d'analyser les liens entre les génotypes de la *NAT2*, les maladies inflammatoires de l'intestin (MII) et les effets indésirables de la SASP, utilisée en traitement de première intention dans les MII.

PATIENTS ET MÉTHODES : L'allèle sauvage (*NAT2*4*) et ses trois variantes (*NAT2*5B*, *NAT2*6A* et *NAT2*7B*) du gène *NAT2* ont été identifiés chez 101 patients souffrant de MII (84 patients atteints de colite ulcéreuse et 17 atteints de la maladie de Crohn) et chez 109 sujets témoins en bonne santé au moyen de la méthode d'analyse du polymorphisme de la longueur des fragments de restriction des produits de la réaction en chaîne de la polymérase. Soixante-huit patients atteints de MII et traités par sulfasalazine ont été suivis et on a pris note de leurs réactions indésirables.

RÉSULTATS : Onze patients (16 %) ont manifesté des réactions indésirables à la sulfasalazine, dont neuf cas d'effets indésirables liés à la dose de sulfapyridine (SP) et deux cas d'hypersensibilité (éruptions cutanées). Les patients qui présentaient les génotypes acétylateurs lents, sans l'allèle *NAT2*4*, ont manifesté plus d'effets secondaires (36 %) que les porteurs des génotypes acétylateurs rapides, qui avaient au moins un allèle *NAT2*4* (11 %), mais les résultats ne se sont pas révélés significativement différents (RR 0,26, IC 95 %, 0,065 à 1,004; P = 0,051). Par contre, les sujets qui présentaient les génotypes acétylateurs lents ont manifesté plus d'effets indésirables liés à la dose de SP que les porteurs de gènes acétylateurs rapides (36 %, contre 8 %, RR 0,17, IC 95 % 0,039 à 0,749; P = 0,019).

CONCLUSIONS : Le polymorphisme du gène *NAT2* n'a pas été associé à une propension aux MII dans les populations chinoises, mais les génotypes acétylateurs lents du *NAT2* ont été significativement associés aux effets indésirables liés à la dose de SP avec la sulfasalazine dans le traitement des MII.

The human *N*-acetyltransferase 2 (*NAT2*) gene is located on human chromosome 8p22, and is highly polymorphic. To date, one allele coding for fast acetylator genotype (wildtype *NAT2*4*) and several mutated alleles coding for impaired acetylator genotype have been identified (1). Polymorphism of the NAT2 enzyme causes interindividual variations of the metabolism of drugs, arylamine and potential carcinogens (2). Studies on the polymorphism of *NAT2* to evaluate genetic predisposition for development of certain diseases or to individualize drug therapy are now underway.

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is an entity of chronic intestinal inflammation with unknown etiology. Genetic susceptibility of this disease has been demonstrated by epidemiological studies, such as increased familial aggregations, an increased concordance in monozygotic twins and ethnic variations. Furthermore, environmental factors also contribute to the etiology of IBD. Xenobiotics that could initiate the development of IBD have been identified. Some of them are believed to be activating factors involved in the initiation of the disease. NAT2 is an important xenobiotic-metabolizing enzyme and is expressed in the intestinal mucosa (3). Current studies have shown an association between *NAT2* gene polymorphisms and various autoimmune diseases such as systemic lupus erythematosus (4), rheumatoid arthritis (5)

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TABLE 1
Restriction sites and lengths of the nucleotide fragments produced by the digestion of the 535 base pair (bp) amplification products with Kpn I, Bam HI and Taq I enzymes

NAT2 alleles	Position (mutation)	Restriction enzymes	Recognition motify	Fragment pattern (bp); alleles (wt)	Fragment pattern (bp); point mutation
*NAT2*5B*	481 (C→T)	Kpn I	G-GTAC'C	483;52	535
*NAT2*6A*	590 (G→A)	Taq I	T'CG-A	205;170;160	330;205
*NAT2*7B*	857 (G→A)	Bam HI	G'GATC-C	428;107	535

A Adenine; C Cytosine; G Guanine; NAT2 N-*acetyltransferase 2; T Thymine; wt Wildtype*

and Behcet's disease (6). *NAT2* gene polymorphisms may also be involved in the pathogenesis of IBD, but the association between *NAT2* gene polymorphisms and IBD has not been studied yet.

Sulfasalazine (SASP) is the first-line therapy for induction and maintenance of remission in patients with UC and patients with CD, and is widely used in China. SASP is split into 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP) by bacterial azoreductases in the colon. 5-ASA is effective in the treatment of IBD and inactivated to *N*-acetyl-5-ASA via *NAT1* in the colon mucosa. SP is well absorbed from the colon, does not have beneficial effects in patients with IBD and results in adverse effects. It is inactivated to *N*-acetyl-SP via *NAT2*, subsequently hydroxylated and conjugated with glucuronide, and excreted in the urine.

It is well known that interindividual variability of therapeutic effectiveness and/or adverse reactions of drugs are often influenced by polymorphisms of metabolic enzymes. Inheritance of a slow acetylator genotype for *NAT1* could potentially lead to increased efficacy. Similarly, adverse effects may occur more frequently in patients with slow acetylator *NAT2* genotypes who are treated with SASP. Patients with rheumatoid arthritis with slow acetylator genotypes *NAT2* have been shown to have more adverse effects than those with fast acetylator genotypes when taking SASP (7). We are interested in studying whether we can find the same results in patients with IBD when they take SASP.

In the present study, we investigated the association between *NAT2* gene polymorphism and the susceptibility to IBD, as well as the association between the *NAT2* genotype and the incidence of adverse effects of SASP in patients with IBD.

PATIENTS AND METHODS

Subjects

One hundred one Chinese patients with IBD (55 males and 46 females, age range 17 to 69 years, and mean age 37.8±1.38 years) were studied. The diagnosis of IBD was established according to previously published criteria (8). Of the 101 patients with IBD, 84 patients had UC and 17 patients had CD (CD is not common in China). The patients were recruited from the Departments of Internal Medicine and Geriatrics of Wuhan University Zhongnan Hospital (Wuhan, People's Republic of China) between 2000 and 2003. One hundred nine healthy physical examiners (62 men and 47 women, age range 25 to 76 years, mean age 45.82±8.73 years) made up the control group and were all affiliated with the same hospital. All subjects were from the Hubei province, were unrelated to each other and were of Chinese Han nationality. The study protocol was approved by the ethics committee of Wuhan University Zhongnan Hospital, and all subjects gave informed consent.

Determination of *NAT2* genetic polymorphism

The most common mutations in the Chinese population, at positions C481T, G590A and G857A (nucleotides: adenine [A], thymine [T], cytosine [C] and guanine [G]) of the *NAT2* gene, were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described previously (9). According to the nomenclature of the *NAT2* gene, the wildtype and three variant alleles were defined as *NAT2*4*, and *NAT2*5B*, *NAT2*6A* and *NAT2*7B*. Determination of the *NAT2* genotype was performed by individuals who were blind to the clinical data of the patients.

Briefly, genomic DNA was isolated from 5 mL of venous blood by conventional proteinase K and phenol/chloroform method. The primers were as follows: *NAT2*(+) 5'-GCCTCAGGTGC-CTTGCATTT-3' and *NAT2*(–) 5'-CGTGAGGGTAGAGAG-GATAT-3'. Each PCR cycle was performed in a total volume of 50 µL (containing 30 ng of DNA, 10 mM of tris hydrochloride, 1.5 mM of magnesium chloride, 200 µM of each deoxyribonucleotide triphosphate, 20 pmol of primer and two units of Taq I polymerase). After initial denaturation at 95°C for 5 min, 30 cycles of amplification were carried out with denaturation at 95°C for 50 s, annealing at 55°C for 50 s and extension at 72°C for 50 s, followed by the final extension at 72°C for 5 min. To establish the presence or absence of each single nucleotide polymorphism, PCR products were digested with 10 U Kpn I, Bam HI or Taq I (TaKaRa Biotechnology Inc, Dalian, People's Republic of China) at 37°C for 3 h and the fragments were separated on 2% agarose gel. Restriction sites and lengths of the nucleotide fragments produced by the digestion of the 535 base pair PCR products with Kpn I, Bam HI and Taq I enzymes, are shown in Table 1.

Acetylator genotype

NAT2 acetylator genotypes were produced according to previously published data (10). Homozygotes (*NAT2*4/NAT2*4*) or heterozygotes (*NAT2*4/NAT2*5B*, *NAT2*4/NAT2*6A* and *NAT2*4/NAT2*7B*) for the dominant *NAT2*4* wildtype allele were classified as fast acetylator genotypes, and homozygotes of the mutant alleles (*NAT2*5B, NAT2*6A* and *NAT2*7B*) were classified as slow acetylator genotypes.

Definition of adverse effects

All of the 101 patients with IBD were followed by telephone, mail or interview every three months. The clinical characteristics, drug histories and adverse effects of the medications of each patient were carefully recorded on the registry form of IBD patients. This form was designed and validated by the IBD Collaborative Study Group in China supervised by the Chinese Association of Gastroenterology. The definition of adverse effects was according to the criteria proposed by Edwards and Aronson (11). Namely, the adverse effects were classified as 'certain', 'probable', 'possible', 'unlikely', 'conditional' and 'unassessable' according to the definition.

Statistical analysis

The data were analyzed using SPSS (SPSS Inc, USA) statistical software. The departure of the Hardy-Weinberg equilibrium was tested using the χ^2 test. Frequency of the allele and acetylator genotypes in each group was compared by χ^2 test or Fisher's exact tests if needed. The association between *NAT2* acetylator genotype and adverse effects of SASP was assessed by binary logistic regression analysis. ORs and 95% CIs were also calculated. $P<0.05$ was considered to be statistically significant.

TABLE 2
Distribution of *N*-acetyltransferase 2 (*NAT2*) alleles and acetylator genotypes in inflammatory bowel disease (IBD) patients and in healthy controls (con)

	Alleles				Acetylator genotypes	
	*NAT2*4* n (%)	*NAT2*5B* n (%)	*NAT2*6A* n (%)	*NAT2*7B* n (%)	Fast n (%)	Slow n (%)
Con (n=109)	130 (59.6)	12 (5.5)	51 (23.4)	25 (11.5)	86 (78.9)	23 (21.1)
IBD (n=101)	116 (57.4)	9 (4.5)	49 (24.3)	28 (13.9)	79 (78.2)	22 (21.8)
UC (n=84)	98 (58.3)	8 (4.8)	40 (23.8)	22 (13.1)	68 (80.9)	16 (19.1)
CD (n=17)	18 (52.9)	1 (2.9)	9 (26.5)	6 (17.6)	11 (64.7)	6 (35.3)

CD Crohn's disease; UC Ulcerative colitis

RESULTS

Distribution of the *NAT2* allele and acetylator genotypes

The distribution of allele and acetylator genotypes of *NAT2* in patients with IBD and healthy controls is shown in Table 2. The allele frequencies of *NAT2*4, NAT2*5B, NAT2*6A* and *NAT2*7B* were 0.57, 0.05, 0.24 and 0.14, respectively, in patients with IBD, and the allele frequencies of *NAT2*4, NAT2*5B, NAT2*6A* and *NAT2*7B* were 0.60, 0.06, 0.23 and 0.12, respectively, in healthy controls. *NAT2* slow acetylator genotypes were detected in 21.8% of patients with IBD and 21.1% of healthy controls. The frequencies of *NAT2* alleles and slow acetylator genotypes of the IBD patients were not significantly different from those of the controls.

Association between *NAT2* acetylator genotype and adverse effects of SASP

Of the 101 patients with IBD, 68 patients (67.3%) were treated with SASP. Adverse effects of SASP occurred in 11 patients (16%). Nine patients experienced SP dose-related adverse effects (four patients had severe gastrointestinal disorders, four had headaches and one patient had renal dysfunction). The other two patients experienced hypersensitivity (skin rash).

As shown in Table 3, patients with the slow acetylator genotypes who had no *NAT2*4* allele experienced adverse effects more frequently than those with the fast acetylator genotypes who had at least one *NAT2*4* allele, but the results were not significantly different (36% versus 11%, OR of 0.26, 95% CI 0.065 to 1.004; P=0.051). When the overall adverse effects were subdivided into two groups – SP dose-related adverse effects and hypersensitive reactions – those with the slow acetylator genotypes experienced SP dose-related adverse effects more frequently than the fast acetylator genotypes (36% versus 8%, OR of 0.17, 95% CI 0.039 to 0.749; P=0.019).

TABLE 3
Risk factors for adverse effects of sulfasalazine (SASP) in the treatment of inflammatory bowel disease

	Overall adverse effects		Serum SP dose-related adverse effects	
Risk factors	With adverse effects	Without adverse effects	With adverse effects	Without adverse effects
Male/female, n	33/24	5/6	33/24	3/6
Age, years (mean ± SD)	39.19±11.74	42.36±17.81	39.19±11.74	44.78±18.38
SASP dose (mean ± SD)	2.92±0.82	2.55±0.68	2.92±0.82	2.55±0.72
Treatment time, months (mean ± SD)	13.00±8.52	16.73±7.54	13.00±8.52	16.67±7.66
Fast/slow acetylator genotypes, n	48/9	6/5*	48/9	4/5†

**36% versus 11%, OR of 0.26, 95% CI 0.065 to 1.004, P=0.051; †36% versus 8%, OR of 0.17, 95% CI 0.039 to 0.749, P=0.019. SP Sulfapyridine*

DISCUSSION

The *NAT2* gene is highly polymorphic. Each mutant allele contains a combination of one or more nucleotide substitutions, which occur at the following positions: 191 (G→A [Arg64→Glu]), 282 (C→T [Tyr94]), 341 (T→C [Ile114→Thr]), 481 (C→T [Leu161]), 590 (G→A [Arg197→Gln]), 803 (A→T [Lys268→Arg]) and 857 (G→A [Gly286→Glu]). Of the 25 *NAT2* mutant alleles, three mutant alleles (*NAT2*5A, NAT2*6A* and *NAT2*7B*) have been shown to be commonly associated with slow acetylator status, and can be identified by the detection of the mutations at positions 481, 590 and 857 of the coding exon of the *NAT2* gene. By detecting these three main alleles, many studies (12-14) have observed a high correlation between the genotype and phenotype of *NAT2*. The prediction rate has been reported to be between 88% and 100%. Gao et al (15) determined these three mutant alleles by real-time fluorescence light-cycle technique, and found that the frequencies of *NAT2*4, NAT2*6A, NAT2*7B* and *NAT2*5B* among the Chinese were 60.4%, 24.1%, 13.7% and 1.8%, respectively. Our results have shown that the frequencies of *NAT2*4, NAT2*6A, NAT2*7B* and *NAT2*5B* were 59.6%, 23.4%, 11.5% and 5.5%, respectively, and were similar to the results found by Gao et al (15).

NAT2 slow acetylator genotypes have been linked with increased susceptibility to certain autoimmune diseases (4-6). It has been suggested that nonacetylated xenobiotics may accumulate in slow acetylators and are subsequently metabolized by other enzymes into reactive intermediates. These reactive intermediates may alter self-proteins presented to the immune system and stimulate T cells, which in turn initiate pathological and clinical signs of autoimmunity (16). However, our study failed to detect any association between the *NAT2* slow acetylor genotypes and susceptibility to IBD in the Chinese population, suggesting that the *NAT2* slow acetylator genotypes may not be an independent risk factor in the pathogenesis of IBD.

The incidence of adverse effects of SASP among the Western populations has ranged from 25% to 40% (14,17), but in our study, the incidence of adverse effects of SASP among the Chinese people was only 16%. This difference may be explained by the low frequency of slow acetylator genotypes in the Asian population (18). Surprisingly, 36% of those with slow acetylator genotypes who were treated with SASP had experienced adverse effects, while adverse effects occurred in only 11% of those with fast acetylator genotypes. It is suggested that those with slow acetylator genotypes are more likely to manifest SP dose-related effects than those with fast acetylator genotypes. However, these data also suggest that other factors may be involved, and prospective studies are needed to determine whether closer monitoring of those with slow acetylator genotypes will lead to a decrease in SP adverse events.

In general, the adverse effects of SASP were divided into two groups: adverse events (eg, vomiting and headache) appeared to be dependent on the serum SP concentration (greater than 50 μg/mL) (19), and hypersensitive reactions. The latter are independent of SP concentration, and are often

TABLE 4
***N*-acetyltransferase 2 (*NAT2*) allele frequencies in the Chinese population and other ethnic populations**

Population (reference)	2n	*NAT2*4* (%)	*NAT2*5B* (%)	*NAT2*6A* (%)	*NAT2*7B* (%)	*NAT2*14* (%)
Chinese (present case)	218	59.6	5.50	23.4	11.5	0.0
Japanese (20)	316	64.0	1.90*	23.1	11.1	0.0
Caucasian (21)	744	25.0†	45.0†	28.0	1.30†	0.0
African (21)	256	36.0†	30.0†	22.0	2.00†	9.0†

**P<0.05; †P<0.01 compared with the Chinese population*

manifested as skin rash and fever. In our study, we found no significant association between the *NAT2* slow acetylator genotypes and the overall adverse effects of SASP. However, when we subdivided the overall adverse effects into two groups – SP-related adverse effects and hypersensitive reactions – we found a strong association between the *NAT2* slow acetylator genotypes and adverse effects of SASP. Tanaka et al (7) reported a strong association between the *NAT2* slow acetylator genotypes and overall adverse effects of SASP, while Kumagai et al (14) and Ricart et al (17) did not find these overall associations significant, but they found that adverse effects occurred more frequently in those with slow acetylator genotypes. In their studies, adverse effects of hypersensitive reactions were very common (61% and 52%, respectively). Although slow acetylator genotypes are also suspected to be a risk factor for development of hypersensitivity reactions (9,17), the results are controversial (13). In our study, only two patients had hypersensitivity reactions and it was difficult to evaluate the associations. We found that the genetic polymorphism of *NAT2* showed great differences among different ethnic groups.

The comparisons of *NAT2* allele frequencies between the Chinese population and other ethnic populations are shown in Table 4. The frequency of the *NAT2*4* allele in the Chinese population was similar to that of the Japanese population (20), and was significantly higher than the Caucasian and African populations (21). Compared with the Caucasian (*NAT2*5B*, 45%; *NAT2*7B*, 1.3%) and African (*NAT2*5B*, 30%; *NAT2*7B*, 2.0%) populations, the Chinese exhibited a very low-frequency of *NAT2*5B* (5.5%) and a relatively higher frequency of *NAT2*7B* (11.5%). The frequency in the Chinese population was similar to that in the Japanese population. *NAT2*14* only existed in the African population. It had been found that the frequency of the slow acetylator activities presented in almost 50% of Caucasians and only 10% in Asians (18). The genetic differences among the populations may cause this difference in the phenotype of the populations. This difference should be considered when drug medication is developed by internationally harmonized clinical trials and marketed in various countries.

CONCLUSION

The *NAT2* genetic polymorphism was not associated with the susceptibility to IBD in Chinese patients, but the slow acetylator genotypes were significantly associated with SP dose-related adverse effects of SASP in IBD treatment.

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