Influence of *CYP2C9* and *COX-2* Genetic Polymorphisms on Clinical Efficacy of Non-Steroidal Anti-Inflammatory Drugs in Treatment of Ankylosing Spondylitis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABC Yu Wang
BDE Xiao-Dong Yi
EF   Hai-Lin Lu

Department of Orthopaedics, Peking University First Hospital, Beijing, P.R. China

Corresponding Author: Yu Wang, e-mail: wangyu5011@126.com
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Background: The aim of this study was to evaluate the relationships of *CYP2C9* and *COX-2* genetic polymorphisms with therapeutic efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) in treatment of ankylosing spondylitis (AS).

Material/Methods: We enrolled 130 AS inpatients and outpatients in the Arthritis and Rheumatism Department of Peking University First Hospital and 106 healthy people getting routine check-ups between September 2013 and July 2014. *CYP2C9* and *COX-2* genetic polymorphisms were detected by PCR-RFLP. All AS patients underwent medical treatment and 12-week follow-up treatment. Score differences of BASDAI, ASAS20, ASAS50, and ASAS70 for AS patients with different genotypes before and after treatment were compared.

Results: In terms of *COX-2*-1290A/G and -1195G/A gene polymorphism genotype and allele frequency, the case group and control group were obviously different (all $P<0.05$), but CYP2C9*3 polymorphism genotype and allele frequency were not statistically different between the 2 groups ($P>0.05$). AS patients had improved BASDAI, ASAS20, ASAS50, and ASAS70 scores after they received NSAID treatment (all $P<0.05$). Furthermore, the efficacy of NSAID in treatment of AS and *COX-2* gene -1290A/G and -1195G/A polymorphism were associated (all $P<0.05$), but it is not associated with CYP2C9 *3 polymorphism (all $P>0.05$).

Conclusions: *COX-2*-1290A/G and -1195G/A polymorphism may increase AS risk and they both can be considered as biological indicators for prediction of efficacy of NSAIDs in treatment of AS.

MeSH Keywords: **Antirheumatic Agents • Polymorphism, Genetic • Spondylitis, Ankylosing • Treatment Outcome**

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Background

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease that mainly causes inflammation and ossification of sacroiliac joints and axial skeleton, affecting peripheral joints, the sites of attachment of the ligaments or tendons to the bone, and other tissues [1]. The overall prevalence of AS in the general population is 1% and AS predominantly affects young men who are more than 15 years old [1,2]. AS is a complex disease with genetic factors and environmental factors, but the genetic-immune pathogenesis of this disease remains unclear [3]. A study has shown that non-steroidal anti-inflammatory drugs (NSAIDs) and physiotherapy are cornerstones of AS treatment [4]. NSAIDs are drugs that can non-selectively block cyclooxygenases (COX) to inhibit the synthesis of prostaglandins, thereby exerting their pharmacological actions such as reducing fever, pain, inflammation, rheumatism, and platelet aggregation. NSAIDs are widely used in treatment of osteoarthritis, immune disorders, inflammatory bowel disease, and reliving various pains [5,6]. Recent studies have proved that drug reactions depend largely on the drug metabolic enzyme activities, and genetic polymorphisms of drug metabolic enzyme occupy an important position among factors influencing enzyme activities [7,8].

CYP2C9 (cytochrome P450 2C9), which accounts for 20% of P450 protein in liver microsomes, is an important member of the CYP second subfamily [9]. Clinically, some drugs are oxidatively metabolized via CYP2C9, including glibenclamide, losartan, irbesartan, and NSAIDs like ibuprofen, lornoxicam, and diclofenac [10,11]. COX is a bifunctional enzyme that exhibits both cyclooxygenase and peroxidase activities and is a key enzyme catalyzing the oxidation of arachidonic acid to prostaglandin [12]. COX-2 is an inducible enzyme induced by pro-inflammatory genes in inflammation, trauma, and pain [13]. The high polymorphism of *CYP2C9* is related to the efficacy of NSAIDs [14]. Recently, much research attention has been focussed on investigating the efficacy of NSAIDs in treatment of AS, but few studies have been conducted on the efficacy of *CYP2C9* and *COX-2* gene polymorphism with NSAIDs in treatment of AS. This study aimed to assess the effect of *CYP2C9* and *COX-2* gene polymorphism on NSAIDs in treatment of AS.

Material and Methods

Ethical statement

This study was performed with the approval of the Clinical Management Committee of Peking University First Hospital. Informed consents were collected from all patients in this research.

Study subjects

This study included 130 AS inpatients and outpatients at Peking University First Hospital between September 2013 and July 2014 as the case group, which included 98 males and 32 females, and 106 healthy people who underwent routine physical examination in Peking University First Hospital were included as the control group, including 68 males and 38 females. All the study subjects were from 20 to 42 years old and the average age was 30.81±6.92 years old (Table 1). None of the study subjects were related by blood. Exclusion criteria were: patients who had taken hormones or other immunosuppressive drugs in the last 12 weeks; patients who had cardiovascular disease, cranial vascular disease, thrombus, thromboembolic disease, diabetes, viral hepatitis, cirrhosis of liver, serious renal, or liver dysfunction; patients with serious malnutrition; patients who had thyroid disease; pregnant women; and patients who had history of organ transplantation or had active tuberculosis. The diagnostic criteria for each AS patient conformed to New York criteria as modified in 1984 [15].

Single-nucleotide polymorphism (SNP) sequence

Five mL of venous blood was collected from the elbow of each subject and were anticoagulated by using dipotassium dihydrogen ethylenediaminetetraacetate (EDTA-K). Nal method was used to extract leucocyte genome DNA and a spectrophotometer was used to measure DNA content. DNA was stored at –20°C. The PCR-RFLP method was used for gene polymorphism analysis. Genotypes of 1290A/G and -1195G/A in the gene promoter region of CYP2C9*3 and *COX-2* were determined by PCR-RFLP method, taking a previous study as a reference [14]. PCR amplification was conducted according to the following conditions: 50 μl of reaction system, including 5 μl of 10×PCR buffer, 3 μl of 25 mmol/LMgCl2, 0.5 μl of 10 mmol/L dNTP, and 0.2 μl of 5 U/μl Taq DNA polymerase, then ultrapure water was added to a total system volume of 50 μl. PCR amplification conditions were: denaturation for 3 min at 95°C and 45 cycles (95°C 30 s, 57°C 30 s, and 72°C 15 s), followed by an extension at 72°C for 3 min. Amplified products were stored at 4°C. Primer sequences and lengths are shown in Table 2.

Therapeutic plans

AS patients who underwent genotyping were given NSAID treatment. Administration of medication consisted of a dose of NSAID, followed by 50 mg/d of indomethacin or 90 mg/d of acemetacin sustained release capsules. Patients who had risks of gastrointestinal adverse effects or incomplete response or low tolerance were given NSAIDs (200 mg of celecoxib and 75 mg of meloxicam). The dosage was gradually reduced after symptoms were controlled. Patients still needed to take the minimum effective dose for a period of time until NSAIDs

Table 1. Clinical data of case group.

Clinical items	Case group (n=130)	
Age of treatment (median, years old)	25	
Age of onset (median, years old)	21	
Course of disease (median, years old)	4	
Male n (%)	98	(75.38)
Female n (%)	32	(24.62)
HLA-B27 positive rate n (%)	115	(88.46)
Hip joint involvement n (%)	41	(31.54)
Peripheral joints involvement n (%)	59	(45.40)
Enthesitis n (%)	22	(16.92)
Extra-articular manifestations (iritis or urethritis) n (%)	9	(6.92)
Family history of spondyloarthropathy n (%)	33	(25.30)
History of axial joints and peripheral joints trauma n (%)	9	(6.92)
Bilateral inflammation <II n (%)	8	(6.15)
Bilateral inflammation in SIJ II n (%)	45	(34.62)
Bilateral or unilateral inflammation in SIJ III n (%)	57	(43.85)
Bilateral l or unilateral inflammation in SIJ IV n (%)	20	(15.38)

Table 2. Primer sequences of CYP2C9*3, *COX-2*-1290A/G and *COX-2*-1195G/A polymorphism.

SNP	Primer sequences
CYP2C9*3	5'-TGCACGAGGTCCAGAGGTAC-3'
	5'-AAACATGGATTGCAGTGTAG-3'
-1290A/G	5'-CAGGTTTTATGCTGTCATTTTCC-3'
	5'-TAGTGCTCAGGGAGGAGCAT-3'
-1195G/A	5'-CCCTGAGCACTACCCATGAT-3'
	5'-GCCCTTCATAGGAGATACTGG-3'

reduction. Patients with more severe conditions had to take more than 2 DMARDs at the beginning, as well as gastric mucosal protective drugs if necessary.

Efficacy evaluation

During 12 weeks of treatment, the AS patients receiving NSAID treatment were assessed according to efficacy criteria, which were ASAS20 response ratio, ASAS40 response ratio, and BASDAI score, based on the following 4 items: 1) function (BASFI), 2) morning stiffness (mean value of the fifth and sixth item of BASDAI), 3) overall evaluation of patients, and 4) pain. According to ASAS20 improvement criteria, at least 3 of the above-mentioned 4 items should be improved by at least 20% and 1 point,

and the remaining 1 item should not be deteriorated compared with the first diagnosis. According to ASAS40 improvement criteria, at least 3 of the above-mentioned 4 items should be improved by at least 40% and 2 points, and the remaining 1 item should not be deteriorated compared with the first diagnosis.

Statistical analysis

All data were analyzed using SPSS13.0 for Windows. $P<0.05$ was the standard for significance testing. The observed values are presented statistically. Improvement proportion and percentage were conducted using the X test. Score improvements of AS patients with different genotypes were compared before and after treatment. The results are presented as X±S. Data were tested for normality of distribution. Data with normal distribution were analyzed using the t test, while data with abnormal distribution were analyzed using the Kruskal-Wallis test.

Results

The electrophoresis of PCR production

Genotypes were classified according to enzyme electrophoresis: (a) 1290A/G mutation: -1290G allele contained an *RsaI* restriction enzyme cutting site, but the -1290A allele did not contain a *RsaI* restriction enzyme cutting site. Sites 2, 3, 4, and 8 only had a 173-bp segment; sites 1, 5, and 6 had a 20-bp segment
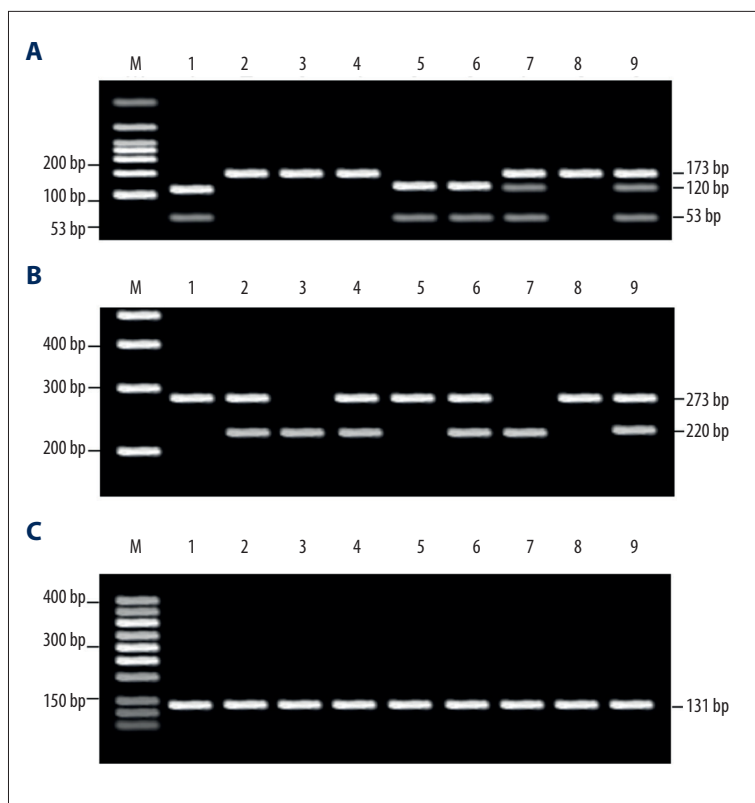
Figure 1. (**A–C**) Electrophoresis of PCR-RFLP production. PCR-RFLP – restriction fragment length polymorphism,

and 53-bp segment; and sites 7 and 9 had a 173-bp segment, 120-bp segment, and 53-bp segment. Therefore, the AA genotype only had a 173-bp segment, the GG genotype produced a 120-bp segment and 53-bp segment, and the AG heterozygous genotype had a 173-bp segment, 120-bp segment, and 53-bp segment. (b) In the -1195G/A mutation, the -1195G allele contained a *Pvu*II restriction enzyme cutting site, but the 1195A allele did not contain a *Pvu*II restriction enzyme cutting site. Sites 1, 5, and 8 only had a 273-bp segment; sites 3 and 7 had a 220-bp segment and 53-bp segment; and sites 2, 4, 6, and 9 had a 273-bp segment, 220-bp segment, and 53-bp segment. In -1195G/A, the AA genotype only had a 273-bp segment and GG genotype that produced a 220-bp segment and a 53-bp segment, while the AG genotype had a 273-bp segment, 220-bp segment, and 53-bp segment. (c) PCR production of CYP2C9*3 genotype was electrophoresed in 2% agarose gel and 131-bp amplified products were acquired. PCR amplified products were digested in 5 μ of KpnI restriction enzyme at 37°C overnight and genotypes were determined using 40 g/L of agarose gel electrophoresis. The results are shown in Figure 1.

Differences in genotype and allele frequency of CYP2C9*3, -1290A/G, and -1195G/A polymorphism

The differences in genotype and allele frequency of CYP2C9*3, *COX-2*-1290A/G, and *COX*-2-1195G/A polymorphism between the case group and control group are listed in Table 3. Genetic

equilibrium was achieved among AS patients of different genotypes and alleles and were group-representative. The differences in genotype and allele frequency of *COX-2* gene -1290A/G and -1195G/A polymorphism between the case group and control group were statistically significant (all *P*<0.05). There was no difference in genotype or allele frequency of CYP2C9 *3 polymorphism between the case group and control group (all *P*>0.05).

Comparisons of clinical indicator before and after NSAIDs treatment

Among the 130 patients who received NSAIDs treatment, 114 patients were followed up for 12 weeks. About 7.02% (8/114) complained that the symptoms were not significantly improved. As shown in Table 4, morning stiffness, function indicators, ESR or CRP level, and BASDAI and BASFI were all obviously improved after 3-month treatment. The proportion of nocturnal pain, peripheral joints involvement, BASDAI ≥4, and abnormal proportion of ESR or CRP were all obviously decreased. A total of 63 patients received BASDAI and BASFI evaluation before and after treatment, among which, 36 patients (57.1%) met ASAS20 improvement criteria and 14 patients (22.2%) met ASAS40 improvement criteria. The symptoms for 92.98% of patients (106/114) were improved or significantly improved and their indicators of morning stiffness, function check, laboratory examination, BASDA, and BASFI were all significantly improved. The remission rates of ASAS20 and

Table 3. The differences in genotype and allele frequency of CYP2C9*3, *COX-2*-1290A/G and *COX-2*-1195G/A polymorphism.

Genotype or allele	Control group (n=106)		Case group (n=130)		χ^2	*P* value
CYP2C9*3						
CYP2C9* 1/* 3	8	(0.08)	12	(0.09)	0.039	0.413
CYP2C9* 3/* 3	98	(0.92)	118	(0.91)	0.039	0.413
CYP2C9*1	8	(0.04)	12	(0.05)	0.038	0.415
CYP2C9*3	204	(0.96)	248	(0.95)	0.038	0.415
COX-2-1290A/G						
AA	96	(0.91)	99	(0.76)	8.449	0.003
AG	9	(0.08)	23	(0.18)	4.218	0.030
GG	1	(0.01)	8	(0.06)	4.322	0.036
A	201	(0.95)	221	(0.85)	11.869	<0.001
G	11	(0.05)	39	(0.15)	11.869	<0.001
COX-2-1195G/A						
GG	48	(0.45)	23	(0.18)	21.132	<0.001
GA	44	(0.42)	73	(0.56)	5.009	0.017
AA	14	(0.13)	34	(0.26)	6.040	0.010
G	140	(0.66)	119	(0.46)	19.374	<0.001
A	72	(0.34)	141	(0.54)	19.374	<0.001

ASAS40 reached 58.2% and 22.4%, respectively. The results are supported by previous reports and confirm the efficacy of NSAID in treatment of AS.

The association of CYP2C9*3, *COX-2*-1290A/G, and -1195G/A polymorphism with the efficacy of NSAIDs in treatment of AS

As show in Table 5, BASDAI scores of AS patients with different genotypes of *COX-2*-1290A/G and -1195G/A polymorphism were all significantly decreased after NSAIDs treatment. There were statistically significant differences in BASDAI scores of different genotypes after treatment (all *P*<0.05). However, the post-treatment BASDAI scores of AS patients with different genotypes of CYP2C9*3 polymorphism were not significantly different from before NSAIDs treatment (all *P*<0.05), suggesting that CYP2C9*3 polymorphism may not be related with NSAIDs treatment of AS.

As show in Table 6, after 12 weeks, ASAS20/50/70 scores of AS patients with different genotypes of *COX-2*-1290A/G and -1195G/A polymorphism were all significantly decreased after NSAIDs treatment. The post-treatment ASAS20/50/70 scores of different genotypes of *COX-2*-1290A/G and -1195G/A

polymorphism were significantly different from before treatment (all *P*<0.05). In *COX-2*-1290A/G genotypes, the proportions of patients with AA and AG+GG genotypes that met ASAS20 evaluation criteria were 94.7% and 71%, respectively, after 12 weeks (AA *vs*. AG+GG, *P*<0.05). In *COX-2*-1290A/G genotypes, the proportions of patients with AA and AG+GG genotypes who met ASAS50 evaluation criteria were 46.7% and 45.2%, respectively, after 12 weeks and the differences were not statistically significant (*P*>0.05). In *COX-2*-1290A/G genotypes, the proportions of patients with AA and AG+GG genotypes who met ASAS70 evaluation criteria were 24.6% and 35.5%, respectively, after 12 weeks and the differences were statistically significant (*P*<0.05). In *COX-2*-1195G/A genotypes, the proportions of patients with GG and GA+AA genotypes who met ASAS20 evaluation criteria were 69.4% and 94.6%, respectively, after 12 weeks and the differences were statistically significant (*P*<0.05). In *COX-2*-1195G/A genotypes, the proportions of patients with GG and GA+AA genotypes who met ASAS50 and ASAS70 evaluation criteria were compared after 12 weeks and the differences were statistically significant (all *P*< 0.05). However, the proportions of ASAS20/50/70 score improvement for AS patients with different genotypes of CYP2C9*3 polymorphism were not significantly different between post-treatment score and pre-treatment score (all *P*>0.05).

Table 4. Clinical indicator changes before and after NSAIDs treatment.

Indicator	Cases	Before treatment	After treatment	P
Duration of morning stiffness (h)	72	0.65±0.05	0.32±0.02	<0.001
Nocturnal pain n (%)	77	69 (89.6)	34 (44.2)	<0.001
Number of peripheral joints involvement	107	0.63±0.04	0.25±0.03	<0.001
Peripheral joints involvement n (%)	107	26 (24.3)	10 (9.4)	0.003
Toe-floor distance (cm)	51	21.5±1.52	13±1.48	<0.001
Pilow-wall distance (cm)	47	2.51±0.06	1.46±0.02	<0.001
Degree of breast enlargement (cm)	39	4.85±1.20	4.71±1.48	<0.001
Schober test (cm)	34	3.62±1.65	4.32±1.23	<0.001
Lumbar vertebrae anteflexion	41	42.27±1.23	47.97±1.45	<0.001
Dorsal extension of lumbar vertebrae	41	12.04±3.43	18.99±0.13	<0.001
Left side bending of lumbar vertebrae	41	11.98±2.23	18.07±2.48	<0.001
Right side bending of lumbar vertebrae	41	12.01±2.22	18.05±2.46	<0.001
ESR (mm/h)	42	37.61±6.35	16.88±1.58	<0.001
ESR anomaly n (%)	42	35 (83.3)	26 (61.9)	0.024
CRP (mg/L)	31	17.65±1.08	8.84±1.05	<0.001
CRP anomaly (%)	31	27 (87.1)	18 (58.1)	0.011
BASDAI (VAS)	63	4.96±1.13	3.45±0.45	<0.001
BASDAI ≥4 n (%)	63	47 (74.6)	13 (20.6)	<0.001
BASFI (VAS)	63	4.25±0.15	1.87±0.12	<0.001
Overall evaluation (VAS)	63	4.54±1.21	2.67±0.75	<0.001
Backache/Nocturnal pain (VAS)	63	7.12±1.58	3.82±1.12	<0.001
Pain of peripheral joints (VAS)	63	2.17±0.58	1.1±0.08	<0.001
Degree of morning stiffness (VAS)	60	5.12±0.06	2.25±0.04	<0.001
Duration of morning stiffness (VAS)	60	4.5±0.15	1.85±0.12	<0.001

Table 5. The association of CYP2C9*3, *COX-2*-1290A/G and -1195G/A polymorphism with the efficacy of NSAIDs in treatment of AS.

Different genotypes comparison of efficacy	BASDAI		
	Before treatment	After treatment	P
CYP2C9			
*1/*3	4.17±0.58	4.14±0.12	0.840
*1/*3+*3/*3	4.24±0.46	4.19±0.86	0.158
-1290A/G			
AA	4.59±0.42	3.55±0.15	<0.001
AG+GG	5.47±0.16	2.96±0.58	<0.001
-1195G/A			
GG	5.01±0.62	3.76±0.44	<0.001
GA+AA	4.76±0.79	3.19±0.51	<0.001

Table 6. The association of CYP2C9*3, *COX-2*-1290A/G and -1195G/A polymorphism with the ASAS20/50/70 score changes of NSAIDs treatment for AS patients.

	ASAS20		ASAS50		ASAS70	
	>ASAS20	<ASAS20	>ASAS50	<ASAS50	>ASAS70	<ASAS70
CYP2C9*3						
*1/*3	8 (66.7%)	4 (33.3%)	7 (58.3%)	5 (41.7%)	3 (25%)	9 (75)
*1/*3+*3/*3	89 (68.5%)	41 (31.5%)	68 (52.3%)	62 (47.7%)	35 (26.9%)	95 (73.1%)
COX-2-1290A/G						
AA	94 (94.7%)	5 (5.3%)	46 (46.7%)	53 (53.3%)	24 (24.6%)	75 (75.8%)
AG+GG	22 (71%)*	9 (29%)	14 (45.2%)	17 (54.8%)	13 (41.9%)*	18 (58.1%)
COX-2-1195G/A						
GG	16 (69.4%)	7 (30.6%)	14 (60.9%)	9 (39.1%)	11 (47.8%)	12 (52.2%)
GA+AA	101 (94.6%)*	6 (5.4%)	42 (39.3%)*	65 (60.7%)	25 (23.2%)*	82 (76.8%)

Compare with genotype*1/*3 or AA or GG, * $P<0.05$.

Discussion

Due to its good treatment response and safety, NSAIDs have always maintained an important position in treatment of AS [16]. AS is a polygenic disease, mainly influenced by genetic factors [17,18]. Therefore, this study aimed to investigate the possible association of *CYP2C9* and *COX-2* polymorphism with the efficacy of NSAIDs in treatment of AS.

In this stud, we collected information on clinical indicator changes before and after NSAIDs treatment for AS patients. We found that morning stiffness, function index, laboratory examination, BASDA, and BASFI were all significantly improved. The remission rates of ASAS20 and ASAS40 reached 56.5% and 21.7%, respectively. The results are consistent with previous reports that confirmed the efficacy of NSAIDs in treatment of AS [19,20].

The results of this study show that *COX-2*-1290A/G and -1195G/A polymorphism can increase AS risk, suggesting that NSAIDs increase anti-inflammatory activities by selectively inhibiting *COX-2* [21]. Celecoxib, a selective COX-2 inhibitor, can significantly decrease the risks of adverse reactions in the intestines [22]. The changes in drug-related genes alter the pharmacokinetic process and affect the safety and efficacy of drugs [23]. A report showed that *COX-2* has wide polymorphisms and its mutation influences its transcription and decreases its activity [24]. Decreased enzyme activity disturbs the metabolism of NSAIDs and affects its efficacy. Consistent with the above results, NSAIDs decrease the catalytic capability of COX-2, influencing its catalytic power, thus reducing the synthesis of PGE2 [25,26]; therefore, NSAIDs can reduce pain and inflammation [27]. Clinical studies have found that *COX-2*-1195G/A polymorphism is related to susceptibility to many inflammatory, autoimmune, and neoplastic diseases [28,29]. In the promoter region of *COX-2*, the mutation of *COX-2*-1195G/A generates a c-MYB binding site, and A allele can increase the transcriptional activity of COX-2 and increase susceptibility to illness [30]. *COX-2*-1290A/G polymorphism and functional -1195G/A polymorphism are in unbalanced strong linkage, but *COX-2*-1195G/A polymorphism can obviously increase COX-2 expression and increase AS risks. Therefore, *COX-2*-1290A/G can influence the onset of AS.

Our study also found that CYP2C9*3 polymorphism may not be related with NSAIDs efficacy in the treatment of AS. The *CYP2C9* polymorphism has a great influence on pharmacokinetics. *CYP2C9* polymorphism can easily induce the change of CYP2C9 protein structure and generate drug metabolic genetic polymorphism [31]. CYP2C9*3 is a common allelic mutant generated in the coding region when amino acid residues are replaced because of single-base exchange. Compared with CYP2C9*1, metabolism of mutant CYP2C9*3 is easily induced or inhibited by drugs, thus decreasing its ability to metabolize drugs [32]. Studies show that the mutation of CYP2C9*3 not only decreases metabolic activity of diclofenac acid, but also influences its reaction to other drugs [33,34]. Some reports suggest that CYP2C9*3 causes decreased enzyme activity and inhibits substrate metabolism [35,36]. *CYP2C9* genetic polymorphism is dependent on different substrates, which may be why the results of this study are different from those of other studies.

Conclusions

COX-2-1290A/G and -1195G/A polymorphisms can increase AS risk, and they can be considered as biological indicators for

predicting the efficacy of NSAIDs in treatment of AS. However, the small sample size of our study may have contributed to bias. Therefore, studies with larger samples are needed to confirm the results of our study.

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