Am J Transl Res 2026;18(11):9793-9804
www.ajtr.org /ISSN:1943-8141/AJTR0174412

Original Article

Association of MTHFR C677T TT genotype and T allele with aspirin resistance in young coronary heart disease patients and the construction of a nomogram predictive model

Tao Ding[1*], Lijuan Li[2*], Mingqiang Fan[1], Jing Bai[1], Zhengfei Peng[1], Qiaoke Si[1], Xiangxiang Yang[1], Li Du[1], Hui Dai[3]

[1]Department of Cardiovascular Medicine, Affiliated Hospital of Gansu Medical College (Pingliang People's Hospital), Pingliang 744000, Gansu, China; [2]Department of Gastroenterology, Affiliated Hospital of Gansu Medical College (Pingliang People's Hospital), Pingliang 744000, Gansu, China; [3]Clinical Medical School, Medical College of Gansu, Pingliang 744000, Gansu, China. [*]Co-first authors.

Received June 1, 2026; Accepted September 20, 2026; Epub November 15, 2026; Published November 30, 2026

Abstract: Objective: To investigate the correlation between Methylenetetrahydrofolate Reductase (MTHFR) C677T TT genotype and T allele with aspirin resistance (AR) in young patients with coronary heart disease and to construct a predictive nomogram model. Methods: The clinical data of 150 young patients with coronary heart disease (CHD) admitted to Pingliang People's Hospital from August 2022 to August 2024 were retrospectively analyzed. All patients received aspirin antiplatelet therapy, and they were divided into AR group (n=35) and aspirin sensitive (AS) group (n=115) according to aspirin response. MTHFR C677T polymorphism and incidence of AR in young CHD patients were analyzed. The risk factors of AR in young CHD patients were analyzed by univariate and multivariate logistic regression analyses. The incidence of AR in patients with different genotypes and alleles was compared, and a nomogram model for AR in young patients with CHD was constructed and verified. Results: The results of univariate and multivariate logistic analysis showed that diabetes, hyperhomocysteinemia (HHcy), TT genotype, T allele and platelet aggregation rate were the risk factors, while CC genotype was protective factor, for AR in young patients with CHD (P<0.05). TT carriers had higher LDL-C, platelet aggregation rate and AR rate (P<0.001). The nomogram had a consistency index (C-index) of 0.885, an area under the curve (AUC) of 0.885 (95% confidence interval [CI]: 0.822-0.947). Bootstrap internal validation with 1000 resamples confirmed the model maintained stable discrimination and calibration performance in the validation cohort with minor index attenuation. Conclusion: The occurrence of AR in young coronary heart disease patients is related to the MTHFR C677T gene. TT genotype and T allele may increase the risk of AR in patients. Constructing a nomogram model based on MTHFR C677T gene and combined with other key indicators, has predictive value for AR occurrence.

Keywords: Coronary heart disease, young patients, methylenetetrahydrofolate reductase, gene polymorphism, aspirin resistance

Introduction

Coronary heart disease (CHD) remains the leading cause of cardiovascular mortality in China, with the national overall prevalence ranging from 4% to 10% and imposing a heavy socioeconomic burden on public health systems [1]. Traditionally, CHD was regarded as an age-related disease predominantly affecting middle-aged and elderly populations over 55 years old; however, epidemiological data over the past decade have demonstrated a prominent younger-onset trend, with an increasing number of patients aged 18-50 years being diagnosed with coronary stenosis requiring antiplatelet intervention. Young CHD patients exhibit distinct risk factor profiles, lesion characteristics and drug response patterns compared with elderly counterparts, yet standardized individualized antiplatelet management

https://doi.org/10.62347/ISRI6067

strategies specifically for young populations remain insufficiently established, creating an urgent clinical gap.

Aspirin is the cornerstone long-term antiplatelet therapy recommended by global and domestic guidelines for secondary prevention of CHD, effectively inhibiting platelet cyclooxygenase-1 (COX-1) to reduce thrombotic event risk [2, 3]. Nevertheless, laboratory-confirmed aspirin resistance (AR) occurs in approximately 20%-30% of treated patients, which completely abolishes aspirin's anti-thrombotic efficacy and significantly elevates the 2-5-year risks of recurrent angina, acute myocardial infarction and ischemic stroke [4]. Unlike elderly patients with multiple combined cardiovascular risk factors, young CHD patients generally present fewer chronic comorbidities, which implies that genetic variants may serve as core drivers of AR in this subgroup, rather than acquired metabolic disturbances.

Methylenetetrahydrofolate reductase (MTHFR) is the rate-limiting enzyme of the folate-homocysteine metabolic pathway, encoded by a gene localized to the short arm of human chromosome 1. The C677T missense mutation in exon 5 induces alanine-to-valine substitution, drastically reducing MTHFR catalytic activity, blocking homocysteine (Hcy) degradation and triggering hyperhomocysteinemia (HHcy) - a well-documented independent cardiovascular risk factor [5]. Existing cohort and case-control studies have validated that MTHFR C677T mutation elevates baseline CHD susceptibility [6, 7], but critical knowledge gaps persist: ① Most previous genetic association research focused on overall CHD populations without stratifying by age, and few studies exclusively enroll young CHD patients to dissect the correlation between MTHFR variants and aspirin treatment response; ② It remains unclear whether MTHFR C677T genotype alters platelet inhibition efficacy via regulating Hcy and lipid metabolism, and no predictive model integrating this genetic marker has been constructed for early AR risk screening in young patients.

The innovations and clinical significance of the present study are highlighted as follows: First, this research targets the understudied young CHD subgroup (18-50 years) to clarify the independent predictive effect of MTHFR C677T TT genotype and T allele on AR occurrence, filling the research gap of pharmacogenomic exploration in young CHD antiplatelet therapy. Second, we systematically link genetic polymorphism, homocysteine metabolism, glycolipid indicators and platelet function to interpret the potential intermediate mechanism connecting MTHFR mutation and AR, rather than merely reporting simple correlation results. Third, we construct a visualized nomogram prediction model incorporating MTHFR genotype and clinical biochemical indicators, which enables clinicians to rapidly identify young CHD patients at high AR risk before initiating aspirin therapy. For high-risk carriers of TT genotype/T allele, clinicians can implement targeted early interventions including folic acid supplementation, intensive blood glucose and lipid control, and dynamic platelet function monitoring to avoid ineffective monotherapy and reduce major adverse cardiovascular event risks.

Current clinical guidelines lack gene-based personalized antiplatelet recommendations for young CHD patients. Elucidating the association between MTHFR C677T polymorphism and AR, as well as developing a feasible predictive tool, will provide objective genetic evidence for individualized aspirin treatment formulation and adjustment in young CHD populations. This retrospective cohort study therefore performs genotyping, platelet function testing and multivariate regression analysis to explore the correlation between MTHFR C677T variants and AR, and validate the predictive performance of the established nomogram model, aiming to deliver clinical references for optimized antiplatelet management in young coronary heart disease patients.

Materials, subjects, and methods

Sample size calculation was performed strictly following the EPV (Events Per Variable) principle with EPV=20 as the threshold; a total of 6 candidate predictive variables were planned to be included in multivariate logistic regression, requiring at least 120 AR events (6×20). Considering the expected AR incidence of 25%, the minimum required total sample size was calculated as 480. Due to single-center patient volume limitation, we enrolled 154 consecutive eligible patients during the study period, finally retaining 150 subjects after exclusion.

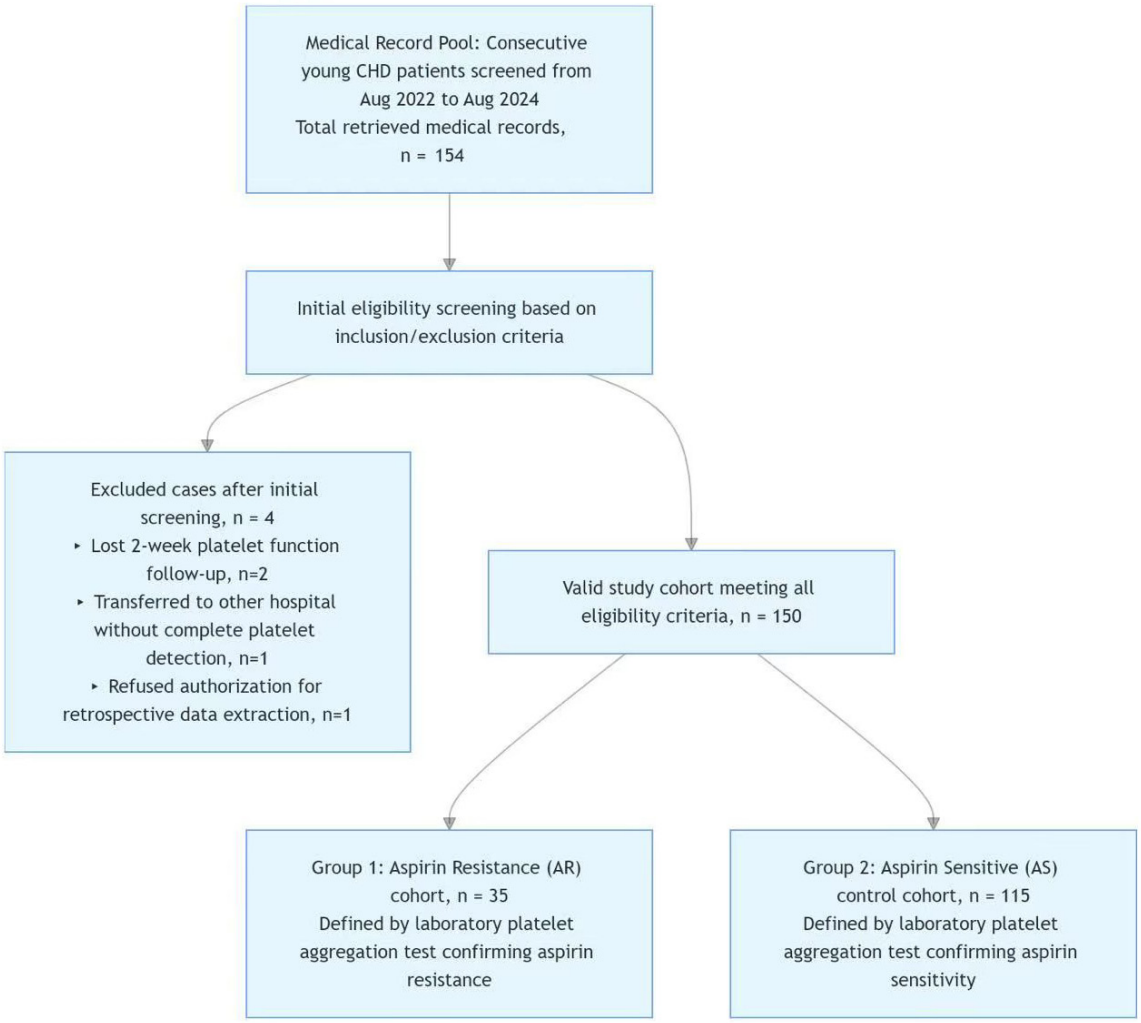


Figure 1. Flow diagram of patient screening and grouping. Notes: CHD, coronary heart disease; AR, aspirin resistance; AS, aspirin sensitive.

We retrieved all electronic medical records of inpatients hospitalized in the Department of Cardiovascular Medicine of Pingliang People's Hospital from August 2022 to August 2024 via the hospital integrated medical record system (**Figure 1**), with retrieval keywords including young, coronary heart disease (CHD), aspirin monotherapy, and MTHFR C677 genetic testing. Two attending physicians independently screened all cases according to unified inclusion and exclusion criteria, and disputed cases were adjudicated by a senior chief physician to reduce selection bias. A total of 154 patients were initially screened. After excluding 4 patients ineligible for inclusion (2 patients lost to follow-up; 1 patient transferred to other hospital without completed platelet detection; 1 patient refused retrospective data authorization), a total of 150 eligible subjects were enrolled (**Figure 1**).

This study was approved by the ethics committee of Affiliated Hospital of Gansu Medical College (Approval No.: Ethics Review 2022-013). Although all patient clinical data were processed anonymously by removing names, ID numbers, contact information and other identifiable personal information before data analysis, Pingliang People's Hospital's medical ethics committee stipulated that all retrospective research using residual clinical test and follow-up data must obtain retrospective written informed consent from patients or their legal guardians, which is an institutional standardized requirement to fully respect patients' right to know and protect medical autonomy,

and avoids potential medical disputes caused by unauthorized use of clinical data. The specific way to obtain informed consent was as follows: After screening eligible discharged patients through the electronic medical record system, our research team contacted patients by the contact telephone number reserved by the hospital; for patients who could not be reached directly, we contacted their immediate family legal guardians. Researchers explained the research purpose, data anonymous processing rules, data storage security measures, and that the study would not interfere with routine clinical diagnosis and treatment to each patient/guardians verbally. After full communication, we mailed or sent electronic informed consent forms for signing, and archived all signed consent documents together with ethical approval materials as supplementary submission files. Patients or guardians had the right to refuse participation at any time, and those who declined to sign informed consent were excluded from the final study cohort. All discharged patients or their legal guardians signed anonymous written informed consent before data extraction; all personal identity information such as name and ID number was desensitized to protect patient privacy. All aspirin treatment regimens were formulated by attending cardiologists based on coronary angiography results, clinical symptoms and bleeding risk stratification without random assignment. Doctors fully explained the antithrombotic benefits, gastrointestinal adverse reactions and limitations associated with aspirin resistance to patients and guardians orally and in writing, and all parties signed dedicated medication informed consent forms.

Inclusion criteria

① CHD diagnosis confirmed by coronary angiography or coronary computed tomography angiography (CCTA), complying with 2019 ACC/AHA guidelines [8]; ② Age at admission of 18-50 years; ③ Clinical treatment plan of single oral aspirin antiplatelet therapy; ④ Complete MTHFR C677T genotyping, serum biochemical and platelet function test data stored in electronic medical records; ⑤ Integrated 2-week outpatient follow-up platelet detection records for aspirin efficacy evaluation; ⑥ Signed informed consent allowing retrospective data analysis.

Exclusion criteria

① Combined acute or chronic systemic thromboembolic diseases; ② Acute coronary syndrome requiring postoperative dual antiplatelet therapy; ③ Valvular heart disease, cardiomyopathy requiring long-term anticoagulation; ④ Long-term antiplatelet use before admission; ⑤ Hematological or autoimmune disorders affecting coagulation; ⑥ Confirmed aspirin contraindications; ⑦ History of cardiac surgery or interventional rotational atherectomy; ⑧ Diagnosed Marfan syndrome or aortic dissection; ⑨ Folate-interfering drugs used within 30 days before admission; ⑩ Pregnancy, lactation or anemia requiring folic acid supplementation; ⑪ Continuous NSAID use during aspirin treatment; ⑫ Missing core laboratory indicators or patients refusing data authorization.

Data extraction method, extracted items and data validation

Two trained researchers independently extracted data from the electronic medical record system and outpatient follow-up database using a unified standardized extraction form, then cross-compared two datasets to eliminate entry errors. All abnormal outlier laboratory values were traced back to original inspection curves; unretrievable extreme values were discarded, and all MTHFR PCR scanning images were rechecked to correct genotype misjudgment. Unified fixed detection time points for all indicators are clearly specified as follows: ① Baseline indicators including MTHFR C677T genotyping, cardiac troponin I (cTnI), CK, creatine kinase-MB isoenzyme (CK-MB), lactate dehydrogenase (LDH), C-reactive protein (CRP), D-dimer, total cholesterol (TC), triglycerides (TG), LDL-C, high-density lipoprotein cholesterol (HDL-C), fibrinogen (FIB), uric acid (UA) and plasma Hcy were detected using fasting venous blood collected within 24 h after admission before aspirin administration; ② Platelet aggregation rate and arachidonic acid (AA) inhibition rate for evaluating aspirin response were uniformly tested after 14 continuous days of standardized aspirin oral treatment.

Outcome measures (primary and secondary outcomes)

The primary outcome of this study was laboratory-verified aspirin resistance (AR), defined as

AA inhibition rate <50% after 2 weeks of standardized aspirin therapy; patients with AA inhibition rate ≥50% were classified as aspirin-sensitive (AS) [9]. The core research goal was to explore the independent correlation between MTHFR C677T gene polymorphism and AR risk. Secondary outcomes included: the distribution difference of MTHFR C677T genotypes and alleles between AR and AS groups; intergroup differences of diabetes, HHcy, LDL-C and platelet aggregation rate across CC/CT/TT genotypes and C/T alleles; independent risk factors for AR identified by regression models; discrimination and calibration performance of the AR prediction nomogram built on genetic and clinical variables.

Statistical analysis

Statistical analyses were conducted by SPSS 25.0 and R 4.2.0. Categorical variables were presented as n (%) and compared using chi-square test; normally distributed continuous variables were expressed as mean ± standard deviation and compared by independent t-test or one-way ANOVA with LSD post hoc test. Hardy-Weinberg equilibrium test was performed for genotype distribution. For logistic regression: univariate logistic regression was first applied to all baseline and laboratory variables; indicators with univariate P<0.05 were incorporated into multivariate backward stepwise logistic regression. The variable entry threshold was set as P_in = 0.05, and the elimination threshold was P_out = 0.10, adopting backward screening to screen independent AR risk factors. After extracting independent predictors, a nomogram prediction model was constructed; 1000 bootstrap resamples were used to calculate C-index, calibration curves and ROC curves to evaluate model discrimination and calibration. A two-tailed α= 0.05 was adopted as the unified significance threshold for all statistical tests.

Results

MTHFR C677T gene polymorphism and incidence of AR in young CHD patients

Genotyping results for MTHFR C677T in the 150 included patients revealed 54 cases of CC genotype, 52 of CT genotype, and 44 of TT genotype. Allele frequency analysis identified 88 C alleles and 62 T alleles. After two weeks of aspirin therapy, 35 patients developed AR, yielding an incidence of 23.33% (35/150).

Comparison of general data between the AR group and the AS group

The proportion of patients with diabetes in the AR group was significantly higher than that in the AS group (P<0.05). There were no significant differences in age, sex, weight, smoking history, alcohol consumption history, the proportion of patients with hypertension/dyslipidemia/hyperuricemia/thyroid disease, the number of diseased vessels, and Gensini score between the AR group and the AS group (P> 0.05; **Table 1**).

Comparison of clinical data between AR group and AS group patients

The proportion of patients with HHcy, the proportion of MTHFR C677T genotype TT type, LDL-C levels, and platelet aggregation rate in the AR group were all significantly higher than those in the AS group. The proportion of the MTHFR C677T CC genotype and the frequency of the MTHFR C677T C allele also differed significantly between the AR and AS groups (P<0.05). In contrast, there were no significant differences in cTnI, CK, CK-MB, LDH, CRP, D-dimer, TC, TG, HDL-C, FIB, or serum UA levels, nor in the proportion of MTHFR C677T CT genotype or the frequency of the MTHFR C677T T allele between the AR and AS groups (P>0.05), as shown in **Table 2**.

Analysis of risk factors for AR in young patients with coronary heart disease

We adopted a two-step logistic regression: univariate logistic regression was conducted on all baseline and laboratory indicators first; variables with univariate P<0.05 were included into the multivariate backward stepwise logistic regression model. The variable entry threshold was set as P_in = 0.05, elimination threshold P_out = 0.10, backward screening mode was applied to automatically filter independent risk factors of AR. All categorical independent variable assignment rules are supplemented in the footnote to **Table 3**, **Table 4** presents the univariate logistic regression results, and **Table 5** presents the multivariate stepwise logistic regression results. LDL-C was not included in the multivariate logistic regression model, but

Table 1. Comparison of general data between the AR group and the AS group

Characteristic		AR Group (n=35)	AS Group (n=115)	t/χ^2	*P*
Age ($\bar{x}$±s, years)		37.72±6.93	39.01±6.28	1.038	0.301
Sex	Male	16 (45.71)	69 (60.00)	2.230	0.135
	Female	19 (54.29)	46 (40.00)		
Body weight	Obese	9 (25.71)	49 (42.61)	3.223	0.200
	Overweight	18 (51.43)	44 (38.26)		
	Normal weight	5 (14.29)	14 (12.17)		
	Underweight	3 (8.57)	7 (6.09)		
Smoking history	Yes	16 (45.71)	47 (40.87)	0.259	0.611
	No	19 (54.29)	68 (59.13)		
Drinking history	Yes	9 (25.71)	26 (22.61)	0.145	0.704
	No	26 (74.29)	89 (77.39)		
Comorbidities	Diabetes	22 (62.86)[a]	37 (32.17)	10.587	0.001
	Hypertension	14 (40.00)	39 (33.91)	0.435	0.509
	Dyslipidemia	21 (60.00)	78 (67.83)	0.732	0.392
	Hyperuricemia	4 (11.43)	7 (6.09)	0.478	0.489
	Thyroid disease	3 (8.57)	10 (8.70)	0.103	0.749
Number of diseased vessels	Single-vessel	13 (37.14)	26 (22.61)	2.966	0.227
	Two-vessel	18 (51.43)	74 (64.35)		
	Multi-vessel	4 (11.43)	15 (13.04)		
Gensini Score ($\bar{x}$±s, points)		38.12±7.01	36.15±7.47	1.385	0.168

Note: CHD, coronary heart disease; AR, Aspirin resistance; AS, Aspirin sensitive. Compared with the AS group, [a]*P*<0.05.

was presented as an intermediate metabolic indicator in the genotype-based subgroup analysis. Variable assignment standard: Dependent variable (AR: 1=AR, 0=AS; Diabetes: 1 = Yes, 0 = No; HHcy: 1 = Yes, 0 = No; MTHFR genotype: CC=0, CT=1, TT=2; Allele: C=0, T=1; Platelet aggregation rate was included as continuous variable without assignment conversion). Taking the incidence of AR as the dependent variable (1=AR, 0=AS), and the indicators with P<0.05 in **Tables 1** and **2** as independent variables, a logistic regression analysis was conducted. The results indicated that diabetes mellitus, HHcy, TT genotype, T allele, and platelet aggregation rate were all risk factors for the occurrence of AR in young patients with CHD, while CC genotype was protective (*P*<0.05; **Tables 3**-**5**).

Occurrence of AR in patients with different genotypes and alleles

The proportions of diabetes and HHcy and the incidence of AR in patients with the TT genotype were higher than those in patients with the CC and CT genotypes (*P*<0.05). The proportions of diabetes and HHcy and the incidence of AR in patients with the T allele were higher than those in patients with the C allele (*P*<0.05). The levels of LDL-C and platelet aggregation rate were elevated in the TT genotype group and the T allele group (*P*<0.05). The Hardy-Weinberg equilibrium test showed that both the AR group (χ^2=5.262, *P*=0.072) and the AS group (χ^2=5.471, *P*=0.065) were in genetic equilibrium. The details are shown in **Tables 6**, **7**.

Nomogram for predicting AR in young CHD patients

A nomogram was constructed incorporating factors potentially associated with AR in young CHD patients, including diabetes, HHcy, C677T genotype, C677T allele, and platelet aggregation rate. The score for each factor was determined based on the point scale at the top of the nomogram, and the total score was calculated by summing individual scores. The probability of aspirin resistance (AR) occurrence in each patient was calculated based on the total score obtained from the prediction model. The unadjusted consistency index (C-index) based on bootstrap validation was 0.885, indi-

Table 2. Comparison of clinical data between AR group and AS group patients

Characteristic		AR Group (n=35)	AS Group (n=115)	t/χ^2	*P*
HHcy	Yes	26 (74.29)[a]	44 (38.26)	13.992	<0.001
	No	9 (25.71)	71 (61.74)		
cTn I ($\bar{x}$±s, μg·L^{-1})		0.21±0.06	0.23±0.08	1.365	0.174
CK ($\bar{x}$±s, U·L^{-1})		142.34±15.26	137.83±13.85	1.647	0.102
CK-MB ($\bar{x}$±s, U·L^{-1})		14.62±4.15	13.53±3.66	1.494	0.137
LDH ($\bar{x}$±s, U·L^{-1})		202.83±21.36	198.54±18.87	1.141	0.256
CRP ($\bar{x}$±s, mg·L^{-1})		7.22±2.15	6.87±1.84	0.946	0.345
D-D ($\bar{x}$±s, μg·L^{-1})		182.26±21.24	187.53±23.54	1.185	0.238
TC ($\bar{x}$±s, mmol/L)		4.62±0.53	4.54±0.41	0.821	0.416
TG ($\bar{x}$±s, mmol/L)		2.10±0.62	2.06±0.57	0.356	0.722
LDL-C ($\bar{x}$±s, mmol/L)		2.81±0.42[a]	2.40±0.35	5.783	<0.001
HDL-C ($\bar{x}$±s, mmol/L)		1.10±0.25	1.04±0.31	1.045	0.298
FIB ($\bar{x}$±s, g/L)		3.22±0.92	3.14±0.87	0.470	0.639
UA ($\bar{x}$±s, μmol/L)		285.65±17.92	281.52±16.35	1.279	0.203
MTHFR C677T genotype*	CC	6 (17.14)[a]	48 (41.74)	7.046	0.008
	CT	9 (25.71)	43 (37.39)	1.615	0.204
	TT	20 (57.14)[a]	24 (20.87)	17.032	<0.001
MTHFR C677T allele	C	12 (34.29)[a]	76 (66.09)	11.191	0.001
	T	23 (65.71)	39 (33.91)		
Platelet aggregation rate ($\bar{x}$±s, %)		41.30±6.51[a]	36.83±5.81	3.873	<0.001

Notes: CHD, coronary heart disease; AR, Aspirin resistance; AS, Aspirin sensitive; HHcy, hyperhomocysteinemia; LDL-C, low-density lipoprotein cholesterol. *The results of the Hardy-Weinberg genetic equilibrium test (χ^2, *P*) for the AR group were 5.262 and 0.072, respectively, and 5.471 and 0.065 for the AS group, respectively. Both groups were in accordance with genetic equilibrium. a: Compared with the AS group (P < 0.05).

Table 3. Variable assignment standard for categorical independent variables

Variable	Classification	Assignment Value
Dependent variable (AR outcome)	Aspirin resistance (AR)	1
	Aspirin sensitive (AS)	0
Diabetes mellitus	Yes	1
	No	0
Hyperhomocysteinemia (HHcy)	Yes	1
	No	0
MTHFR C677T genotype	CC	0
	CT	1
	TT	2
MTHFR C677T allele	C allele	0
	T allele	1

Note: Platelet aggregation rate was incorporated as continuous variable without assignment conversion.

cating good discrimination ability (**Figure 2**). The calibration curve showed that the predicted probability is highly consistent with the observed results, and the deviation from the ideal curve is minimal, indicating a strong predictive performance (**Figure 3**). ROC curve analysis showed that the area under the curve (AUC) was 0.885 (95% confidence interval: 0.822-0.947), the sensitivity was 0.926, the specificity was 0.795, and the Youden index was 0.721, confirming that the model has good accuracy in predicting AR in young patients with coronary heart disease (**Figure 4**).

Comparison of predictive performance between training cohort and bootstrap validation cohort

After constructing the nomogram model based on the original training cohort (n=150), bootstrap resampling was repeated 1000 times for internal validation. All quantitative predictive evaluation indicators of the two cohorts were sorted into **Table 8**, which intuitively compares the discrimination, sensitivity,

Table 4. Univariate logistic regression analysis of ar risk factors

Variable	*β*	*S.E*	*Wald*	*P*	*OR*	*95% CI*
Diabetes	1.315	0.312	17.724	<0.001	3.725	2.024-6.856
HHcy	1.463	0.297	24.218	<0.001	4.319	2.417-7.723
CC genotype	-0.814	0.367	4.921	0.026	0.443	0.216-0.908
TT genotype	0.762	0.301	6.423	0.011	2.142	1.187-3.867
T allele	1.391	0.473	8.657	0.003	4.019	1.590-10.148
Platelet aggregation rate	0.142	0.045	9.963	0.002	1.153	1.054-1.261

Note: All categorical variable assignment rules are detailed in **Table 3**.

Table 5. Multivariate backward stepwise logistic regression analysis of independent AR risk factors

Variable	*β*	*S.E*	*Wald*	*P*	*OR*	*95% CI*
Diabetes	1.132	0.295	14.702	0.000	3.103	1.740-5.530
HHcy	1.286	0.281	20.891	0.000	3.618	2.086-6.276
CC genotype	-0.728	0.350	4.332	0.037	0.483	0.243-0.959
TT genotype	0.690	0.289	5.687	0.017	1.994	1.132-3.513
T allele	1.282	0.449	8.142	0.004	3.605	1.495-8.689
Platelet aggregation rate	0.129	0.042	9.492	0.002	1.138	1.048-1.235
Constant	-0.472	0.130	13.274	0.000	-	-

Note: All categorical variable assignment rules are detailed in **Table 3**. This Tab displays multivariate logistic regression results; the second column lists detailed assignment rules for all categorical independent variables as required by reviewers.

Table 6. Occurrence of AR in patients with different genotypes [n (%)]

Characteristic		CC (n=54)	CT (n=52)	TT (n=44)	F/χ^2	*P*
Diabetes	Yes	10 (18.52)	13 (25.00)	22 (50.00)	15.28	<0.001
	No	44 (81.48)	39 (75.00)	22 (50.00)		
HHcy	Yes	12 (22.22)	14 (26.92)	28 (63.64)	27.46	<0.001
	No	42 (77.78)	38 (73.08)	16 (36.36)		
LDL-C (mmol/L)		2.42±0.38	2.45±0.36	2.85±0.41	11.32	<0.001
Platelet aggregation rate (%)		35.98±6.11	36.55±5.57	41.91±5.46$^{*,\#}$	11.61	<0.001
AR	Yes	6 (11.11)	9 (17.31)	20 (45.45)	17.12	<0.001
	No	48 (88.89)	43 (82.69)	24 (54.55)		

Notes: AR, Aspirin resistance; HHcy, hyperhomocysteinemia; LDL-C, low-density lipoprotein cholesterol. Compared to CC, $^{*}P<0.05$; Compared to CT, $^{\#}P<0.05$.

Table 7. Occurrence of AR in Patients with different alleles [n (%)]

Characteristic		C (n=88)	T (n=62)	t/χ^2	*P*
Diabetes	Yes	17 (19.32)	28 (45.16)	12.94	<0.001
	No	71 (80.68)	34 (54.84)		
HHcy	Yes	20 (22.73)	34 (54.84)	19.01	<0.001
	No	68 (77.27)	28 (45.16)		
LDL-C (mmol/L)		2.41±0.37	2.83±0.40	9.9	<0.001
Platelet aggregation rate (%)		35.51±5.68	41.19±5.50^{c}	5.41	<0.001
AR	Yes	12 (13.64)	23 (37.10)	11.27	0.001
	No	76 (86.36)	39 (62.90)		

Note: AR, Aspirin resistance; HHcy, hyperhomocysteinemia; LDL-C, low-density lipoprotein cholesterol. Compared to C, $^{c}P<0.05$.

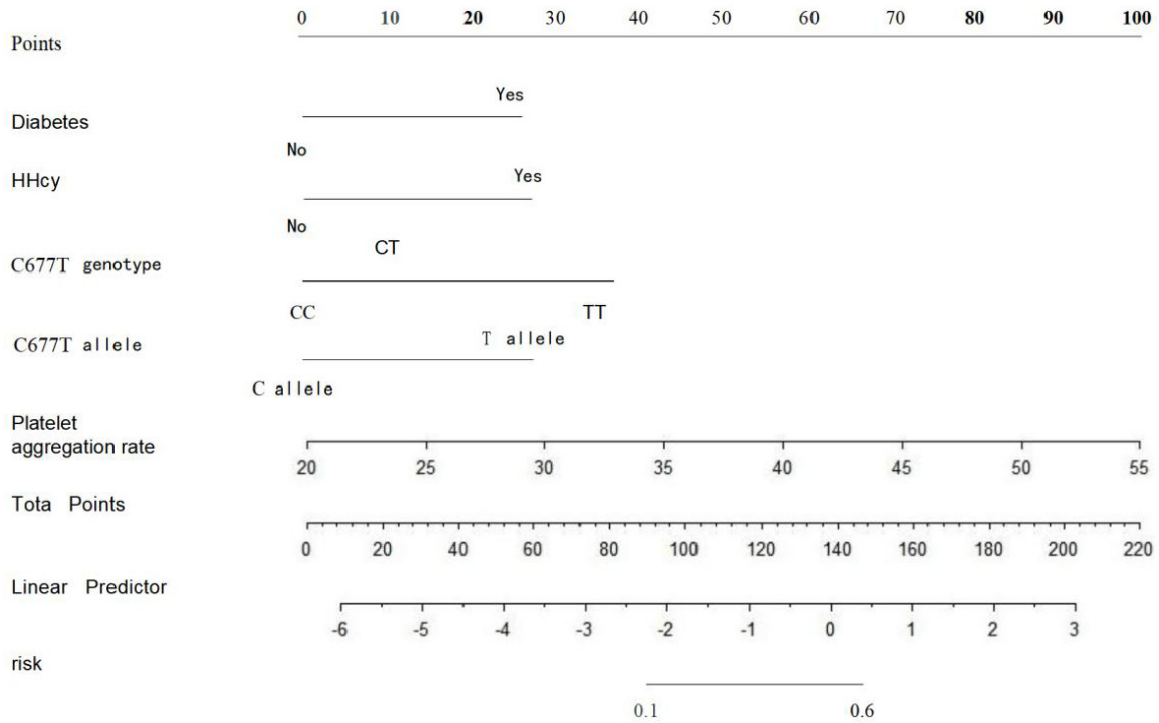


Figure 2. Nomogram model for predicting aspirin resistance (AR) in young patients with coronary heart disease (CHD). Notes: MTHFR, Methylenetetrahydrofolate Reductase; HHcy, Hyperhomocysteinemia; LDL-C, Low-Density Lipoprotein Cholesterol; HHcy, hyperhomocysteinemia; C667T, methylenetetrahydrofolate reductase C667T gene polymorphism; CC, homozygous C-allele genotype; CT, heterozygous C-T genotype; TT, homozygous T-allele genotype.

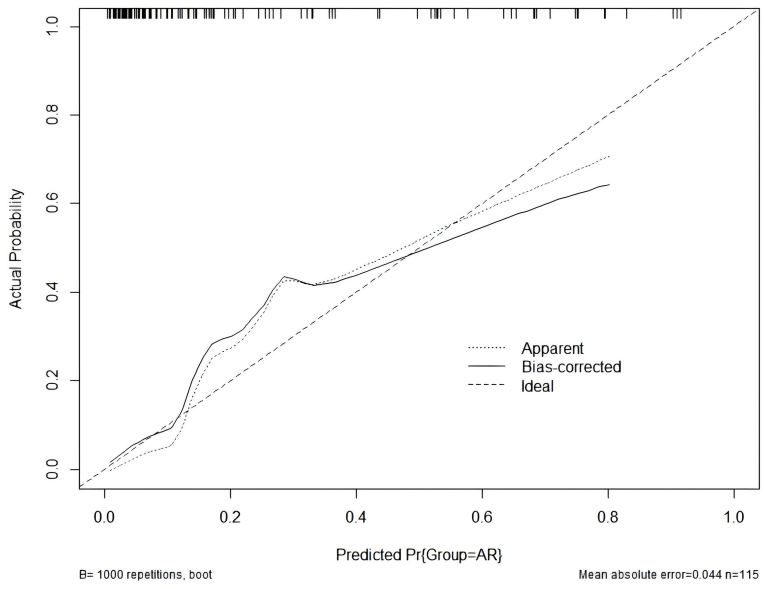


Figure 3. Calibration curve of the nomogram model. Notes: Apparent, uncorrected predicted probability curve; Bias-corrected, bootstrap-corrected curve (B=1000 bootstrap repetitions); Ideal, perfect prediction reference line; C-index, Consistency Index; AUC, Area Under the Curve.

specificity and calibration stability of the model in different cohorts. As shown in **Table 8**, all evaluation indices of the validation cohort were slightly attenuated compared with the training cohort, but the degree of attenuation was small, indicating that the nomogram model had stable internal predictive performance without obvious overfitting.

Discussion

Aspirin resistance (AR) describes the insufficient platelet inhibitory effect of aspirin verified by laboratory detection, which strongly elevates recurrent cardiovascular thrombotic risk during antiplatelet management [10, 11]. Our study observed a 23.33% incidence of AR in young CHD patients, highlighting prominent clinical risks of AR in this understudied subgroup. Unlike elderly patients burdened with multiple overlapping chronic

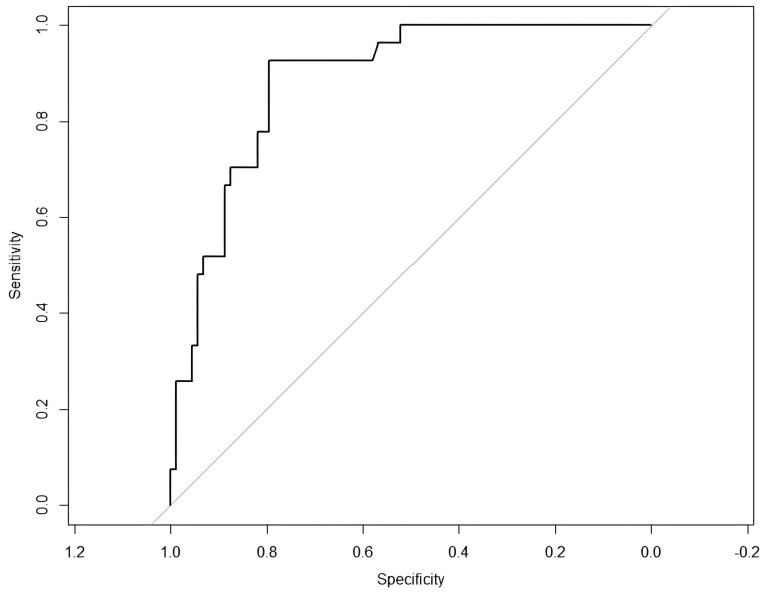


Figure 4. Receiver operating characteristic (ROC) curve analysis for the nomogram in predicting aspirin resistance (AR). Notes: AUC, Area Under the Curve; Sensitivity, true positive rate; Specificity, true negative rate.

metabolic confounders, young CHD cases carry far fewer acquired risk disturbances, making genetic variants like MTHFR C677T the core driving factor of inter-individual aspirin response difference [12].

MTHFR C677T mutation mediates AR risk via a complete linear metabolic pathway: TT genotype reduces MTHFR catalytic activity, blocks homocysteine degradation and induces HHcy [13, 14]; accumulated serum Hcy suppresses platelet COX-1 activity, upregulates thromboxane A2 synthesis and offsets aspirin's irreversible antiplatelet function [15, 16]. Meanwhile, excess Hcy aggravates vascular endothelial injury and elevates circulating LDL-C levels, jointly amplifying platelet aggregation reactivity, which fully explains the significantly higher LDL-C, platelet aggregation rate and AR rate in TT/T allele carriers observed. Hardy-Weinberg equilibrium test verified representative sample genotype distribution, eliminating sampling bias interference for genetic association conclusions.

Consistent with previous population genetic studies [6, 7, 17], our work further innovatively confirms that MTHFR C677T TT genotype and T allele act as independent AR risk factors specifically for young CHD patients. In aged mixed CHD cohorts, the genetic effect of MTHFR mutation is often masked by long-term hypertension, persistent dyslipidemia and multiple comorbidities, while the cleaner risk background of young patients uncovers the pure independent predictive value of this gene variant. Diabetic comorbidity further magnifies AR risk: sustained hyperglycemia interferes with aspirin-mediated COX acetylation and weakens platelet drug sensitivity [18, 19]. Notably, TT genotype carriers simultaneously presented higher diabetes prevalence, implying potential gene-metabolism interaction between MTHFR variant and glucose homeostasis disorder, which requires combined blood glucose and Hcy monitoring in clinical practice.

The constructed nomogram integrated genetic polymorphism, glycolipid metabolism and platelet functional indicators, with bootstrap validation demonstrating favorable discrimination and calibration across training and validation cohorts [20, 21]. This visualized tool enables clinicians to pre-estimate AR risk before aspirin initiation and implement targeted interventions: folic acid supplementation for HHcy, intensive lipid and glucose control, and dynamic platelet aggregation monitoring for TT/T allele carriers to avoid ineffective single-agent antiplatelet therapy.

This study also has some limitations. First, its single-center retrospective design and the limited regional Han Chinese samples restrict the generalizability of the findings to broader populations. Second, the sample size fell short of the theoretical minimum (n=480) required by the EPV=20 rule for multivariate logistic regression, as only 35 AR events were included. With an actual EPV of approximately 5.83, owing to the limited case volume at a single center, the multivariate regression model and nomogram may be at mild risk of overfitting. Future prospective studies with an adequate number of outcome events (≥120 AR cases) are therefore needed to further verify our findings. Third, only laboratory-defined AR was evaluated. Future multi-center, prospective, large-cohort studies encompassing diverse

Table 8. Comparison of predictive performance between training cohort and bootstrap validation cohort

Evaluation Index	Training Cohort	Bootstrap Validation Cohort	Difference Value
C-index	0.885	0.862	0.023
AUC (95% CI)	0.885 (0.822-0.947)	0.863 (0.798-0.928)	0.022
Optimal Sensitivity	0.926	0.904	0.022
Optimal Specificity	0.795	0.771	0.024
Youden index	0.721	0.675	0.046
Mean absolute calibration error	0.044	0.051	0.007

ethnic groups are warranted to externally validate the nomogram and to elaborate the precise gene-molecular mechanism by which MTHFR C677T regulates aspirin response.

Conclusion

MTHFR C677T TT genotype and T allele are independent risk biomarkers of aspirin resistance in young CHD patients, functioning through Hcy-lipid-platelet intermediate pathways. The internally validated nomogram achieves stable predictive efficiency and supports personalized antiplatelet strategy formulation for young patients. Clinicians should prioritize MTHFR genotyping screening for young CHD candidates before aspirin treatment, and deliver comprehensive metabolic interventions for high-risk TT/T allele carriers to reduce AR-related adverse cardiovascular events.

Acknowledgements

This study was supported by the Natural Science Foundation of Gansu Provincial Science and Technology Program (Grant No. 23JRRL0001) and the Pingliang City Science and Technology Program Project (Grant No. PL-STK-2022B-059).

Disclosure of conflict of interest

None.

Address correspondence to: Hui Dai, Clinical Medical School, Medical College of Gansu, Pingliang 744000, Gansu, China. Tel: +86-15825839735; E-mail: Daihui6556@163.com

References

[1] Chagas P, Caramori P, Barcellos C, Galdino TP, Gomes I and Schwanke CH. Disease burden in adult patients with coronary heart disease and its correlation with sociodemographic index. Public Health Prev Med 2024; 35: 79-82.

[2] Dimitriadis K, Lazarou E, Tsioufis P, Soulaidopoulos S and Tsioufis K. Aspirin for primary prevention of cardiovascular diseases: "WALTZ" with the evidence. Curr Cardiol Rep 2022; 24: 1139-1147.

[3] Han Y. Chinese expert consensus statement on dual antiplatelet therapy in patients with coronary artery disease. Eur Heart J 2022; 43: 1283-1285.

[4] Ebrahimi P, Farhadi Z, Behzadifar M, Shabaninejad H, Abolghasem Gorji H, Taheri Mirghaed M, Salemi M, Amin K, Mohammadibakhsh R, Bragazzi NL and Sohrabi R. Prevalence rate of laboratory defined aspirin resistance in cardiovascular disease patients: a systematic review and meta-analysis. Caspian J Intern Med 2020; 11: 124-134.

[5] Raghubeer S and Matsha TE. Methylenetetrahydrofolate (MTHFR), the one-carbon cycle, and cardiovascular risks. Nutrients 2021; 13: 4562.

[6] Zhang DJ, He XL and Wang XY. Correlation between MTHFR gene polymorphism and carotid atherosclerotic plaque stability in patients with hypertension. J Chin Physician 2024; 26: 581-584.

[7] Du C and Li C. Correlation between MTHFR C677T gene polymorphism, serum Hcy level and genetic susceptibility to coronary heart disease in Xiaochang area. Chin J Birth Health Hered 2020; 28: 1170-1173.

[8] Shivkar RR, Gawade GC, Padwal MK, Diwan AG, Mahajan SA and Kadam CY. Association of MTHFR C677T and A1298C polymorphisms and hyperhomocysteinemia with coronary heart disease. Labeled Immunoassays Clin Med 2020; 27: 219-223, 241.

[9] Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, Khera A, Lloyd-Jones D, McEvoy JW, Michos ED, Miedema MD, Muñoz D, Smith SC Jr, Virani SS, Williams KA Sr, Yeboah J and Ziaeian B. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of

the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guideline. Circulation 2019; 140: e596-e646.
[10] Silva GFD, Lopes BM, Moser V and Ferreira LE. Impact of pharmacogenetics on aspirin resistance: a systematic review. Arq Neuropsiquiatr 2023; 81: 62-73.
[11] Wang JJ, Kuang J and Tu JL. Relationship between precision medication of clopidogrel combined with aspirin and short-term prognosis in stroke patients. J Pract Med 2021; 37: 1737-1741.
[12] Li T and Tang CY. Research progress on aspirin resistance and gene polymorphism. Mil Med J Southeast China 2019; 21: 171-175.
[13] Hermann A and Sitdikova G. Homocysteine: biochemistry, molecular biology and role in disease. Biomolecules 2021; 11: 737.
[14] Zhao WJ, Li QT and Sun JZ. Relationship between homocysteine, thromboelastography and aspirin resistance in patients with cerebral infarction. Lab Med 2018; 33: 637-639.
[15] Park EJ, Je J, Dusabimana T, Yun SP, Kim HJ, Kim H and Park SW. The uremic toxin homocysteine exacerbates the brain inflammation induced by renal ischemia-reperfusion in mice. Biomedicines 2022; 10: 3048.
[16] Liu Y, Liu YX and Qu Y. Correlation between cyclooxygenase gene polymorphism and aspirin resistance and cardiovascular events. Chin J Clin Healthc 2021; 24: 320-323.
[17] Qiu W, Chen SM and Liu DS. Relationship between MTHFR C677T polymorphism and Hcy and lipid levels in patients with H-type hypertension. Lab Med Clin 2022; 19: 622-625.
[18] Paven E, Dillinger JG, Bal Dit Sollier C, Vidal-Trecan T, Berge N, Dautry R, Gautier JF, Drouet L, Riveline JP and Henry P. Determinants of aspirin resistance in patients with type 2 diabetes. Diabetes Metab 2020; 46: 370-376.
[19] Zhao YQ, Liang W and Hong YE. Incidence of aspirin resistance in elderly patients and analysis of related risk factors. Basic Clin Med 2021; 41: 1035-1038.
[20] Van Oosterom N, Barras M, Cottrell N and Bird R. Platelet function assays for the diagnosis of aspirin resistance. Platelets 2022; 33: 329-338.
[21] Van Oosterom N, Barras M, Cottrell N and Bird R. Evaluation of Panax notoginseng in the treatment of aspirin resistance using light transmission aggregometry for platelet aggregation rate. Chin J Clin Lab Sci 2023; 41: 35-37.