

RESEARCH ARTICLE

The DNAm levels of CREB5 (cg11301281) were associated with clopidogrel resistance

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Abstract

Purpose: Clopidogrel resistance (CR) is mostly caused by interindividual variability of the platelet inhibition of clopidogrel, which may induce cardiovascular events. The aim of this research was to evaluate whether DNAm levels of CREB5 (cg01534253) are involved in CR among acute coronary syndrome (ACS) patients treated with clopidogrel. **Methods:** 72 patients (36 CR and 36 non-CR) who underwent ACS were included in this study. The VerifyNow P2Y12 assay was selected to evaluate residual platelet reactivity, and bisulfite pyrosequencing methods was used to examine DNA methylation levels on cg01534253. Secondly, CREB5 mRNA expression was analyzed via quantitative real-time PCR. Last, we employed logistic regression to test the interaction between genetic factors of CREB5 methylation and multiple clinical variables in CR patients.

Results: Subunit analysis indicated that for patients whose HbA1c levels were $\geq 6.5\%$ or whose GLU levels were ≥ 7 mmol/L, lower methylation of cg01534253 indicated a poorer clopidogrel response. In addition, CREB5 mRNA expression was increased in CR patients with GLU levels ≥ 7 mmol/L. Moreover, regression analysis indicated that the values of albumin and uric acid were correlated with the incidence of CR.

Conclusions: Our findings were likely to provide fresh understanding for the new mechanism of platelet inhibition failure and promote individualized antiplatelet therapy.

KEYWORDS

clopidogrel resistance, CREB5, DNA methylation

1 | INTRODUCTION

After rupture and bleeding of coronary atherosclerotic plaque, platelets activate rapidly and accumulate at the injured site, leading to thrombosis, which is the main process of acute coronary syndrome (ACS). Therefore, antiplatelet therapy is the cornerstone of drug therapy after percutaneous coronary intervention (PCI) in patients

with coronary heart disease. However, while clopidogrel reduces platelet aggregation induced by adenosine diphosphate by inhibiting the ADP receptor P2Y12, 10%–30% of patients still have ischemic events,¹ which is related to the low degree of platelet inhibition. This phenomenon that clopidogrel cannot effectively inhibit platelet aggregation and activation, resulting in high-residual platelet activity, is defined as clopidogrel low response or clopidogrel resistance

Jiyi Li and Jin Yang are the equal contributors to this article, and they are first coauthors.

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(CR), which will significantly increase the risk of major cardiovascular events after PCI in patients with coronary heart disease.

DNA methylation modification plays a pivotal role in maintaining normal cell function and transmitting genomic genetic imprinting.² Changes in the level of DNA methylation may also lead to the development and progression of certain diseases, such as diabetes and cardiovascular disease.³ In recent years, many studies have found that DNA methylation may be related to CR.⁴ Our team studied the relationship between the methylation levels of ABCB1, P2Y12, and PON1 gene promoters and CR and found that among patients with alcoholism, the hypomethylation of two CpGs of the P2Y12 gene was associated with CR, while in smokers and low protein people, the methylation level of CpG1 was negatively correlated with CR.⁵ In the subgroup of coronary heart disease complicated with hyperlipidemia, the DNAm level of CpG4 in the PON1 promoter is higher and the expression of PON1 mRNA is lower, suggesting that the DNAm will lead to the lower expression of mRNA, resulting in the emergence of CR.⁶ In addition, it has also been reported that hypomethylation of the ABCB1 promoter is associated with a low response to clopidogrel in people with ischemic stroke.⁴

Recently, we conducted genome-wide methylation detection among patients with clopidogrel resistance and nonclopidogrel resistance (NCR) with an 850k methylation chip (Illumina human methylation epic beadchip) and found that methylation differential genes were enriched in the insulin secretion pathway (hsa04911); it is suggested that this pathway gene may be involved in the regulation of different antiplatelet reactivities of clopidogrel.⁷ Among them, cAMP-response element binding protein (CREB5) is a key molecule in insulin secretion. It plays an important role in blood glucose control and insulin secretion. A recent study revealed that leukocyte DNAm levels of CREB5 (cg11301281) were associated with obesity and metabolic syndrome traits.⁸ However, the effects of DNA methylation in CREB5 on CR are poorly understood. Hence, according to our former 850k methylation chip results, in this research, we attempted to evaluate whether DNAm levels of CREB5 (cg01534253) are involved in CR among ACS patients administered clopidogrel.

2 | MATERIALS AND METHODS

2.1 | Participants

The participants were 72 ACS patients who attended Ningbo No. 1 Hospital from 2019 to 2021 (36 CR and 36 NCR, Figure S1). The patients were underwent percutaneous coronary intervention (drug-eluting stents) and were all older than 18 years in the two groups. The patients were taking 600mg clopidogrel, as well as 300mg aspirin as loading-doses before stenting and 75 mg clopidogrel, as well as 100mg aspirin daily as maintenance-doses. The patients and the controls did not have severe infection, rheumatoid-related diseases, hepatic or kidney function disorders, or a history of active bleeding. Select the case group according to the following diagnostic criteria of CR: reaction units of P2Y12 (PRU) measured by the VerifyNow

P2Y12 assay (Accumetrics, Inc.,) $\geq 240^9$ 4 weeks after PCI (at that time, the platelet reactivity was much stable compared with those just post-PCI¹⁰), and meanwhile, there is indeed a reality of antiplatelet failure under clopidogrel treatment in clinical practice. After the patient or guardian signed the informed consent form, blood samples and clinical information were obtained. The research process was approved by the Ethics Committee of Ningbo NO.1 Hospital, which is in line with the Helsinki Declaration.

2.2 | Genomic DNA extraction

A -80°C freezer was used to store peripheral blood samples, which were collected from 36 CR and 36 NCR subjects in hospital. First, we thawed the sample before DNA purification. A QIAamp DNA Blood Mini Kit (Qiagen,) was used to extract DNA from human whole blood cells. Then, we used a NanoDrop2000 system (NanoDrop, Wilmington, DE) to quantify the concentration and purity of DNA by detecting the optical density ratio of the maximal absorbent wavelengths from 260nm to 280nm (OD260/280). The requirements for qualified samples are as follows: the OD260/280 ratio is from 1.60 to 2.10 and the concentration is greater than 50ng/ μl . The qualified DNA sample can be used for the next step of detection. Finally, qualified DNA sample tubes were stored in a -20°C refrigerator to ensure that DNA would not be broken down. DNA integrity was determined with agarose gel electrophoresis.

2.3 | DNA methylation assay

In this research, bisulfite pyrosequencing was used to detect DNA methylation of cg01534253. First, EpiTech Bisulfite Kits was selected to carry out sodium bisulfite DNA conversion. Second, PCR amplification on the targeted region was performed by the PyroMark PCR kit (Qiagen,). We designed pyrosequencing primers and PCR primers based on PyroMark Assay Design software platform (Table S1). Last, PyroMark Qiagen Q96 reagents was used to sequence DNAm of the targeted CpG island.

2.4 | Level of CREB5 mRNA determination

We used qRT-PCR to validate the relative mRNA expression of the CREB5 gene corresponding to the loci above. The RNA of the samples was extracted with RNeasy Plus Universal Kit (Qiagen). The synthesis process of cDNA required the PrimeScript™ RT Reagent Kit and gDNA Eraser (TaKaRa Bio), and 1 μg RNA was applied. Template cDNAs were diluted 1:4. The relative expression of CREB5 gene was quantified with ABI 7500 Quantitative Real-time PCR (qRT-PCR) System (Applied Biosystems). The GAPDH gene was selected for normalization. The primers for qRT-PCR amplification were designed with Primer Premier 5 (Table S2). After the samples were run in triplicate, the mean result was determined. The relative

quantitative method was applied to calculate the mRNA levels of CREB5.

2.5 | Statistical analysis

PASW 21.0 software (SPSS, Inc.) was selected to establish a database for statistical breakdown. The graphics were drawn with GraphPad Prism 7. Association analyses between clinical indices, CREB5 DNA methylation, mRNA expression, and CR were performed. The normal distribution of measurement data was tested by Kolmogorov-Smirnov. The variables of normal distribution were described by the mean with standard deviation and compared by the independent sample *t*-test, and the variables that were not normally distributed are shown as medians with interquartile ranges (IQRs), which will be compared with nonparametric tests. Meanwhile, categorical variables are described as the mean \pm standard deviation and were compared by chi-square tests or Fisher's exact tests between groups. The correlations analysis between clinical index and CREB5 methylation was performed with logistic regression. It was considered to be statistically significant when two-sided *p*-value <0.05.

3 | RESULTS

3.1 | Patient characteristics

The comparison of demographic and clinical characteristics of the CR and NCR groups is shown in Table 1. There was no significant difference in clinical variables between the two groups, except for uric acid and albumin. The findings indicated that clopidogrel resistance was observed for lower albumin levels (case and control group: 37.86 ± 5.47 vs. 40.61 ± 3.96 , *p* = 0.017) and higher uric acid levels (case and control group: 335.4 ± 134.5 vs. 240.1 ± 159.2 , *p* = 0.008).

3.2 | Association of CR and CREB5 DNA methylation

In our study, we chose a CpG dinucleotide (probe ID cg01534253) to investigate the association of DNA methylation levels in the CREB5 gene between CR and NCR patients. Table 2 shows that the methylation levels of cg01534253 in CREB5 were not associated with CR significantly.

TABLE 1 The comparison of demographic and clinical characteristics between CR and NCR patients

Index	CR(n = 36)	NCR(n = 36)	<i>z</i> / <i>t</i> / χ^2	<i>p</i> value
HbA1c(%)	6.37 \pm 1.30	6.28 \pm 0.99	-0.315	0.753
Blood sugar	5.98 \pm 2.05	5.52 \pm 1.34	-1.141	0.258
BUN(mmol/L)	5.990 \pm 2.067	5.544 \pm 2.569	-0.741	0.462
Age (years)	63.8 \pm 10.0	59.3 \pm 10.4	-1.86	0.067
Left ventricular ejection fraction(%)	58.6 \pm 10.8	62.3 \pm 6.8	1.77	0.081
ALT (umol/L)	35.2 \pm 27.3	37.4 \pm 32.6	0.31	0.758
Uric acid(ummol/L)	335.4 \pm 134.5	240.1 \pm 159.2	-2.745	0.008
PLT (10 ⁹ /L)	183.5 \pm 53.7	205.7 \pm 67.9	1.539	0.128
TBIL(mmol/L)	14.44 \pm 9.02	13.54 \pm 5.59	-0.508	0.613
Albumin(g/L)	37.86 \pm 5.47	40.61 \pm 3.96	2.449	0.017
Cr (μ mol/L)	73.64 \pm 20.75	69.32 \pm 17.43	-0.958	0.341
MPV(fl)	8.53 \pm 1.62	8.52 \pm 1.38	-0.024	0.981
BMI(kg/m ²)	23.684 \pm 2.604	24.031 \pm 3.059	0.519	0.606
Total cholesterol(mg/dL)	4.385 \pm 0.927	4.596 \pm 1.447	0.737	0.463
HDL(mg/dL)	1.029 \pm 0.306	0.963 \pm 0.249	-1.011	0.316
LDL(mg/dL)	2.554 \pm 0.889	2.721 \pm 1.036	0.734	0.466
PCT(%)	0.162 \pm 0.036	0.175 \pm 0.049	1.313	0.193
Triglyceride (mg/dL)	1.514 \pm 0.806	1.808 \pm 1.240	1.195	0.236
HsCRP(mg/dL)	3.190(0.953,6.068)	1.795(0.850,7.400)	-0.591	0.554
AST (umol/L)	30.5(18.5,82.3)	24.5(18.0,75.0)	-0.344	0.731
Male Gender, n(%)	11(30.6)	9(25.0)	0.277	0.599
Alcohol abuse, n(%)	31(86.1)	29(80.6)	0.400	0.527
Hypertension, n(%)	11(30.6)	12(33.3)	0.064	0.800
Diabetes, n(%)	23(63.9)	25(69.4)	0.250	0.617
Dyslipidemia, n(%)	25(69.4)	22(61.1)	0.551	0.458
Current smoking, n(%)	23(63.9)	20(55.6)	0.520	0.471

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; Cr, creatinine; HDL, High density lipoprotein; LDL, low density lipoprotein; MPV, mean platelet volume; PCT, platelet hematocrit; PLT, platelet; TBIL, Total bile acid.

Additionally, we performed a subunit analysis based on differences in clinical baseline data related to diabetes mellitus between the two groups, including DM (Figure 1A,B), HbA1c (Figure 1C,D), and GLU levels (Figure 1E,F), to evaluate whether the DNAm levels of CREB5 were associated with CR. The results showed that for patients whose HbA1c levels were $\geq 6.5\%$ (case vs. control: 98.01 ± 2.55 vs. 95.04 ± 3.84 , $p = 0.050$) (Figure 1D) or whose GLU levels were ≥ 7 mmol/L (case vs. control: 99.33 ± 1.51 vs. 93.85 ± 4.37 , $p = 0.020$) (Figure 1F), lower methylation indicated a poorer clopidogrel response.

Meanwhile, we also take subunit analysis in various uric acid level and albumin levels, but the results were of no significance.

3.3 | The association of CREB5 mRNA expression and clopidogrel resistance

The mRNA expression of cg01534253 in CREB5 was not significantly associated with CR. Then, we tried to find the correlation between different gene expression and CR via subgroup analysis according to the results of methylation analysis. The results suggested that CREB5 mRNA expression was higher when patients with GLU levels ≥ 7 mmol/L underwent a low clopidogrel response ($p = 0.035$) (Figure 2B). However, in patients with HbA1c levels $\geq 6.5\%$, the result was no significance (Figure 2A).

3.4 | Regression analysis

From the above results, we found that CR results from the confounding effects of multiple factors, so we conducted a regression analysis on gene methylation and clinical characteristics. (Table 3). The results suggest that uric acid level is a risk factor ($p = 0.009$) and that albumin level is a protective factor for clopidogrel resistance ($p = 0.010$).

4 | DISCUSSION

Although new antiplatelet drugs show rapid, sustained and effective P2Y₁₂ inhibition, their bleeding risk is significantly increased.¹¹ Clopidogrel is superior to new antiplatelet drugs in terms of the safety and effectiveness of the primary endpoint in the East Asian population.^{12,13} The COSTIC study conducted 2018 proposed that the Chinese population had more obvious clinical benefits from clopidogrel. Clopidogrel resistance is affected by a variety of clinical factors, including diabetes,¹⁴ chronic kidney disease,¹⁵ smoking,¹⁶ drug interactions (such as proton pump inhibitors),¹⁷ circadian rhythm,¹⁸ age, inflammation, reduced left ventricular ejection fraction, and body mass index.¹⁹

Among them, diabetes mellitus (DM) had the most significant effect on clopidogrel's low response.¹⁴ Compared with nondiabetic

Index	Meth.(%)	NCR	CR	t	p value
All cases		96.639 ± 4.45	96.561 ± 3.520	0.083	0.934
DM					
Yes(11 vs. 13)		95.21 ± 5.63	95.41 ± 3.70	-0.108	0.915
No(25 vs. 23)		97.27 ± 3.78	97.21 ± 3.32	0.058	0.954
HbA1c(%)					
<6.5(26 vs. 24)		96.11 ± 4.93	97.32 ± 3.16	-1.023	0.311
≥ 6.5 (10 vs. 12)		98.01 ± 2.55	95.04 ± 3.84	2.090	0.050
GLU(mmol/L)					
<7(31 vs. 27)		96.21 ± 4.63	97.47 ± 2.72	-1.240	0.220
≥ 7 (5 vs. 9)		99.33 ± 1.51	93.85 ± 4.37	2.677	0.020

TABLE 2 Comparison of CREB5 gene promoter DNA methylation levels between CR and NCR

Abbreviations: DM, diabetes mellitus; GLU, glucose.

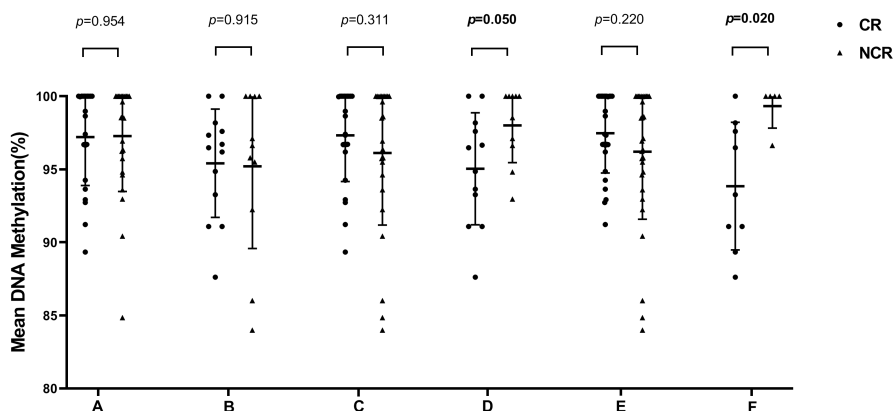


FIGURE 1 Comparison of CREB5 gene DNA methylation levels between cases and controls in the subgroups. (A, patients with no DM; B, patients with DM; C, patients with HbA1c < 6.5%; D, patients with HbA1c levels $\geq 6.5\%$; E, patients with GLU levels < 7 mmol/L; F, patients with GLU levels ≥ 7 mmol/L)

patients, the antiplatelet effect of clopidogrel in DM patients was lower.²⁰ If blood glucose is well controlled, the antiplatelet effect in DM patients is significantly improved.²¹ This is mainly due to the following reasons. (1) Abnormal secretion of insulin can lead to increased platelet activity. This may be related to insulin receptor substrates; some studies also believe that this is caused by the reduction of nitric oxide (no) and prostaglandin I₂ (PGI₂) molecules. (2) Hyperglycemia induces the expression of P-selectin by glycosylating the protein on the surface of platelets, activating the protein kinase C pathway, and damaging the fluidity of the cell membrane to increase the adhesion of platelets. (3) DM can cause dysfunction of vascular endothelial cells and reduce the production of no and PGI₂ and release tissue factors, leading to a prothrombotic state to enhance platelet activity. (4) The disorder of calcium metabolism regulation, the upregulation of the P2Y₁₂ receptor pathway and the increase in ADP exposure in DM patients will also increase the activity of platelets.¹⁴

cAMP-response element (CRE) can be activated by Cyclic AMP response element binding protein (CREB) and start transcription. Basic leucine zipper (bZIP) and Zinc-finger DNA-binding domains are included in the members of this CRE-binding protein family. CREB belongs to the family too. The encoded protein binds to CRE as a heterodimer or a homodimer with CRE-BP1 or c-Jun specifically, and play an important role as a CRE-dependent transactivator. Our results suggested that there is no significant correlation between the cg01534253 methylation level of the CREB5 gene and CR. Fortunately, the results of the subgroup analysis suggested that lower methylation of CREB5 was positively associated with CR for patients whose HbA1c levels were $\geq 6.5\%$ or whose

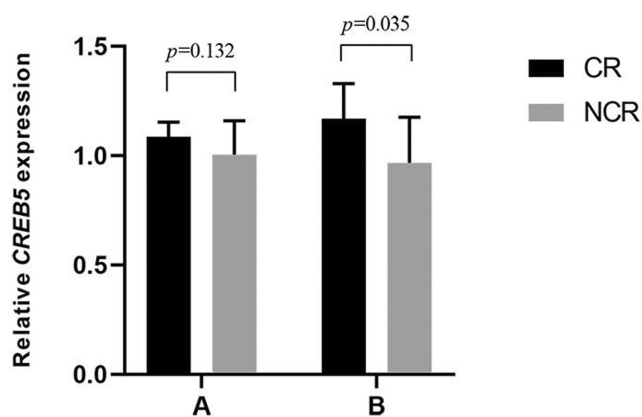


FIGURE 2 Comparison of CREB5 mRNA expression between CR and NCR patients in the subgroups. (A, patients with HbA1c levels $\geq 6.5\%$; B, patients with GLU levels ≥ 7 mmol/L)

TABLE 3 Multiple logistic regression analysis

	B	Std. Error	Wald	p value	Exp(B)	Exp(B) 95% CI
Uric acid	0.006	0.002	6.816	0.009	1.006	1.001-1.011
Albumin	-0.205	0.8	6.641	0.010	0.814	0.697-0.952
CREB5 Meth	0.05	0.07	0.516	0.473	1.052	0.917-1.206

GLU levels were ≥ 7 mmol/L. Methylation of CpG sites plays an important role in blood glucose control. A study found that 7 CpG sites were related to fasting blood glucose, and 10 CpG sites were related to HbA1c levels through an analysis correlation between fasting plasma glucose levels, HbA1c levels and DNA methylation in adult twins.²² According to the results of the subgroup analysis, CREB5 may be an important pathway for clopidogrel hyporeactivity in patients with abnormal blood glucose. No similar results were found in the DM group, which may be because diabetic patients generally treated standardly and whose blood sugar is usually maintained at a stable level. The real blood glucose level is the key factor leading to the effect of the CREB5 pathway. The mechanism may be that the hyperglycemic state stimulates the hypomethylation of CREB5, which affects the body's response to clopidogrel. This mechanism was almost verified by our mRNA expression detection in the subgroup analysis of patients with GLU levels ≥ 7 mmol/L.

CREB5 and its DNA methylation participate in differences in pathological processes and drug efficacy. After genome-wide DNA methylation analysis combined with genome-wide gene expression in decidual tissue, one study highlighted CREB5 as a novel risk gene contributing to recurrent pregnancy loss,²³ and another integration analysis of gene promoter and exon DNA methylation revealed that the CREB5 gene was hypomethylated in colon cancer development.²⁴ In addition, an observational study identified CREB5 as one mechanism that drives resistance to androgen receptor antagonists in prostate cancers.²⁵ Another study pointed out that genes with continuously increasing or decreasing methylation, such as CREB5, may also affect the tumor-repopulating cell screening process.²⁶ Moreover, DNAm levels of CREB5 (cg11301281) are related to metabolic syndrome traits [8], so its expression may be involved in the process of blood glucose control and metabolic response.

Some studies suggest that in patients with insulin resistance, an abnormal insulin-mediated signaling pathway is related to a reduction in the platelet inhibitory effect.²⁷ These abnormalities can be divided into IRS-related factors and nonrelated factors. Insulin can regulate platelet activity through IRS1 on platelets. The IRS1 SNP (rs956115 and rs13431554) was associated with increased platelet activity and adverse cardiovascular events in patients with coronary heart disease and diabetes mellitus.^{28,29} Furthermore, some scholars also focus on RNA in exploring the genetic factors of clopidogrel resistance. A latest investigation suggested that NONHSAT083775.2 and NONHSAT107804.2 lncRNAs were upregulated in leukocytes in CR group, and NONHSAT133455.2 lncRNA was downregulated,³⁰ through lncRNA microarray and real-time RT-PCR analyses. Furthermore, another recent study

on T2D patients showed differentially expressed lncRNAs in megakaryocytes and confirmed that lncRNA metallothionein 1 pseudogene 3 (MT1P3) was upregulated; moreover, the research identified that MT1P3 played a key role of regulation by sponging miR-126 in upregulation of P2Y12 expression, which might lead to platelet activation.³¹ In addition, with the advancement of RNA methylation and exosomal miRNA research, exploring the role of RNA methylation (m6A or m5C) and exosomes in CR may provide new enlightenment in the future.^{32,33}

5 | CONCLUSIONS

In summary, this research found that the DNAm levels of CREB5 (cg11301281) were associated with CR. Moreover, for patients whose HbA1c levels were $\geq 6.5\%$ or GLU levels ≥ 7 mmol/L, lower methylation of CREB5 was positively associated with CR. Finally, logistic regression revealed that higher levels of UA may be positively associated with CR, and the values of albumin were correlated with a decreased risk of CR. Our findings were likely to provide fresh understanding for the new mechanism of platelet inhibition failure and promote individualized antiplatelet therapy. An advanced studies and approach with larger samples are necessary to further validate findings above in future investigation.

FUNDING INFORMATION

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Udell JA, Bonaca MP, Collet JP, et al. Long-term dual antiplatelet therapy for secondary prevention of cardiovascular events in the subgroup of patients with previous myocardial infarction: a collaborative meta-analysis of randomized trials. *Eur Heart J*. 2016;37(4):390-399.
- Aavik E, Babu M, Yla-Herttuala S. DNA methylation processes in atherosclerotic plaque. *Atherosclerosis*. 2018;281:168-179.
- Willmer T, Johnson R, Louw J, Pfeiffer C. Corrigendum: blood-based DNA methylation biomarkers for type 2 diabetes: potential for clinical applications. *Front Endocrinol*. 2019;10:1.
- Yang J, Zhou JS, Zhang YD, Tian YY, Zou JJ. The association of ABCC3 promoter methylation with clopidogrel response in Chinese ischemic stroke patients. *Pharmazie*. 2014;69(10):764-768.
- Su J, Li X, Yu Q, et al. Association of P2Y12 gene promoter DNA methylation with the risk of clopidogrel resistance in coronary artery disease patients. *Biomed Res Int*. 2014;2014:450814.
- Su J, Li J, Yu Q, et al. Association of PON1 gene promoter DNA methylation with the risk of Clopidogrel resistance in patients with coronary artery disease. *J Clin Lab Anal*. 2019;33(5):e22867.
- Yang J, Yu Q, Xu Z, et al. Clopidogrel resistance is associated with DNA methylation of genes from whole blood of humans. *Front Genet*. 2020;11:583215.
- Salas-Perez F, Ramos-Lopez O, Mansego ML, et al. DNA methylation in genes of longevity-regulating pathways: association with obesity and metabolic complications. *Aging*. 2019;11(6):1874-1899.
- Marcucci R, Gori AM, Paniccia R, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay a 12-month follow-up. *Circulation*. 2009;119(2):237-242.
- Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation*. 2004;109(25):3171-3175.
- Wiviott SD, Steg PG. Clinical evidence for oral antiplatelet therapy in acute coronary syndromes. *Lancet*. 2015;386(9990):292-302.
- Goto S, Huang CH, Park SJ, Emanuelsson H, Kimura T. Ticagrelor vs. clopidogrel in Japanese, Korean and Taiwanese patients with acute coronary syndrome -- randomized, double-blind, phase III PHILO study. *Circ J*. 2015;79(11):2452-2460.
- Kang J, Han JK, Ahn Y, et al. Third-Generation P2Y12 Inhibitors in East Asian acute myocardial infarction patients: a nationwide prospective multicentre study. *Thromb Haemost*. 2018;118(3):591-600.
- Faggioni M, Baber U, Chandrasekhar J, et al. Use of prasugrel vs clopidogrel and outcomes in patients with and without diabetes mellitus presenting with acute coronary syndrome undergoing percutaneous coronary intervention. *Int J Cardiol*. 2019;275:31-35.
- Tabata N, Hokimoto S, Akasaka T, et al. Chronic kidney disease status modifies the association of CYP2C19 polymorphism in predicting clinical outcomes following coronary stent implantation. *Thromb Res*. 2014;134(5):939-944.
- Bossard M, Granger CB, Tanguay JF, et al. Double-dose versus standard-dose clopidogrel according to smoking status among patients with acute coronary syndromes undergoing percutaneous coronary intervention. *J Am Heart Assoc*. 2017;6(11):e006577.
- Abraham NS, Hlatky MA, Antman EM, et al. ACCF/ACG/AHA 2010 expert consensus document on the concomitant use of proton pump inhibitors and thienopyridines: a focused update of the ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American college of cardiology foundation task force on expert consensus documents. *Circulation*. 2010;122(24):2619-2633.
- Zheng N, Yin F, Yu Q, et al. Associations of PER3 polymorphisms with clopidogrel resistance among Chinese Han people treated with clopidogrel. *J Clin Lab Anal*. 2021;35(4):e23713.
- Cayla G, Hulot JS, O'Connor SA, et al. Clinical, angiographic, and genetic factors associated with early coronary stent thrombosis. *JAMA*. 2011;306(16):1765-1774.
- Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes*. 2005;54(8):2430-2435.
- Gajos G, Konieczynska M, Zaleski J, Undas A. Low fasting glucose is associated with enhanced thrombin generation and unfavorable

- fibrin clot properties in type 2 diabetic patients with high cardiovascular risk. *Cardiovasc Diabetol*. 2015;14:44.
22. Wang Z, Gao W, Wang B, et al. Correlation between fasting plasma glucose, HbA1c and DNA methylation in adult twins. *Beijing Da Xue Xue Bao Yi Xue Ban*. 2020;52(3):425-431.
 23. Yu M, Du G, Xu Q, et al. Integrated analysis of DNA methylome and transcriptome identified CREB5 as a novel risk gene contributing to recurrent pregnancy loss. *EBioMedicine*. 2018;35:334-344.
 24. Molnar B, Galamb O, Peterfia B, et al. Gene promoter and exon DNA methylation changes in colon cancer development - mRNA expression and tumor mutation alterations. *BMC Cancer*. 2018;18(1):695.
 25. Hwang JH, Seo JH, Beshiri ML, et al. CREB5 promotes resistance to androgen-receptor antagonists and androgen deprivation in prostate cancer. *Cell Rep*. 2019;29(8):2355-2370.
 26. Huang W, Hu H, Zhang Q, Wang N, Yang X, Guo AY. Genome-Wide DNA methylation enhances stemness in the mechanical selection of tumor-repopulating cells. *Front Bioeng Biotechnol*. 2020;8:88.
 27. Westerbacka J, Yki-Jarvinen H, Turpeinen A, et al. Inhibition of platelet-collagen interaction: an in vivo action of insulin abolished by insulin resistance in obesity. *Arterioscler Thromb Vasc Biol*. 2002;22(1):167-172.
 28. Zhang D, Zhang X, Liu D, et al. Association between insulin receptor substrate-1 polymorphisms and high platelet reactivity with clopidogrel therapy in coronary artery disease patients with type 2 diabetes mellitus. *Cardiovasc Diabetol*. 2016;15:50.
 29. Angiolillo DJ, Jakubowski JA, Ferreiro JL, et al. Impaired responsiveness to the platelet P2Y12 receptor antagonist clopidogrel in patients with type 2 diabetes and coronary artery disease. *J Am Coll Cardiol*. 2014;64(10):1005-1014.
 30. Xie W, Huang B, Yin Q, Chen S. Differential expression of lncRNA in patients with coronary artery disease plus clopidogrel resistance. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 2019;44(1):9-13.
 31. Zhou M, Gao M, Luo Y, Gui R, Ji H. Long non-coding RNA metallothionein 1 pseudogene 3 promotes p2y12 expression by sponging miR-126 to activate platelet in diabetic animal model. *Platelets*. 2019;30(4):452-459.
 32. Zheng N, Su J, Hu H, Wang J, Chen X. Research progress of N6-methyladenosine in the cardiovascular system. *Med Sci Monit*. 2020;26:e921742.
 33. Su J, Li J, Yu Q, et al. Exosomal miRNAs as potential biomarkers for acute myocardial infarction. *IUBMB Life*. 2020;72(3):384-400.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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