Journal of Huazhong University of Science and Technology ［Med Sci］
DOI 10.1007/s11596-007-0611-2
27 (6): 664-667, 2007

Association of the Platelet Membrane Glycoprotein Ⅰa C807T Gene Polymorphism with Aspirin Resistance

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Summary: To explore the correlation between the C807T polymorphism of platelet membrane glycoprotein Ⅰa (GPⅠa) gene and aspirin resistance in Chinese people, 200 patients with high-risk of atherosclerosis took aspirin (100 mg/d) for 7 days. Platelet aggregation function was detected using adenosine diphosphate (ADP) and arachidonic acid (AA) before and after the administration of aspirin. Then the subjects were divided into three groups according to the results of platelet aggregation function: an aspirin resistant (AR) group, an aspirin semi-responder (ASR) group and an aspirin-sensitive (AS) group. Platelet GPⅠa gene 807CT polymorphism was examined by means of polymerase chain reaction-sequence specific primers (PCR-SSP). The results showed that T allelic frequency in AR group and ASR group were higher that of AS group (P<0.005), and the prevalence of genotypes (TT+TC) of these two groups was significantly higher than that in AS group (P<0.05). Platelet GPⅠa T allele was significantly associated with aspirin resistance as revealed by multiple logistic regression (OR=3.76, 95% CI: 2.87–9.58). The results suggest that inherited platelet GPⅠa variations may have an important impact on aspirin resistance and the presence of GPⅠa T allele may be a marker of genetic susceptibility to aspirin resistance.
Key words: platelet membrane glycoprotein; aspirin resistance; genetic polymorphism; atherosclerosis

Aspirin is a potent anti-platelet drug which is widely used for primary and secondary prevention of ischemic vascular diseases. It is believed that aspirin can reduce about 20%–40% of ischemic vascular diseases. Despite its well-documented benefits, approximately 30% of the total population has been shown to develop an inadequate response to the platelet inhibitory effect of aspirin, which was named "Aspirin Resistance".

Recent studies have showed that several prothrombotic genetic variations may contribute to aspirin resistance, and increased risk of cardiovascular events[1] and they include (1) the C807T or A873G polymorphism allied with increased density of platelet GPⅠa-Ⅱa collagen-receptor gene, (2) polymorphism PLA1/A2 of the gene encoding glycoprotein Ⅲa and (3) polymorphism on the cyclooxygenase-1 (COX-1) gene affecting Ser529. Because of the possible increased risk of ischemic vascular events, carriers of these genetic polymorphisms may be resistant to the anti-platelet effects of aspirin.

However, the reports have been scanty on the relationship between the C807T polymorphism of platelet membrane GPⅠa gene and the clinical effects on aspirin. The aim of this study was to further investigate the possible association between the platelet GPⅠa gene polymorphism and the occurrence of aspirin resistance, as well as the prevalence of patients with aspirin-insensitive platelet aggregation of Chinese population.

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1 SUBJECCTS AND METHODS

1.1 Samples

The prevalence of the C807T polymorphism of platelet membrane GPⅠa gene was assessed in 200 patients with high-risk of atherosclerosis (86 females, 114 males, with age ranging from 54－76 y). They were selected from the Medical Examination Center of the Third Affiliated Hospital of He'nan University of Science and Technology, Luoyang, China, and was compared with 100 healthy blood donors from Luoyang City, from January 2004 to June 2005.

The patients with high-risk of atherosclerosis took aspirin (100 mg/d) for 7 days. Approximately 5 mL of blood was obtained in the morning after an overnight fasting, and then platelet aggregation function was detected with 5 μmol/L adenosine diphosphate (ADP) and 500 μmol/L arachidonic acid (AA) serving as inductor before and after taking aspirin.

Key demographic and clinical features of the subjects were recorded for all recruited individuals, including age, gender, history of diabetes, primary hypertension, hyperlipemia, current smoking habit, platelet count and recently used medication (include ticlopidine, clopidogrel, low molecular weight heparin and other drugs that can effect the function of platelet).

Exclusion criteria included the history of haemorrhage in the individual or family, platelet count ≥450×10^9/L or ≤100×10^9/L, haematoglobin ≤80 g/L, myelodysplastic syndrome, malignant plasma cell dyscrasia, having receiving operation within the past month; taking drugs that can effect the function of platelet such as ticlopidine, NSAID, clopidogrel, low molecular heparin, warfarin etc within the preceding 1 week.

1.2 Methods

1.2.1 The Laboratory Criteria for Maximum Agglutination of Platelets Healthy blood donors (*n*=100) were taken as control group, and platelet aggregation function was detected by using 5 μmol/L ADP and 500 μmol/L AA as inductor[2]. The normal values of ADP-induced and AA-induced maximum platelet aggregation for healthy adults was set at (52.1±9.0)% and (60.3±5.7)%, respectively.

1.2.2 Evaluation Criteria for Aspirin Resistance The aspirin resistance was defined as ADP-induced platelet maximum agglutination≥(52.1±9.0)% and AA-induced platelet maximum agglutination≥(60.3±5.7)% after the patients with high-risk of atherosclerosis took aspirin (100 mg/d) for 7 days. Aspirin semi-responder (ASR) was defined as those who satisfied either of the above-mentioned criteria.

1.2.3 Genotyping of GP Ⅰa C807T Dimorphism Leukocyte DNA was isolated from whole blood by using standard procedures. The GP Ⅰa-specific polymerase chain reaction (PCR) primers used in this study were constructed on the basis of the published GP Ⅰa cDNA15 and GP Ⅰa gene sequences[3]. About 0.1–1.0 μg genomic DNA was added to a 50 μL of reaction mixture containing 10 mmol/L Tris (pH 8.0), 50 mmol/L KCl, 2.75 mmol/L $MgCl_2$, 0.125 mmol/L of each dNTP, 0.25 μmol/L each of sense primer (5'-GACAGCCCATTA ATAAATGTCTCCTCTG-3') and sequence-specific anti-sense primer (5'-CCTTGCATATTGAATTGCTA CG-807-3' or 5'-CCTTGCATATTGAATTGCTACA-807-3') and 2.5 U Taq DNA polymerase. After initial denaturation at 95°C for 10 min, amplification was performed in a DNA thermocycler for 35 cycles (denaturation at 93°C for 50 s, annealing at 56°C for 30 s, and extension at 72°C for 15 s). A final extension of 5 min at 72°C completed the PCR. The PCR products were analyzed by electrophoresis on 2.0% agarose gels using Tris-acetate/EDTA buffer for about 30 min (8 V/cm) and visualized by ethidium bromide staining. DNA molecular marker Ⅰ was used as the standard (Beijing Tianwei Corporation, China). Amplification of genomic DNA yielded 184 bp specific products (detected by United Gene Holdings Ltd, Shanghai, China) (fig. 1).

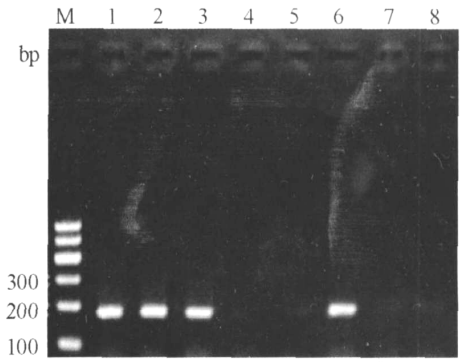

Fig. 1 Representative results of GP Ⅰa genotypes determined by PCR-SSP of three individuals
lanes 1, 2: person 1 (heterozygous CT); lanes 3, 4: person 2 (homozygous TT); lanes 5, 6: person 3 (homozygous CC; lanes 7, 8 negative controls
Genomic DNA was amplified by specific primer for C807 (right panel) or for T807 allele (left panel) and was analyzed on 2.0% agarose gel electrophoresis. DNA molecular marker Ⅰ was used as standard (lane M)

1.3 Statistical Analysis

Statistical analysis was performed with SPSS software (Version 12.0). Established risk factors of aspirin resistance were identified by multiple logistic regression. The *Chi*-square test was used to test deviation of genotype distribution from Hardy-Weinberg equilibrium (α=0.05) to determine the significance of the difference in allele or genotype frequencies in ASR, AS and AR patients. The relationship between the C807T polymorphism and aspirin resistance was determined by multiple logistic regression with adjustment for other aspirin resistance risk factors. A two-sided probability value of less than 0.05 was considered to indicate statistical significance.

2 RESULTS

2.1 Epidemiological Features of the Patients with High-risk of Atherosclerosis

Risk factors such as age, diabetes, primary hypertension, platelet count in ASR and AR patients had no significant differences from AS patients. The number of current smokers and women in ASR or AR patients were significantly higher than that in AS patients, which were demonstrated in table 1 (*P*<0.005).

Table 1 Epidemiological features of aspirin semi-responder (ASR), aspirin-sensitive (AS) and aspirin-Resistant (AR) patients

Features	AR (*n*=9)	ASR (*n*=41)	AS (*n*=150)	χ^2	*P*
*Age (y)	68.7±10.2	65.1±8.7	64.6±10.6		
**Women（%）	44.4	34.1	14.7	11.156	0.004
**Current smokers (%)	33.3	31.7	11.3	11.639	0.003
Diabetes (%)	22.2	19.5	15.3	0.632	0.729
Hypertension (%)	55.6	53.7	51.3	0.117	0.943
*Platelet count (10^9/L)	276±103	237±97	265±112		

*P>0.05 (analysis of variance, $\bar{x}\pm s$) **P<0.005 (*Chi*-square test, χ^2=12.848)

2.2 Investigation of Group Heritage

Distribution of GP Ⅰa 807C/T allelic and genotypic frequencies in ASR, AS and AR patients evaluated by chi-square analysis was complied with Hardy–Weinberg equilibrium (data not shown).

2.3 Genotypic and Allelic Frequencies in the AR, ASR, and AS groups

T allelic frequency in AR group and ASR group were significantly higher than that in AS group (χ^2=12.848, P<0.005) (table 2). Also, the prevalence of homozygous or heterozygous T allele carriers (genotypes TT+TC) in AR and ASR group were higher than that in AS group (χ^2=7.138, P<0.05) (table 3). The C807T polymorphism of platelet membrane GP Ⅰa gene was significantly correlated with the presence of aspirin resistance.

Table 2 Genomic frequency of the GP Ⅰa C807T polymorphisms in aspirin semi-responder (ASR), aspirin-sensitive (AS) and aspirin-resistant (AR) patients

Groups	*n*	Genotype (*n*)			Genomic frequency	
		TT	TC	CC	T (%)*	C (%)
AR	9	5	2	2	12（66.7%）	6（33.3%）
ASR	41	8	20	13	36（43.9%）	46（56.1%）
AS	150	20	53	77	93（31.0%）	207（69.0%）
Total	200	33	75	92	141（35.2%）	259（64.8%）

*P=0.002 (*Chi*-square test, χ^2=12.848)

Table 3 The correlation of genotype of the GP Ⅰa and risk of aspirin resistance

Genotypes	AR Group		ASR Group		AS Group	
	n	%	*n*	%	*n*	%
TC+TT△	7	77.8	28	68.3	73	48.7
CC	2	22.2	13	31.7	77	51.3
Total	9	100.0	41	100.0	150	100.0

△P=0.028 (*Chi*-square test, χ^2=7.138)

2.4 Multivariate Logistic Regression Analysis for Aspirin Resistance

After multivariable adjustment for other risk factors of aspirin resistance by logistic regression, which included age, gender, history of diabetes or hypertension, smoking etc, the relation between T alleles and aspirin resistance remained significant (OR=3.76; 95%CI, 2.87–9.58; P<0.05). Consequently, platelet membrane GP Ⅰa T807 allele may be an independent risk predictor of aspirin resistance.

3 DISCUSSION

Platelets play a pivotal role in the onset of arterial thrombotic disease. Because of its low price, safety and effectiveness, aspirin has been used as the most popular first-line antiplatelet agent. However, the exact mechanism of "aspirin resistance" is still unknown. Recently, increasing evidence shows that platelet GP Ⅰa–Ⅱa polymorphism, as a genetic risk factor for arterial thrombosis, is a new area of human genomics that has been intensively investigated in recent years. GP Ⅰa-Ⅱa is the main collagen receptor on the platelet membrane, and can mediate platelet adhesion to types Ⅰ–Ⅷ collagens[4]. Moreover, the binding of platelet GP Ⅰa–Ⅱa and collagens also play a key role in platelet activation and the development of thrombosis.

Based on the results from eight individuals, Kawasaki *et al*[5] demonstrated that aspirin responders could be separated from aspirin non-responders by lower fixed-dose collagen. In addition, high platelet sensitivity in aspirin responders could weaken the efficacy of aspirin. Meanwhile, one study[6] identified that polymorphisms within the GP Ⅰa gene were associated with variations in platelet GP Ⅰa–Ⅱa expression levels. Platelets from individuals bearing the T807 allele expressed high levels of GP Ⅰa–Ⅱa, whereas individuals who carried the C807 allele exhibited a lower density of the platelet integrin. Moreover, Cambria-Kiely *et al*[7] showed that T807 allele could increase the density of the platelet membrane GP Ⅰa–Ⅱa, which may be a potential risk factor of thrombogenesis that can promote the development of aspirin resistance. Homoncik *et al*[8] studied the relationship between platelet function and pharmacodynamics of aspirin, and found that the patients who had the highest GP Ⅰa–Ⅱa on platelet surface exhibited the shortest collagen epinephrine closure time (CEPI-CT). Thus, the investigators hypothesized that the genetically determined collagen receptor density could influence both basal CT and aspirin induced CT.

Further clinical research by Macchi *et al*[9] explored the correlation between the C807T polymorphism of GP Ⅰa gene and aspirin resistance, but their sample size was small. Nevertheless, the relationship between them remains controversial. Our study showed that platelet membrane GP Ⅰa T807 allele and C807 allele frequencies in Chinese patients with high risk of atherosclerosis were 35.2% and 64.8% respectively, which was consistent with the findings of previous study[3]. T allelic frequency in AR group and ASR group was higher than that in AS group (P<0.005), while the prevalence of homozygous or heterozygous T allele carriers (genotypes, TT+TC) in AR and ASR group were significantly higher than that in AS group (P<0.05). After multivariable ad-

justment for other risk factors of aspirin resistance by logistic regression, the relation between T alleles and aspirin resistance remained positively significant (OR=3.76，95% CI 2.87–9.58).

In conclusion, our findings suggest that inherited platelet GP Ⅰa C807T polymorphism variations may have an important impact on the occurrence of aspirin resistance, and we suppose that T807 allele may enhance platelet sensitivity to collagen, and shorten aspirin induced CT. Because the pathogenetic mechanisms of aspirin resistance are complicated and multifactorial, further researches on platelet-associated genetic factors and pharmacological study should be conducted in a larger population.

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(Received Sept. 27, 2007)