Original Article

Study of Clinical and Genetic Risk Factors for Aspirin-induced Gastric Mucosal Injury

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Abstract

Background: Current knowledge about clinical and genetic risk factors for aspirin-induced gastric mucosal injury is not sufficient to prevent these gastric mucosal lesions.

Methods: We recruited aspirin takers as the exposed group and healthy volunteers as the control group. The exposed group was categorized into two subgroups such as subgroup A as gastric mucosal injury diagnosed by gastroscopy, including erosion, ulcer or bleeding of the esophagus, stomach, or duodenum; subgroup B as no injury of the gastric mucosa was detected by gastroscopy. Clinical information was collected, and 53 single nucleotide polymorphisms were evaluated.

Results: Among 385 participants, 234 were in the aspirin-exposed group. According to gastroscopy, 82 belonged to subgroup A, 91 belonged to subgroup B, and gastroscopic results of 61 participants were not available. Using the Chi-square test and logistic regression, we found that peptic ulcer history (odds ratio [*OR*] = 5.924, 95% confidence intervals [*CI*]: 2.115–16.592), dual anti-platelet medication (*OR* = 3.443, 95% *CI*: 1.154–10.271), current *Helicobacter pylori* infection (*OR* = 2.242, 95% *CI*: 1.032–4.870), male gender (*OR* = 2.211, 95% *CI*: 1.027–4.760), GG genotype of rs2243086 (*OR* = 4.516, 95% *CI*: 1.180–17.278), and AA genotype of rs1330344 (*OR* = 2.178, 95% *CI*: 1.016–4.669) were more frequent in subgroup A than subgroup B. In aspirin users who suffered from upper gastrointestinal bleeding, the frequency of the TT genotype of rs2238631 and TT genotype of rs2243100 was higher than in those without upper gastrointestinal bleeding.

Conclusions: Peptic ulcer history, dual anti-platelet medication, *H. pylori* current infection, and male gender were possible clinical risk factors for aspirin-induced gastric mucosal injury. GG genotype of rs2243086 and AA genotype of rs1330344 were possible genetic risk factors. TT genotype of rs2238631 and TT genotype of rs2243100 may be risk factors for upper gastrointestinal bleeding in aspirin users.

Key words: Aspirin; Gastric Mucosal Injury; Risk Factors; Single Nucleotide Polymorphisms

Introduction

Aspirin (acetylsalicylic acid) was invented in 1897 and has been widely used as an anti-inflammatory drug for over 100 years. Due to the discovery of its anti-platelet function, low-dose aspirin, commonly defined as 75–325 mg daily, is now strongly recommended for primary or secondary prevention of cardiovascular events.[1,2]

However, physicians are concerned about the adverse effects of this drug. The most common adverse effects of aspirin are gastric mucosal injury and gastrointestinal bleeding,[3] which are often painless. Interestingly, among aspirin users, development of gastrointestinal symptoms and mucosal injury is variable, which indicates that some factors may promote this process. Thus, identification of factors related to aspirin-induced gastric mucosal injury could provide information needed to evaluate patients before aspirin use and possibly reduce or minimize aspirin-induced gastric mucosal injury and bleeding.

Recent studies indicate that aging and male gender are risk factors for aspirin-induced gastric mucosal injury and other factors, such as drug dose, drug combination, *Helicobacter pylori* infection, can also affect gastric mucosal injury.[4-6] However, the relationship between these injuries and other

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clinical features or laboratory results (e.g., clinical history, liver and kidney function, and hyperlipidemia) has not been investigated. In addition, studies have demonstrated that some genetic polymorphisms are related to aspirin-induced gastric ulcer and bleeding.[6-8] However, current knowledge about risk factors for aspirin-induced gastric mucosal injury is not sufficient to present patients with effective early assessment or prevent these gastric mucosal lesions. Therefore, this study collected information about basic clinical features and laboratory results of patients, including their genetic polymorphisms, and aimed to identify possible risk factors for aspirin-induced gastric mucosal injury.

Methods

We considered patients who visited the Peking University People's Hospital from March 2009 to March 2012 and reported taking aspirin as potential members of the exposed group. The inclusion criteria were as follows: (1) age ≥18, (2) had been using aspirin for at least 3 months or had stopped using aspirin within 3 months because of gastrointestinal bleeding (positive fecal occult blood test, melena, hematochezia, or hematemesis) or other severe gastrointestinal adverse effects (intolerable upper abdominal pain after taking aspirin). Exclusion criteria were as follows: (1) gastroscopy was contraindicated due to conditions such as acute myocardial infarction, uncontrolled angina, or acute cerebral infarction, (2) portal hypertension, (3) aspirin use was combined with use of nonsteroidal anti-inflammatory drugs (NSAIDs) or an anticoagulant, such as warfarin, (4) any ethanol consumption, and (5) previous upper abdominal surgery. Healthy volunteers with no history of aspirin use, no use of other medicine, no ethanol consumption, and no gastrointestinal diseases or gastrointestinal symptoms were recruited from hospital staff and the community as the control group. Informed consent was obtained from all subjects before their enrollment. Study procedures did not harm patients' health and were permitted by the Ethics Committee of Peking University People's Hospital.

Physicians who were blind to the status of the patients and had over 10 years of endoscopic experience performed the gastroscopies. The exposed group was categorized into two subgroups based on gastroscopy results. Subgroup A: Gastric mucosal injury was detected by gastroscopy, including erosion, ulcer or bleeding of the stomach or duodenum. Gastroduodenal ulcer was defined as a mucosal break ≥5 mm in diameter, and erosion was defined as a mucosal change < 5 mm in diameter covered with the white necrotic matter.[5] Subgroup B: No injury of gastric mucosa was detected by gastroscopy. Their gastroscopy results were normal or only showed basic manifestations of nonatrophic gastritis, such as erythema (point-like, sheet, or strip), rough mucous membrane, bleeding point, or mucosal edema.

Gastrointestinal bleeding was also evaluated in subgroup A (mucosal injury group), which was defined as one or more of these following status: (1) occult blood positive, (2) melena, and (3) hematemesis.

Data assessment

At enrollment, each participant's information was collected, including age, gender, clinical history (cardiac disease, cerebrovascular disease, hypertension, diabetes mellitus, fatty liver, gastrointestinal tumor, and peptic ulcer history), antacid medication, details of aspirin use (dose, time), dual anti-platelet medication (the combination of aspirin and clopidogrel), and laboratory results (white blood cell, hemoglobin, platelets, coagulation factors including prothrombin time, activated partial thromboplastin time, international normalized ratio, fibrinogen, liver function, kidney function, blood lipids, and serum gastrin). The presence of *H. pylori* infection in patients was determined by one of the following assays: (1) positive rapid urease test or positive findings on histologic examination of biopsies obtained during endoscopy procedures or (2) positive ^{13}C urea breath test.

Genotyping

Both exposed group and control group were evaluated for 53 single nucleotide polymorphisms (SNPs); reference SNP numbers (rs number), a specific number listed for each SNP, were searched and confirmed using PubMed (http://www.ncbi.nlm.nih.gov/snp). Some SNPs were selected based on previous research reports. For instance, SNPs of cyclo-oxygenase-1 (*COX-1*), *COX-2*, tumor necrosis factor-α (*TNF-α*), and interleukin-1β (*IL-1β*), which had been reported to be possibly related to aspirin-induced peptic ulcer,[6-8] were chosen. Then, we used Chinese Beijing (CHB) SNP genotype data downloaded from HapMap database (http://www.hapmap.ncbi.nlm.nih.gov) to exclude those SNPs with a genetic frequency of <0.05 in the Chinese population. Some experts have demonstrated that genetic polymorphisms of platelet membrane glycoproteins (GPI1, GPIba, GPIIIa, and GPIV) may influence the efficacy of aspirin or platelet responsiveness, and genetic polymorphisms of the thromboxane A2 receptor (TBXA2R), platelet-activating factor acetylhydrolase, and coagulation factor XIII were associated with platelet aggregation. However, clinical studies have not investigated whether these polymorphisms are risk factors that affect or thrombosis in patients receiving aspirin. Thus, we considered these genes and screened the tag SNPs of these genes using the program Haploview (http://www.broad.mit.edu/mpg/haploview/). A total of 53 SNPs of 11 genes were selected as listed in Table 1. SNPs were genotyped using the high-throughput Sequenom genotyping platform (Bio Miao Biological Company, Beijing, China). DNAs from exposed and control subjects were randomly assigned to the 96 well plates, and genotyping was performed blind to the status of the samples.

Statistical analysis

Values are presented as mean ± standard deviation (SD) or *n* (%). The genotyping quality of each SNP was first checked for Hardy–Weinberg equilibrium. SNPs that violated the equilibrium were excluded from further consideration. Haplotypes were reconstructed by SHEsis program

Table 1: SNP evaluated, listed by gene, rs number, and base pair

Gene	rs number	Base pair
COX-1	rs3842787	C/T
	rs1330344	T/C
	rs3842788	G/A
	rs5788	C/A
COX-2	rs5275	T/C
	rs20417	C/G
	rs689466	A/G
TNF-α	rs1800629	A/G
	rs361525	A/G
	rs1799964	T/C
CYP2C9	rs1057910	A/C
IL-1β	rs16944	C/T
PLA2G7	rs1362931	A/C
	rs9395208	C/G
	rs3799862	A/G
	rs1805017	A/G
	rs1421378	A/G
GP2	rs4078023	A/C
	rs4643330	A/G
	rs7185876	C/T
	rs4780877	C/T
	rs12922283	C/G
	rs7188098	C/T
GP1BA	rs2243100	C/T
	rs2243086	G/T
	rs9914087	A/G
	rs2243102	C/T
F13B (gene of coagulation factor XIII)	rs1412635	C/T
	rs10801586	C/T
	rs2990510	G/T
	rs13375369	A/G
F13A1 (gene of coagulation factor XIII)	rs4960171	C/T
	rs7766109	A/G
	rs3844196	A/G
	rs7770172	G/T
	rs1050782	C/T
	rs414247	C/T
	rs3024443	A/G
	rs3778355	C/G
	rs714408	A/G
	rs2295753	A/C
	rs900401	A/G
	rs3024429	C/T
	rs1742926	C/T
	rs434602	C/T
	rs6928884	A/G
	rs6597195	A/T
	rs3116567	A/G
	rs1267913	A/G
	rs7740009	C/T
	rs2274393	A/G
TBXA2R	rs2238631	C/T
	rs4807491	A/G

rs number: A specific number that identifies each SNP. SNP: Single nucleotide polymorphisms; *COX-1*: Cyclo-oxygenase-1; *COX-2*: Cyclo-oxygenase-2; *TNF-α*: Tumor necrosis factor-α; *CYP2C9*: Cytopigment 2C9; *IL-1β*: Interleukin-1β; *TBXA2R*: Thromboxane A2 receptor.

(http://analysis.bio-x.cn/SHEsisMain.htm, Bio-X Research Institute, Shanghai Jiao Tong University, Shanghai, China), which implemented an expectation maximization algorithm. Continuous variables were analyzed using the *t*-test, and categorical variables were analyzed using the Chi-square test. The risk of gastric mucosal injury was estimated by the odds ratio (*OR*) with 95% confidence intervals (*CI*) using univariate and multivariate logistic regression models. For all analyses, the level of significance was set at $P < 0.05$. Statistical analyses were performed using SPSS 19.0.0 (IBM Company, Chicago, IL, USA).

Results

Demographic and clinical characteristics

In the exposed group, 234 participants were enrolled, 110 were males and 124 were females, and their mean age was 64.91 ± 9.15 years old. Based on gastroscopy results, 82 patients were categorized as subgroup A, 91 patients were categorized as subgroup B, and the gastroscopy results of the other 61 participants were unavailable. Among subgroup A, 13 patients suffered from upper gastrointestinal bleeding. Among the 151 healthy volunteers in the control group, the mean age was 57.86 ± 10.74 years old.

Clinical features and aspirin-induced gastric mucosal injury

To analyze the clinical factors which could influence gastric mucosal injury in aspirin users, we compared the clinical characteristics of subgroup A and subgroup B, including age, gender, and medical history [Table 2]. Results indicated that the proportion of male gender ($P = 0.021$), previous cerebrovascular disease ($P = 0.028$), previous peptic ulcer ($P < 0.010$), and dual anti-platelet medication (the combination of aspirin and clopidogrel) ($P = 0.016$) in mucosal injury group were higher than that in the noninjury group.

Laboratory results of the two exposed subgroups were also compared but did not differ significantly.

Genetic polymorphisms and aspirin-induced gastric mucosal injury

We performed genotyping for 53 SNPs in 385 individuals as 234 aspirin users and 151 healthy control subjects. After checking for Hardy–Weinberg equilibrium, 5 SNPs (rs1805017, rs689466, rs2295753, rs3799862, and rs7188098) were excluded from further analysis.

The composition of rs1330344, rs361525, rs2990510, and rs3778355 genotype differed significantly between exposed and control groups [Table 3]. The proportions of AA genotype of rs1330344, GG genotype of rs361525, TT genotype of rs2990510, and CC genotype of rs3778355 in the exposed group, aspirin users were higher than in the healthy controls.

To identify possible adverse prognostic factors for gastric mucosal injury in aspirin users, further analysis between the two subgroups of the exposed group was

Table 2: Association between clinical characteristics and aspirin-induced gastric injury

Factors	Subgroup A, $n = 82$	Subgroup B, $n = 91$	χ^2/t	P
Age (years)	63.83 ± 8.95	65.03 ± 9.01	0.880	0.380
Male, gender	49 (59.7)	34 (37.4)	8.301	0.004
Previous cardiac disease	32 (39.0)	25 (27.5)	2.605	0.107
Previous cerebrovascular disease	15 (18.3)	30 (33.0)	4.826	0.028
Previous hypertension	50 (61.0)	49 (53.8)	0.896	0.344
Previous diabetes mellitus	19 (23.2)	23 (25.3)	0.104	0.747
Gastrointestinal tumor	0 (0)	2 (2.2)	–	0.498
Previous peptic ulcer	29 (35.4)	7 (7.7)	20.045	<0.010
Antacid medication[†]	31 (37.8)	34 (37.4)	0.004	0.952
Previous fatty liver	14 (17.1)	11 (12.1)	0.867	0.352
Current smoking	13 (15.9)	12 (13.2)	0.283	0.595
Aspirin dose ≥100 mg	70 (85.37)	81 (89.01)	0.516	0.472
Aspirin use duration >1 year	57 (69.51)	58 (63.74)	0.646	0.422
Dual antiplatelet medication[‡]	18 (21.95)	8 (8.79)	5.850	0.016
Helicobacter pylori current infection	37 (50.68)	29 (35.37)	3.707	0.054

Values are presented as mean ± SD or *n* (%). Continuous variables were analyzed using *t*-test, and categorical variables were analyzed using Chi-square test. *P*: To compare the clinical characteristics between aspirin users with and without gastric mucosal injury. The significance level was defined as $P<0.05$; [†]Proton pump inhibitor and H2 receptor antagonist; [‡]Patients were taking both aspirin and clopidogrel. SD: Standard deviation.

Table 3: The comparison of genetic frequencies between the exposed and control groups

SNP	A1	A2	Exposed group, $n = 233$[†] (A1A1/A1A2/A2A2)	Control group, $n = 151$ (A1A1/A1A2/A2A2)	χ^2	P
rs1330344	A	G	98/96/34	39/85/21	11.237	0.004
rs361525	G	A	221/11/0	131/15/0	4.282	0.039
rs2990510	G	T	2/48/178	1/48/96	6.738	0.034
rs3778355	C	G	79/105/44	33/82/30	6.209	0.045

P: Comparison of the frequencies of the genetic polymorphisms between aspirin users (exposed group) and healthy volunteers (control group). The significance level was defined as $P<0.05$; [†]Among the blood sample of the 234 participants of the aspirin-exposed group, 1 sample failed to meet the standard of the SNP test, thus, the number of samples tested was 233. SNP: Single nucleotide polymorphisms: Variations in nucleotides found in some locations of a gene in the human genome. A1, A2: The two different alleles that can be detected at the SNP location.

performed. Haplotypes were constructed using the program SHEsis, but no relationship was found between haplotypes and the gastric mucosal injury. rs12922283 and rs1330344 genotype frequency distribution between the two subgroups differed significantly ($P = 0.017$ and $P = 0.049$, respectively, Chi-square test). The frequency of GG genotype of rs12922283 was 73.75% in the gastric mucosal injury group, significantly higher than that in the noninjury group (59.09%). The frequency of AA genotype of rs1330344 was 58.75% in the gastric mucosal injury group, higher than that in the noninjury group (39.73%). The genotype frequency distribution of the other SNPs did not differ between subgroups.

Possible risk factors of aspirin-induced gastric mucosal injury

Logistic regression was then performed. Both clinical features and genetic polymorphisms possibly associated with gastric mucosal injury (based on $P < 0.200$ in univariate logistic regression) were entered into the multivariate models to not miss any possible risk factors. Statistical analysis showed that aspirin users with previous peptic ulcers, dual anti-platelet medication, current *H. pylori* infection, male gender, GG genotype of rs2243086, or AA genotype of rs1330344 had a higher risk of gastric mucosal injury [Table 4], indicating that these factors may be risk factors for aspirin-induced gastric mucosal injury.

Genotypes and gastrointestinal bleeding

In subgroup A, 13 subjects suffered from gastrointestinal bleeding. Compared with those who had only mucosal injury but no bleeding, we found that the TT genotype of rs2238631 ($\chi^2 = 6.239$, $P = 0.044$) and TT genotype of rs2243100 ($\chi^2 = 6.841$, $P = 0.033$) were more frequent in the bleeding group, indicating that the polymorphisms of these 2 SNPs might be related to gastrointestinal bleeding caused by aspirin.

Discussion

Gender and age

In an overview of epidemiologic studies published in the 1990s, Hernández-Díaz *et al*.[4] pointed out that male NSAIDs users developed gastrointestinal bleeding more often than female users. van Oijen *et al*.[7] also supported this association. We reached a similar conclusion that male gender may be an adverse prognostic factor of aspirin-induced gastric mucosal injury (*OR* = 1.980, 95% *CI* = 1.017–3.856).

Table 4: Possible adverse prognostic factors for aspirin-induced gastric mucosal injury

Factors	Wald statistic	*P*	*OR* (95% *CI*)
Previous peptic ulcer	11.461	<0.010*	5.924 (2.115–16.592)
Dual antiplatelet medication	4.913	0.027*	3.443 (1.154–10.271)
Current *Helicobacter pylori* infection	4.164	0.041*	2.242 (1.032–4.870)
Male, gender	4.115	0.043*	2.211 (1.027–4.760)
rs2243086 (GG/GT + TT)	4.850	0.028*	4.516 (1.180–17.278)
rs1330344 (AA/AG + GG)	4.000	0.045*	2.178 (1.016–4.669)

P: Based on Wald test, backward logistic regression. *The significance level was defined as $P<0.05$. *OR*: The relative odds of gastric mucosal injury given exposure to a specific clinical or genetic factor, among aspirin users. *OR* >1 indicates that the variable is a risk factor for gastric mucosal injury among aspirin users. *OR*: Odds ratio; *CI*: Confidence interval.

Though some experts hold the opinion that age was an important risk factor for gastriculcer caused by NSAIDs or aspirin, some others still treat this opinion with reserve. In a study of 903 aspirin users,[9] age group (higher vs. lower than 65 years of age) was not associated with upper gastrointestinal bleeding in the population. In our study, we also found no relationship between age and the risk of aspirin-induced gastric mucosal injury.

Previous peptic ulcer history

Some experts found that a history of peptic ulcer is an important adverse prognostic factor in NSAIDs-related gastric mucosal injury. In a meta-analysis that summarized 16 primary studies between 1975 and 1990, Gabriel *et al*.[10] found that history of gastrointestinal events was associated with an increased risk of peptic ulcers in NSAIDs users (*OR* = 4.800, 95% *CI*: 4.100–5.600). In addition, in a study of low-dose aspirin users, the author concluded that a history of peptic ulcer or upper gastrointestinal bleeding was associated with higher risk of upper gastrointestinal bleeding.[11] This study observed a similar association; aspirin users with a history of peptic ulcer had a greater risk of gastric mucosal injury (*OR* = 5.593, 95% *CI*: 2.180–14.348). It is worth noting, however, that a previous peptic ulcer will affect the risk of gastrointestinal events for all patients, not just the patients who take aspirin or other NSAIDs drugs. A large-scale retrospective case–control study concluded that patients with a history of peptic ulcer experienced a higher risk for gastrointestinal bleeding events, regardless of whether they used NSAIDs.[12]

Combined medicine

Studies have suggested that the combination of anticoagulants such as warfarin, corticosteroid, or other NSAIDs with aspirin can increase the risk of gastrointestinal damage and bleeding.[13] Because use of these combination medications is related to complex comorbidities that imply confounding factors, we only investigated one combination of anti-platelet medications, specifically the combination of aspirin and clopidogrel. Two studies[14,15] provided evidence on the bleeding risk associated with dual anti-platelet therapy, reporting relative risks of 1.38(95% *CI*: 1.130–1.670) and 1.57 (95% *CI*: 1.290–1.920), with the most common site of hemorrhage in gastrointestinal tract. Our study indicated that patients who took both aspirin and clopidogrel had a greater risk of mucosal injury than those who only took aspirin (*OR* = 3.458, 95% *CI*: 1.241–9.333). The combination of anti-platelet agents is widely used after percutaneous coronary intervention; thus, patients prescribed this combination merit greater attention strategies to avoid adverse gastrointestinal events.

Helicobacter pylori infection

The relationship between the aspirin-induced gastric mucosal injury and *H. pylori* infection remains controversial. Some experts believe that NSAIDs, such as aspirin, and *H. pylori* infection are two independent risk factors for gastric mucosal injury, and the coexistence of these two factors may significantly increase the risk of peptic ulcer bleeding. In their studies, both Pilotto *et al*.[16] and Lanas *et al*.[17] suggested that in aspirin users, the occurrence of peptic ulcer significantly was greater in those with *H. pylori* test positive. Similarly, in our study, we observed that among aspirin users those with *H. pylori* infection had a higher risk of gastric mucosal injury (*OR* = 2.094, 95% *CI*: 1.030–4.256). However, in another study, the authors[18] came to a conclusion that *H. pylori* eradication in long-term users of NSAIDs with past or current peptic ulcer or troublesome dyspepsia led to impaired healing of gastric ulcers and did not affect the rate of peptic ulcers or dyspepsia over 6 months. However, these studies differ in many ways, including goals, study design, methods, and definitions. Based on the aggravation on mucosal damage caused by *H. pylori* infection in aspirin users, the ACCF/ACG/AHA 2008 expert consensus document[19] recommended testing for and eradicating *H. pylori* in patients with a history of ulcer disease before starting long-term anti-platelet therapy.

Genetic polymorphisms and gastric mucosal injury caused by aspirin

In our comparison of genetic polymorphisms between aspirin users and healthy volunteers, the percentages of AA genotype of rs1330344, GG genotype of rs361525, TT genotype of rs2990510, and CC genotype of rs3778355 were higher in aspirin users, indicating that these genotypes may be related to some diseases in aspirin users.

COX-1 is a constitutively expressed enzyme that generates prostaglandins (PGs) and thromboxanes from arachidonic acid. PGs have a protective effect in the stomach, including acid secretion, production of mucus, regulation of mucosal blood flow, epithelial cell turnover and repair, and mucosal immunocyte function.[20] Aspirin prevents the production of PGs by irreversibly inhibiting platelet COX-1, causing gastric mucosal damage. Rs1330344 is located in the promoter of COX-1 gene. G is the major allele, and A is the minor allele. Arisawa *et al*.[6] reported that the genetic frequency of the A allele of rs1330344 was higher in patients who suffered from peptic ulcer than nonulcer patients (*OR* = 2.860, 95% *CI*: 1.290–6.340), and in NSAIDs users, the A allele was

also an important risk factor associated with peptic ulcer (*OR* = 5.800, 95% *CI*: 1.590–21.100). In contrast, Shiotani *et al*.[8] found that in aspirin users, the frequency of A allele did not differ significantly between the ulcer group and nonulcer group. Arisawa *et al*.[6] also found that in non-NSAIDs users with peptic ulcer, the frequency of the A allele of rs1330344 was also higher than in non-NSAIDs users without peptic ulcer, suggesting that this SNP may also be a risk factor for gastric mucosal injury itself, in the absence of NSAID use. Our analysis of this study data suggested that the AA homozygous genotype was associated with gastric mucosal injury in aspirin users (*OR* = 3.458, 95% *CI*: 1.241–9.333). However, further study is needed to understand the mechanism of this phenomenon. In other investigations, experts did not find that this allele was associated with mucosal atrophy or infiltration of inflammatory cells in the mucosa and speculated that the allele might cause gastric mucosal injury by destroying mucosal integrity rather than aggravating gastrointestinal inflammation.

The anti-platelet effects of aspirin may not be equal in all individuals. Some patients prescribed aspirin suffer recurrent thromboembolic vascular events, giving rise to the term "aspirin resistance." Recently, a number of studies have examined the association between aspirin resistance and several receptors on the surface of platelets,[21] such as genetic polymorphisms of platelet membrane glycoproteins, genetic mutations of TBXA2R, the platelet-activating factor acetylhydrolase, and coagulation factor XIII. In the presence of gastric mucosal injury caused by *H. pylori* infection or other factors, the anti-platelet function of aspirin contributes to gastrointestinal bleeding. It is hypothesized that when aspirin resistance occurs, the anti-platelet function of aspirin is reduced, and the risk of bleeding may be less than that of aspirin-sensitive patients.

Rs2243086 is an SNP located on *GP1BA* (gene for platelet membrane glycoprotein Ibα [GPIbα]). GPIbα, GPIX, and GPV form a complex that binds Von Willebrand factor, and thus plays an important role in the initial process of platelet aggregation. However, relevant studies that showing rs2243086 is associated with aspirin resistance are still lacking. Our study found that the GG genotype was associated with gastric mucosal injury in aspirin users (*OR* = 3.458, 95% *CI*: 1.241–9.333), but the mechanism remained unknown. Since GPIbα mainly influences platelet aggregation, we may evaluate the platelet aggregation ability between different genotypes to understand better the mechanisms involved.

Other SNPs were reported to be associated with peptic ulcer or mucosal damage in aspirin users. Polymorphisms of cytopigment 2C9, an enzyme related to aspirin metabolism, have been suggested to be responsible for gastrointestinal bleeding in patients taking aspirin,[22] and rs1057910 is a risk factor for gastrointestinal bleeding. However, in our study, the frequency of this SNP did not differ between the two subgroups. A possible reason for this finding is that the C allele of this SNP has a low frequency in the Chinese Han population; thus, it is difficult to reach a meaningful conclusion about its role in the Chinese population.

Some inflammatory cytokines are known to be associated with peptic ulcer disease, such as TNF-α, IL-1β, and IL-1RN, but there were few studies of their relationship with aspirin-induced peptic mucosal injury. The T allele of rs 16944 (an SNP of IL-1β) was detected in low-dose aspirin users,[8] and it was detected in 43.8% of the ulcer group and 52.1% of the nonulcer group, indicating that the T allele of rs16944 was a possible protective factor for aspirin-induced peptic ulcer. However, our study did not support this observation.

The up-regulation of TNF-α has been recognized as a risk factor for aspirin-induced mucosal injury, based on animal experiments.[23] Both Sugimoto *et al*.[24] and Lu *et al*.[25] found that some SNPs of the TNF-α gene may be associated with peptic ulcer and gastric cancer; however, there have been no investigations of the relationship between these SNPs and aspirin-induced gastric mucosal injury. In our study, we took rs1800629, rs361525, and rs1799964 (SNPs of the TNF-α gene) into consideration, but no meaningful differences were observed.

Genetic polymorphisms and gastrointestinal bleeding caused by aspirin

It is known that one reason aspirin can induce gastrointestinal bleeding is that this drug can inhibit platelet aggregation. Therefore, if a patient's gastric mucosal is already injured by alcohol, *H. polyri* infection, or other factors and is taking aspirin at the same time, the patient will be more likely to develop gastrointestinal bleeding with a genotype that can enhance the inhibition effect of platelet aggregation. In our study, analysis showed that rs2243100 and rs2238631 may be related to upper gastrointestinal bleeding caused by aspirin. rs2243100 is an SNP located on *GP1BA*, and rs2238631 is located on *TBXA2R* (gene for TBXA2R). Both genes are associated with platelet aggregation. However, the function of rs2243100 and rs2238631 in gene regulation remains unknown, and further study is still needed.

Our study has several limitations that should be taken into consideration when interpreting the data. The use of polypharmacotherapy in the treatment of patients with complex, comorbid illnesses may have influenced the outcome, despite the use of multivariate analysis. In addition, selection biases may be present in cross-sectional studies because exposure is measured in prevalent cases, whereas cohort studies and randomized controlled trials measure exposure in incident cases. In addition, we did not evaluate the frequencies of these gene polymorphisms in patients who did not take aspirin and suffer from gastric mucosal injury or peptic ulcer, so could not distinguish whether these SNPs are related to aspirin-induced gastric mucosal injury or mucosal injury itself.

In conclusion, history of peptic ulcer, dual anti-platelet medication, male gender, current *H. Pylori* infection, GG genotype of rs2243086, and AA genotype of rs1330344

were possible risk factors for gastric mucosal injury caused by aspirin. These findings may help us evaluate the risk of gastric mucosal injury in aspirin users more accurately, thus prevent adverse effect of using aspirin.

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Conflicts of interest

There are no conflicts of interest.

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