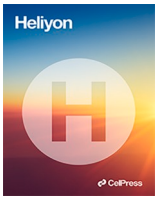
Heliyon 10 (2024) e34552

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Genetic factors related to aspirin resistance using the Multiplate® device in Hong Kong Chinese patients with stable coronary heart disease

Weiwei Zeng [a,1], Tanya TW. Chu [b,1], Elaine YK. Chow [b], Miao Hu [b], Benny SP. Fok [b], Juliana CN. Chan [b], Bryan PY. Yan [b], Brian Tomlinson [b,c,*]

[a] Shenzhen Longgang Second People's Hospital, 518112, China
[b] Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China
[c] Faculty of Medicine, Macau University of Science & Technology, Macau, 999078, China

ARTICLE INFO

Keywords:
Aspirin
PTGS1/COX1
PTGS2/COX2
Platelet function
TXB2
ASPI measurements

ABSTRACT

Objective: Associations between single nucleotide polymorphisms (SNPs) and aspirin resistance (AR) have been studied with variable results. The associations of genetic variants with AR may be helpful to explain why some individuals demonstrate aspirin insensitivity with this anti-platelet therapy. The purpose of this research was to investigate the effect of different genotypes in candidate genes on aspirin response in patients taking long-term aspirin therapy by measuring the serum thromboxane B2 (TXB2) and platelet function using the Multiplate® analyser.
Methods: A total of 266 patients with stable coronary heart disease (CHD) taking low-dose aspirin for long periods of time and without any other anti-platelet drugs medications were enrolled into the study. They were required to take 80 mg of aspirin every morning for a week including the day before blood tests. Blood samples were collected 24 h after the last dose. The 80 mg dose of aspirin was taken orally and blood samples were collected again 1 h later. The serum TXB2 levels were measured in samples at 24 h post-dose and 1 h post-dose using the EIA kit and platelet activity was determined using the Multiplate® Impedance Platelet Aggregometry (ASPI) assay. Genotyping assays were performed by the TaqMan SNP genotyping technique.
Results: Of the 266 patients, only 251 patients were enrolled in the present study. The *PTGS1/COX1*-1676 A > G (rs1330344) and the *PTGS2/COX2*-765 G > C (rs20417) SNPs showed significant associations with the ASPI measurements in samples taken at 24 h post-dose, but not with the values at 1 h post-dose or with the TXB2 levels ($P < 0.05$).
Conclusions: Our results suggest that polymorphisms in the *PTGS1/COX1* and the *PTGS2/COX2* genes may be associated with reduced anti-aggregatory effects and increased the risk of AR, but future larger-scale cohort studies are necessary for further validation.

1. Introduction

Aspirin is widely utilized as an antithrombotic drug for prevention and treatment of cardiovascular diseases (CVDs), especially for

* Corresponding author. Faculty of Medicine, Macau University of Science & Technology, Avenida Wai Long, Taipa, Macau, 999078, China.
E-mail address: btomlinson@must.edu.mo (B. Tomlinson).
[1] These authors contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.heliyon.2024.e34552
Received 29 August 2023; Received in revised form 9 July 2024; Accepted 11 July 2024
Available online 14 July 2024

stable coronary heart disease (CHD) [1,2]. Aspirin exerts pharmacodynamic effects via irreversibly inhibiting the activation of platelet cyclooxygenase-1 (COX-1) to prevent the production of thromboxane A2 (TXA2) from arachidonic acid (AA), thereby, suppressing platelet activation and aggregation [3–6]. Studies found that taking long-term low-dose aspirin decreased the chances of experiencing cardiovascular events [7]. Aspirin was shown to reduce the risk of recurrent ischaemic stroke within 12 weeks by approximately 60 % [8]. However, not all patients benefit from aspirin as there still are many patients who suffer adverse cardiovascular events when taking aspirin [1]. A prospective pilot study of 80 patients in Poland showed that 17.3 % of acute stroke patients exhibited reduced sensitivity to aspirin [9].This phenomenon has been termed "Aspirin Resistance" (AR) and its physiopathologic and molecular mechanisms have not been fully elucidated [3,10]. Researchers speculate that it may be related to various factors such as genetic polymorphisms in platelet glycoprotein (GP) and COX-1 genes, collagen, smoking, and dyslipidemia. As researchers delve deeper, there is growing evidence that certain single nucleotide polymorphisms (SNPs) are associated with AR [5].

Currently, studies on the relationship between AR and gene polymorphisms have focused on the following aspects: (1) Polymorphisms in the genes encoding COX-1 and COX-2 in the thromboxane activation pathway [11]. Multiple polymorphic loci in the COX genes have been identified, and SNPs in different COX genes can affect the protein structure or conformation of COX-1 making it highly heterogeneous in its susceptibility to the inhibitory effects of aspirin, which may be the structural basis of AR in some patients [12]. (2) An integrin-family member of cell adhesion receptors, platelet glycoprotein GPIIb/IIIa is involved in platelet adhesion and aggregation, as well as the ultimate common pathway for platelet activation. Changes in the GPIIb/IIIa genes, whether through mutations, deletions, or insertions, result in changes in phenotype that ultimately affects platelet function. Thus, AR may be associated with polymorphisms in the platelet membrane GPIIb/IIIa receptor complex [13]. (3) Adenosine kinase (ADK) is an important regulator in the cell that can catalyze the formation of adenosine 5′-phosphate (AMP) [14,15]. A study showed that the ADK rs16931294 variant was highly correlated with the reduction of aspirin sensitivity and higher post-aspirin inosine levels in participants with the G allele [16]. (4) Platelet Endothelial Aggregation Receptor 1 (PEAR1), as a transmembrane molecule, plays a role in the process of triggering platelet activation. The gene is polymorphic, and PEAR1 gene variants can affect platelet aggregation function, leading to AR, thereby affecting the efficacy of aspirin [17,18].These genetic factors were considered as implicated in AR, but the effects of these genotypes on AR have not always been reproducible, which may be attributed to differences in ethnically specific genetic characteristics and none of these variants have been shown to have consistent effects in different studies using different methodologies [19]. Further investigation into the connection between SNPs and AR is necessary. The relationship between genetic variants and AR may help to explain the insensitivity of some patients to aspirin antiplatelet therapy.

In the clinic, AR is usually characterized by the failure of aspirin to adequately prevent platelet aggregation in vitro. Therefore, it is crucial to determine the impact of aspirin on the aggregation of platelets. Platelets are an essential part of maintaining homeostasis in the body [20]. Although there are many clinical methods to detect the impact of aspirin on platelet aggregation, most of these methods have inconsistent results on the response of aspirin to platelet aggregation. It has been proposed that it was preferable to directly measure the ability of platelets to produce TXA2 [21,22]. In particular, the levels of 11-dehydro-TXB2 in urine serve as a general indicator of TXA2 biosynthesis the body. However, this method lacks precision in assessing the impact of aspirin on platelet COX-1 due to the fact that around 30 % of urinary metabolites originate from sources outside of platelets [23]. Thromboxane B2 (TXB2), the primary product of platelet TXA2 metabolism, originates exclusively from platelet TXA2. Therefore, serum TXB2 was deemed the most precise indicator of aspirin's impact on platelets [24]. The serum TXB2 assay was moderately correlated with other tests detecting platelet function and inadequate aspirin response. However, platelets might be activated by various pathways besides TXA2. Therefore, measuring platelet response to aspirin using this method may potentially underestimate the prevalence of AR or "aspirin non-responders".

In order to improve the accuracy of detection of AR, in our previous study, we assessed serum TXB2 levels and platelet function with the Multiplate® analyzer in individuals receiving prolonged aspirin therapy. We found that AR was detected in 46 % of patients within 24 h of the last aspirin dose, with a higher likelihood of AR in patients with elevated white blood cell counts. It was also found that AR was not associated with diabetes, but could be connected to levels of white blood cells, hemoglobin, platelets, and smoking habits [25]. However, in previous studies, we did not explore the impact of aspirin administration on platelet aggregation response in patients with different genetic polymorphisms. At the same time, mutations at the *PTGS1*, *COX2*, *ITGA2*, *ITGA2B*, *PEAR1* and *ADK* genes have been reported as possible associations with AR, but their specific relevance needs to be further confirmed [26,27]. In this study, serum TXB2 levels and platelet activity were measured by EIA kit and Multiplate® impedance platelet aggregometry (ASPI), respectively, in patients with stable CHD who were taking low-dose aspirin for a long time and did not take any other antiplatelet drugs. In addition, the effect of candidate SNPs on AR in these Hong Kong, China patients was investigated after genomic DNA was extracted from the patients' blood. It was expected that the Multiplate® device will improve the diagnostic accuracy of AR and further clarify the effect of genetic polymorphisms on AR.

2. Methods

2.1. Patient recruitment

Eligible patients aged over or equal to 18 years, with stable CHD, and receiving plain aspirin (80 mg once daily) for at least 3 months were invited to participate in the study at the outpatient clinic of the Prince of Wales Hospital in Hong Kong. Details of patient recruitment could be found in our previous publication [25].

2.2. Ethics

The study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee under reference number CRE-2014.516-T, in accordance with the Principles outlined in the Declaration of Helsinki. All participants signed the informed consent at enrolment.

2.3. Sample collection

A 10 ml blood sample was collected in an EDTA tube and used for DNA extraction.

2.4. Multiplate analyzer

Platelet function in the samples was assessed using hirudin blood samples and the Multiplate® analyzer from Roche (Roche Diagnostics International Ltd, CH-6343 Rotkreuz, Switzerland) according to the manufacturer's instructions for ASPI measurements. The analysis was performed within 0.5–3 h after blood collection.

2.5. Serum TXB2 assay

Serum samples were assayed for TXB2 using enzyme-linked immunoassay (EIA) kits from Cayman (Item No. 501020, Cayman Chemical, MI, USA) following the product inserts.

2.6. Genetic studies

The patients' genomic DNA was obtained from EDTA blood through the use of phenol chloroform extraction. The following six SNPs were investigated: *PTGS1* rs1330344, *COX2* rs20417, *ITGA2* rs1126643, *ITGA2B* rs5911, *PEAR1* rs12041331 and *ADK* rs16931294. All the SNPs with the exception of *COX2* rs20417 were tested using TaqMan SNP genotyping assays from Applied Biosystems (Applied Biosystems, Foster City, CA, USA), conducted by Thermo Fisher Scientific following the provided instructions.

The *COX2* rs20417 variant was analyzed using the Applied Biosystems Custom TaqMan SNP genotyping assays from Thermo Fisher Scientific. The forward and reverse primers sequences were 5′-CGTGGAGCTCACATTAACTATTTACAG- 3′ and 5′-CCCTCCTTGTTTCTTGGAAAGA- 3′, respectively.

2.7. Statistics

All the statistical analyses were conducted with SPSS version 17.0 for windows (SPSS Inc., Chicago, IL, USA). The recorded data are displayed as the mean ± standard error of the mean (SEM). The chi-squared test was used to evaluate the observed allele and genotype frequencies in relation to Hardy-Weinberg equilibrium. Genetic polymorphisms in the various groups before and after aspirin administration were compared using the chi-squared or Fisher's exact tests and one-way ANOVA analysis, and the odds ratio were described with the 95 % confidence intervals (CI). All probability values were two-tailed tests, and the P-value less than 0.05 was considered statistically significant. The data were analyzed by GraphPad Prism version 8.0.2 for windows (GraphPad software, Inc., San Diego, CA, USA).

Table 1
Frequencies of genotypes and alleles of the polymorphisms studied.

Polymorphism	Genotype frequency			Hardy–Weinberg analysis		Allele frequency	
ADK rs16931294	AA	AG	GG	X^2 =	0.981188	A	G
	211	50	5	X^2 p value =	0.321906	0.887218	0.112782
	79.30 %	18.80 %	1.90 %				
ITGA2 rs1126643	AA	AG	GG	X^2 =	0.306786	A	G
	131	114	21	X^2 p value =	0.579659	0.293233	0.706767
	49.20 %	42.90 %	7.90 %				
PEAR1 rs12041331	CC	CT	TT	X^2 =	1.288484	C	T
	75	141	50	X^2 p value =	0.256327	0.546992	0.453008
	28.20 %	53.00 %	18.80 %				
PTGS1 rs1330344	TT	TG	GG	X^2 =	3.807284	T	G
	88	143	35	X^2 p value =	0.05103	0.400376	0.599624
	33.10 %	53.80 %	13.20 %				
ITGA2B rs5911	GG	GA	AA	X^2 =	0.174386	G	A
	79	135	52	X^2 p value =	0.676243	0.449248	0.550752
	29.70 %	50.80 %	19.50 %				
COX2 rs20417	GG	GC		X^2 =	0.405884	G	C
	246	20		X^2 p value =	0.524066	0.962406	0.037594
	92.50 %	7.50 %					

3. Results

3.1. The effect of genotype on serum TXB2 and ASPI AUC

With the exception of *PTGS1* rs1330344, which was marginally significant at P = 0.05103, the frequencies of the other six SNPs were not significantly different from the Hardy-Weinberg equilibrium (Table 1).

The analyses of these 6 SNPs in correlation with serum TXB2 levels and the ASPI Area Under the Curve [AUC] measures in the samples 24 h and the samples 1 h post-dose were shown in Table 2. The *PTGS1/COX1* –1676 A > G (rs1330344) and *PTGS2/COX2* -765 G > C (rs20417) showed significant associations with ASPI measurements in the samples 24 h post-dose, but not with the values 1 h post-dose or with the TXB2 levels (Figs. 1–2, and Table 2).

4. Discussion

The mechanisms of AR remain uncertain. It may be related in part to genetic polymorphisms resulting in aspirin insensitivity [28]. There are various methods to test the effects of aspirin on platelet aggregation, but the results are inconsistent [29,30]. Therefore, it is crucial to precisely identify how aspirin impacts platelet reaction in individuals with different genetic variants. In this study, the ASPI was used to measure platelet aggregation, the EIA kit was utilized to determine serum TXB2 levels, and SNP genotyping was performed. The results of the relevant experiments demonstrated that only the *PTGS2/COX2*-765 G > C (rs20417) and the *PTGS1/COX1*-1676 A > G (rs1330344) SNPs showed significant associations with the ASPI measurements in the samples pre-dose ($P < 0.05$) but not with the values 1 h after the dose or with the TXB2 levels. The other SNPs, *ITGA2* rs112643, *ITGA2B* rs5911, *ADK* rs16931294, *PEAR1* rs1204133, had no significant effect on either the ASPI measurements or the TXB2 levels ($P > 0.05$). These genetic findings are also supported by some previous studies. Research conducted on Chinese individuals found that the occurrence of the *PTGS2-765* G > C genetic variation was notably elevated in the AR or semi-resistant categories in contrast to the aspirin sensitive group [31]. In that study, there were no significant association between AR and several polymorphisms in the *PTGS1* gene (*A842G*, *C50T*, *C22T*, *G128A*, *C644A* and *C714A*) [31]. A meta-analysis found that the COX-2 gene rs20417 variant showed significant association with aspirin insensitivity, the risk allele of rs20417 in Chinese patients was up to 19.59 % compared with healthy humans [13]. In addition, Yi et al. reported that the interaction between the COX-1 gene rs3842787 and COX-2 gene rs20417 variants was crucial in determining genetic susceptibility to AR [32]. However, in some other studies the results obtained were inconsistent with our findings. Vadana et al.'s research in India with around 900 participants found that the -756 C allele of COX-2 was significantly associated with a poorer prognosis in stroke patients receiving aspirin therapy [33]. Moreover, Deniz et al. obtained a similar result in a study in 74 patients in Turkey [34]. These findings may not be opposed to our results, because their study populations are different from the present study. However, two studies involving 97 and 850 acute ischemic stroke Chinese patients, respectively, showed that COX-2 gene −765 G > C was not related with AR [35,36]. Those findings are contradictory with our results.

Table 2
Associations of genotype polymorphisms with the serum TXB2 and ASPI AUC in patients with stable CHD.

Gene and genotype	n	Serum TXB2 (pg/ml) Mean ± SD				ASPI AUC (AU*min) Mean ± SD			
		pre-dose	post-dose	P_1	P_2	pre-dose	post-dose	P_1	P_2
PTGS1/COX1: (−1676) A > G (rs1330344)									
TT	84	94.1 ± 12.2	55.0 ± 4.8	0.91	0.54	348.9 ± 21.5	277.6 ± 13.2	0.034*	0.26
TG	137	90.6 ± 7.0	54.4 ± 3.0			299.5 ± 11.9	254.8 ± 10.4		
GG	30	86.1 ± 10.8	63.3 ± 9.1			273.2 ± 29.2	242.9 ± 19.6		
PTGS2/COX2: (−765) G > C (rs20417)									
GG	232	91.4 ± 6.1	55.1 ± 2.6	ns	ns	303.5 ± 10.3	257.0 ± 7.7	0.004**	0.08
GC	19	89.4 ± 12.2	62.3 ± 11.8			418.5 ± 51.6	306.7 ± 32.6		
ITGA2 (rs1126643)									
AA	121	91.6 ± 91.0	53.6 ± 39.9	0.64	0.47	316.7 ± 168.5	265.4 ± 129.2	0.28	0.41
AG	109	94.2 ± 94.4	59.0 ± 41.8			316.7 ± 166.4	261.9 ± 112.1		
GG	20	73.2 ± 66.9	49.7 ± 35.8			255.7 ± 165.6	227.1 ± 128.1		
ITGA2B (rs5911)									
GG	71	95.9 ± 88.9	56.0 ± 40.2	0.30	0.54	320.9 ± 166.9	273.7 ± 136.2	0.61	0.53
GA	128	95.8 ± 102.5	57.6 ± 41.9			301.8 ± 176.1	253.8 ± 118.4		
AA	49	73.8 ± 53.9	50.3 ± 37.0			324.4 ± 145.3	260.4 ± 109.6		
PEAR1 (rs12041331)									
CC	69	90.2 ± 76.2	56.3 ± 36.7	0.47	0.63	317.9 ± 167.5	263.4 ± 112.9	0.77	0.69
CT	132	96.8 ± 95.2	57.2 ± 41.7			304.9 ± 162.7	264.2 ± 134.1		
TT	47	78.3 ± 97.1	50.8 ± 42.2			322.5 ± 183.2	247.1 ± 98.7		
ADK (rs16931294)									
AA	200	93.1 ± 40.6	56.0 ± 40.6	0.75	0.97	316.3 ± 171.2	266.8 ± 123.1	0.69	0.29
AG	46	85.7 ± 40.3	54.6 ± 40.3			294.0 ± 157.6	238.7 ± 118.4		
GG	4	68.6 ± 41.7	53.2 ± 41.7			296.3 ± 74.8	226.8 ± 106.6		

*The asterisk indicates statistical significance between bars (*p < 0.05, **p < 0.01, ***p < 0.001).

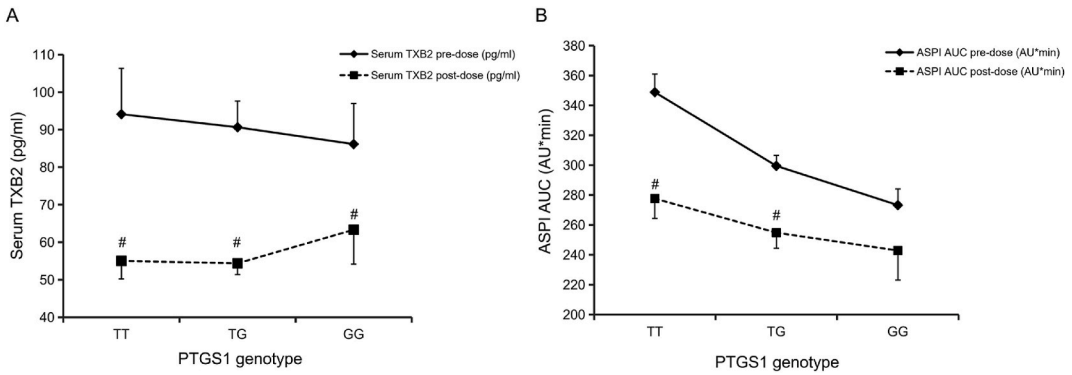


Fig. 1. Comparisons of serum TXB2 and ASPI AUC *PTGS1/COX1*: (−1676) A > G (rs1330344) genotypes (mean ± SEM). A. Serum TXB2 at pre-dose and 1 h post-dose; B. ASPI AUC at pre-dose and 1 h post-dose; #*P* < 0.0001, as compared to corresponding pre-dose value.

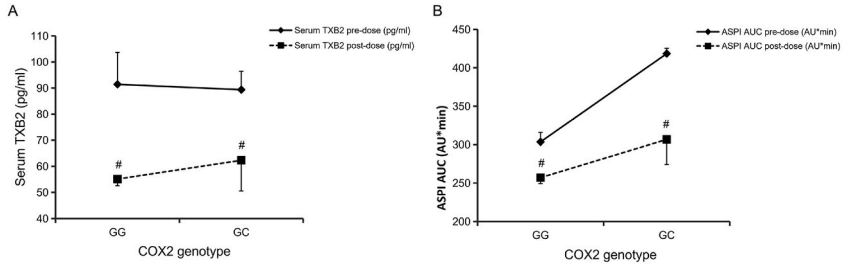


Fig. 2. Comparisons of serum TXB2 and ASPI AUC among *COX2/PTGS2*: (−765) G > C (rs20417) genotypes (mean ± SEM). A. Serum TXB2 at pre-dose and 1 h post-dose; B. ASPI AUC at pre-dose and 1 h post-dose; #*P* < 0.0001, as compared to corresponding pre-dose value.

Another research conducted on Chinese individuals with CVD found that AR, as measured by light transmission aggregometry (LTA) and thromboelastography platelet mapping assay (TEG) using AA as a stimulus, was linked to a higher likelihood of experiencing combined cardiovascular events. Additionally, the presence of the G-allele variant of *PTGS1* rs1330344 (-1676 A/G) was significantly associated with an increased the risk of AR [11]. Another study observed that the frequency of the C-allele in rs1330344 was higher among patients, and the T-allele and CC genotype increased the association with AR [34].

For the other variant genes, however, some findings in the present study appear to be inconsistent with results from several studies. It is known that the *ITGA2* and *ITGA2B* genes encoding GPIa and GPIIb, respectively, are important glycoprotein-coupled receptors in the cellular membrane binding with TXA2 that can lead to the activation of platelets [36,37]. The present study found the G-allele frequency of the rs1126643 variant was up to 70.68 %, but the variant genes were not related to AR. In contrast to our results, recent meta-analyses also found that the *ITGA2B* rs5911 and *ITGA2* rs1126643 SNPs might be associated with AR in Asians, especially in Chinese populations [13,26,37]. Furthermore, Ningrum et al. reviewed studies about the *ITGA2* rs1126643 SNP among various population, and found that the variant in Chinese and Asians increased the risk of ischemic stroke or other bleeding events more than in Caucasians [38]. In that study it was also shown that the C-allele of the rs1126643 SNP in Indonesia was similar to other Asians, but the TT genotype and T-allele were more associated with vascular events [38]. An earlier study suggested that rs5911 was verified as a biomarker of AR in a Chinese population, especially the C-allele carriers [37]. However, the A-allele carriers were the most frequent in all the recruited patients, while the patients did not carry the C-allele. This may be a reason why our findings are inconsistent with some others.

In addition, we found that the *PEAR1* rs12041331 and *ADK* rs16931294 SNPs were not significant AR factors in Hong Kong Chinese. Similarly, in 283 ischemic stroke patients included in a clinical study with aspirin, there was no association observed between in the *PEAR1* rs12041331 SNP and AR [39]. AA homozygotes have been considered as a risk factor for increasing adverse cardiovascular events [40]. Lewis et al. showed that the genetic variation *PEAR1* rs12041331 was not correlated with cardiovascular events in patients taking low-dose aspirin [28] Our results were consistent with these findings. However, a study of Xiang et al. on genetic variants of *PEAR1* did suggest that rs12041331 is associated with a greater platelet aggregation [41]. ADK as a modulator of intracellular adenosine concentration, is considered to regulate the development of blood vessels in vivo [42]. Recently, it was demonstrated that the *ADK* rs16931294 G-allele and GG genotype were significantly correlated with platelet aggregation [16]. Contrasting with that study, our patients were more often carrying the A-allele and AA genotype than the G-allele and GG genotype. Perhaps for this reason, the *ADK* rs16931294 SNP had no association with AR in our patients.

Although there are some positive findings in this study, there are several limitations. We recruited only 251 patients, a relatively small sample size that may not be sufficient to reveal the specific effects of certain SNPs on AR in Hong Kong Chinese patients with

stable CHD. The sample size limitation may lead to insufficient validity of the statistical analyses, thus affecting the reliability and generalizability of the results. Moreover, our genetic study design did not include an in-depth analysis of specific subgroups, and in this analysis, we did not differentiate between subjects with and without diabetes mellitus. Diabetes is a disease that is known to affect drug metabolism and tolerance; therefore, failure to consider this factor may have biased our results, although our earlier study did not show any difference between subjects with and without diabetes with regard to AR. In addition to these limitations, we did not consider possible gene-gene interactions, because we felt the sample size was insufficient. In genetic studies, interactions between different genes may have an important impact on drug response, and ignoring this may miss some important biological information. To overcome these limitations, future studies should expand the sample size to enhance the validity of the statistical analyses and to improve the generalizability and credibility of the findings. Meanwhile, designing more detailed stratified analyses, such as grouping subjects according to diabetes status or other clinical characteristics, may reveal differences between subgroups. In addition, more complex genetic models including gene-gene interactions should be considered to more fully understand how genetic factors influence AR.

In summary, these variant genotypes, *PTGS2/COX2* rs20417 and *PTGS1/COX1* rs1330344, *ITGA2* rs112643, *ITGA2B* rs5911, *PEAR1* rs1204133 and *ADK* rs16931294, have inconsistent impact on AR measurements. We speculate that the inconsistent findings may result from studies in different ethnic groups, different methods, the size of the study population, environmental and gene-gene interactions in the polymorphisms and other reasons. Currently, the specific mechanism and influence of genetic polymorphisms on AR need further investigation. If AR is confirmed, clinicians have the option of modifying the aspirin dosing regimen or using other antiplatelet therapies. These clinical features and genetic tests can be utilized to identify individuals at higher risk for AR, which can then be tested with the Multiplate® analyzer. This approach may be logical, but has not been tested in randomized clinical trials. Thus, further clinical validation is necessary. In addition, further studies will assist in determining whether an increased dose or frequency of aspirin administration would improve the measurement of platelet aggregation. Following this, large-scale clinical trials will be essential to demonstrate the benefits of this approach.

5. Conclusion

This study revealed a significant association between polymorphisms in the *PTGS1/COX1* and *PTGS2/COX2* genes and the pharmacodynamics of ASPI. Specifically, we found statistically significant associations between variants in these genes and the patients' ASPI measurements 24h after dosing, which may imply that the genetic variants have an effect on aspirin antiplatelet aggregation effects at the end of the dosing period. However, this association was not significant in the measurements taken 1h after dosing, which may suggest that the effect of genetic polymorphisms on drug response may be time-dependent. Alternatively, other factors may be more important in the early stages of drug action. In addition, the findings suggest that polymorphisms in the *PTGS1/COX1* and *PTGS2/COX2* genes may be associated with reduced antiplatelet aggregation effects and increased risk of AR. It suggests that an individual's genetic makeup may influence their response to aspirin therapy and thus have an impact on cardiovascular disease prevention outcomes. In summary, genetic polymorphisms have a significant impact on aspirin efficacy, which means that considering a patient's genetic polymorphisms may be critical to developing a personalized treatment plan in clinical practice. With genetic testing, physicians can more accurately predict a patient's response to aspirin and adjust the treatment regimen accordingly to improve efficacy and reduce the risk of adverse effects. Therefore, this study highlights the value of genetic testing in individualized medicine, suggesting that optimizing treatment strategies for aspirin-resistant patients by taking into account genetic polymorphisms may help to avoid treatment failures and associated serious health consequences, thereby improving clinical outcomes for patients.

Data availability statement

Data associated with this study are not deposited in a publicly available repository. Further information could be accessed upon reasonable requests by contacting the corresponding authors.

Ethics declarations

The study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee with reference number CRE-2014.516-T and followed the Principles of the Declaration of Helsinki.

Funding

This work was supported by the Health and Medical Research Fund of the Food and Health Bureau of the Hong Kong SAR Government (Project No. 12130981).

CRediT authorship contribution statement

Weiwei Zeng: Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Tanya TW. Chu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Elaine YK. Chow:** Writing – review & editing. **Miao Hu:** Validation, Software, Methodology, Conceptualization. **Benny SP. Fok:** Writing – review & editing, Supervision, Investigation. **Juliana CN. Chan:** Writing – review & editing. **Bryan PY.**

Yan: Writing – review & editing, Investigation, Conceptualization. **Brian Tomlinson:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The investigators would like to thank Ms. Kathy YC Tsang and Ms. Emily WM Poon for technical support, Ms. Hermia WF Kwong, Ms. Eliza CY Choy, Ms. Winnie MN Yeung, Ms. Maybo SL Lin and Ms. Swen YW Ip for nursing support, Dr. Tanya TW Chu for statistical support and Prof. Juliana CN Chan for general advice for the project.

References

[1] R.W. Godley, E. Hernandez-Vila, Aspirin for primary and secondary prevention of cardiovascular disease, Tex. Heart Inst. J. 43 (4) (2016) 318–319.
[2] C. Baigent, L. Blackwell, R. Collins, et al., Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials, Lancet 373 (9678) (2009) 1849–1860.
[3] G. Du, Q. Lin, J. Wang, A brief review on the mechanisms of aspirin resistance, Int. J. Cardiol., 2202016) 21-26.
[4] M. Dovizio, A. Bruno, S. Tacconelli, P. Patrignani, Mode of action of aspirin as a chemopreventive agent,Recent Results Cancer Res. 1912013) 39-65.
[5] M. Ferreira, M. Freitas-Silva, J. Assis, R. Pinto, J.P. Nunes, R. Medeiros, The emergent phenomenon of aspirin resistance: insights from genetic association studies, Pharmacogenomics 21 (2) (2020) 125–140.
[6] K.T. Hall, T. Kessler, J.E. Buring, et al., Genetic variation at the coronary artery disease risk locus gucy1a3 modifies cardiovascular disease prevention effects of aspirin, Eur. Heart J. 40 (41) (2019) 3385–3392.
[7] J.M. Guirguis-Blake, C.V. Evans, L.A. Perdue, S.I. Bean, C.A. Senger, Aspirin use to prevent cardiovascular disease and colorectal cancer: updated evidence report and systematic review for the us preventive services task force, JAMA 327 (16) (2022) 1585–1597.
[8] P.M. Rothwell, A. Algra, Z. Chen, H.C. Diener, B. Norrving, Z. Mehta, Effects of aspirin on risk and severity of early recurrent stroke after transient ischaemic attack and ischaemic stroke: time-course analysis of randomised trials, Lancet 388 (10042) (2016) 365–375.
[9] M. Morton, K. Kubiak-Balcerewicz, A. Sarnowska, U. Fiszer, Biochemical aspirin resistance in acute stroke patients and its association with clinical factors: a prospective pilot study, Folia Neuropathol. 59 (3) (2021) 271–275.
[10] H. Khan, O. Kanny, M.H. Syed, M. Qadura, Aspirin resistance in vascular disease: a review highlighting the critical need for improved point-of-care testing and personalized therapy, Int. J. Mol. Sci. 23 (19) (2022), https://doi.org/10.3390/ijms231911317.
[11] L. Fan, J. Cao, L. Liu, et al., Frequency, risk factors, prognosis, and genetic polymorphism of the cyclooxygenase-1 gene for aspirin resistance in elderly Chinese patients with cardiovascular disease, Gerontology 59 (2) (2013) 122–131.
[12] L. Cao, Z. Zhang, W. Sun, et al., Impacts of cox-1 gene polymorphisms on vascular outcomes in patients with ischemic stroke and treated with aspirin, Gene 546 (2) (2014) 172–176.
[13] Z. Weng, X. Li, Y. Li, J. Lin, F. Peng, W. Niu, The association of four common polymorphisms from four candidate genes (cox-1, cox-2, itga2b, itga2) with aspirin insensitivity: a meta-analysis, PLoS One 8 (11) (2013) e78093.
[14] U. Nayar, J. Sadek, J. Reichel, et al., Identification of a nucleoside analog active against adenosine kinase-expressing plasma cell malignancies, J. Clin. Invest. 127 (6) (2017) 2066–2080.
[15] M. Murugan, D. Fedele, D. Millner, E. Alharfoush, G. Vegunta, D. Boison, Adenosine kinase: an epigenetic modulator in development and disease, Neurochem. Int. 1472021)105054, https://doi.org/10.1016/j.neuint.2021.105054.
[16] L.M. Yerges-Armstrong, S. Ellero-Simatos, A. Georgiades, et al., Purine pathway implicated in mechanism of resistance to aspirin therapy: pharmacometabolomics-informed pharmacogenomics, Clin. Pharmacol. Ther. 94 (4) (2013) 525–532.
[17] N. Nanda, M. Bao, H. Lin, et al., Platelet endothelial aggregation receptor 1 (pear1), a novel epidermal growth factor repeat-containing transmembrane receptor, participates in platelet contact-induced activation, J. Biol. Chem. 280 (26) (2005) 24680–24689.
[18] N. Ansari, S. Najafi, S. Shahrabi, N. Saki, Pear1 polymorphisms as a prognostic factor in hemostasis and cardiovascular diseases, J. Thromb. Thrombolysis 51 (1) (2021) 89–95, https://doi.org/10.1007/s11239-020-02149-w.
[19] C. Bellenguez, S. Bevan, A. Gschwendtner, et al., Genome-wide association study identifies a variant in hdac9 associated with large vessel ischemic stroke, Nat. Genet. 44 (3) (2012) 328–333.
[20] G.A. Mason, D.J. Rabbolini, The current role of platelet function testing in clinical practice, Semin. Thromb. Hemost. 47 (7) (2021) 843–854.
[21] N. Van Oosterom, M. Barras, N. Cottrell, R. Bird, Platelet function assays for the diagnosis of aspirin resistance, Platelets 33 (3) (2022) 329–338.
[22] C. Brun, Y. Daali, C. Combescure, et al., Aspirin response: differences in serum thromboxane b2 levels between clinical studies, Platelets 27 (3) (2016) 196–202.
[23] C. Patrono, B. Rocca, Measurement of thromboxane biosynthesis in health and disease, Front. Pharmacol.102019) 1244.
[24] G. Kidson-Gerber, J. Weaver, R. Gemmell, A.M. Prasan, B.H. Chong, Serum thromboxane b2 compared to five other platelet function tests for the evaluation of aspirin effect in stable cardiovascular disease, Heart Lung Circ. 19 (4) (2010) 234–242.
[25] W. Zeng, T. Chu, E. Chow, et al., Factors associated with aspirin resistance in Hong Kong Chinese patients with stable coronary heart disease using the multiplate ((r)) analyzer and serum thromboxane b2, Pharmaceutics 14 (10) (2022).
[26] J. Yang, X. Chen, J. Zhou, S. Hu, Y. Tang, Associations of candidate gene polymorphisms with poor responsiveness to aspirin: a meta-analysis, Clin. Exp. Pharmacol. Physiol. (2018).
[27] A. Ikonnikova, A. Anisimova, S. Galkin, et al., Genetic association study and machine learning to investigate differences in platelet reactivity in patients with acute ischemic stroke treated with aspirin, Biomedicines 10 (10) (2022), https://doi.org/10.3390/biomedicines10102564.
[28] J.P. Lewis, M. Riaz, S. Xie, et al., Genetic variation in pear1, cardiovascular outcomes and effects of aspirin in a healthy elderly population, Clin. Pharmacol. Ther. 108 (6) (2020) 1289–1298.
[29] G. Renda, M. Zurro, G. Malatesta, B. Ruggieri, R. De Caterina, Inconsistency of different methods for assessing ex vivo platelet function: relevance for the detection of aspirin resistance, Haematologica 95 (12) (2010) 2095–2101.
[30] S.T. Lim, S. Murphy, S.M. Murphy, et al., Assessment of on-treatment platelet reactivity at high and low shear stress and platelet activation status after the addition of dipyridamole to aspirin in the early and late phases after tia and ischaemic stroke, J. Neurol. Sci. 4412022) 120334.
[31] Z.H. Xu, J.R. Jiao, R. Yang, B.Y. Luo, X.F. Wang, F. Wu, Aspirin resistance: clinical significance and genetic polymorphism, J. Int. Med. Res. 40 (1) (2012) 282–292.
[32] X. Yi, W. Cheng, J. Lin, Q. Zhou, C. Wang, Interaction between cox-1 and cox-2 variants associated with aspirin resistance in Chinese stroke patients, J. Stroke Cerebrovasc. Dis. 25 (9) (2016) 2136–2144, https://doi.org/10.1016/j.jstrokecerebrovasdis.2016.05.039.
[33] V. Sharma, S. Kaul, A. Al-Hazzani, A.A. Alshatwi, A. Jyothy, A. Munshi, Association of cox-2 rs20417 with aspirin resistance, J. Thromb. Thrombolysis 35 (1) (2013) 95–99.

[34] D. Kirac, A.E. Yaman, T. Doran, M. Mihmanli, E.C. Keles, Cox-1, cox-2 and cyp2c19 variations may be related to cardiovascular events due to acetylsalicylic acid resistance, Mol. Biol. Rep. 49 (4) (2022) 3007–3014.
[35] X. Yi, C. Wang, Q. Zhou, J. Lin, Interaction among cox-2, p2y1 and gpiiia gene variants is associated with aspirin resistance and early neurological deterioration in Chinese stroke patients, BMC Neurol. 17 (1) (2017) 4.
[36] H. Wang, X. Sun, W. Dong, et al., Association of gpia and cox-2 gene polymorphism with aspirin resistance, J. Clin. Lab. Anal. 32 (4) (2018) e22331.
[37] M. Xue, X. Yang, L. Yang, et al., Rs5911 and rs3842788 genetic polymorphism, blood stasis syndrome, and plasma txb2 and hs-crp levels are associated with aspirin resistance in Chinese chronic stable angina patients, Evid.-based Complement Alternative Med. 20172017) 9037094.
[38] V.D.A. Ningrum, R. Istikharah, A.H. Sadewa, Genetic polymorphism of itga2 c807t collagen receptor encoding gene of aspirin therapy among Javanese-Indonesian healthy respondents, Open Access Macedonian Journal of Medical Sciences 9 (A) (2021) 1067–1073.
[39] L.L. Peng, Y.Q. Zhao, Z.Y. Zhou, et al., Associations of mdr1, tbxa2r, pla2g7, and pear1 genetic polymorphisms with the platelet activity in Chinese ischemic stroke patients receiving aspirin therapy, Acta Pharmacol. Sin. 37 (11) (2016) 1442–1448.
[40] K. Xu, S. Ye, S. Zhang, et al., Impact of platelet endothelial aggregation receptor-1 genotypes on platelet reactivity and early cardiovascular outcomes in patients undergoing percutaneous coronary intervention and treated with aspirin and clopidogrel, Circ. Cardiovasc. Interv. 12 (5) (2019) e7019.
[41] Q. Xiang, S. Zhou, J.P. Lewis, A.R. Shuldiner, G. Ren, Y. Cui, Genetic variants of pear1 are associated with platelet function and antiplatelet drug efficacy: a systematic review and meta-analysis, Curr. Pharmaceut. Des. 23 (44) (2017) 6815–6827.
[42] Y. Xu, Y. Wang, S. Yan, et al., Intracellular adenosine regulates epigenetic programming in endothelial cells to promote angiogenesis, EMBO Mol. Med. 9 (9) (2017) 1263–1278.