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

MIN CHEN PhD1, BING XIA MD PhD1,2, BIXIAO CHEN MD2, QIUSHUA GUO PhD2, JIN LI MD PhD1, MEI YE MD PhD1, ZHENGGUO HU MD3

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 NAT2*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

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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 toindividual 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)
TABLE 1

<table>
<thead>
<tr>
<th>NAT2 alleles</th>
<th>Position (mutation)</th>
<th>Restriction Recognition enzymes</th>
<th>Fragment pattern (bp); (bp); point alleles (wt) mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT2*5B</td>
<td>481 (C→T)</td>
<td>Kpn I</td>
<td>G-GTAC'C 483;52 535</td>
</tr>
<tr>
<td>NAT2*6A</td>
<td>590 (G→A)</td>
<td>Taq I</td>
<td>TCGA-A 205;170;160 330;205</td>
</tr>
<tr>
<td>NAT2*7B</td>
<td>857 (G→A)</td>
<td>Bam HI</td>
<td>G-GATC-C 428;107 535</td>
</tr>
</tbody>
</table>

A: Adenine; C: Cytosine; G: Guanine; NAT2 N-acetylartransferase 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 azaeoductases 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 of 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 45.8±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'-GCCCTACGGTCCTTGCAATT-3' and NAT2(−) 5'-CGTGAAGGTTAGAGGATAT-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 30 s, annealing at 55°C for 20 s and extension at 72°C for 40 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 $\chi^2$ test. Frequency of the allele and acetylator genotypes in each group was compared by $\chi^2$ test or Fisher's exact test 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.
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*4A, and NAT2*7B were 0.57, 0.05, and 0.12, respectively, in healthy controls. The frequencies of NAT2*4, 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*4, NAT2*6A, and NAT2*7B genotypes were detected in 21.8% of patients with IBD and 21.1% of healthy controls. The frequencies of NAT2*4, NAT2*6A, and NAT2*7B genotypes 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, 65 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 1.044, 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).

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 [Thr94]), 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 major 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*4A, 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 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 adverse 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) that those with slow acetylator genotypes are more likely to experience adverse effects and hypersensitive reactions – those with the fast acetylator genotypes who had no slow acetylator genotypes experienced SP dose-related adverse effects. 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.
TABLE 4

<table>
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<th>Population (reference)</th>
<th>2n (%)</th>
<th>NAT2*4 (%)</th>
<th>NAT2*5B (%)</th>
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<th>NAT2*7B (%)</th>
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<tr>
<td>Chinese (present case)</td>
<td>218</td>
<td>19.6</td>
<td>95.0</td>
<td>93.1</td>
<td>72.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Japanese (20)</td>
<td>316</td>
<td>64.0</td>
<td>6.0*</td>
<td>3.1</td>
<td>11.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Caucasian (21)</td>
<td>744</td>
<td>25.0</td>
<td>45.0</td>
<td>28.0</td>
<td>1.30</td>
<td>0.0</td>
</tr>
<tr>
<td>African (21)</td>
<td>256</td>
<td>36.0</td>
<td>30.0</td>
<td>22.0</td>
<td>2.00</td>
<td>9.0†</td>
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*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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