Adverse events in cardiovascular disease patients taking clopidogrel: impact of CYP2C19 genotype polymorphisms

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Abstract

Background: Clopidogrel combined with aspirin in antiplatelet therapy is the first-line clinical regimen for cardiovascular diseases. The CYP2C19 gene influences the absorption and metabolism of clopidogrel and its polymorphisms affect antiplatelet therapy drug efficacy, which may lead to adverse events including stent thrombosis and haemorrhage. The main objective of this study was to explore the impact of CYP2C19 polymorphisms on adverse events in cardiovascular disease patients.

Methods: We recruited 350 patients taking clopidogrel and performed CYP2C19 genotype testing. Adverse event information was collected through telephone follow-up. According to CYP2C19 genotype results, patients were divided into three groups: poor metabolism (PM) group, extensive metabolism (EM) group and intermediate metabolism (IM) group. The number of adverse events was compared between the three groups using the chi-squared test and the onset time of adverse events was analysed using the log-rank test. The main factors affecting adverse events were analysed using binary logistic analysis.

Results: In total, 326 patients were included in the analysis: 143 patients were in the EM group, 129 patients were in the IM group and 54 patients were in the PM group. In this cohort, 127 adverse events were noted, which occurred in 88 patients. There was no significant difference in the occurrence of adverse events between the EM group and PM group (P=0.185). The median survival times of adverse events in the EM, IM and PM groups were 112 days, 137.5 days and 169 days, respectively, with no significant differences between the three groups (P=0.8713).

Conclusion: We found that CYP2C19 polymorphisms were not necessarily associated with adverse events in patients with cardiovascular diseases taking clopidogrel. Rather, the main factors influencing the occurrence of adverse events were concomitant diseases such as hypertension, diabetes and hyperlipidaemia.

Background

In China, with the ageing of society and acceleration of urbanisation, unhealthy lifestyles have become increasingly prevalent. As a result, cardiovascular disease (CVD) risk factors have increased, showing rapid growth and individual aggregation trends particularly in younger, lower-income groups. At present, CVD is the top cause of total deaths in China, accounting for 45.01% of deaths in rural areas and 42.61% of deaths in urban areas. With the burden of CVD increasing, the CVD mortality rate among rural residents has increased significantly [1]. P2Y12 platelet receptor inhibitor drugs play an important role in the prevention and treatment of cardiovascular diseases [2]. Of these, clopidogrel is the most widely used drug to prevent antiplatelet aggregation. Its use in combination with aspirin is the standard therapy for the prevention and treatment of ischemic events in cardiovascular patients with acute coronary syndrome (ACS), percutaneous coronary intervention (PCI) and acute myocardial infarction in China [3]. With the expansion of clinical use and research of these drugs, several large multi-centre clinical trials have reported individual differences in the outcomes of antiplatelet therapy with clopidogrel [4]. Specifically, some patients with cardiovascular disease or PCI have experienced adverse events such as haemorrhage and thrombus re-formation while taking the standard therapeutic dose of clopidogrel. Studies have shown that the occurrence of these adverse events is related to the absorption and metabolism rate of clopidogrel [5]. Patients with fast absorption and metabolism of

clopidogrel are prone to bleeding events, and so adverse events like thrombosis re-formation are more likely to occur in patients with slow absorption and metabolism [6].

Clopidogrel is an inactive thienopyridine prodrug that is absorbed by regulating P-glycoprotein in the intestine[7]. It is metabolised by the hepatic cytochrome P450 (CYP) system to form the active product, and its active metabolite selectively and irreversibly binds to the platelet P2Y12 receptor, thereby exerting anti-platelet activity [8,9]. In the CYP metabolic system, clopidogrel is first metabolized by CYP1A2, CYP2B6 and CYP2C19 into 2-O-clopidogrel, and then catalysed by CYP2C19, CYP2C9, CYP3A4 and CYP2B6 to generate thiol derivatives that have drug activity. These thiol derivatives can cause irreversible antagonism of the ADP receptor P2Y12 platelet membrane, cannot make fibrinogen glycoprotein □ b/□ a combine with receptors and inhibit platelet activation and aggregation. The CYP2C19 gene is thought to play an important role in this transformation process [10].

Like many other CYP450 superfamily members, the CYP2C19 gene is highly polymorphic and has more than 25 known variant alleles. Different CYP2C19 genotypes can lead to an inconsistent conversion of clopidogrel [11]. CYP2C19 polymorphisms are mainly caused by two types of mutations: one type reduces enzyme activity, such as CYP2C19*2, CYP2C19*3, CYP2C19*4, CYP2C19*5, CYP2C19*7 and CYP2C19*8; the other type enhances enzyme activity, and CYP2C19*17 is the only know mutant allele of this type [12]. The most common CYP2C19 loss-of-function allele is *2 (c.681G > A; rs4244285), with allele frequencies of 15% in Caucasians and Africans and 29–35% in Asians. Other CYP2C19 variant alleles with reduced or absent enzymatic activity have been identified (e.g.,*3-*8); however, their allele frequencies are typically < 1%, with the exception of CYP2C19*3(c.636G > A; rs4986893) which is present in Asians at 2–9% [13]. Correspondingly, in Asian populations there are four metabolic rate types for clopidogrel: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM). PM individuals carry two loss-of-function alleles (*2-*8), e.g., CYP2C19*2/*2, CYP2C19*2/*3 and CYP2C19*3/*3; IM individuals carry one functional allele (*1) plus one loss-of-function allele (*2–*8), e.g., CYP2C19*1/*2 and CYP2C19*1/*3; EM individuals carry two functional (*1) alleles, e.g., CYP2C19*1/*1; and UM individuals carry two increased-activity alleles (*17) or one functional allele (*1) plus one increased-activity allele (*17), e.g., CYP2C19*1/*17, CYP2C19*17/*17 [14,15].

In clinical practice, it has been found that the use of clopidogrel for the prevention and treatment of cardiovascular disease can lead to adverse events, such as haemorrhage and thrombosis, leading to stroke, acute myocardial infarction and revascularisation, ultimately resulting in treatment instability and uncontrollability [16]. On March 2, 2010, the US Food and Drug Administration (FDA) issued a warning to guide anti-platelet therapy using clopidogrel by assessing CYP2C19 genotype [17]. A correlation between CYP2C19 genetic polymorphisms and adverse events caused by clopidogrel has been reported in Asian Han patients with cardiovascular disease[18]. However, whether CYP2C19 genotyping can be used as a basis for guiding anti-platelet therapy with clopidogrel remains to be determined. To address this question, we performed a single-centre, non-blinded, non-randomised and open-label study to assess CYP2C19 genetic polymorphisms and adverse events caused by clopidogrel in patients with cardiovascular disease.

Methods

Study Design

This was a single-centre, non-blinded and non-randomised open-label trial. We aimed to enrol 350 patients diagnosed with a cardiovascular disease, such as myocardial infarction, ACS or PCI, who were receiving conventional clopidogrel (75 mg qid) for antiplatelet therapy for more than 1 year. After patients signed the informed consent form, blood samples (1 mL) were collected for CYP2C19 genotyping. According to their CYP2C19 genotype, patients were divided into different metabolic groups for clopidogrel. To obtain information about any adverse events, telephone interviews were performed on days 30, 90, 180 and 360 after enrolment. The time of the first adverse event was the end of study follow-up. The occurrence of adverse events was then compared between the different groups. Survival analysis was used to compare the time of the first adverse event in different metabolites. The factors that may influence adverse events, including CYP2C19 genotype, age, the type of disease being treated with clopidogrel and concomitant diseases, were analysed by multiple factor regression analysis.

Study Population

Patients meeting the following inclusion criteria were eligible to participate in this study.

a diagnosis of cardiovascular disease, such as ACS, PCI or acute myocardial infarction;

undergoing clopidogrel antiplatelet therapy for more than one year, with a clopidogrel dose of 75 mg per day;

over the age of 18 years;

voluntarily signed the informed consent form;

able to undergo venous blood collection;

available for follow-up by telephone.

Patients with haemophilia, mental illness or those currently pregnant or breastfeeding were excluded from participation in this study. In some cases, other features that hampered compliance with the study requirements, according to the clinical judgment of the investigators, resulted in exclusion.

Genotyping

Patients' CYP2C19 genotypes were determined using a CYP2C19 gene detection kit and a fully automated medical PCR analysis system (JY-1000, JY-1000A) produced by Chongqing Jingin Biotechnology Co., Ltd. The CYP2C19*2 and CYP2C19*3 alleles were detected by the fluorescent probe method (molecular beacon technology). The gene detection kit contained a cell lysate that directly lysed cells to release DNA from the nuclei and amplify the target fragments (including the CYP2C19 gene sites C.636 and C.681) using two specific primers. After amplification, specific hybridisation was carried out with the amplified fragments using two pairs of molecular beacon probes marked with different fluorescent groups and complementary to the different bases at the CYP2C19 sites. The hybridisation process caused the fluorescent group to move away from the quenching group, thus releasing the fluorescent signal. With the accumulation of the amplified fragments, the fluorescent signal increased correspondingly and was monitored by the PCR system. The mutant genotypes of CYP2C19 were interpreted according to the difference in the fluorescent signals.

Adverse Events

Adverse events were collected during telephone follow-up. The association between adverse events and clopidogrel was judged according to the criteria set in the International Conference on Harmonization (ICH), and only relevant or possibly relevant adverse events were analysed. Patients' first drug-related adverse events were focused on, especially the onset times of the adverse events. Known adverse events associated with

clopidogrel use in patients with cardiovascular disease include haemorrhage, thrombosis and death.[19] Haemorrhage adverse events include stroke, gastrointestinal haemorrhage (abdominal pain, melena, etc.) and minor haemorrhage (gingival haemorrhage, eye haemorrhage, epistaxis, skin haemorrhage, etc.). Thrombotic adverse events include stent thrombosis, angina pectoris, myocardial infarction and chest tightness[20].

Statistical analysis

Data are expressed as number (percentage) or mean ± standard deviation (SD). Adverse events in the three clopidogrel metabolism groups, i.e. the EM, PM and IM groups, were compared using the chi-squared test. The onset times of the adverse events in the three groups were analysed using the log-rank test. The main factors affecting adverse events were analysed using binary logistic analysis. Statistical analyses were performed using SPSS version 20 (SPSS, IL, USA). The level of statistical significance was set at $p < 0.05$.

Results

Study Population

Ultimately, 347 patients were included in the analysis, including 203 males (58.5%) and 144 females (41.5%). The age distribution was: 23 patients under 50 years old, 97 patients between 50 and 60 years old, 138 patients between 60 and 70 years old and 89 patients over 70 years old. Of the total sample, 209 patients were taking clopidogrel for coronary syndrome and 138 patients were receiving clopidogrel antiplatelet therapy after PCI surgery. Co-morbidities included 193 patients with hypertension, 67 with diabetes, 5 with hyperlipidaemia, 38 with gastrointestinal diseases, 5 with hepatic diseases and 13 with renal diseases. Due to the attention of the elderly population on a diet, the proportion of hyperlipidaemia in this sample was lower than the epidemic level. The demographic characteristics of the included patients are shown in Fig. 2.

CYP2C19 Polymorphisms

CYP2C19 genotype was successfully determined for 347 of 350 patients. Genotype could not be determined for three patients, who were excluded from further analysis. The mutant genotype CYP2C19*17 was not detected. The mutation rate of CYP2C19*4/*5/*8 is low in the Asian population and was not detected in the sample. The genotype test results of 151 patients showed no alleles with decreased function (CYP2C19*1/*1). Twenty patients had the CYP2C19*1/*2 genotype, 122 patients had the CYP2C19*1/*3 genotype, 15 patients had the CYP2C19*2/*2 genotype, 11 patients had the CYP2C19*2/*3 genotype and 28 patients had the CYP2C19*3/*3 genotype. The baseline characteristics of the patients grouped by the presence of CYP2C19 reduced function alleles are presented in Fig. 3.

Adverse Events

Follow-up to determine adverse events was incomplete for 21 of the 347 patients and they were withdrawn from further analysis. A total of 127 adverse events were reported from the remaining 326 patients, of which 88 adverse events were included in the correlation analysis. Among the events, 28 patients had a first adverse event of haemorrhage, 115 patients had a first adverse event of thrombus, 11 patients had a stroke-related adverse event, 17 patients had a minor haemorrhage, 7 patients had stent thrombosis, 13 patients had angina pectoris, 11 patients had myocardial infarction and 29 patients had chest tightness. The distribution of adverse events is shown in Fig. 4.

Metabolic Groups and Adverse Events

The 326 patients were divided into three metabolic groups according to their CYP2C19 genotype: EM (CYP2C19*1/*1), IM (CYP2C19*1/*2, CYP2C19*1/*3) and PM (CYP2C19*2/*2, CYP2C19*2/*3, CYP2C19*3/*3). There were 33 adverse events in the EM group, including 15 haemorrhage events and 18 thrombosis events; 42 adverse events in the IM group, including 11 haemorrhage events and 31 thrombosis events; and 13 adverse events in the PM group, including 3 haemorrhage events and 10 thrombosis events. These events are shown in Table 1 and Fig. 5. There was no significant difference in the occurrence of adverse events between the three groups (chi-squared test, p = 0.185), and no significant differences between pairs of groups (p = 0.085/0.883/0.254). There was also no difference in the occurrence of bleeding or thrombotic adverse events among the three groups (p = 0.545/0.167).

Table 1
Metabolic groups and adverse events

Metabolic type	Genotype	Total AE	Bleeding AE	Thrombotic AE
EM	CYP2C191*/1*	33	15	18
IM	CYP2C191*/2*	42	11	31
	CYP2C191*/3*			
PM	CYP2C192*/2*	13	3	10
	CYP2C192*/3*			
	CYP2C193*/3*			

To further investigate the relationship between the onset time of adverse events and CYP2C19 genotype, a log-rank survival analysis was conducted with the occurrence of adverse events as the end point of survival time. Survival after adverse events is shown in Fig. 6. The median survival times of adverse events in the EM, IM and PM groups were 112 days, 137.5 days and 169 days, respectively. The p-value was 0.8713 using the survival chi-squared test, indicating no significant difference among the three groups. The median survival times of the three groups regarding thrombotic adverse events were 207.5 days, 186 days and 177 days, respectively. The p-value was 0.7454, indicating no significant difference between the three groups. The survival times of the EM and IM groups were 53 days and 47 days, respectively. The p-value was 0.4545, indicating no difference between the two groups.

Factor Analysis of Adverse Events

Factors that may affect the occurrence of adverse events, including age, cardiovascular disease type, concomitant disease and metabolic group, were analysed by binary logistic multivariate analysis. The prediction accuracy of the binary logistic regression fitting equation was 82.5%. The p-value of the Hosmer-Lemeshow test was 0.72. As the logistic regression equation might better fit the influencing factors, regression analysis was carried out. The results showed that hypertension, hyperlipidaemia, diabetes and gastrointestinal diseases had a significant impact on the occurrence of adverse events (Table 2).

Table 2
Variables predicting adverse events

Factor	B	S.E.	Wals	df	Sig.	Exp (B)	EXP(B) 95% C.I.	
							Lower	Upper
Disease types (1)	− .417	.334	1.561	1	.211	.659	.342	1.268
Age			1.383	3	.710			
Age (1)	− .025	.670	.001	1	.970	.975	.262	3.628
Age (2)	.315	.410	.590	1	.442	1.370	.614	3.058
Age (3)	.393	.373	1.111	1	.292	1.481	.714	3.074
Hepatic Disease			2.690	2	.261			
Hepatic Disease (1)	18.785	40193.290	.000	1	1.000	143979259.519	.000	.
Hepatic Disease (2)	21.101	40193.290	.000	1	1.000	1459517080.501	.000	.
Metabolic Type			3.151	2	.207			
Metabolic Type (1)	− .008	.454	.000	1	.987	.992	.408	2.415
Metabolic Type (2)	− .559	.458	1.494	1	.222	.572	.233	1.402
Renal Disease (1)	-2.749	.895	9.423	1	.002	.064	.011	.370
Gastrointestinal Disease (1)	-1.591	.454	12.282	1	.000	.204	.084	.496
Hyperlipidaemia (1)	1.542	.901	2.932	1	.087	4.676	.800	27.333
Diabetes (1)	-2.029	.374	29.503	1	.000	.131	.063	.273
Hypertension (1)	-1.350	.346	15.183	1	.000	.259	.131	.511
Constant	-18.217	40193.290	.000	1	1.000	.000		

Discussion

CYP2C19 Genetic Polymorphisms

The gene coding for the CYP2C19 enzyme is located on chromosome 10 (10q24.1-q24.3) in the CYP2C gene cluster. The gene consists of nine exons and five introns, and the full-length of cDNA is 1.94 kb, of which the coding region is 1.473 kb [21]. Twenty-five alleles, including CYP2C19*1 to CYP2C19*25, have been identified,

of which 23 encode proteins [22]. The gene encoding normal enzyme activity is CYP2C19*1. According to changes in enzyme metabolic activity caused by changes in CYP2C19, the population can be divided into two categories according to ability to metabolise S-mephenytoin: extensive metabolisers and poor metabolisers [23]. CYP2C19 gene polymorphisms show individual and ethnic differences. In the Chinese Han population, the CYP2C19 gene mutations are mainly CYP2C19*2 and CYP2C19*3. In the Asian population, the CYP2C19 mutations that occur in almost all poor clopidogrel metabolisers are CYP2C19*2 and CYP2C19*3 [24]. The CYP2C19*17 mutation was not found in the patients in the present study. In our patient sample, the mutation rate of CYP2C19*2 was 13.25%, the mutation rate of CYP2C19*3 was 46.39% and the overall mutation rate was 58.48%, which is consistent with previous reports for the Han population [25]. Thus, this study confirmed the phenomenon of a high mutation rate of the CYP2C19 gene in the Han population.

Factors Influencing Adverse Events

The causes of adverse events in patients with cardiovascular diseases taking clopidogrel are complex. Many studies have shown that clopidogrel resistance due to CYP2C19 gene mutations are the dominant factor[26]. However, as research proceeds, it is becoming clear that the absorption and metabolism of clopidogrel are regulated by multiple genes. The absorption of clopidogrel occurs mainly in the small intestine and is regulated by the ABCB1 gene encoding P-glycoprotein.[27] Only 15% of clopidogrel enters the liver and is metabolised into active products through the CYP system; the other 85% is metabolised into inactive substances by esterase (mainly regulated by the CES1 gene) and discharged from the body[28]. Not only can esterase rapidly hydrolyse the absorbed clopidogrel into inactive carboxylic acid metabolites, it can also hydrolyse 2-O-clopidogrel and active metabolites to carboxylic acids [29; thus, when patients with cardiovascular diseases also have intestinal diseases, changes in gastrointestinal homeostasis will affect the stability of the related metabolic enzymes. Clopidogrel is metabolised mainly by CYP1A2, CYP2B6 and CYP2C19 into 2-O-clopidogrel, which is then catalysed by CYP2C19, CYP2C9, CYP3A4 and CYP2B6 to generate thiol derivatives with drug activity [30]. Thiol derivatives irreversibly antagonise the ADP receptor P2Y12 on the platelet membrane, making fibrinogen unable to bind with its receptor glycoprotein IIb/IIIa, which ultimately inhibits platelet activation and aggregation. Patients suffering from liver disease are likely to have altered metabolism of related substances due to weakened liver metabolic capacity[31].

In the present study, based on their CYP2C19 genotype, patients were divided into PM, EM and IM groups. The occurrence of adverse events did not differ significantly between these groups. Furthermore, taking the onset time of adverse events as the end point, log-rank survival analysis showed no significant differences between the three groups. Binary logistic analysis of factors thought to affect the occurrence of adverse events revealed that the combination of diseases, including hypertension, gastrointestinal diseases and liver diseases, had significant effects on adverse events. Gastrointestinal diseases are known to affect the absorption of drugs, whereas liver diseases affect the metabolism of drugs. Hypertension, hyperlipidaemia and other diseases interfere with the antiplatelet effect of clopidogrel. Therefore, this study does not find any evidence of a connection between CYP2C19 gene polymorphisms and adverse events caused by clopidogrel resistance.

Limitations

This study is subject to several limitations. First, our 326 patients were divided into PM, EM and IM groups based on their genotype. This sample size is too small to be used as a representative point in a regional

population study. Secondly, we lack blood and platelet count data for the patients taking clopidogrel, which would reflect the collective absorption and metabolism of clopidogrel and the real anti-platelet aggregation effect. Third, this study did not consider the effect of combined medications on the occurrence of adverse events. Furthermore, this was an investigational study and we did not alter the dose of clopidogrel according to patients' CYP2C19 genotype. Whether altering clopidogrel dose based on genotype can reduce adverse events warrants a follow-up study.

Conclusion

Due to differences in the absorption and metabolic processing of clopidogrel between individuals and populations, bleeding and thrombotic adverse events may occur when clopidogrel is taken at the conventional dose. Adverse events occurring with clopidogrel use are thought to relate to genes that regulate the absorption and metabolism of clopidogrel: CYP2C19 is considered the key regulatory gene for clopidogrel conversion into active antiplatelet compounds. Through this single-centre, non-blinded and non-randomised investigation, we find no evidence that CYP2C19 polymorphisms are related to adverse events caused by clopidogrel resistance. Rather, in our sample, the occurrence of adverse events was affected by concomitant diseases such as hypertension, hyperlipidaemia, gastrointestinal diseases and liver diseases.

Declarations

Ethics approval and consent to participate

This study was conducted at the Shiyan Taihe Hospital (Hubei, China). Approval was granted by Taihe Hospital Medical Ethics Committee prior to initiation of the study, the approval document No.2017(62),and all patients provided written informed consent. This study was conducted in accordance with Chinese Medical Device Clinical Trial Quality Management Standards, and the investigators complied with all applicable regulatory and legal requirements of the Chinese GCP guidelines.

Consent for publication

Not applicable

Availability of data and materials

All data generated and analyzed are included in this research article.

Competing interests

We declare that there are no conflicting interests as regards the contents and data presented in the manuscript under reference.

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Authors' contributions

JFC collected subject information and test samples.JLM and LT mainly carry out subject follow-up work and collect adverse events.QH andHPL detected and analyzed the polymorphism of CYP2C19 genotype.CZ signed informed consent and judge the correlation of adverse events.ZGL was the study designer and research data collector and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Abbreviations

CVD :Cardiovascular disease; ACS:Acute coronary syndrome; PCI:Percutaneous coronary intervention;PM:Poor metabolizers;IM:Intermediate metabolizers:EM: Extensive metabolizers;UM: ultrarapid metabolizers;FDA: Food and Drug Administration ;ICH:International Conference on Harmonization .

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Figures

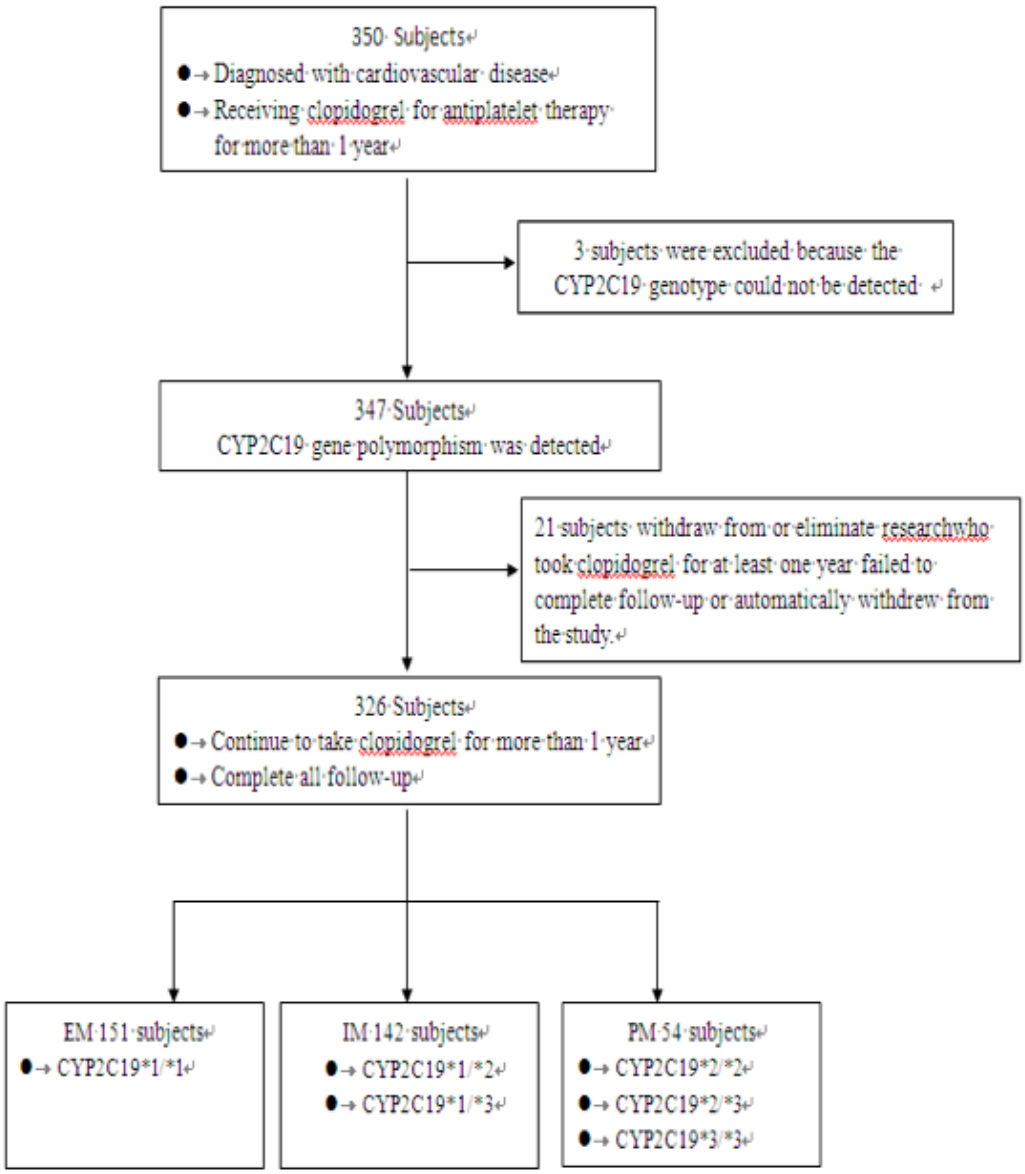

Figure 1

Study population flow chart

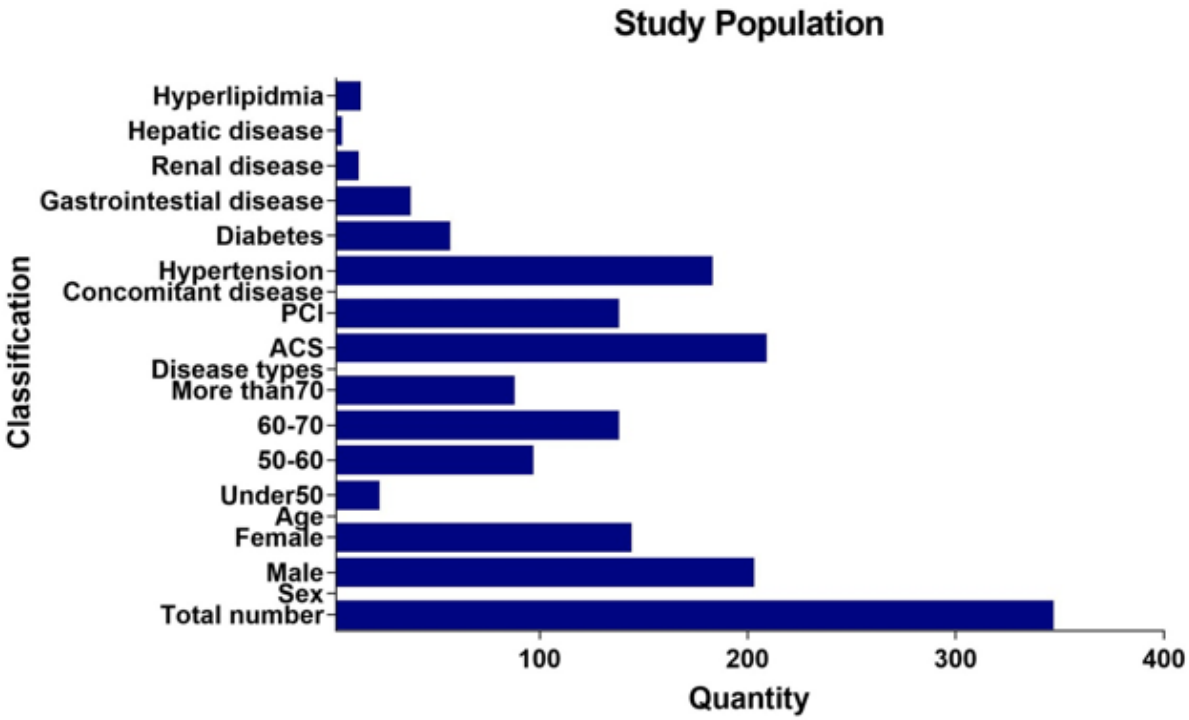

Figure 2

Study population

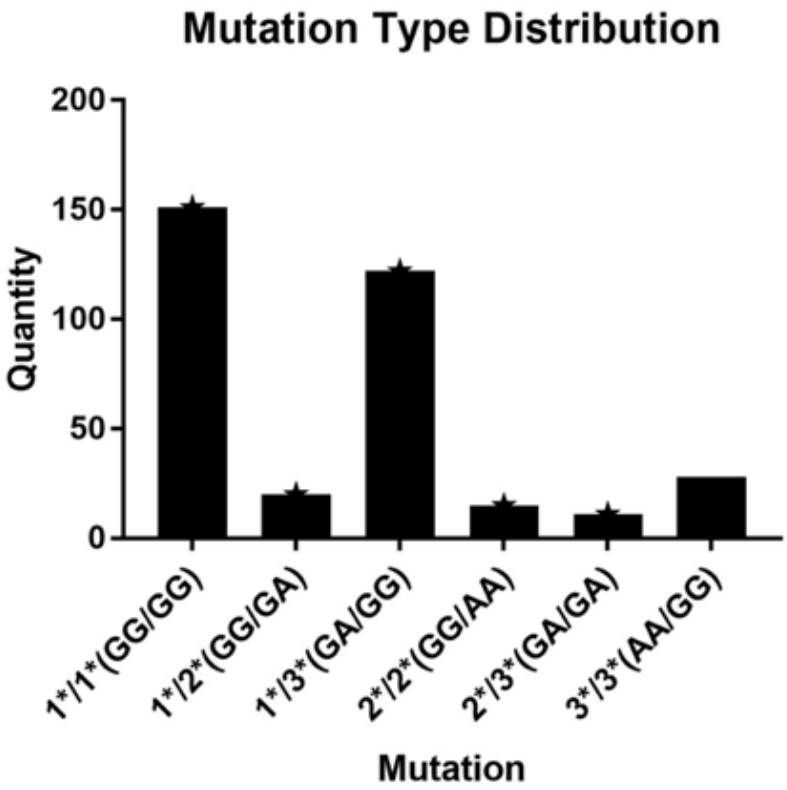

Figure 3

Genotype distribution

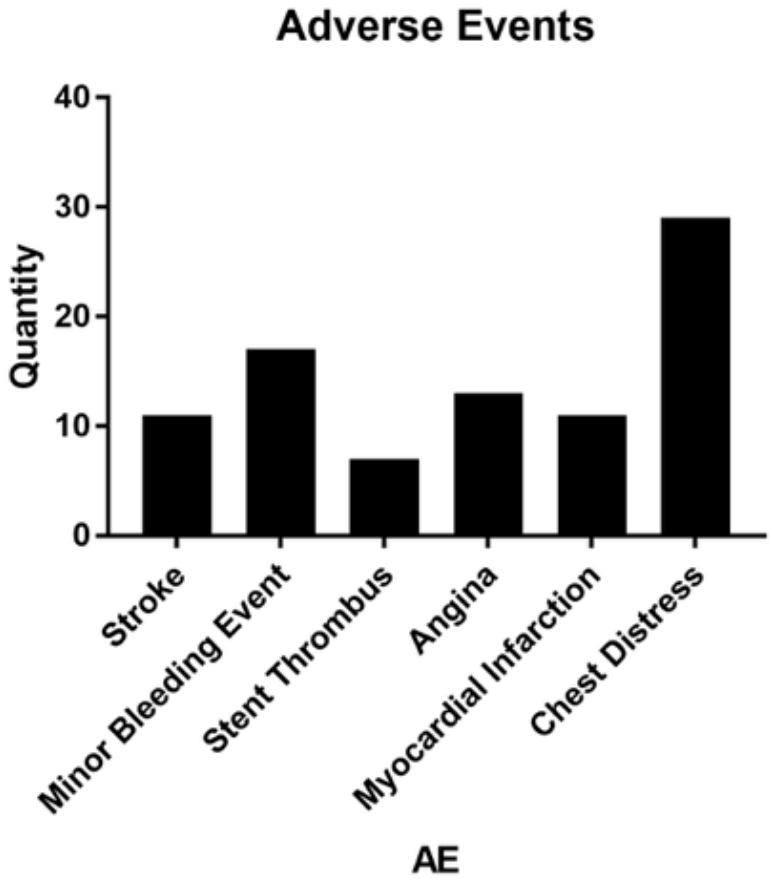

Figure 4

Adverse event distribution

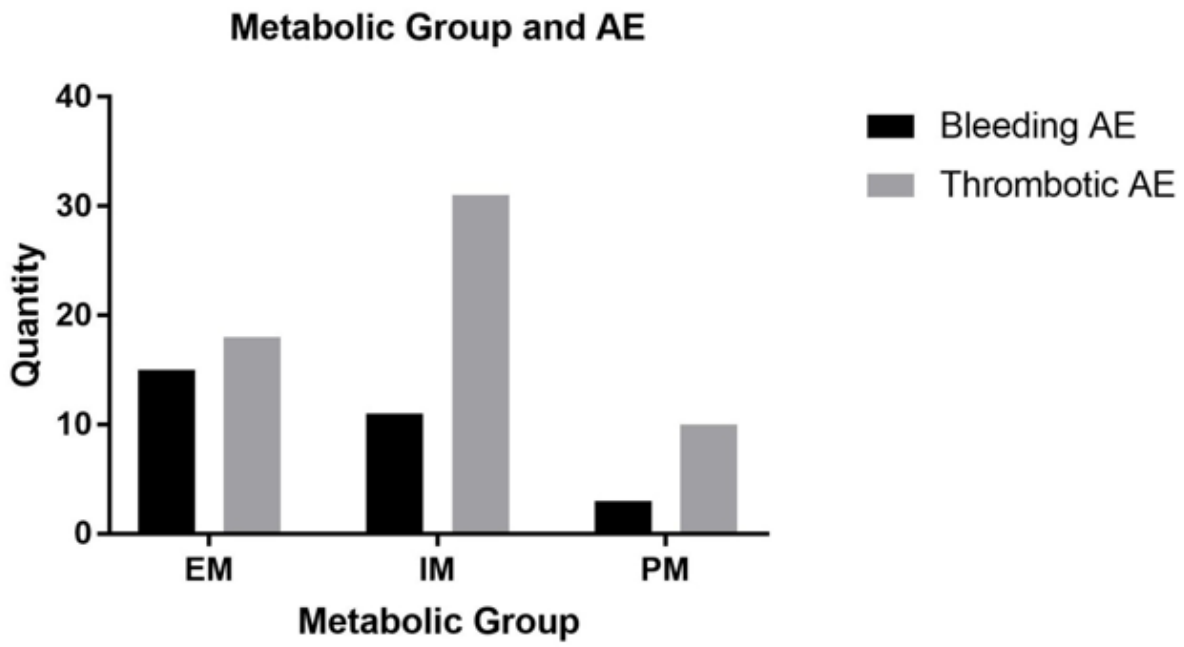

Figure 5

Metabolic groups and adverse events

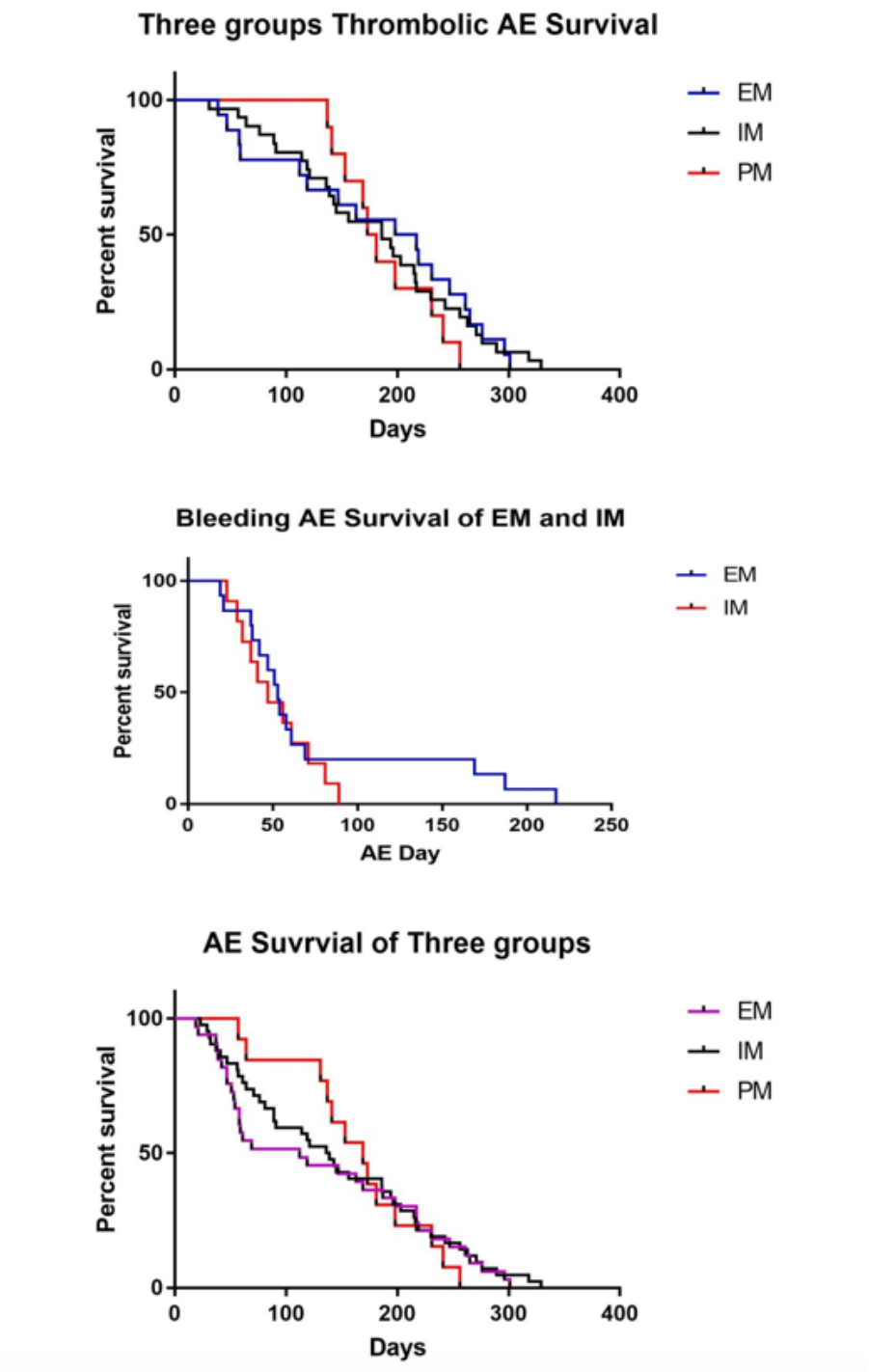

Figure 6

Survival analysis following adverse events