Frequency, Risk Factors, Prognosis, and Genetic Polymorphism of the Cyclooxygenase-1 Gene for Aspirin Resistance in Elderly Chinese Patients with Cardiovascular Disease

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Key Words

Aspirin resistance • Frequency • Risk factors • Prognosis • Cyclooxygenase-1 gene • Elderly Chinese patients • Cardiovascular disease

Abstract

Background: Cardiovascular disease (CVD) is an important cause of mortality in elderly patients worldwide. Aspirin resistance has been well reported in CVD. **Objective:** The frequency, risk factors, prognosis, and genetic polymorphism of the cyclooxygenase-1 (COX-1) gene for aspirin resistance have not been reported in elderly patients with CVD. We therefore undertook this study to evaluate these associations among elderly Chinese patients with CVD. **Methods:** Four hundred thirty-one elderly Chinese patients with CVD receiving daily aspirin therapy (≥ 75 mg) over 1 month were enrolled. Platelet aggregation was measured by light transmission aggregometry (LTA) and thromboelastography platelet mapping assay (TEG) using arachidonic acid (AA) as a stimulus. The median follow-up was 1.8 years. **Results:** After the median follow-up, aspirin-resistant patients were at an increased risk of the composite endpoint compared to nonresistant patients by $LTA_{AA} + TEG_{AA}$ (23.7 vs. 9.2%, $p =$

0.025). Additionally, Cox proportional hazards regression modeling demonstrated that aspirin resistance and cerebrovascular disease were associated with major adverse long-term outcomes (HR for aspirin resistance = 2.31, 95% CI 1.11–4.81, $p = 0.026$). The variant G-allele of COX-1 rs1330344 (–1676 A/G) significantly increased the risk of aspirin resistance defined by $LTA_{AA} + TEG_{AA}$ (OR = 1.82, 95% CI 1.13–2.92, $p = 0.01$). **Conclusions:** Aspirin resistance, evaluated by $LTA_{AA} + TEG_{AA}$, is associated with an increased risk of adverse clinical events in elderly Chinese patients with CVD. The variant G-allele of COX-1 rs1330344 is significantly associated with aspirin resistance defined by $LTA_{AA} + TEG_{AA}$.

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Introduction

Cardiovascular disease (CVD) is an important cause of mortality in elderly patients worldwide [1, 2]. Aspirin is the cornerstone of antiplatelet therapy in patients with CVD and has been reported to reduce the risk of ischemic

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events by 32% [3]. However, some studies have suggested that the antiplatelet effect of aspirin may not be uniform in all patients [4, 5]. Epidemiological surveys have discovered that 0.4–70.1% of patients have a poor response to aspirin [6], which is defined as aspirin resistance by different methods.

Recently, several meta-analyses revealed that aspirin-resistant patients had a significantly increased risk of death, myocardial infarction, or cerebrovascular events compared to nonresistant patients [7–10]. Possible mechanisms of aspirin resistance include poor compliance or an inadequate dose, increased platelet activity, upregulation of a nonplatelet source of thromboxane production, decreased bioavailability, drug interactions, smoking, hyperlipidemia, hyperglycemia, and genetic variability [11–15]. Despite ongoing research, there is currently no clear consensus on the definition and treatment of aspirin resistance.

Recently, we published our observations demonstrating that aspirin resistance or semiresponders, defined by light transmission aggregometry (LTA), are associated with an increased risk of adverse clinical events in elderly male patients with CVD [16]. However, there was no investigation of the association between aspirin resistance and polymorphisms of the Cyclooxygenase-1 (COX-1) gene. In the present study, we explored the relationship between aspirin resistance and polymorphisms of COX-1 and evaluated other associations with aspirin resistance among elderly Chinese patients with CVD.

Materials and Methods

Ethical Approval of the Study Protocol

This study complied with the Declaration of Helsinki. It was approved by the Scientific and Ethics Review Board of the Chinese PLA General Hospital (Beijing, PR China). All patients provided written informed consent prior to inclusion in the study.

Participants

Initially, we prospectively enrolled 479 patients from April 2008 to June 2010. Patients were recruited from the Wangshoulu area of Beijing. Patients were aged ≥ 65 years and were being treated for coronary heart disease, hypertension, peripheral arterial disease, or stroke; all patients were on regular treatment with aspirin (75–100 mg daily over 1 month). Patients' use of aspirin was determined by patient interview both at study enrolment and at follow-up. The exclusion criteria included: use of clopidogrel, ticlopidine, dipyridamole or other nonsteroidal anti-inflammatory drugs, or heparin or low-molecular-weight heparin; a major surgical procedure within 1 week of study enrolment; a family or personal history of bleeding disorders; platelet count $<150 \times 10^3/\mu\text{l}$ or $>450 \times 10^3/\mu\text{l}$; hemoglobin <8 g/dl; a history of myelo-

proliferative disorders, or a history of drug-induced thrombocytopenia. After enrollment, patients with poor compliance ($n = 22$), an inadequate dose (≤ 75 mg) ($n = 15$), and a history of taking other antiplatelet medications ($n = 11$) were excluded. Thus, 431 patients were eventually included in the present study.

Blood Sampling

Blood samples were collected from the antecubital vein into Vacutainer tubes 2–12 h after the administration of the last dose of aspirin. The first 2 ml of blood drawn by venipuncture was discarded. Four tubes of whole blood, anticoagulated with 3.2% sodium citrate and lithium heparin, were used for LTA and thromboelastography (TEG). One tube which contained lithium heparin was also used for TEG. In addition, one tube of blood anticoagulated with CTAD (a mixture of citrate, theophylline, adenosine, and dipyridamole) was used for measurements of CD62P (P-selectin) and PAC-1 (activated GP IIb/IIIa receptors). The tubes were filled to capacity and gently inverted 3–5 times to ensure complete mixture of the anticoagulant. Moreover, four conventional tubes were collected to determine: high-sensitivity C-reactive protein, type-B natriuretic peptide, and protein C; the percentage activity of antithrombin III; routine measurements of blood components and blood lipids, and other biochemical measurements. All assays were processed within 2 h of blood sampling.

Light Transmission Aggregometry

Blood samples were centrifuged at 800 rpm for 5 min to obtain native platelet-rich plasma and further centrifuged at 4,000 rpm for 8 min to obtain platelet-poor plasma. The platelet count of the platelet-rich plasma was assessed and adjusted to between $200 \times 10^3/\mu\text{l}$ and $300 \times 10^3/\mu\text{l}$ using platelet-poor plasma. Aggregation was performed using arachidonic acid (AA; 0.5 mM) with a Chrono-Log aggregometer (Chrono-Log, Havertown, Pa., USA).

TEG Platelet Mapping Assay

The TEG platelet mapping assay (Hemoscope, Niles, Ill., USA) relies on the measurement of platelet function through clot strength. AA (1 mmol/l) was added to activator F to measure the degree of thromboxane A_2 (TXA $_2$)-induced platelet aggregation. This methodology is described elsewhere [17].

Other Laboratory Analyses

The hemoglobin assay and platelet counts were performed on a SYSMEX-XE2100 analyzer (SYSMEX, Kobe, Japan). Serum creatinine, blood lipid, homocysteine, high-sensitivity C-reactive protein, type-B natriuretic peptide and uric acid, and other basic biochemical blood tests were measured by standard chemical and enzymatic commercial methods in a cobas 6000 analyzer series (Roche Diagnostics). The percentage activity of antithrombin III and protein C was tested by a STA-R Evolution coagulation analyzer (Stago, Paris, France).

Single-Nucleotide Polymorphism Detection

DNA samples were extracted from peripheral blood obtained from all patients by a standard phenol-chloroform method. Single-nucleotide polymorphism (SNP) detection was performed using the Sequenom MassARRAY iPLEX platform (Sequenom, USA). PCR primers and single base extension probes were designed using Assay Design 3.1 software from Sequenom. SNPs

were genotyped by the Sequenom MassArray system according to the manufacturer's instructions. PCR was performed using iPLEX chemistry according to the recommendations of the manufacturer as found in the MassArray iPLEX standard operating procedure. Data analysis was performed using MassArray Typer software version 4.0.

Definition of Aspirin Resistance

The definition of aspirin resistance was $\geq 20\%$ AA aggregation according to LTA [18]. Using TEG, aspirin resistance was defined as $\geq 50\%$ aggregation induced by AA [17]. Additionally, we used the combination of LTA ($\geq 20\%$ AA-aggregation) and TEG ($\geq 50\%$ AA aggregation) criteria to define aspirin resistance.

Study Endpoints

The primary end point was the composite of adverse clinical events, including death, myocardial infarction, stroke, transient ischemic attack, and unstable angina. Death was defined as death from vascular and nonvascular causes. Myocardial infarction was defined by the European Society of Cardiology/American College of Cardiology criteria [19]. Stroke was defined as an acute neurologic vascular event lasting more than 24 h. Transient ischemic attack was defined as an acute neurologic vascular event lasting less than 24 h. Unstable angina was defined according to the American College of Cardiology Foundation/American Heart Association criteria [20].

Follow-Up

The follow-up was completed in all patients by telephone interviews and review of medical records. Personnel performing data collection were blind to aspirin responsiveness. The follow-up time ranged from 1 to 3 years. The median follow-up time was 1.8 years.

Statistical Analyses

Continuous variables are expressed as means $\pm$ SD. Univariate tests or Kruskal-Wallis tests (if the distribution was not normal) were used to compare the continuous variables among the three groups defined by aggregation. For continuous variables, differences between the two groups were evaluated using Student's *t* test or Mann-Whitney *U* two-sample tests (if the distribution was not normal). Categorical data and proportions were analyzed using Pearson's χ^2 test or, in the case of small expected cell frequencies, exact tests. All SNPs evaluated in this study were tested for deviation from Hardy-Weinberg equilibrium with a χ^2 test. The association between COX-1 polymorphisms and aspirin resistance was analyzed in logistic regression models after adjustment for age and gender by SNP status in web and the pairwise *D'* and *r*² values were analyzed for linkage disequilibrium. Haploview 4.2 software was used to generate the maximum likelihood estimates of haplotype frequencies. OR and 95% CI were used to estimate associations of alleles and genotypes between cases and controls. $p < 0.05$ was considered statistically significant. Cumulative survival curves for both aspirin-resistant and nonresistant groups were constructed using the Kaplan-Meier method. Parameters significantly related to the presence of aspirin resistance were determined using binary logistic regression analyses. A Cox proportional hazards regression model was generated to describe risk factors for clinical events (SPSS, Windows, version 14.0; Chicago, Ill., USA).

Results

Patient Characteristics

Demographics, as well as a comparison of aspirin-resistant patients and nonresistant patients by LTA_{AA}, TEG_{AA}, and LTA_{AA} + TEG_{AA}, are provided in table 1. There were no differences between aspirin-resistant patients and nonresistant patients regarding sex, age, BMI and cardiovascular risk factors, and other biochemical measurements by LTA_{AA}.

According to TEG_{AA}, there were no significant differences between the aspirin-resistant and nonresistant groups when comparing: age; being a current smoker; having hypertension, coronary artery disease, cerebrovascular disease, or peripheral arterial occlusive disease, and the baseline platelet count. Aspirin-resistant patients had higher levels of fasting serum glucose, CD62P, and LDL cholesterol compared to nonresistant patients ($p = 0.006$, $p = 0.003$, and $p = 0.047$, respectively). Patients with aspirin resistance were less likely to take nitrate therapy than were nonresistant patients ($p = 0.018$).

Aspirin resistance and aspirin nonresistance as measured by LTA_{AA} + TEG_{AA} were not associated with significant differences with regard to: age, being a current smoker, having hypertension, coronary artery disease, or peripheral arterial occlusive disease, and the baseline platelet count. The number of patients with cerebrovascular disease was significantly higher in aspirin-resistant patients compared to nonresistant patients ($p = 0.035$).

Testing of Platelet Aggregation

According to LTA_{AA}, 59 (13.7%) elderly patients were found to be resistant to aspirin therapy. According to TEG_{AA}, 102 patients (23.7%) were aspirin resistant. Of the 102 patients who were aspirin-resistant by TEG_{AA}, 38 patients were aspirin resistant by LTA_{AA}. The kappa statistic between these two methods was 0.361. According to LTA_{AA} + TEG_{AA}, 38 (8.8%) patients were aspirin resistant. The kappa statistic between the two former methods and the combined methods was 0.757.

Multiple Logistic Regression Analysis

We did not find significant risk factors using LTA_{AA}. TEG_{AA} revealed that CD62P levels (OR = 1.01, 95% CI 1.00–1.02, $p = 0.028$), fasting serum glucose levels (OR = 1.32, 95% CI 1.09–1.59, $p = 0.003$), and administration of nitrates (OR = 0.44, 95% CI 0.23–0.85, $p = 0.016$) were significantly associated with aspirin resistance (table 2). Using LTA_{AA} + TEG_{AA}, cerebrovascular disease (OR = 1.67, 95% CI 1.17–2.39, $p = 0.005$) was a risk factor for as-

Table 1. Patient demographics by different methods

	AR (LTA _{AA}) (n = 59)	Non-AR (LTA _{AA}) (n = 372)	p value	AR (TEG _{AA}) (n = 102)	Non-AR (TEG _{AA}) (n = 329)	p value	AR (LTA _{AA} + TEG _{AA}) (n = 38)	Non-AR (LTA _{AA} + TEG _{AA}) (n = 393)	p value
Age, years	74.88 ± 9.01	73.98 ± 7.92	0.428	74.21 ± 8.21	74.08 ± 8.04	0.888	75.37 ± 9.92	74.98 ± 7.87	0.314
Current smoker, n (%)	5 (8.5)	31 (8.3)	0.566	6 (5.9)	30 (9.1)	0.302	4 (10.5)	32 (8.1)	0.612
Female, n (%)	19 (32.2)	129 (34.7)	0.710	43 (42.2)	105 (31.9)	0.057	11 (28.9)	137 (34.9)	0.464
Hypertension, n (%)	46 (77.9)	255 (68.5)	0.143	74 (72.5)	227 (69.0)	0.495	28 (73.7)	273 (69.5)	0.588
Coronary artery disease, n (%)	38 (64.4)	205 (58.1)	0.181	60 (58.8)	183 (55.6)	0.569	23 (60.5)	220 (60)	0.589
Cerebrovascular disease, n (%)	26 (40.7)	133 (35.8)	0.219	39 (38.2)	120 (36.5)	0.747	20 (52.6)	139 (35.4)	0.035
Diabetes, n (%)	12 (20.3)	123 (33.1)	0.050	34 (33.3)	101 (30.7)	0.616	9 (23.7)	126 (32.1)	0.288
PAOD, n (%)	5 (8.5)	35 (9.4)	0.524	12 (11.8)	28 (8.5)	0.322	5 (13.2)	35 (8.9)	0.388
BMI	25.35 ± 3.44	25.15 ± 3.56	0.691	25.10 ± 3.39	25.13 ± 3.81	0.943	24.95 ± 3.81	25.14 ± 3.71	0.765
Homocysteine, μmol/l	17.36 ± 6.71	17.17 ± 8.66	0.883	17.24 ± 7.53	17.19 ± 8.67	0.957	17.19 ± 5.04	17.20 ± 8.65	0.997
BNP, pg/ml	166.74 ± 214.02	124.38 ± 220.96	0.205	128.60 ± 189.57	130.25 ± 229.29	0.950	128.73 ± 162.63	129.95 ± 224.76	0.976
Hs-CRP, mg/dl	0.29 ± 0.26	0.47 ± 1.15	0.284	0.54 ± 1.49	0.42 ± 0.92	0.355	0.34 ± 0.30	0.45 ± 1.12	0.576
CD62P, %	17.29 ± 21.05	15.74 ± 20.86	0.600	21.20 ± 21.16	14.26 ± 19.42	0.003	19.46 ± 23.24	15.61 ± 20.62	0.278
PAC-1, %	37.88 ± 30.56	43.79 ± 27.90	0.141	46.93 ± 29.79	41.67 ± 27.75	0.104	42.43 ± 31.92	43.02 ± 27.98	0.903
Protein C activity, %	117.87 ± 41.885	118.19 ± 66.76	0.984	114.75 ± 36.31	119.04 ± 69.75	0.742	100.08 ± 31.04	119.73 ± 66.11	0.311
Antithrombin III activity, %	104.15 ± 14.59	105.02 ± 14.04	0.677	105.61 ± 14.34	104.70 ± 14.05	0.586	102.30 ± 16.51	105.13 ± 13.87	0.269
Creatinine, μmol/l	82.64 ± 25.90	78.74 ± 20.40	0.191	77.76 ± 22.33	81.59 ± 39.60	0.191	83.73 ± 25.29	80.38 ± 37.17	0.587
Fasting serum glucose, mmol/l	6.14 ± 1.21	6.07 ± 1.41	0.764	6.41 ± 1.58	5.98 ± 1.30	0.006	6.23 ± 1.32	6.07 ± 1.39	0.498
Total cholesterol, mmol/l	4.80 ± 1.04	5.04 ± 2.53	0.484	5.16 ± 1.24	4.95 ± 2.64	0.438	4.86 ± 0.98	5.02 ± 2.48	0.689
Triglyceride, mmol/l	1.74 ± 0.82	1.58 ± 0.71	0.128	1.71 ± 0.83	1.58 ± 0.69	0.130	1.76 ± 0.93	1.59 ± 0.71	0.169
HDL cholesterol, mmol/l	1.28 ± 0.36	1.31 ± 0.36	0.495	1.30 ± 0.36	1.31 ± 0.36	0.841	1.28 ± 0.37	1.31 ± 0.36	0.664
LDL cholesterol, mmol/l	2.81 ± 0.84	2.88 ± 0.86	0.581	3.02 ± 1.04	2.82 ± 0.79	0.047	2.85 ± 0.85	2.87 ± 0.86	0.857
Uric acid, μmol/l	325.71 ± 96.47	325.65 ± 86.42	0.997	328.87 ± 85.15	325.59 ± 88.43	0.981	357.09 ± 77.51	323.40 ± 87.88	0.075
Platelet count, × 10 ³ /μl	217.57 ± 77.62	206.87 ± 55.19	0.206	215.00 ± 67.57	206.32 ± 55.80	0.206	220.00 ± 79.82	207.25 ± 56.43	0.220
Medications taken									
Statins, n (%)	18 (30.5)	94 (25.3)	0.394	30 (29.4)	82 (24.9)	0.367	13 (34.2)	99 (25.2)	0.226
ACEIs/ARBs, n (%)	11 (18.7)	80 (21.5)	0.617	27 (26.5)	64 (19.5)	0.102	9 (23.7)	82 (20.8)	0.684
Nitrates, n (%)	10 (16.9)	86 (23.1)	0.290	14 (13.7)	82 (24.9)	0.018	6 (15.8)	90 (22.9)	0.314
CCBs, n (%)	25 (42.4)	132 (35.5)	0.307	36 (35.3)	121 (36.7)	0.786	12 (31.6)	145 (36.9)	0.537
Daily aspirin dose, n (%)									
75 mg	26 (44.1)	131 (35.2%)	0.189	44 (36)	113 (34.3)	0.107	15 (39.5)	142 (36.1)	0.683
100 mg	33 (55.9)	241 (64.8%)	0.189	58 (64)	216 (65.7)	0.107	23 (60.5)	251 (63.9)	0.683

AR = Aspirin resistance; non-AR = non-aspirin resistance; PAOD = peripheral arterial occlusive disease; Hs-CRP = high-sensitivity C-reactive protein; BNP = type-B natriuretic peptide; ACEIs = angiotensin-converting enzyme inhibitors; ARBs = angiotensin receptor blockers; CCBs = calcium channel blockers.

pirin resistance (table 2). Additionally, Cox proportional hazards regression modeling demonstrated that aspirin resistance (HR = 2.31, 95% CI 1.11–4.81, $p = 0.026$) and cerebrovascular disease (HR = 1.71, 95% CI 1.38–2.12, $p < 0.001$) were associated with major adverse long-term outcomes by LTA_{AA} + TEG_{AA} (table 2).

Follow-Up, Clinical Vascular Events

During the follow-up period, the frequency of cardiovascular-related events was high in aspirin-resistant patients compared with nonresistant patients by LTA_{AA} (16.9 vs. 9.4%). By TEG_{AA}, aspirin-resistant patients had more cardiovascular events compared with nonresistant patients (12.7 vs. 9.7%). The frequency of cardiovascular-related events was higher in patients with aspirin resis-

tance as compared to nonresistant patients by LTA_{AA} + TEG_{AA} (23.7 vs. 9.2%, $p = 0.025$) (table 3). The Kaplan-Meier curves of the probability of survival without clinical events reached a significant difference between the two groups by LTA_{AA} + TEG_{AA} ($p < 0.001$) (fig. 1).

Genetic Association with Aspirin Resistance

The correlation of aspirin resistance with 6 SNPs, including rs1888943 (–8759C/T), rs1330344 (–1676A/G), rs3842787 (exon 2, 50C/T), rs5787 (exon 4, 323G/A), rs5789 (exon 7, 709C/A), and rs5794 (exon 10, 1330G/A), in COX-1 genes was investigated. rs1888943, rs3842787, rs5787, rs5789, and rs5794 variants were rare in the present study and did not reach statistical significance between aspirin-resistant patients and nonresistant patients

by the different modes. The variant G-allele of COX-1 rs1330344 (–1676 A/G) showed an increased risk tendency for aspirin resistance defined by LTA_{AA} or TEG_{AA} ($p = 0.06$) (table 4). However, the variant G-allele of rs1330344 significantly increased the risk of aspirin-resistant patients compared with nonresistant patients ($p = 0.01$) by LTA_{AA} + TEG_{AA} (table 4).

Discussion

Among patients with CVD, the present study showed, for the first time, that the frequency of aspirin resistance by LTA_{AA} + TEG_{AA} was 8.8% in elderly patients. However, the frequency of aspirin resistance evaluated by LTA_{AA} or TEG_{AA} was reported as 13.7 and 23.7%, respectively, which was high compared with the combined methods. Because of the lack of a standardized approach to diagnosis, a different frequency of aspirin resistance has been observed in previous studies when using different methods for the detection of aspirin resistance.

Some studies showed that the frequency of aspirin resistance by LTA ranged from 4 to 60% [11, 18]. The frequency of aspirin resistance measured by TEG was reported to be from <1 to 33% [21–23]. The frequency of aspirin resistance assessed by PFA-100 was 9.5–46.8% [24, 25]. On the other hand, several studies revealed that aspirin resistance may be overestimated using methods that do not use AA as a stimulus [16, 20]. We therefore used the combined methods to evaluate the frequency of aspirin resistance. Interestingly, the frequency of cardiovascular-related events was high in aspirin-resistant patients by LTA_{AA} + TEG_{AA} compared with aspirin-resistant patients by LTA_{AA} or TEG_{AA}. However, the present study demonstrated that aspirin-resistant patients defined by LTA_{AA} + TEG_{AA} had a significantly increased risk of clinical events. Furthermore, when aspirin resistance was defined by a single method, LTA_{AA} or TEG_{AA}, aspirin resistance showed an association with COX-1 variants. However, aspirin resistance defined by LTA_{AA} + TEG_{AA} was significantly associated with COX-1 variants. These results indicate that aspirin resistance may not be overestimated by the combined methods of LTA_{AA} + TEG_{AA}.

Using multivariate logistic regression analysis, we demonstrated that cerebrovascular disease, fasting serum glucose levels, CD62P levels, and administration of nitrates was associated with aspirin resistance in elderly patients. The number of patients with cerebrovascular disease was significantly higher in those with aspirin re-

Table 2. Results of multiple logistic regression and Cox proportional hazards regression analysis by different methods

a OR for aspirin resistance by TEG_{AA}

	OR (95% CI)	P value
Univariate analysis		
Sex	1.55 (0.98–2.45)	0.058
CD62P levels	1.01 (1.00–1.02)	0.004
Fasting serum glucose levels	1.22 (1.05–1.41)	0.009
LDL cholesterol levels	1.30 (1.00–1.68)	0.048
Nitrates therapy	0.43 (0.23–0.81)	0.009
Multivariate analysis		
CD62P levels	1.01 (1.00–1.02)	0.028
Fasting serum glucose levels	1.32 (1.09–1.59)	0.003
Nitrates therapy	0.44 (0.23–0.85)	0.016

b OR for aspirin resistance by LTA_{AA} + TEG_{AA}

	OR (95% CI)	P value
Univariate analysis		
Cerebrovascular disease	1.44 (1.09–1.91)	0.010
Uric acid	1.01 (1.00–1.02)	0.072
Multivariate analysis		
Cerebrovascular disease	1.67 (1.17–2.39)	0.005

c HR of the composite endpoint according to LTA_{AA} + TEG_{AA} by Cox proportional hazards regression

	OR (95% CI)	P value
Univariate analysis		
Diabetes	1.27 (1.01–1.59)	0.034
Aspirin resistance	2.44 (1.36–4.39)	0.003
Cerebrovascular disease	1.72 (1.39–2.11)	<0.001
Multivariate analysis		
Aspirin resistance	2.31 (1.11–4.81)	0.026
Cerebrovascular disease	1.71 (1.38–2.12)	<0.001

sistance than in nonresistant patients in the present study. Previous studies showed that the frequency of aspirin resistance was not low in a stroke population (12–56%) [26–28]. Furthermore, Englyst et al. [29] demonstrated that aspirin resistance was associated with stroke severity. Unfortunately, one study demonstrated that clinical events (including myocardial infarction, vascular death, and stroke) occurred in 40% of aspirin-resistant patients compared with 4.4% of nonresistant patients ($p < 0.001$), which enrolled 180 post-ischemic stroke patients and followed them over 2 years [30]. The present study showed

Table 3. Occurrence of composite endpoints in aspirin-resistant and nonresistant patients

	LTA _{AA}		p value	TEG _{AA}		p value	LTA _{AA} + TEG _{AA}		p value
	AR (n = 59)	non-AR (n = 372)		AR (n = 102)	non-AR (n = 329)		AR (n = 38)	non-AR (n = 393)	
Events, n (%)	8 (13.6)	37 (9.9)	0.399	13 (12.7)	32 (9.7)	0.384	9 (23.7)	36 (9.2)	0.025
MI, n	3	7	0.145	3	7	0.434	2	8	0.218
Hospitalization for UA, n	2	7	0.355	2	7	0.662	2	7	0.184
Stroke, n	2	15	0.642	5	12	0.565	4	13	0.053
Hospitalization for TIA, n	1	8	0.645	3	6	0.362	1	8	0.568

MI = Myocardial infarction; UA = unstable angina; TIA = transient ischemic attack; AR = aspirin resistance; non-AR = non-aspirin resistance.

Fig. 1. Kaplan-Meier curves of the probability of freedom from composite endpoints according to response to aspirin therapy. Composite endpoints included myocardial infarction, stroke, transient ischemic attack, and unstable angina. Patients were defined as aspirin resistant by different methods.

that a history of cerebrovascular disease was an independent risk factor for clinical events. These results suggest that we should not ignore patients with a history of cerebrovascular disease.

Several studies have shown an association between diabetes and aspirin resistance in humans and in animal models [31, 32]. In most of these studies, an association between aspirin resistance and elevated HbA1c and increased fasting serum glucose levels was reported in diabetic patients [33–35]. The present study also found that fasting serum glucose level was a risk factor for aspirin resistance. Hyperglycemia may interfere with the acetylation process of platelets by aspirin, which may contribute

to aspirin resistance in the presence of diabetes [13]. These studies suggest that glycemic control in CVD patients complicated by diabetes may improve the anti-thrombotic effects of aspirin.

CD62P is used as a marker of platelet activation. Dropinski et al. [36] revealed that P-selectin (CD62P) expression was increased in aspirin-treated aspirin-resistant patients with coronary artery disease compared with aspirin-sensitive patients or aspirin-treated healthy subjects. Andersen et al. [25] showed that the levels of soluble P-selectin (CD62P) were significantly higher in aspirin-resistant patients as compared to nonresistant patients in a group of 129 patients with acute myocardial

Table 4. Comparison of the SNP allele changes in the COX-1 gene by different diagnostic criteria

	LTA _{AA}			TEG _{AA}			LTA _{AA} + TEG _{AA}		
	AR vs. non-AR			AR vs. non-AR			AR vs. non-AR		
	case (n = 59)	control (n = 372)	p value OR (95% CI)	case (n = 102)	control (n = 329)	p value OR (95% CI)	case (n = 38)	control (n = 393)	p value OR (95% CI)
rs1888943									
Allele			0.49			0.33			0.59
C	118	741		204	655		76	783	
T	0	3		0	3		0	3	
rs1330344									
Allele			0.06			0.06			0.01
A	61	451	0.69 (0.47–1.02)	110	402	0.74 (0.54–1.02)	35	477	0.55 (0.34–0.88)
G	57	291	1.45 (0.95–2.14)	94	254	1.35 (0.99–1.86)	41	307	1.82 (1.13–2.92)
rs3842787									
Allele			0.57			0.43			0.66
C	118	740		204	654		76	782	
T	0	2		0	2		0	2	
rs5787									
Allele			0.37			0.21			0.49
G	118	737		202	653		76	783	
A	0	5		0	5		0	1	
rs5789									
Allele			0.69			0.58			1
C	118	741		1	655		76	383	
A	0	1		0	204		0	0	
rs5794									
Allele			1			1			1
G	118	742		204	656		76	784	

AR = Aspirin resistance; non-AR = non-aspirin resistance.

infarction. The present study agreed with these findings. These results imply that aspirin resistance may be associated with increased platelet reactivity.

Holmes et al. [37] showed that chronic therapy with either isosorbide 5' mono-nitrate or transdermal nitroglycerine is associated with the development of vascular tolerance. Despite the induction of vascular tolerance, platelet responsiveness to nitroglycerine and sodium nitroprusside remains unaffected [37]. The present study demonstrated that administration of nitrates was a protective factor for aspirin resistance in elderly patients. This may be associated with increased production of exogenous nitric oxide, which can inhibit platelet aggregation. These findings suggest that nitrate therapy may improve the antiplatelet effects of aspirin.

Recently, Krasopoulos et al. [7] evaluated 2,930 patients with CVD receiving aspirin therapy for secondary prevention from 20 studies. The frequency of cardiovascular-related events including death, acute coronary syn-

dromes, new cerebrovascular episodes, or failure of vascular intervention was higher in aspirin-resistant patients than in aspirin-sensitive patients (39 vs. 16%; OR = 3.85), as measured by various laboratory methods [7]. Snoep et al. [8] evaluated the rate of recurrence of cardiovascular events in 2,296 patients from 16 studies. The pooled OR for clinical ischemic events and the resulting OR for laboratory aspirin resistance was 4.37 (95% CI 2.19–8.73), as assessed by different methods. When studies with all clinical events were pooled, the pooled OR was 3.78 (95% CI 2.34–6.11) in aspirin-resistant patients [8]. Two meta-analyses have associated the occurrence of adverse clinical events with laboratory aspirin resistance defined by the PFA-100. A systematic review of 53 studies with 6,450 patients showed that aspirin-resistant patients were more likely to have vascular events than were nonresistant patients (relative risk 1.63; 95% CI 1.16–2.28) [9]. Similarly, in another meta-analysis of 8 studies (n = 1,227), the global OR was 2.1 (95% CI 1.4–3.4) for the recurrence of an

ischemic event in aspirin-resistant patients, as evaluated by the PFA-100 [10]. The present study also found that aspirin-resistant patients had a significantly increased risk of cardiovascular events compared with nonresistant patients by $LTA_{AA} + TEG_{AA}$. These results suggest that laboratory aspirin resistance may be associated with the occurrence of adverse clinical outcomes.

Many studies focus on pharmacogenetic factors related to aspirin resistance. Genetic variants of COX-1, COX-2, platelet glycoproteins GPIa, GPIb, and GPIIb/IIIa, coagulation factor XIII, and TXA2 receptor have been reported in patients with aspirin resistance [38–40]. However, the data are inconsistent regarding C50T polymorphisms of COX-1 rs3842787, which may be because of race, inadequate assessment of phenotype and risk factors, or lack of a gold standard method for diagnosis. A much larger number of subjects is needed to clarify the association between aspirin resistance and pharmacogenetics.

COX-1 is a target enzyme of aspirin. COX-1 gene polymorphisms may affect the action of the enzyme, as well as its interaction with aspirin [41]. Aspirin resistance detected by COX-1-specific tests should be completely consistent with the aspirin resistance phenotype, as well as with COX-1 genetic variants, according to the primary principle that phenotype is determined by genotype. In addition, the gene difference in COX-1 associated with aspirin resistance significantly varies with racial status. Data from a review by Agúndez et al. [42] suggested that the major COX-1 targets in aspirin resistance are rs3842787 and rs5789 for European subjects, and rs3842789 and rs3842792 for African subjects. The present study showed that COX-1 rs1330344 is the target SNP for the Chinese Han population. rs1888943, rs3842787, rs5787, rs5789, and rs5794 are rare in Han. The promoter –1676 G allele appears to contribute significantly to aspirin resistance in the Chinese Han population. rs1330344 has a high frequency in the Chinese population. The frequency of minor allele –1676G is 0.40. In the Japanese population, rs1330344 is also highly prevalent, and the –1676C allele has a protective effect against functional dyspepsia in Japanese women; –1676A is a risk factor for aspirin-induced peptic ulcer in Japanese [43, 44]. Moreover, the gene differences for COX-1 are highly consistent between the Chinese and Japanese populations. Thus, rs1330344 may be a common tag SNP in the COX-1 gene for Asian patients with aspirin resistance. We should pay more attention to elderly Chinese patients with gene differences for COX-1 and evaluate the genetic polymorphism of COX-1 in aspirin-resistant patients. Establishing the relationship between aspirin resistance and ge-

netic polymorphism of COX-1 may provide more effective and less hazardous treatment strategies in patients with aspirin resistance.

There are several possible therapeutic options of intervention in genetic polymorphism of COX-1 for aspirin resistance. Firstly, increasing the dose of aspirin may possibly reduce the rate of the poor response to aspirin in individual patients [45]. Secondly, aspirin may be switched to clopidogrel, prasugrel, or cilostazol, which have been found to result in decreased rates of clinical events in patients with CVD [46–48]. Thirdly, adding clopidogrel or other antiplatelet drugs to aspirin is another approach. Further studies are needed to focus on the relationship between aspirin dose and genetic polymorphism of the COX-1.

The present study had several limitations. Firstly, the frequency of aspirin resistance in elderly male patients with CVD was valid for the dose of 75–100 mg/day of aspirin, but we did not investigate the other suggested doses of 162 mg/day and 325 mg/day. Secondly, the study was conducted in the Chinese Han population, and the relevance and applicability of these results to other populations is unknown. Thirdly, the relatively low number of subjects may have reduced the ability of this study to detect and relate rarer gene variants with aspirin resistance. Fourthly, there is no universally agreed upon way to assess aspirin resistance, and this study relied on 3 different measures.

In conclusion, we found that the frequency of aspirin resistance in elderly Chinese patients with CVD is considerably higher in patients with cerebrovascular disease, higher serum levels of fasting serum glucose and CD62P, and changes in the medication taken. Aspirin resistance, evaluated by $LTA_{AA} + TEG_{AA}$, is associated with an increased risk of clinical events in elderly Chinese patients with CVD. The variant G-allele of COX-1 rs1330344 is significantly associated with aspirin resistance defined by $LTA_{AA} + TEG_{AA}$ in the present study. These findings may provide important information for individualized aspirin therapy.

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Disclosure Statement

The authors have no conflicts of interest with this paper.

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