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Candidate gene and pathway analyses identifying genetic variations associated with prasugrel pharmacokinetics and pharmacodynamics

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ABSTRACT

Aim: We aimed to investigate the genetic polymorphisms and pharmacogenetic variability associated with the pharmacodynamics (PD) and pharmacokinetics (PK) of prasugrel, in healthy Han Chinese subjects.

Patients & methods: Healthy, native, Han Chinese subjects (n = 36) aged 18 to 45 years with unknown genotypes were included. All subjects received a loading dose (LD) on day 1 and a maintenance dose (MD) from day 2 until day 11. Candidate gene association and gene-set analysis of biological pathways related to prasugrel and platelet activity were analyzed.

Results: 28 SNPs of 17 candidate genes previously associated with prasugrel or platelet activity were selected after a literature search. In the 30 mg LD groups (n = 24), *ITGA2*-rs28095 was found to be significantly associated with the P2Y12 reaction unit (PRU) value at 24 h after the LD (p = 0.015). 165 study genes related to platelet activation-related processes and prasugrel activity were selected from the MSigDB database, including curated gene sets from KEGG, Bio Carta, and Gene Cards. 14 SNPs of 9 genes were found to be significantly correlated both at 24 h and 12 days after LD: *ADAMTSL1, PRKCA, ITPR2, P2RY12, P2RY14, PLCB4, PRKG1, ADCY1, and LYN*. Seven SNPs of 6 protein-coding genes associated with area under the concentration-time curve (AUC0-tlast) were significantly identified among the 47 selected genes, including *ADAMTSL1, CD36, P2RY1, PCSK9, PON1,* and *SCD*.

Conclusion: These results show that genetic variation affects the PK and PD of prasugrel in normal individuals. Further studies with larger sample sizes are required to explore whether the SNPs are associated only with prasugrel activity or also with cardiovascular events and all-cause mortality.

1. Introduction

Thrombotic diseases, such as acute coronary syndrome (ACS), stroke, and peripheral vascular thrombosis disease, represent a major health problem worldwide [1,2]. Platelet adhesion, activation, and aggregation play critical roles in the formation of pathological thrombosis, and thus antiplatelet therapy has become the cornerstone for inhibiting platelet aggregation. P2Y12-adenosine diphosphate (ADP) receptor inhibition has proven to be an effective way to prevent and treat thrombotic diseases [2–4]. Guidelines formulated by both the American College of Cardiology/American Heart Association and the European Society of Cardiology recommend the use of dual antiplatelet therapy with aspirin in combination with a P2Y12 receptor inhibitor in patients with ACS and in those undergoing percutaneous coronary intervention (PCI) [5]. At present, three oral *P2Y12* receptor antagonists (clopidogrel, prasugrel, and ticagrelor) are commonly used in clinical practice. Prasugrel is an orally administered third-generation thienopyridine that achieves a faster, higher, and more consistent level of inhibition of platelet function than ticagrelor and clopidogrel [6–8], as well as significantly lower rates of high on-treatment platelet reactivity [9].

Nevertheless, some intrinsic and extrinsic factors may contribute to an individual's response profile to the pharmacokinetics (PK) and pharmacodynamics (PD) of prasugrel, including age; sex; body weight; body fat; alcohol consumption; concomitant drug use; nutritional status; cardiovascular, liver, and renal function; and environmental pollutants [10–15]. Among genetic factors, a number of genes coding for enzymes or receptors that may be involved in the PK or PD profiles of prasugrel have been investigated previously [16,17]. Genetic testing is an attractive option as a strategy to personalize antiplatelet therapy

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[18–20], but data supporting the role of genotype-based personalized antiplatelet therapy are limited and largely derived from registry data or small randomized studies [21–24]. Candidate gene association studies identify genetic differences that influence platelet function in normal individuals [25]. Target genes were rationally selected as candidates based on their established or logical involvement in the important molecular events that contribute to normal platelet function. Gene-set analysis was mainly developed to analyze large-scale profiling data, which facilitate the interpretation of experiment results and help in identifying important biological findings [26,27]. Candidate gene association studies and gene-set analysis were widely used to determine a cumulative effect on platelet function via modifying basic platelet parameters, by altering the expression or activity of key platelet receptors, or by influencing downstream effector pathways utilized by these receptors [28–30].

To preliminarily explore the effects of genetic variation on the PK and PD of prasugrel in normal individuals, candidate gene association studies and gene-set analysis were used in this study.

2. Methods

2.1. Candidate gene selection

Candidate genes previously associated with prasugrel or platelet activity were selected after a search of the literature. Several candidate genes have been identified and extensively investigated for their functional role in healthy individuals undergoing antiplatelet therapy. SNPs in the selected glycoprotein genes were consistently associated with differences in platelet function among normal individuals. Regarding receptor genes, 28 SNPs of 17 genes were selected (Appendix Table A1), of which the most notable SNPs were of the following genes: *ITGA2, CD36, P2RY12, GNAZ, MA2K2, MAPK14, P2Y1, 9p21.3, ADRA2A, PIK3CG, JMJD1C, SHH, TBXA2R,* and *FCGR2A* [31–37].

2.2. Pathway selection

Pathways related to platelet activation-related processes and prasugrel activity were selected from the MSigDB database [38], which included curated gene sets from KEGG [39], Bio Carta [40], and Gene Cards [41]. A total of 165 genes from platelet activation pathway (KEGG), platelet amyloid precursor protein pathway, eph kinases and ephrins support platelet aggregation, aspirin blocks signaling pathway involved in platelet activation (Bio Carta), and prasugrel related gene (Gene Cards) were selected for this study (Appendix Table A2).

2.3. Study population

Healthy, native, Han Chinese subjects (n = 36) between 18 and 45 years of age with a BMI of 19 kg/m^2 to 24 kg/m^2 and unknown genotypes were included, after obtaining written informed consent. The study was conducted in compliance with the Declaration of Helsinki. Detailed inclusion and exclusion criteria and the study design have been published previously [42].

All subjects received a loading dose (LD) on day 1 and a maintenance dose (MD) from day 2 until day 11. The dose regimen we designed for healthy Chinese subjects included a standard regimen of 60 mg LD with 10 mg MD and 30 mg LD with a 7.5 mg MD or 5 mg MD.

2.4. Blood sample collection

A total of nine blood samples (2 ml each) were collected through venipuncture into citrate-containing tubes from all the subjects on day 1 pre-dose and at 0.5, 1, 2, 4 and 24 h post-dose; day 10 pre-dose; and day 11 pre-dose and 24 h post-dose.

2.5. Pharmacodynamic evaluations

Platelet aggregation was assessed using a VerifyNow-P2Y12 (VN-P2Y12) assay (Accumetrics, San Diego, CA, USA). The data were recorded directly from the VerifyNow device as P2Y12 reaction unit (PRU) values. The PD parameters were summarized by dose group using distributive statistics at 24 h and at day 12 after LD.

2.6. Pharmacokinetic parameters

The PK parameter for the active metabolite assessed included the area under the concentration-time curve (AUC), which was calculated using the sampling time of the last quantifiable serum concentration (AUC0-tlast). The AUC0-tlast was estimated at each dose level using a validated liquid chromatography with tandem mass spectrometric detection (LC/MS/MS) method.

2.7. Genetic test

Genomic DNA was extracted from peripheral whole-blood samples of each subject using a DNA Purification kit (Wizard®; Promega, WI, USA). After the PCR products were obtained, all samples were sequenced by NGS (Next Generation Sequencing, NGS) to determine the SNPs in the candidate genes and selected pathways (Life Technologies Biotechnology Co., Ltd., Shanghai, China).

2.8. Statistical analysis

The association between the genotype and the PRU value was assessed by the Kruskal-Wallis test or the Mann-Whitney *U* test. All statistical analyses were performed using the Statistical Package for Social Sciences version 22.0 for Windows. A p-value < 0.05 indicated significance. Testing a single-nucleotide polymorphism (SNP) marker is calculated to require 90 cases, achieving 80% power under a dominant model [43]. However, by using candidate gene association studies and a gene-set analysis of biological pathways firstly, we included 36 healthy subjects in the study, as a preliminary exploration of gene polymorphisms associated with pharmacogenetics variability in the PD and PK of prasugrel. The findings could serve as a hypothesis-generating effort, and could be validated in further studies with larger sample sizes.

2.9. Genomic sequence analysis

Statistical analyses were performed using the set-based test of PLINK v1.07 [44]. In the set-based test of PLINK, first, a single SNP analysis of all SNPs within the set is performed. Subsequently, a mean SNP statistic is calculated from the single SNP statistic of a maximum number of independent SNPs below a p-value threshold of 0.01, and the SNP with the lowest p-value in the single SNP analysis is selected. This analysis is repeated at certain permutations of the phenotype. An empirical p-value for the SNP set is computed by calculating the number of times the test statistic of the simulated SNP set exceeds that of the original SNP set.

Initially, all 28 SNPs of 17 candidate genes and 165 study genes were tested as a whole to determine associations with platelet function (PRU), and 47 protein-coding genes for enzymes were analyzed to determine the relationship between genes and AUC0-tlast.

3. Results

3.1. Demographic characteristics

In total, 36 healthy, native Chinese subjects (19 males, 17 females) were enrolled, and 35 subjects completed the study. The mean age, body weight, and BMI were similar across the treatment groups

Table 1
Baseline demographic characteristics of participants.

	Prasugrel (LD60/MD10 mg)	Prasugrel (LD30/MD7.5 mg)	Prasugrel (LD30/MD5 mg)
N(Males)	12(5)	12(7)	12(7)
Age, years mean ± SD	36.3 ± 3.7	34.0 ± 3.9	34.8 ± 3.6
Weight, kg mean ± SD	57.8 ± 4.9	59.8 ± 6.6	60.8 ± 6.3
BMI, kg/m^2 mean ± SD	22.2 ± 1.5	22.3 ± 1.6	22.3 ± 1.8

Abbreviations: BMI = body mass index; N = number of subjects; SD = standard deviation.

(Table 1).

3.2. Effects of polymorphisms on the PD of prasugrel

3.2.1. Candidate gene variations

A total of 28 genetic polymorphism sites in the candidate genes were identified among the studied Han Chinese population. The PRU value was assessed by using the VN-P2Y12 assay. No significant differences were observed between SNPs and PRU values after prasugrel administration for 11 days (n = 36) based on the SPSS analysis. However, in the 30 mg LD groups (including 30 mg LD/7.5 mg MD and 30 mg LD/5 mg MD groups; n = 24), one SNP of *ITGA2* was found to be significantly associated with the PRU value at 24 h after the LD (P value < 0.05; Table 2). In the 30 mg LD groups, *ITGA2* rs28095 CC and CT carriers had higher PRU values than TT carriers at 24 h after the LD (p = 0.015, CC vs CT vs TT: 29.80 ± 26.66 vs 31.43 ± 42.62 vs 3.00 ± 1.73, respectively; Table 2).

A comparison of PRU values following a prasugrel LD and MD in different rs28095 genotype groups is shown in Fig. 1. In the 30 mg LD/5 mg MD group, the PRU values in rs28095 CT carries were the highest, and rs28095 TT carries had the lowest PRU values (Fig. 1a). However, the rs28095 CC carriers had higher PRU values than CT and CC carriers during the entire period on medication in the 30 mg LD/7.5 mg MD group (Fig. 1b). Owing to the limited number of subjects in each group (n = 12), our findings only show the probable effects of SNPs on prasugrel PD in patients on a MD.

PRU values in rs28095 TT carriers are much lower than CC carries, remaining the lowest in both maintenance dose groups. Thus, we speculate that T allele seem to be more sensitive to prasugrel therapy than C allele, with better platelet inhibition and lower PRU values. However, PRU values in heterozygotes (CT) carriers correlated with different dosage: the greater the dose, the stronger the platelet inhibition and the lower the PRU value of CT heterozygous carriers. In the 5 mg MD group, the PRU values in rs28095 CT carriers were the highest (Fig. 1a). However, PRU values were about 50 units lower in the 7.5 mg MD group than the 5 mg MD group (Fig. 1b), while effect of dosage on PRU values of TT and CC carriers was comparatively smaller. This indicates us that gene detection can be useful to guide escalation/de-escalation during prasugrel therapy, especially for *ITGA2* rs28095 CT carriers.

Table 2
Association between candidate gene SNPs and PRU values at 24 h after administration of 30 mg LD.

GENE	SNP	Genotypes	Number	PRU (mean ± SD)	P-value
ITGA2	rs28095	CC	5	29.80 ± 26.66	0.015
		CT	14	31.43 ± 42.62	
		TT	5	3.00 ± 1.73	

ITPR2, inositol 1, 4, 5-triphosphate receptor type 2.

Low platelet reactivity (LPR) is usually used in to investigate personalized antiplatelet therapy, and PRU lower than 85 is defined as the LPR [45] in the study. The relationship between LPR and genotypes was explored in the candidate genes. Rs3821667 (*P2RY12*), rs2046934 (*P2RY12*) and rs28095 (*ITGA2*) were found significantly associated with LPR at 24 h, with a p value of 0.009, 0.009 and 0.003 respectively. The results were consistent with the results above, with more SNPs found. However, no significant association between LPR and SNPs was found in the 165 genes from different pathways. More outcomes index might be utilized in association exploring between antiplatelet therapy and genotypes in the future study.

3.2.2. Pathways gene variations

A total of 165 genes from different pathways were analyzed in the current study. We explored the joint effect of genetic variation on PRU values at 24 h and 12 days post LD, at p ≤ 0.01 in PLINK. Further, 136 SNPs of 47 genes at 24 h and 116 SNPs of 38 genes at 12 days were identified to show a significant association at p ≤ 0.01. Finally, 14 SNPs of 9 genes were found to show a significant association at both 24 h and 12 days; the details of the genes and p-min-values are listed in Table 3. The genes identified included the following: *ADAMTSL1, PRKCA, ITPR2, P2RY12, P2RY14, PLCB4, PRKG1, ADCY1,* and *LYN*, based on KEGG, Bio Carta, or Gene Cards.

Among SNPs related to *ADAMTSL1* described by Gene Cards, rs10156461 was most significantly associated with PRU values, with a minimum p-value of 0.004067 at 24 h and 0.004940 at 12 days (Table 3). The population distribution of the AA/AG/GG allele was 3, 12, and 17, respectively, with one subject carrying the AG genotype. This subject was excluded from the study 24 h post LD due to an allergic reaction to prasugrel. Five SNPs related to *PRKCA* from aspirin blocks signaling pathway involved in platelet activation were found to be significantly associated with PRU values (p-24 h = 0.00303, p-12d = 0.00714), including rs77445472, rs11653415, rs10491174, rs4641779, and rs9910778. The association with *PLCB4* from platelet activation pathway was driven by two SNPs, rs2179798 and rs962349 (p = 0.00387). In addition to the genes mentioned above, *ITPR2, P2RY12, P2RY14, PRKG1, ADCY1, and LYN* were also significantly associated with PRU values (p < 0.01). The strongest association at 24 h was observed for an SNP in *ITPR2* from platelet activation pathway (rs10771282, p = 0.000026), whereas rs62516880 located in *LYN* from platelet activation pathway, eph kinases and ephrins support platelet aggregation pathway showed the most significant association at 12 days (p = 0.00089).

All the SNPs found suggest that the PRU values were significantly association with gene variations, which might be the potential target for the treatment of thromboembolisms and other clotting disorders.

3.3. Effect of enzyme-coding genes on prasugrel pharmacokinetics

A total of 47 genes coding enzymes described by Gene Cards were analyzed in this study. We explored the joint effect of protein-coding genetic variation on AUC0-tlast with a p-value below 0.01 in PLINK. Seven SNPs of six protein-coding genes associated with AUC0-tlast were identified, including *ADAMTSL1, CD36, P2RY1, PCSK9, PON1,* and *SCD*. Information on SNPs, p values in PLINK, gene types, and population distribution is shown in Table 4.

ADAMTSL1 was also found to significantly influence the PK of prasugrel, and the minimum p value was 0.001383 for rs526826. Two SNPs located in *SCD* were associated with AUC0-tlast, rs11190486, and rs1393491 (p = 0.003636). In addition, rs2495477 showed the strongest association and was located in *PCSK9*. Moreover, rs940541, rs9826760, and rs854552, located in *CD36, P2RY1,* and *PON1* respectively, were also considered as significant SNPs related to prasugrel action.

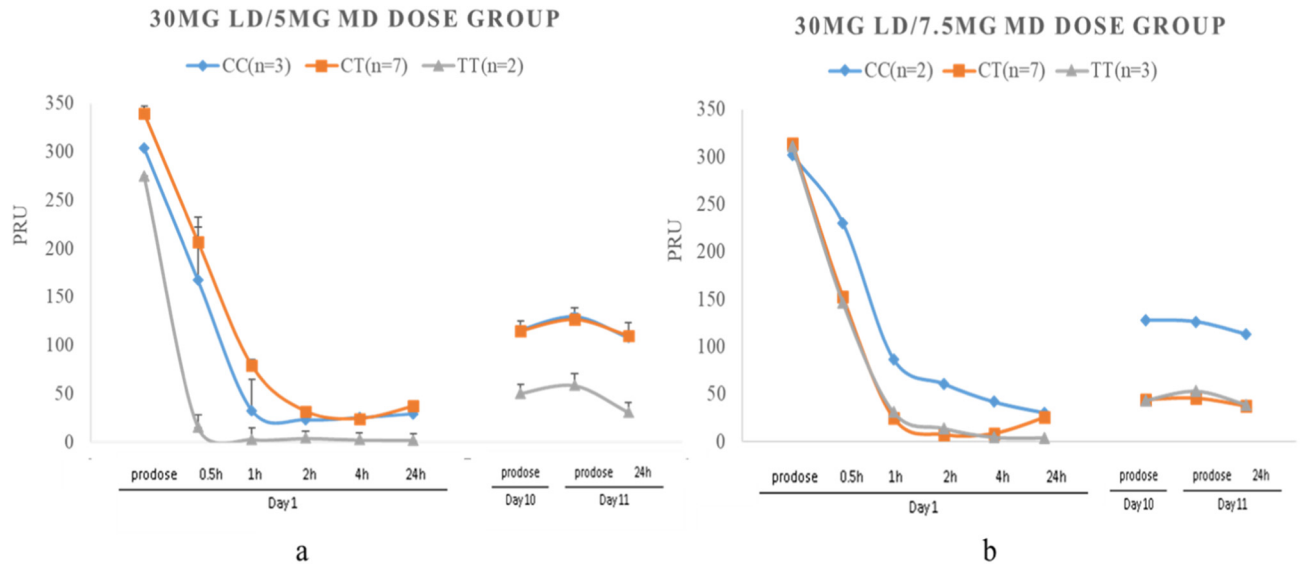

Fig. 1. Comparison of the PRU values following a prasugrel LD and prasugrel MD in different *ITGA2* rs28095 genotype groups. One subject carrying the rs28095 TT genotype in the 30 mg LD/7.5 mg MD group was excluded from the study after 24 h post LD due to an allergic reaction to prasugrel. LD: Loading dose; MD: Maintenance dose.

4. Discussion

To our knowledge, this is the first study to determine gene polymorphisms associated with pharmacogenetic variability in the PD and PK of prasugrel using candidate gene association studies and a gene-set analysis of biological pathways. Using a healthy Chinese population, we found several important SNPs that were associated with prasugrel activity.

Among the 28 SNPs of 17 candidate genes tested in this study, *ITGA2* rs28095 showed a significant association with prasugrel PK, indicating that different SNPs might influence drug therapy with prasugrel in cardiovascular disease. In agreement with our findings, several studies [45–48] have indicated that rs28095 is known to attenuate *ITGA2* transcription, leading to diminished a2b1 expression and reduced platelet adhesion to collagen I, which can also accelerate platelet formation and decrease mean platelet volume. Genetic variability within *ITGA2* may confer risk for ischemic stroke independent of conventional risk factors [49]. Nine key genes described based on KEGG, Bio Carta, or Gene Cards were significantly associated with prasugrel PD in the gen-set analysis. Among them, *P2RY12* is a protein-coding gene on platelet activation pathway and plays a key role in platelet aggregation, and is known as a potential target for the treatment of thromboembolisms and other clotting disorders [50]. Moreover, polymorphisms in *P2RY12* were found [51] to be associated with platelet reactivity in patients with cardiovascular disease treated with aspirin. Rs38211667 was located in the non-coding region of *P2Y12* exon 2 [52], and it's not clear about its effect on protein structure. But rs38211667 was confirmed complete linkage disequilibrium with the *P2Y12* H_2 haplotype SNPs in a large population (n = 295) study (D' = 1.0, r^2 = 0.936–1.0) [53]. *P2Y12* H_2 haplotype is associated with increased ADP-induced platelet aggregation [54]. Rs38211667 was also confirmed complete linkage disequilibrium with *P2RY13* rs1466684 (r^2 = 1.0), missense mutation in which is the most common type of mutation causing protein structure changes [55]. Thus, we speculate that rs3821667 might influence *P2RY12* protein by two ways: (1) although located in non-coding region of *P2Y12*, it might involves in gene activation, gene silencing, protein synthesis, functional regulation and other processes. (2) It would affect protein function by strong linkage with other SNPs. In our study, the *P2RY12* rs3821667 was significantly associated with PRU values at 24 h and 12 d post LD (24 h p = 0.002932, 12d p = 0.007415), indicating that polymorphisms in *P2RY12* were associated with platelet reactivity following prasugrel treatment. Another study [56] also revealed *APOH* as a new candidate gene associated with thrombosis, whereas 5 SNPs of *APOH/PRKCA* were identified as potential factors influencing prasugrel PD (rs77445472, rs11653415, rs10491174, rs4641779, and rs9910778; 24 h p = 0.003034, 12 day p = 0.007144) in this study. Moreover, it has been suggested previously [57] that *PLCB* proteins may be potential targets for the development of novel therapies for the treatment of cardiovascular diseases, and *PLCB4* was identified as a potential target in our analysis.

The joint effect of protein-coding genetic variation on prasugrel PK was also explored in this study, and 6 protein-coding genes (*ADAMTSL1, CD36, P2RY1, PCSK9, PON1 and SCD*) were identified. The loss of function of *PCSK9* is known to reduce the formation and stability of arterial thrombi and platelet function in mice [58]. Furthermore, increased *PCSK9* levels are associated with higher platelet reactivity, and are a possible predictor of ischemic events in patients undergoing treatment with novel *P2Y12* inhibitors, prasugrel or ticagrelor [59,60]. Similar to the above findings, rs2495477 of *PCSK9* was also proven to be significantly associated with the PK of prasugrel (p = 0.00119) among Han Chinese population in this study.

ADAMTSL1 was found to have an effect on both the PK and PD of prasugrel, and the protein resembles members of the *ADAMTS* family of proteases. A genome-wide association study [61] identified that *ADAMTS* in combination with misbalanced coagulation signals plays a major role in postnatal vascular injury with subsequent thrombus formation, which is the leading cause of pediatric stroke. *ADAMTS7* expression correlated with atherosclerosis and vascular calcification, and levels above the median values were associated with increased risk for postoperative cardiovascular events [62,63]. Versican is a component of the extracellular matrix and is degraded by *ADAMTS1, ADAMTS4, ADAMTS5,* and *ADAMTS9*; this degradation is of importance in atherosclerosis because versican accumulation was observed in blood vessels susceptible to atherosclerosis [64,65]. Immunohistochemical analysis also demonstrated that *ADAMTS1*, *−4, −5* and *− 8* are present within human carotid lesions and advanced coronary atherosclerotic plaques [66]. Thus, we hypothesized that *ADAMTS* genes might be a promising treatment target for atherosclerosis and vascular calcification.

At present, there are quite a lot of different platelet detection methods, including light-transmission aggregometry (LTA), thromboelastometry (TEG), VerifyNow, vasodilator-stimulated phosphoprotein

Table 3
Results of individual gene set-based analysis of genes previously associated with 24 h and 12d PRU in PLINK.

Gene symbol	Database	Total SNPs	Plink-P min 24 h	Plink-P min 12d	SNP	Chr	BP	Gene types	GENO-24 h	GENO-12d	Pathways/Gene
ADAMTSL1	GeneCards	506	0.00407	0.00494	rs10156461	9	18,818,890	AA/AG/GG	3/12/17	3/11/17	Prasugrel related gene
PRKCA	BioCarta	261	0.00303	0.00714	rs77445472	17	64,256,243	GG/GA/AA	0/11/21	0/11/20	Aspirin Blocks Signaling Pathway Involved in Platelet Activation
					rs11653415		64,279,331	CC/CA/AA			
					rs10491174		64,254,559	GG/GA/AA			
					rs4641779		64,283,326	AA/AC/CC			
					rs9910778		64,261,974	CC/CA/AA			
ITPR2	KEGG	213	0.000026	0.00986	rs10771282	12	26,533,574	GG/GA/AA	0/6/26	0/6/25	Platelet activation pathway
P2RY12	GeneCards/KEGG	4	0.00293	0.00742	rs3821667	3	151,058,441	AA/AG/GG	2/7/23	2/7/22	Prasugrel related gene/Platelet activation pathway
P2RY14	GeneCards	18	0.00145	0.00281	rs2141631	3	150,970,104	GG/GA/AA	2/5/25	2/5/24	Prasugrel related gene
PLCB4	KEGG	159	0.00054	0.00387	rs2179798	20	8,891,346	AA/AG/GG	0/13/19	0/13/18	Platelet activation pathway
					rs962349		8,891,686	GG/GA/AA			
PRKG1	KEGG	401	0.00312	0.00466	rs4935293	10	53,693,565	CC/CA/AA	4/13/15	4/13/14	Platelet activation pathway
ADCY1	KEGG	210	0.00249	0.00322	rs6463268	7	45,245,316	GG/GA/AA	0/7/25	0/7/24	Platelet activation pathway
LYN	KEGG/BioCarta	37	0.00108	0.00089	rs62516880	8	56,789,408	AA/AG/GG	1/4/27	1/4/26	Platelet activation pathway/Eph Kinases and ephrins support Platelet aggregation

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; BP, base pair; GENO, gene type number; ADAMTSL1, a disintegrin and metalloproteinase with thrombospondin motif Like 1; PRKCA, Protein kinase C, alpha; ITPR2, inositol 1,4,5-triphosphate receptor type 2; P2RY12, Purinergic Receptor P2Y12; P2RY14, Purinergic Receptor P2Y14; PLCB, Phospholipase C, beta (phosphoinositide-specific); PRKG1, cGMP-dependent protein kinase 1; ADCY1, adenylate cyclase 1; LYN, V-yes-1 Yamaguchi sarcoma viral related oncogene homolog;

Table 4
Results of individual gene set-based analysis of genes previously associated with AUC0-tlast in Gene Cards database.

Gene symbol	Total SNPs	Plink-P min	SNP	Chr	BP	Gene types	GENO	Gene location
ADAMTSL1	506	0.001383	rs526826	9	18,721,962	CC/CA/AA	1/12/19	INTRON
CD36	59	0.005485	rs940541	7	80,150,594	AA/GA/GG	2/9/21	INTERGENIC
P2RY1	143	0.0091	rs9826760	3	152,852,188	GG/GA/AA	2/4/16	INTERGENIC
PCSK9	38	0.00119	rs2495477	1	55,518,467	GG/GA/AA	5/11/16	INTRON
PON1	228	0.009472	rs854552	7	94,927,924	GG/GA/AA	5/13/12	UTR
SCD	82	0.003636	rs11190486	10	102,131,267	AA/GA/GG	1/4/27	INTERGENIC
			rs1393491		102,127,147	GG/GA/AA		

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; BP, base pair; *GENO*, gene type number; *ADAMTSL1*, a disintegrin and metalloproteinase with thrombospondin motif Like 1; *PRKCA*, Protein kinase C, alpha; *P2RY1*, Purinergic Receptor P2Y1; *CD36*, *CD36* Molecule; *PCSK9*, Proprotein Convertase Subtilisin/Kexin Type 9; *PON1*, Paraoxonase 1; *SCD*, Stearoyl-CoA Desaturase.

(VASP), platelet function analyzers (PFA), and others. The VerifyNow P2Y12 is a point-of-care device that measures platelet reactivity to adenosine diphosphate and the assay is sensitive to the detection of antiplatelet drug resistance [67], which gives results in terms of P2Y12 Reaction Units (PRU) and %. VerifyNow P2Y12 test was performed in prasugrel related clinical trials (e.g., NCT01684813 [68], NCT01852214 [69], and NCT01115738 [70]), thus we used the assay to assess platelet reactivity so as to be comparable to other studies. But it has a relatively poor relationship with PK and LTA in cases of high platelet inhibition. Thus, we might use more platelet function test methods in order to measure platelet reactivity during prasugrel treatment in the future study design, to better exploring the genetic variations associated with prasugrel pharmacodynamics and clinical outcomes.

We investigated the genetic polymorphisms associated with PD and PK of prasugrel in the study. The analysis also suggested the roles of multiple genes (*ADAMTSL1, PRKCA, ITPR2, P2RY12, P2RY14, PLCB4, PRKG1, ADCY1*, and *LYN*) on prasugrel effect, thus genotyping-guided approach had its own limited value at some point. There is no suitable statistical method to integrate multifactorial, multigenomic effects on antiplatelet drug therapy, so we can explore the possible factors affecting PD and PK of prasugrel by determining phenotypes, such as PRU values or other characteristics of patients. Phenotyping as a sum of the whole effect might be more useful to guide escalation/de-escalation during prasugrel than genotyping-guided approach. Several methods for phenotyping of the pharmacodynamics effect of antiplatelet drugs might be a good risk predictor [71–73]. At the same time, it is hoped that better statistical methods can be used to integrate the effects of multiple genomics, phenotypes and other factors to predict the efficacy of antiplatelet drugs before and during medication.

We found that genetic variation affected the PK and PD of prasugrel in Han Chinese individuals, and some new, not reported SNPs were discovered in the study. This might be the results of ethnic difference. Actual and weight-normalized estimates of prasugrel's active metabolite exposure were 31% to 47%, and 19% higher in Asians than in Caucasians after a 30 mg dose of prasugrel in the study of Small DS et al. [74,75], resulting in greater platelet inhibition in Asian subjects. Thus, we speculate that genotypes might be one of the underlying mechanisms of the ethnic difference. A study of Small DS et al. and the findings of this study provide a good clue for future studies on the potential mechanism of ethnic differences affecting PK/PD.

Owing to the limited number of subjects included in this study, the results obtained signify a probable effect of SNPs on prasugrel PD and PK. These findings could serve as a hypothesis-generating effort, and should be validated in further studies with larger sample sizes to determine whether the SNPs are associated with the effect of prasugrel only or also with cardiovascular events and all-cause mortality.

5. Conclusion

The results show that genetic variation may affect the PK and PD of prasugrel in normal individuals. These SNPs may not only be associated prasugrel activity but also with cardiovascular events and all-cause mortality.

Author contributions

All authors conceived and drafted the study, and analyzed and interpreted the data. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2018.11.013.

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