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The Impact of Cardiovascular Disease Gene Polymorphism and Interaction with Homocysteine on Deep Vein Thrombosis

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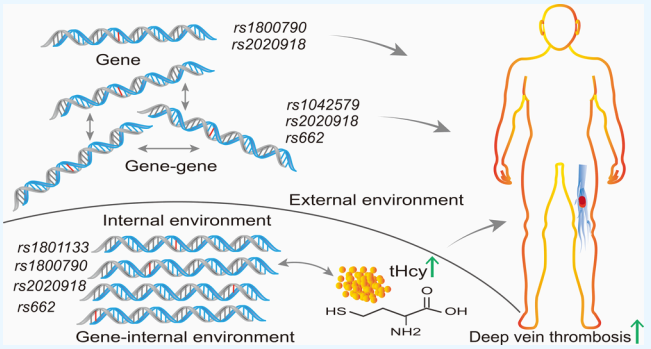
ABSTRACT: Deep vein thrombosis (DVT) affects vascular health and can even threaten life; however, its pathogenesis remains unclear. Cardiovascular disease (CVD) and DVT share common risk factors, such as dyslipidemia, aging, etc. We aimed to investigate the loci of published CVD susceptibility genes and their association with environmental factors that might be related to DVT. Genotyping by Kompetitive Allele Specific PCR (KASP), collection of lifestyle information, and determination of blood biochemical markers were performed in 165 DVT cases and 164 controls. The impact of six single nucleotide polymorphisms (SNPs) and additional potential variables on DVT morbidity was evaluated using unconditional logistic regression (ULR). To explore the high-order interactions related to genetics and the body's internal environment exposure that affect DVT, ULR, crossover analysis, and multifactor dimensionality reduction/generalized multifactor dimensionality reduction (MDR/GMDR) were employed. Sensitivity analyses were performed using the EpiR package. The polymorphisms of *FGB* rs1800790 and *PLAT* rs2020918 were significantly associated with DVT. The optimum GMDR interaction model for gene−gene (G × G) consisted of *THBD* rs1042579, *PLAT* rs2020918, and *PON1* rs662. The *PLAT* rs2020918 and *MTHFR* rs1801133 polymorphisms together eliminated the maximum entropy by the MDR method. The optimum GMDR interaction model for gene−environment (G × E) consisted of *MTHFR* rs1801133, *FGB* rs1800790, *PLAT* rs2020918, *PON1* rs662, and total homocysteine (tHcy). Those with high tHcy levels and three risk genotypes significantly increased the DVT risk. In conclusion, certain CVD-related SNPs and their interactions with tHcy may contribute to DVT. These have implications for investigating DVT etiology and developing preventive treatment plans.



1. INTRODUCTION

Deep vein thrombosis (DVT) and its potentially fatal consequent pulmonary embolism (PE) are the primary symptoms of venous thromboembolism (VTE), prevalent globally.[1] Therefore, preventing PE and the sudden cardiac death it causes, early detection and treatment of DVT are crucial. DVT is fundamentally related to the elements of Virchow's Triad, including vascular endothelial damage, stasis of flow, and hypercoagulability.[2] However, the molecular mechanisms of DVT are ill-defined.[3] Genetic research on environmental impacts may provide new insights into the etiology of DVT.

Current evidence suggests that DVT is associated with single nucleotide polymorphisms (SNPs).[4] Pathogenicity frequently exhibits conflicting results in different ethnic populations. Many studies have explained the risk genetic factors of DVT in some areas, and most of them are based on the assessment of single-locus effects or multiple genetic variants within a gene. In addition, Chinese populations are underrepresented in higher-order combinatorial effects on DVT. In gene-environment (G × E) interactions, the body's internal environment is rarely mentioned as exposure. Few biomarkers like total homocysteine (tHcy) can match over 100 diseases or conditions, of which cardiovascular disease (CVD) is the most common association reported; however, whether tHcy is an independent risk predictor remains controversial. Therefore, in this study, we prefer to use tHcy as an essential internal environmental exposure.

World Health Organization classified CVD as a collection of conditions that include cerebrovascular illness, DVT, PE, coronary heart disease, etc.[5] VTE and CVD are strongly associated conditions with similar risk factors.[6] We assumed that partial potential overlaps may be due to similar genetic susceptibility. To our knowledge, this is the first time selecting

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six hotspot CVD-related gene loci (*THBD* rs1042579, *MTHFR* rs1801131, *MTHFR* rs1801133, *FGB* rs1800790, *PLAT* rs2020918, *PON1* rs662) to detect the role of SNPs, gene−gene (G × G) and gene-tHcy interactions in DVT risk. Additionally, using statistically significant variables as potential confounders, such as blood biochemical indicators and lifestyle risk factors, were rarely included in previous studies. These are of great significance for revealing the genetic mechanism of DVT and developing screening models for the DVT susceptibility population.

An economical and high throughput Kompetitive allele-specific PCR (KASP) technique has been established to detect SNPs.[7] Multifactor dimensionality reduction/generalized multifactor dimensionality reduction (MDR/GMDR) are nongenetic models and nonparametric methods appropriate for processing high-order data, especially for sparse data;[8,9] however, compared to unconditional logistic regression (ULR), they can not perform comparisons between different genotypes. GMDR does not present an interactive entropy graph, and MDR does not support adjustment for covariates, etc. To avoid missing some true interactions, besides the traditional univariate analyses, we have, for the first time, used multiple methods to analyze these associations in various dimensions, generating an interaction entropy graph and displaying the optimal interaction models.

2. MATERIALS AND METHODS

2.1. Subjects. Subjects in total (165 DVT cases and 164 controls) were included. They are all Han Chinese from Shanxi province, and their ages range from 20 to 91. From November 2014 to November 2015, newly diagnosed cases of primary DVT were recruited from the Vascular Surgery Departments of the Second Hospital of Shanxi Medical University and Shanxi Provincial People's Hospital (Taiyuan, Shanxi, China); controls consisted of healthy staff from Datong Coal Industry Group Co, Ltd. (Datong, Shanxi, China) who underwent annual physical examinations. Neither cases nor controls had a prior history of DVT. For every research subject, 2 mL of peripheral whole blood and written informed consent were acquired. This research was conducted ethically following the World Medical Association Declaration of Helsinki. This study received ethical approval from Shanxi Medical University's ethical committee (approval no. 2014046) on March 12, 2014.

Subjects were included in this study if they met the following conditions: (1) Local clinical manifestations with thrombosis; (2) Plasma D-dimer > 0.5 mg/L; (3) Objective examination reveals signs of thrombosis, and pressurized ultrasound or venography is used to diagnose deep vein thrombosis; Participants were excluded if any of the following criteria were present: (1) Thrombotic diseases; (2) Malignant tumors; (3) Fractures; (4) Pregnancy; (5) Autoimmune diseases; (6) Blood system diseases, etc. The control group and the case group should be matched as closely as possible in terms of age, gender, etc.

2.2. Epidemiological Investigation. We interviewed people with confirmed DVT and healthy controls to collect data on demographic traits, native places, drinking, and smoking routines. During the inquiry, monitoring reduction, interviewer, social desirability, and recall bias were well considered. In addition, we obtained clinical laboratory data, such as body mass index (BMI), high-density lipoprotein (HDL), fibrinogen (FIB), total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG), tHcy, d-dimer (DD), fibrinogen degradation product (FDP). The maximum value of the clinical reference range was defined as the cutoff value.

2.3. Gene Selection and Genotyping. Based on the hypothesis that there are common risk factors for CVD and DVT, we identified genes closely related to CVD. We confirmed their strong correlation with multiple risk factors such as hypertension, hyperlipidemia, and obesity. The SNPs we selected are located in gene regions related to processes such as coagulation, fibrinolysis, and inflammation. We hypothesize that specific variations (SNPs) in these genes may also affect the risk of DVT, as they can regulate similar pathophysiological pathways.

Threshold Settings for SNP Selection: (1) Minor allele frequency (MAF) > 0.1; (2) Effect size: odds ratios (OR) > 1.5; (3) Significance threshold: *P* values < 0.05; (4) Consistency: We prioritize SNPs, known or suspected SNPs with functional effects, such as variations located in gene coding regions and regulatory regions, that consistently show significant associations with CVD (including DVT) in multiple independent studies.

Functional Annotation: We further refined our selection by integrating the chromosomal locations of the SNPs, primer sequences for amplification, and their known or inferred functions (Table S1).

Two milliliters of peripheral blood containing EDTA from the subject was kept at −80 °C. DNA was isolated from whole blood using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). Genotyping was conducted using KASP technology with SNPLine plat form (LGC Genomics, the United Kindom) by Shanghai Baygene Biotechnology Company Limited. A total of six SNPs from genes including *THBD*, *MTHFR*, *FGB*, *PLAT*, and *PON1* were considered for this study. The accuracy for all SNPs was over 99.8% based on independent assessment. The primer sequence information for the six SNPs is shown in Table S2.

2.4. Statistical Analysis. Using the online program SHEesis, the Hardy−Weinberg equilibrium (HWE) in controls was tested. Using ULR, the impacts of each potential variable were evaluated on DVT. With the wild genotype serving as a reference, the genotype variable was investigated as a categorical variable. In different genetic models, for selected loci, the odds ratios (ORs) and 95% confidence intervals (CIs) on the risk of DVT were estimated using ULR and adjusting for smoking, gender, age, DD, FDP, and tHcy. Based on the genotype frequencies of loci, the Power and Sample Size Program indicates that our sample size has above 80% (99.4%, 99.9%, 91.7%, 93.1%, 83.2%, and 85.3%, respectively) statistical power to detect an OR of around 1.5. ULR analyzes the first-order interaction between G × G and G × E using the previously mentioned adjustment parameters. All the genetic and environmental variables included in the study were split as dichotomized variables, and risk factors were represented by 1 and 0 otherwise. By crossover analysis, four groups were created in a dummy variable: exposed to one risk factor (OR_{10} or OR_{01}), exposed to both risk factors (OR_{11}) and not exposed to any risk factors (OR_{00}), as a reference. The calculation of indicators for analyzing multiplicative and additive interactions involves OR_{00}, OR_{10}, and OR_{01}. ULR calculated the OR for multiplicative interaction and *P* value. The epiR package in R statistical software version 3.3.1 was used to compute the synergy index (S), the attributable proportion of interaction (AP), and the relative excess risk of interaction (RERI) to evaluate additive

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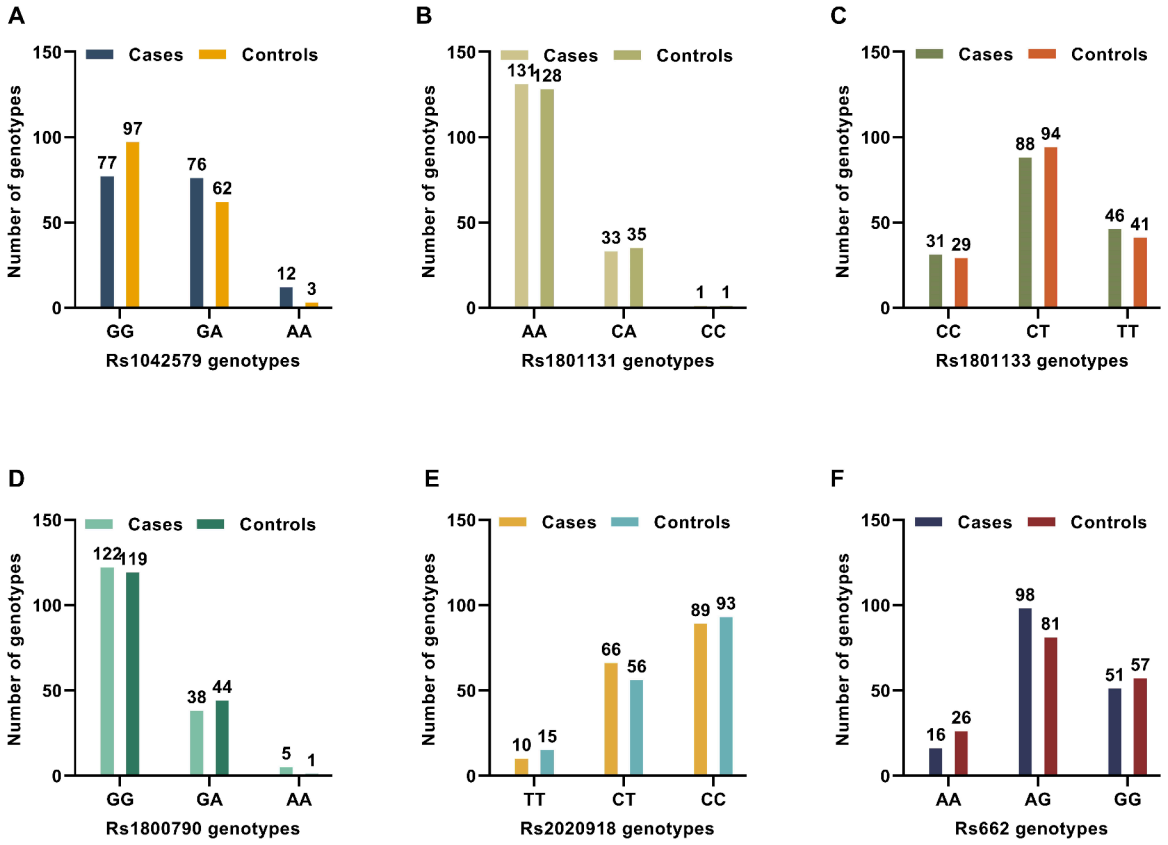

Figure 1. Genotype frequency of SNPs. The column's top number indicates the number of individuals who possess this genotype. (A) Genotype frequency for rs1042579 in the cases or controls is GG > GA > AA. (B) Genotype frequency for rs1801131 in the cases or controls is AA > CA > CC. (C) Genotype frequency for rs181133 in the cases or controls is CT > TT > CC. (D) Genotype frequency for rs1800790 in the cases or controls is GG > GA > AA. (E) Genotype frequency for rs2020918 in the cases or controls is CC > CT > TT. (F) Genotype frequency for rs662 in the cases or controls is AG > GG > AA. KASP genotyped all the loci.

interaction. The 95% CI for S does not include 1, and RERI and AP do not include 0, indicating an additive interaction.[10]

Using MDR/GMDR, high-order G × G and G × E interactions are detected and characterized. Parameters including sign test *P* value, cross-validation (CV) consistency, and testing balanced accuracy (Testing Bal. Acc) were acquired. It was decided that the optimal model was the one with the highest Testing Bal. Acc, lower sign test *P* value (≤0.05), and highest CV consistency score. Add smoking, gender, age, DD, FDP, and tHcy as covariates to the GMDR model for adjustment. MDR can offer interaction entropy analysis. IBM SPSS Statistics 22, GMDR v0.7, and MDR 3.0.2 were used for statistical analysis.

2.5. Gene−gene Interactions within the Structure of a Network. To extend and investigate the possible linked genetic effects of the five genes (*THBD*, *MTHFR*, *FGB*, *PLAT*, and *PON1*) with other genes, we used the STRING database (https://string-db.org/) to exhibit their interactions in the network and further built up the protein−protein interaction (PPI) graph through Cytoscape software 3.6.1.

3. RESULTS

3.1. Genotypes. The genotype frequency of SNPs is shown in Figure 1. All the loci reached the Hardy−Weinberg equilibrium. *P* values in controls (0.1858, 0.3964, 0.0508, 0.1494, 0.1326, 0.7546, respectively) > 0.05 indicate that SNPs are in HWE.

3.2. Characteristics of Subjects. The mean ages of the subjects were 41.7 ± 9.3 years old in the controls, 55.5 ± 14.5 years old in the cases (t = 10.262, $P < 0.05$), with 240 (72.9%) males and 89 (27.1%) females. ULR indicated that gender, DD, FDP, and tHcy were associated with increased DVT risk, while male smoking reduced DVT risk. In addition, no association was identified between drinking, BMI, age, FIB, TC, HDL, LDL, TG, and DVT (Figure 2), so we removed them in the later analysis.

The genotype distributions of the six selected SNPs and their associations with DVT are shown in Table 1. Allele A in *FGB* rs1800790 was found to be associated with a 2.9 and 3.2 times higher risk of DVT in the additive and dominant models, respectively; allele C in *PLAT* rs2020918 was found to be associated with a 7.1 times higher risk of DVT in dominant model. After adjusting for multiple comparisons, these associations continued to have statistical significance. However, the other SNPs were not associated with DVT (95% CIs including 1 or $P > 0.05$).

3.3. High-Order Interaction. As shown in Table S5, no multiplicative G × G interaction was detected by ULR analysis. The unadjusted data can be shown in Table S6. Using the EpiR package, the additive interaction for *PLAT* rs2020918 and *PON1* rs662 (RERI = 1.298, 95% CI 0.053−2.544; AP = 1.673, 95% CI −3.148−6.495; and S, 95% CI is unachievable) was significant, while none was detected in other G × G pairs (95% CIs for S including 1 and RERI and AP including 0). Table 2 presents the optimal G × G interaction models to explain DVT risk by GMDR. The optimal interaction models included the

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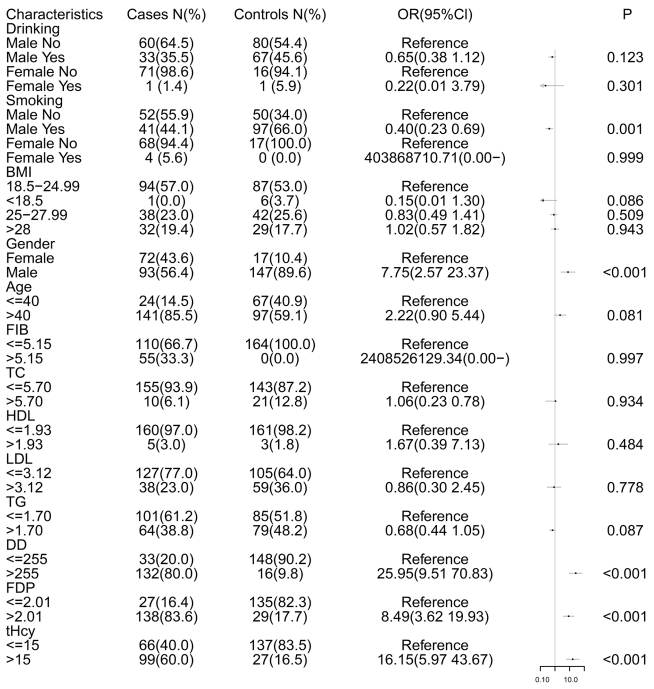

Figure 2. Subgroup analysis of the primary DVT potential variables. OR, odds ratio.

combination of *THBD* rs1042579, *PLAT* rs2020918, and *PON1* rs662. Another significant model was the combination of *THBD* rs1042579, *MTHFR* rs1801133, *PLAT* rs2020918, and *PON1* rs662.

As shown in Table S7, a significant multiplicative G × E interaction between *THBD* rs1042579 and tHcy was detected by ULR, while none was detected in the other five SNPs-tHcy pairs. The unadjusted data can be shown in Table S8. Using the EpiR package, no additive interaction was detected in all G × E combinations. Table 2 and Figure S1 show the optimal G × E interaction models (rs1801133 rs1800790 rs2020918 rs662 tHcy) to explain DVT by GMDR. Figure 3 shows four dimensions of G × E interaction models (rs1801133 rs1800790 rs662 tHcy) to explain DVT by GMDR. The other three significant models will not be elaborated on here.

We used MDR interaction entropy analysis to depict the observed interactions and ascertain whether they were additive or nonadditive after determining the combinations that posed a

Table 2. GMDR Interaction Models on DVT Risk

model[a]	training Bal.Acc	testing Bal.Acc	*P*	cross-validation consistency
gene−gene interaction				
rs1800790	0.612	0.492	0.623	8/10
rs1800790 rs662	0.666	0.543	0.623	9/10
rs1042579 rs2020918 rs662	0.745	0.648	0.010	10/10
rs1042579 rs1801133 rs2020918 rs662	0.792	0.544	0.010	8/10
rs1042579 rs1801133 rs1800790 rs2020918 rs662	0.845	0.560	0.171	10/10
rs1042579 rs1801131 rs1801133 rs1800790 rs2020918 rs662	0.878	0.513	0.377	10/10
gene−environment interaction				
tHcy	0.759	0.767	0.001	10/10
rs1801131 tHcy	0.768	0.728	0.001	5/10
rs1800790 rs662 tHcy	0.809	0.765	0.001	6/10
rs1801133 rs1800790 rs662 tHcy	0.843	0.785	0.001	9/10
rs1801133 rs1800790 rs2020918 rs662 tHcy	0.877	0.769	0.001	10/10
rs1042579 rs1801133 rs1800790 rs2020918 rs662 tHcy	0.905	0.685	0.010	10/10
rs1042579 rs1801131 rs1801133 rs1800790 rs2020918 rs662 tHcy	0.926	0.703	0.054	10/10

[a]Adjusted by covariate, including smoking habit, gender, age, DD, and FDP. The unadjusted data can be seen in Table S4.

Table 1. SNPs in *THBD*, *MTHFR*, *FGB*, *PLAT*, and *PON1* Genes and Risk of DVT[a]

				dominant		additive		recessive	
SNPs	genotypes	controls	cases	OR[a] (95% CI)	*P*[a]	OR[a] (95% CI)	*P*[a]	OR[a] (95% CI)	*P*[a]
rs1042579	GG	97	77	1.79(0.78−4.08)	0.167	1.74(0.86−3.51)	0.122	2.56(0.40−16.08)	0.315
	GA	62	76						
	AA	5	12						
rs1801131	AA	128	131	1.35(0.52−3.49)	0.533	1.23(0.52−2.91)	0.637	0.60(0.02−12.65)	0.748
	CA	35	33						
	CC	1	1						
rs1801133	CC	29	31	0.68(0.23−1.98)	0.481	0.79(0.43−1.45)	0.455	0.78(0.31−1.94)	0.606
	CT	94	88						
	TT	41	46						
rs1800790	GG	119	122	3.28(1.24−8.61)	0.016[b]	2.94(1.27−6.76)	0.011[b]	6.28(0.56−70.05)	0.135
	GA	44	38						
	AA	1	5						
rs2020918	TT	15	10	7.12(1.05−48.18)	0.044[b]	1.51(0.75−3.05)	0.240	1.22(0.53−2.79)	0.632
	CT	56	66						
	CC	93	89						
rs662	AA	26	16	1.78(0.51−6.17)	0.364	0.89(0.47−1.67)	0.720	0.58(0.23−1.43)	0.242
	AG	81	98						
	GG	57	51						

[a]ORs and *P* values were adjusted by smoking habit, gender, age, DD, FDP, and tHcy. The unadjusted data can be seen in Table S3. [b]After using the FDR (false discovery rate) method to adjust for multiple comparisons, the associations were statistically significant at the 0.20 levels.

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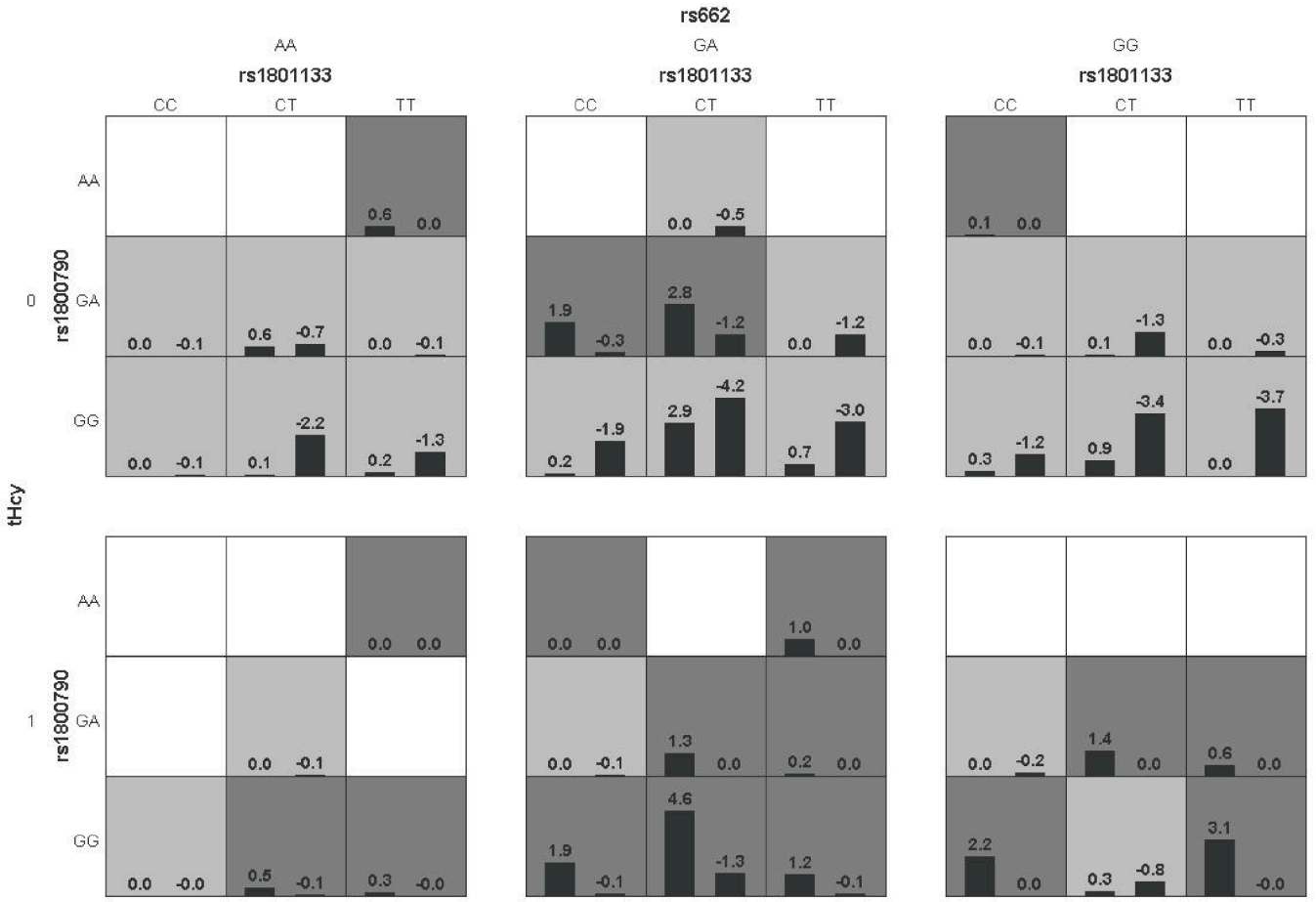

Figure 3. Graphical presentation of the four-dimensional interaction pattern obtained by GMDR. Each square shows a negative score on the right bar and a positive score on the left bar. Light gray squares indicate low risk, while dark gray squares indicate high risk. "0" means tHcy levels ≤ 15 μmol/L, "1" means tHcy levels > 15 μmol/L. White squares indicate no subjects.

high risk (Figure 4). The entropy circle graph indicates the independent main effects of *THBD* rs1042579 and tHcy are 1.47% and 15.15%, respectively. Furthermore, in addition to the respective main effects of both SNPs, *PLAT* rs2020918 and *MTHFR* rs1801133 polymorphisms have a significant synergistic relationship on DVT risk, which eliminates an extra 1.16% of the overall entropy. Likewise, the dendrogram showed their strongest synergic effect. MDR discovered three other minor interactions, all of which are additive.

Table 3 shows the combined effects of three risk SNPs and tHcy levels on DVT risk. Table 1 and Figure 4 results show that potential pathogenic genotypes include *FGB* rs1800790 GA +AA, *PLAT* rs2020918 CT+CC, and *THBD* rs1042579 GA +AA. Four groups were created according to the number of risk genotypes among the subjects. A significant dose effect (*P* trend = 0.015) was found between the number of risk genotypes carried by subjects and developing DVT. Subjects with three risk genotypes and high tHcy levels had a 271.5 times increase in developing DVT compared to those with low tHcy levels and no risk genotypes. Figure 5 shows that subjects with these three risk genotypes and high tHcy levels are associated with high DVT risk.

3.4. Network for Protein−Protein Interaction (PPI).

Figure 6 shows that the STRING database was performed to search for known and predicted protein−protein interactions, and network diagrams of protein interactions were constructed.

4. DISCUSSION

Environment, behavior, and genetic factors all have a role in causing illness.[11] In fact, complex diseases or phenotypes are often affected by the combined action of multiple genes.[12] Therefore, identifying G × G interactions is essential for understanding the fundamental mechanisms and genetic susceptibility of diseases. In organisms, gene expression may be affected by the environment, including not only the external surroundings where the organism lives or develops but also the internal factors such as metabolism and hormones. G × E interaction can reveal that some genotypes are much more sensitive to environmental effects than others.

This study demonstrated that gene polymorphisms related to CVD (*THBD, MTHFR, FGB, PLAT, and PON1*), together with tHcy levels, could be more effective in predicting DVT using MDR/GMDR analysis. It also revealed, for the first time, associations between polymorphisms of *FGB* rs1800790, *PLAT* rs2020918, and DVT.

As stated in the introduction, one prerequisite for our hypothesis is that DVT and CVD have common risk factors, indicating that they may have partially similar genetic backgrounds. In this study, the genetic polymorphisms of *FGB* and *PLAT* are closely linked to DVT, providing additional support for our hypothesis. However, the potential molecular mechanisms underlying its association with DVT are still unclear. A reasonable hypothesis might be that the recognized *PLAT* and

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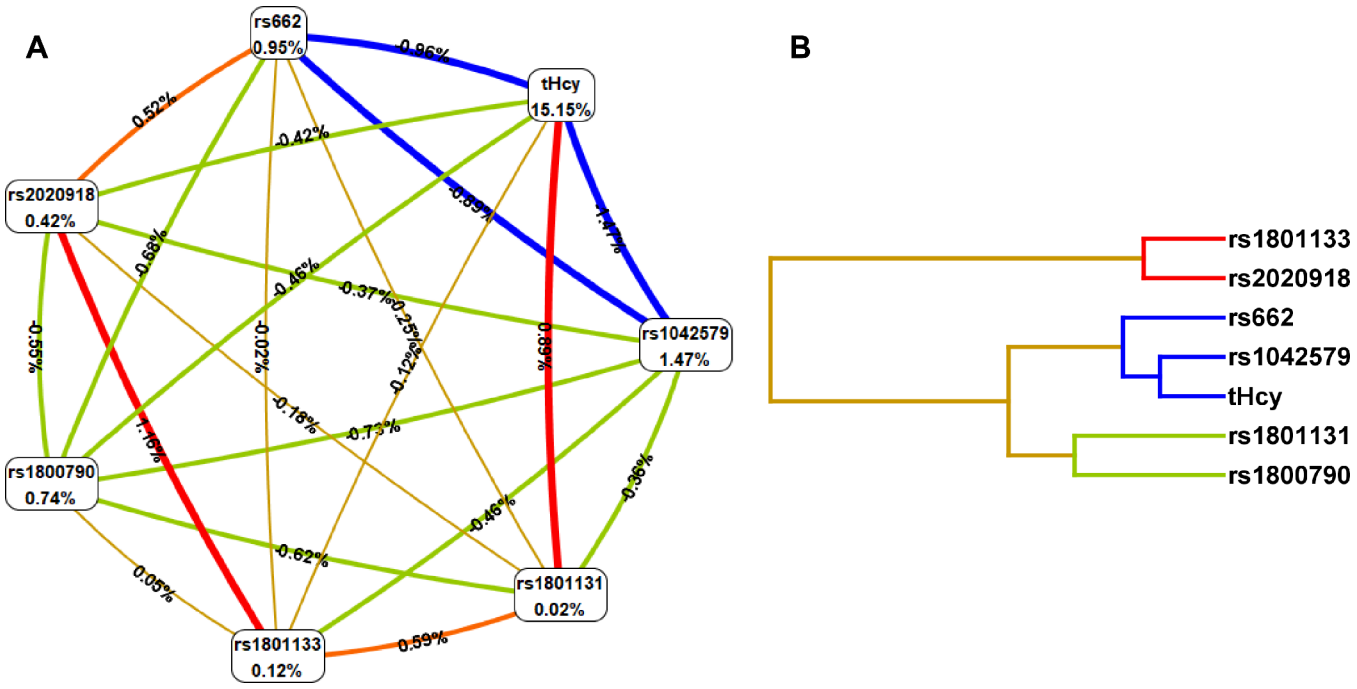

Figure 4. Circle graph and dendrogram of interaction entropy. (A) The circle graph displays the entropy of each SNP as a percentage at the bottom, while the percentage on each line indicates the entropy interaction percentage between two SNPs. The interaction of redundancy and synergy is represented by the blue and red lines, respectively. (B) According to the interaction dendrogram, a weaker synergistic interaction is represented by the orange line, and a stronger interaction is represented by a red line. The interaction intensifies from left to right.

Table 3. Cumulative Effects of Risk Genotypes of *THBD* rs1042579 GA+AA, *FGB* rs1800790 GA+AA, and *PLAT* rs2020918 CT +CC Combined with tHcy Levels on DVT Risk

	total			low-tHcy (≤15 μmol/L)			high-tHcy (>15 μmol/L)		
no.[a]	Ca/Co[b]	OR[c] (95% CI)	P^c	Ca/Co[b]	OR[c] (95% CI)	P^c	Ca/Co[b]	OR[c] (95% CI)	P^c
0	5/8	reference		2/6	reference		3/2	reference	
1	58/69	7.63(0.56−103.06)	0.126	23/57	3.95(0.04−345.89)	0.547	35/12	18.47(0.52−651.7)	0.109
2	78/69	12.92(0.96−173.77)	0.054	28/57	4.68(0.05−398.83)	0.496	50/12	95.42(1.64−5546.31)	0.028
3	24/18	55.18(3.05−998.45)	0.007	13/17	20.61(0.19−2156.02)	0.202	11/1	271.57(2.02−36511.5)	0.025

[a]Number of risk genotypes (*FGB* rs1800790 GA+AA, *PLAT* rs2020918 CT+CC, *THBD* rs1042579 GA+AA). [b]Case group and control group. [c]Adjusted by smoking habit, gender, age, DD, and FDP. The unadjusted data can be seen in Table S9.

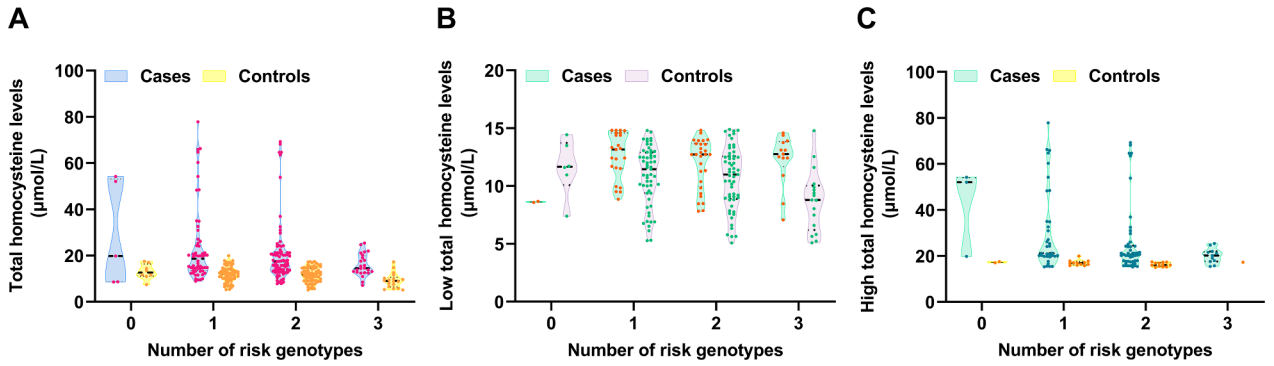

Figure 5. Distribution state and probability density of different tHcy levels with the other numbers of risk genotypes. (A) Violin plot with all tHcy levels. (B) Violin plot with low tHcy levels. (C) Violin plot with high tHcy levels.

FGB gene variants (leading to an increase in plasma fibrinogen[13] and a lower vascular release of Tissue-type plasminogen activator,[14] respectively) can result in CVD. *FGB* rs1800790 (−455G/A) alteration occurs in the promoter region.[15] *PLAT* rs2020918 (−7351C/T) is located within an enhancer region, which results in a decreased affinity of Sp1 transcription factors for this site;[16] therefore, these polymorphisms lead to functional changes at the transcriptional level. An increase in plasma fibrinogen levels can lead to hypercoagulability. At the same time, a decrease in *t-PA* release can impair the natural breakdown of blood clots and increase the risk of thrombus persistence and growth, which may contribute to DVT.

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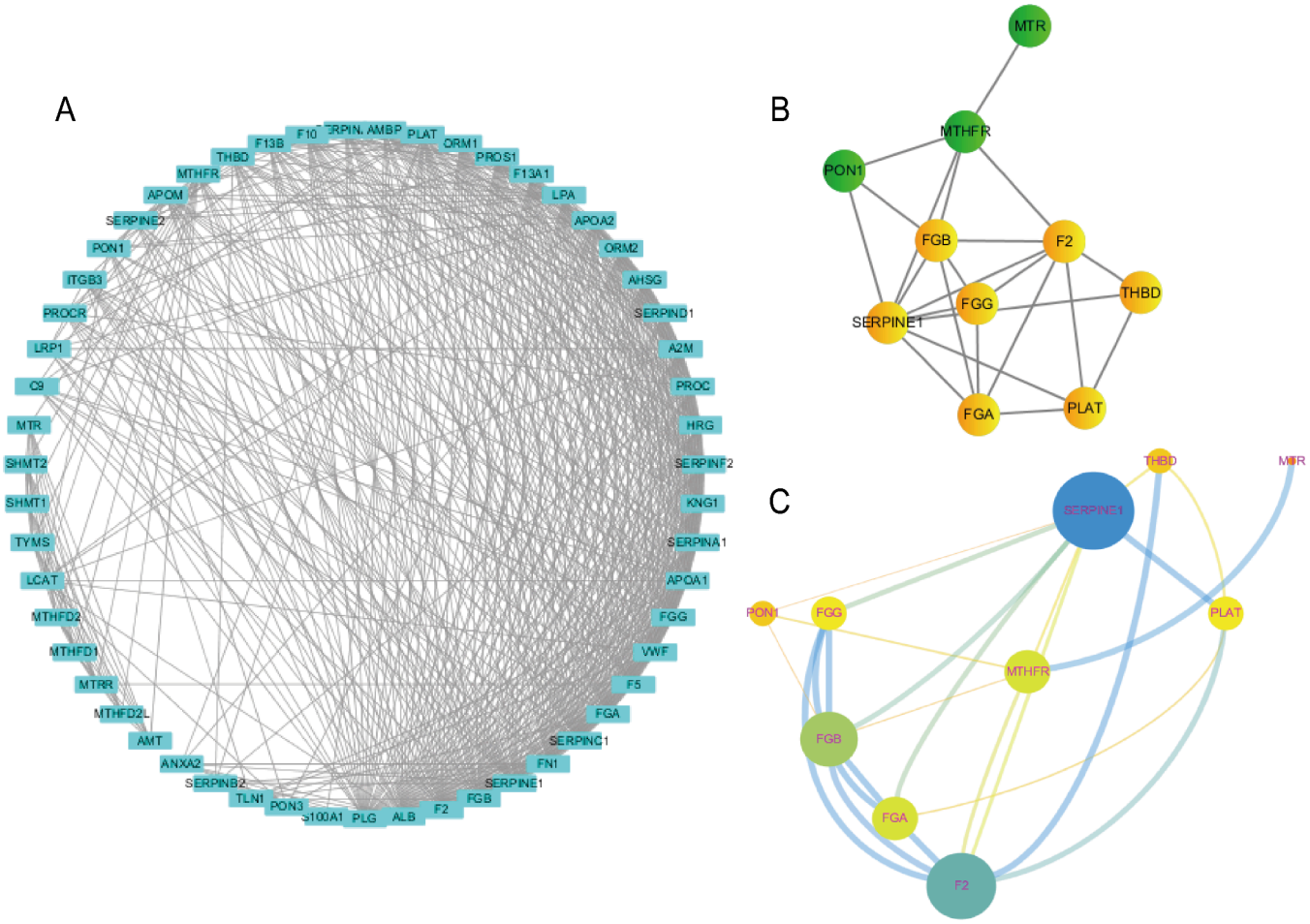

Figure 6. The network of PPI was constructed by STRING version 11.5 and displayed by Cytoscape 3.6.1. (A) PPI network of different genes. STRING analysis identified 55 nodes and 579 edges. (B) A significant module (a clustered protein interaction network diagram of yellow color) studied in this paper was selected from PPI network A. (C) STRING analysis identified 10 nodes and 23 edges. The nodes with greater degrees in the network are shown by larger sizes and darker circles. The lines represent the interaction relationship between nodes, and the larger size and darker lines indicate the nodes with the higher combined score values in the network.

For G × G, GMDR identified two significant three- and four-dimensional interaction models (rs1042579 rs2020918 rs662 and rs1042579 rs1801133 rs2020918 rs662); however, it was not stated whether a synergistic effect existed. ULR showed the interaction between *PLAT* rs2020918 and *PON1* rs662 on an additive scale. MDR generated an interactive entropy graph; most notably, *PLAT* rs2020918 or *MTHFR* rs1801133 alone does not play any role in predicting DVT, but their interaction increases the risk by 1.16%. Additionally, minor additive interactions were observed between *PLAT* rs2020918 and *PON1* rs662, and between *MTHFR* rs1801131 and *MTHFR* rs1801133. Based on the above results, we found that all significant SNP interaction pairs fell within the GMDR four-dimensional risk model, indicating that first-order synergistic interactions may play a role in higher-order genetic networks. *FGB*, *THBD*, *MTHFR*, and *PLAT* are involved in the various biological processes: negative regulation of blood coagulation (GO:0030195), negative regulation of fibrinolysis (GO:0051918), zymogen activation (GO:0031638), blood coagulation(GO:0007596), and blood coagulation, fibrin clot formation (GO:0072378). These biological processes are all related to blood coagulation and fibrinolysis. A reasonable hypothesis is that the network jointly impacts the onset and progression of DVT through complex regulatory mechanisms.

PON1 rs662 is located in the coding region,[17] while *MTHFR* rs1801133 and *MTHFR* rs1801131 are located at the folate binding site and within a presumptive regulatory domain.[18] Variations in the *PON1 and MTHFR* genes can result in changes in serum *PON1* enzyme activity[19] and plasma tHcy levels.[20] These can lead to increased oxidative stress and disorders in folate and homocysteine metabolism, respectively, subsequently causing abnormal lipid levels, ischemic stroke, and CVD, such as coronary artery disease etc.[21−25] Although current research and this study have shown that these three SNPs are not independently associated with DVT risk, the network's functional enrichments reveal that *PLAT* and *MTHFR* belong to the Folate metabolism (WP176) pathways, representing the most relevant biochemical processes related to folate in the context of metabolism, oxidation, and inflammation. They also participate in Vitamin B12 metabolism (WP1533) pathways. Central B12 metabolism involves key nodes in folate metabolism (WikiPathways). Both of these two genes are associated with vascular diseases (DISEASE DOID:178). PLAT and PON1 belong to both the complement and coagulation cascades, as well as the protein−lipid complex network (STRING CL:18726). These findings can also provide support for the interaction between these SNPs.

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In PPI analysis, the STRING database supports both *PLAT* and *PON1* interactions with *SERPINE1* (Plasminogen activator inhibitor 1) *and both PLAT* and *MTHFR* interact with *F2* (Coagulation Factor II), respectively. The MCL clustering algorithm effectively clusters proteins with similar functions, revealing *that PLA*T, F2, and *SERPINE1* belong to the same subnet, while *MTHFR and PON1* belong to another subnet. This suggests that potential interactions could exist between *PLAT* rs2020918 and *PON1* rs662, as well as between *PLAT* rs2020918 and *MTHFR* rs1801133. These findings are consistent with our statistical predictions.

The other interactions are negative in the MDR graph, but THBD rs1042579 alone has a risk of 1.47%, exhibiting a major effect. It is located in the coding region that activates protein C and binds thrombin, and its polymorphism may have the ability to regulate thrombomodulin functions.[26] Some studies in the white population did not observe any associations between this polymorphism and soluble thrombomodulin levels or a significant susceptibility to thrombosis.[27,28] However, another study suggested that *THBD* rs1042579 and *THBD* rs3176123 had virtually identical strength of association with DVT in Japanese men.[29] *THBD* rs1042579 is significantly associated with DVT in both dominant and additive gene models when unadjusted. Also, *FGB* belongs to WikiPathways, such as complement and coagulation cascade (WP558) and coagulation cascade (WP272). It plays a role in biological processes related to coagulation and fibrinolysis (Gene Ontology). Therefore, we used a relaxed significance level (P = 0.12) to avoid missing true associations in a small sample. Additionally, we included *THBD* rs1042579 in the model to examine the cumulative effects of risk genotypes.

In this study, FDP, DD, and tHcy were independently associated with the risk of DVT. FDP and DD are well-recognized markers for coagulation generated by the fibrinolytic system, and their levels have been found to correlate with thrombosis.[30−32] Numerous prospective studies have reported that elevated tHcy levels are an independent risk factor for CVD.[33,34] Genetic abnormalities, enzyme dysfunction, diet (vitamin intake deficiency, coffee, tea consumption, drinking, strict vegetarians, etc.), unhealthy living habits (cigarette smoking, acute exercise, etc.), elderly persons, drug, renal function, etc. can result in a rise in tHcy levels.[35−40] Our research revealed no association between the *MTHFR* polymorphism and DVT. Thus, high tHcy levels may be caused by factors other than genetic polymorphism. Although we tested the interactions between FDP, DD, and the six selected SNPs, these two markers did not induce a significant synergistic effect; therefore, we reported the effect of tHcy as the body's internal environment exposure.

For G × E, ULR showed the interaction between *THBD* rs1042579 and tHcy on a multiplicative model. GMDR has identified five risk interaction models. The six-dimensional model (rs1042579 rs1801133 rs1800790 rs2020918 rs662 tHcy) covers all factors of the G × G risk interaction model. It is worth noting that tHcy alone has a risk of 15.15%, exhibiting a major effect, and *MTHFR* rs1801131 alone does not play any role; however, their interaction eliminates an extra 0.89% of overall entropy in the MDR graph. Under normal circumstances, tHcy *is* quickly converted into glutathione and S-adenosylmethionine,[41] which are closely related to liver detoxification, elimination of waste-free radicals, and DNA repair, and are crucial for cellular growth, differentiation, and function.[42−45] An increase in tHcy indicates a deficiency of these two essential substances in organisms, which can cause severe health damage.[46,47] The association between high tHcy levels and DVT may be due to blocked methylation, oxidative injury to the vascular endothelium caused by inhibited nitric oxide, elevated asymmetric dimethylarginine levels, and imbalance of coagulation and fibrinolysis, etc.[48,49] Our research suggests that high tHcy levels above 15 μmol/L are a significant risk factor for DVT. Therefore, it is crucial to determine sources of tHcy and reduce them, as well as develop risk models for its interaction with genetic factors, in order to prevent and treat DVT.

One potential limitation of our study is the relatively small sample size. Certain categorical variables display uneven frequency distribution, *such as* the scarcity of cases within a specific age group, the high prevalence of smoking among males in East Asia, the high proportion of males in the mining area, and particular genotypes with a frequency of 0. These factors may lead to a small number of outcomes with OR values significantly deviating from 1 and wider CIs or an inability to determine the upper limit of the CIs. However, these analysis models have all been adjusted for confounding factors in order to minimize bias. There is no evidence of an association between additional SNPs and DVT, except for relatively small sample sizes, possibly due to ethnic differences and unconditional statistics.

Furthermore, the research findings on interactions are not entirely consistent due to the applicability and limitations of each method, which need to be interpreted with caution. These "interactions" refer to statistical interactions, and it is unclear whether they have true biological significance. Therefore, additional genetic association studies and functional experiments need to be conducted in a larger, representative population to verify the results.

5. CONCLUSION

In summary, we observed for the first time an association between DVT and CVD-related genes (*FGB* rs1800790 and *PLAT* rs2020918) polymorphisms. We found that individuals with high tHcy levels and carrying these three specific risk genotypes (rs1042579 GA+AA, rs1800790 GA+AA, rs2020918 CT+CC) are at higher risk of developing DVT. Using the GMDR methods, this significant model (rs1042579, rs1801133, rs1800790, rs020918, rs662, tHcy) covers all risk factors, including the two optimal G × G and G × E combinations, and can effectively predict DVT. *PLAT* rs2020918 and *MTHFR* rs1801133 showed the strongest synergistic effect according to MDR entropy analysis. To further understand these associations and ascertain whether there is a real biological correlation, more research is required. Therefore, this study will significantly improve our chances of unraveling the etiology of DVT. It will provide information for developing screening kits for populations at risk of DVT, which is essential for clinical prevention, early diagnosis, and personalized treatment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c05204.

Functional description and pathway for the six selected SNPs; primer sequences for the six selected SNPs; SNPs in *THBD*, *MTHFR*, *FGB*, *PLAT*, and *PON1* genes and risk of DVT (crude); GMDR interaction models on DVT risk (crude); gene−gene interaction for *THBD* rs1042579, *MTHFR* rs1801131, *MTHFR* rs1801133, *FGB*

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rs1800790, *PLAT* rs2020918, and *PON1* rs662 on DVT risk; gene−gene interaction for *THBD* rs1042579, *MTHFR* rs1801131, *MTHFR* rs1801133, *FGB* rs1800790, *PLAT* rs2020918, *PON1* rs662 on DVT risk (crude); gene−environment interaction for *THBD* rs1042579, *MTHFR* rs1801131, *MTHFR* rs1801133, *FGB* rs1800790, *PLAT* rs2020918, *PON1* rs662, and tHcy levels on DVT risk; gene−environment interaction for *THBD* rs1042579, *MTHFR* rs1801131, *MTHFR* rs1801133, *FGB* rs1800790, *PLAT* rs2020918, *PON1* rs662, and tHcy levels on DVT risk (crude); cumulative effects of risk genotypes of *THBD* rs1042579 GA+AA, *FGB* rs1800790 GA+AA, and *PLAT* rs2020918 CT+CC combined with tHcy levels on DVT risk (crude); and graphical presentation of the optimal five-dimensional interaction pattern obtained by GMDR (PDF)

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Author Contributions

L.L.N. conceptualized and designed the study, analyzed and interpreted data, and drafted the manuscript. H.L.F. and J.C. acquired the data and conducted a review. Y.Y.W. and J.H.S. contributed to revising the manuscript critically for important academic content. Q.X.D and Q.Q.J. contributed to resources, project administration, and funding acquisition. All authors approved the final version to be published.

Notes

The authors declare no competing financial interest.

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