Integrated Transcriptome-Wide Profiling And Protein Structure Analysis of Pathogenic Genes In Venous Thromboembolism

Jing Ding  
Shanghai Institute for Biomedical and Pharmaceutical Technologies

Yan Zhang  
Shanghai Institute for Biomedical and Pharmaceutical Technologies

Yan Che (cheyan2004@163.com)  
Shanghai Institute for Biomedical and Pharmaceutical Technologies

Research

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Abstract

**Background:** Genetic factors are considered to determine the balance of the coagulation and anticoagulation processes, yet the genetic variants related to venous thromboembolism (VTE) remain unclear. This study aimed to investigate the potential molecular mechanisms and pathogenic mutations associated with VTE by determining VTE-related differentially expressed genes (DEGs) by transcriptome-wide profiling and assaying protein structure in VTE.

**Methods:** Two gene expression datasets, GSE48000 and GSE19151, were accessed from the Gene Expression Omnibus (GEO) database to obtain gene expression data associated with VTE. We identified the DEGs between VTE patients and healthy people using R and performed functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Then, whole-exome sequencing (WES) was performed for 25 VTE patients and 17 normal cases, and the structural locations of pathogenic missense mutations were identified using pyMOL. Finally, DGIdb database was used to select candidate drugs for the treatment of VTE.

**Results:** A total of 232 DEGs were identified from the GEO database. The significant function of these DEGs was mostly involved in RNA catabolic process and ribosome pathway. Notably, the results of WES for DEGs and protein structure analysis showed that Histamine N-Methyltransferase (HNMT) (chr2: 138759649 C>T, rs11558538) may be a major predisposing factor for VTE. In addition, Amodiaquine, Harmaline, Aspirin, Metoprine, Dabigatran, and Diphenhydramine were screened for VTE therapy.

**Conclusion:** The results showed that HNMT (chr2: 138759649 C>T, rs11558538) may be potential target for the diagnosis and treatment of VTE.

Introduction

Venous thromboembolism (VTE) is the third most common cardiovascular disease worldwide, which manifests as deep-vein thrombosis, pulmonary embolism, or both[1, 2]. Various epidemiological studies have demonstrated that the incidence of VTE is characterized by a remarkable number of genetic and environmental factors. In early epidemiological studies, the highest incidence of VTE was in Africa, followed by Caucasians and was lower for Asian[3]. With increased awareness of the diagnosis and management of VTE, the incidence in Asia has increased in recent years[4]. While VTE is classified as a complex, multifactorial and polygenic disease, common mechanisms driven VTA have been confirmed, such as gene-gene and gene-environment interactions[5]. Stasis, vessel damage, and a hypercoagulable state are three widely accepted mechanisms related to the occurrence of VTE[6]. Genetic epidemiological studies have revealed that genetic conditions are significant risk factors for VTE, accounting for up to 50% of all VTE patients including anticoagulant protein deficiency trapping (deficiency of protein C, protein S, and antithrombin), factor V Leiden mutation (FVL) (c.1601G > A, p.R534Q), prothrombin G20210A mutation (FII G20210A), hyperhomocysteinemia, elevated coagulation factors VIII, IX, X, histidine rich glycoprotein, and ABO blood group[7, 8]. However, only a few genetic factors have been considered and the distribution of FVL and FII G20210A mutation depends on ethnic group, race, or geographical region, suggesting that there is still an urgent need to identify VTE pathogenic genetic factors[9].

With the development of gene chip technology, large-scale deep sequencing and bioinformatics analysis, scientists now have rich datasets for answering biological questions, including information on DEGs, pathways, and even targeted drugs associated with disease development[10]. Whole-exome sequencing and protein structure analysis can detect potentially important mutations that have not been reported, which is, hence, of vital significance for VTE patients to enrich anticoagulation and the use of catheter-directed thrombolytic therapy[11].

In this study, we used the GSE48000 and GSE19151 datasets downloaded from the Gene Expression Omnibus to identify DEGs associated with VTE. GO and KEGG enrichment analyses of these DEGs were then performed. WES for a total of 25 VTE patients and 17 normal people was performed to screen out pathogenic mutations in VTE associated DEGs. Based on our findings, HNMT(chr2: 138759649 C>T, rs11558538) appeared to be a genetic susceptibility risk factor for VTE, representing a novel, potentially druggable target for the treatment of VTE.

Materials And Methods

2.1 Patients samples

Twenty-five VTE and 17 normal patients admitted to participating hospitals from July 2015 to December 2018 were selected as study subjects. All patients were confirmed for VTE by B-ultrasound scan or CT examination, and basic characteristics of the recruited VTE patients were recorded, including gender, age, medication history, disease history, history of cardiovascular diseases, and history of chronic obstructive pulmonary disease. All patients signed informed consent documents, and this study was approved by the ethics committee of the Shanghai Institute of Planned Parenthood Research and ethical committees of participating hospitals.

2.2 Extraction of datasets

Gene expression profiles were mined from the GEO (GEO: www.ncbi.nlm.nih.gov/geo/) database, which distributes high-throughput gene expression and other functional genomics datasets, using the following keywords: ‘VTE’ and ‘Homo sapiens’[12]. Two datasets, GSE48000 and GSE19151, were identified for this study. The gene expression profile dataset of GSE48000 included 40 high-risk VTE cases and 25 healthy controls and had data on whole blood-derived RNA samples sequenced using a GPL10558 Illumina Human HT-12 V4.0 expression bead chip[13]. The GSE19151 dataset was generated on the GPL571[HG-U133A_2] Affymetrix Human Genome U133A 2.0 Array platform. This dataset contained 70 adults with VTE cases and 63 healthy controls[14].

2.3 Preprocessing and repeatability tests of datasets
The original raw expression data at the probe level was downloaded as CEL files and pre-processed and normalized with RMA using the 'affy' package in R version 4.0.2, followed by converting the data into corresponding gene expression data based on the different platform specifications[15]. The Pearson's correlation coefficient was determined to validate the intra-group data repeatability and heatmap generation was visualized based on the 'heatmap' R package[16]. Principal component analysis (PCA) was conducted to view the clustering trends according to sample-to-sample distances using the 'ggord' package in R[17].

2.4 Identification of DEGs

The 'limma' package in R program was applied to screen DEGs between VTE samples and normal samples[18]. A two-tailed t-test was performed to examine DEGs by log2 (Fold Change) > 1 or < -1 and adjusted P value < 0.05. Genes satisfying these conditions were grouped separately as DEGs by volcano plot in R [19].

2.5 Gene ontology and KEGG pathway enrichment analysis

GO is used to describe the biological processes (BPs), molecular functions (MFs), and cellular components (CCs) of gene products in a hierarchical ontology[20]. Signaling pathway analysis was conducted to map DEGS to the Kyoto Encyclopedia of Gene and Genomes (KEGG), which is a pathway-related database for systematic and comprehensive analysis of gene functions[21]. GO and KEGG pathway enrichment analyses were performed using the 'clusterProfiler' package in R version 4.0.2 and P values less than 0.05 were considered statistically significant[22]. A GO network was visualized using the Metascape database (http://metascape.org) to validate our results[23].

2.6 Whole-exome sequencing for DEGs

DNA was extracted from each patient using a DNA extraction kit (Qiagen, Hilden, Germany) from whole blood. Library construction, WES, and data analysis were conducted by iGeneTech in Shanghai. Then, 200 ng of genomic DNA from each individual was sheared by Biorupter (Diagenode, Belgium) to acquire 150–200 bp fragments. The ends of DNA fragment were repaired and Illumina adapters were added (Fast Library Prep Kit, iGeneTech, Beijing, China). After sequencing libraries were constructed, whole exomes were captured using the AExome Enrichment Kit V1 (iGeneTech, Beijing, China) and libraries sequenced on an Illumina NovaSeq 6000 (Illumina, San Diego, CA) Next-Generation sequencing platform in the 150 bp PE mode. Bioinformatics analysis was performed to analyze nonsynonymous mutations including single-nucleotide polymorphisms (SNPs) and insertion-deletions (INDELs) using GATK (Genome Analysis Toolkit). All allele frequency data for DEG mutations were compared with the 1000 Genomes Project (http://www.1000genomes.org) and Exome Aggregation Consortium ExAC (http://exac.broadinstitute.org).

2.7 Protein Structure Modeling and Molecular Docking

The three-dimensional structure diagram of HNMT was generated using swiss model (http://www.swissmodel.expasy.org) and pymol v2.4 (https://pymol.org/2/). The DGIdb database (https://dgidb.org/) was used to select drugs based on the genes that served as promising targets[24].

2.6 Molecular Docking

Ligand docking of HNMT and drugs was performed with default parameters using AutoDock molecular docking software (version 4.2) and the coordinates and box size were finalized according to ligand location[25].

2.8 Statistical Analysis

DEGs were selected based on t test. The whole genome GO categories and pathogenic mutations for these DEGs were identified using Fisher's exact test. The significance level for statistical tests was set at less than 0.05 (P < 0.05).

Results

3.1 Pearson's Correlation Testing and PCA

Pearson's correlation test showed strong correlations between VTE and control samples in the GSE48000 dataset (Fig. 1A). The PCA profile for the GSE48000 data revealed that the distances between samples were small in the VTE groups and control groups, respectively (Fig. 1B). Pearson's correlation analysis also indicated strong correlations for the GSE19151 data among the samples in the VTE group and control group, respectively (Fig. 1C). The close distance in the dimension of PCA illustrated the acceptable data repeatability between samples in the VTE group and control group for the GSE19151 dataset (Fig. 1D).

3.2 Identification of DEGs in VTE

As shown in Fig. 2, a total of 232 genes were designated as DEGs in the VTE group when compared with the control group. The volcano plots in this figure present the DEGs with a cutoff criteria of having an adjusted P-value < 0.05 and |log2 fold change|>1 in the GSE48000 and GSE19151 datasets (Fig. 2A and 2B). As examples of these differences, the top 10 differentially expressed genes are reported in Table 1.
### Table 1

Top 10 most upregulated DEGs and top 10 most downregulated DEGs in VTE

| Gene name | Log2 fold change | Adjusted P-value | Gene Expression |
|-----------|------------------|------------------|----------------|
| EVI2A     | 2.15595960       | 2.46×10^{-13}    | Upregulation   |
| RPL9      | 1.89195402       | 1.48×10^{-18}    | Upregulation   |
| IFI27     | 1.87618320       | 1.88×10^{-5}     | Upregulation   |
| RPL31     | 1.77436415       | 3.44×10^{-14}    | Upregulation   |
| NDUFA4    | 1.74588189       | 7.28×10^{-17}    | Upregulation   |
| IGFBP1    | 1.74512022       | 1.33×10^{-7}     | Upregulation   |
| SNORD8    | 1.71070142       | 5.02×10^{-5}     | Upregulation   |
| RPS7      | 1.68354151       | 2.35×10^{-13}    | Upregulation   |
| XK        | 1.63316845       | 1.00×10^{-11}    | Upregulation   |
| RPS15A    | 1.51993276       | 1.20×10^{-15}    | Downregulation |
| FOS       | -1.37065523      | 1.43×10^{-7}     | Downregulation |
| JMJD1C    | -1.30709903      | 3.65×10^{-6}     | Downregulation |
| ZFP36L2   | -1.30546256      | 9.36×10^{-10}    | Downregulation |
| CD46      | -1.16530934      | 7.51×10^{-6}     | Downregulation |
| SNX10     | -1.14759082      | 2.10×10^{-5}     | Downregulation |
| UBXN4     | -1.14648762      | 1.22×10^{-5}     | Downregulation |
| DICER1    | -1.13523701      | 3.46×10^{-9}     | Downregulation |
| LSP1      | -1.10000543      | 5.98×10^{-24}    | Downregulation |
| TMEM259   | -1.05424088      | 4.66×10^{-13}    | Downregulation |
| DCK       | -1.04088634      | 1.11×10^{-7}     | Downregulation |

### 3.3 Enrichment of DEGs by GO and KEGG Analysis

Gene functional enrichment analysis was performed to analyze the biological connections of these DEGs. Results of Gene Ontology (GO) enrichment analysis revealed that RNA catabolic process, viral gene expression, SRP-dependent cotranslational protein targeting to membrane, and viral transcription were the main biological processes (BPs) (Fig. 3A), and structural constituent of ribosome, cytochrome-c oxidase activity, and heme-copper terminal oxidase activity were the most enriched categories of molecular functions for these DEGs (Fig. 3B). The variations in cell component (CC) of DEGs were enriched largely in ribosome and hemoglobin complex (Fig. 3C). KEGG pathway analysis indicated that these DEGs were mainly involved in particular pathways, such as the ribosome, Huntington disease, and oxidative phosphorylation (Fig. 3D). Metascape was used to visualize these gene enrichment analyses to verify our results from R (Fig. 3E). We found that these DEGs were enriched in amino acid deficiency, ribosomal complex, oxidative phosphorylation, rRNA transcript, and blood coagulation.

### 3.4 Identification of Probable Disease-causing DEGs by WES

WES revealed 48 mutations of DEGs in the VTE group. The mutation types and the log₂ fold change are shown in Table 2. Because nonsynonymous mutations are most likely to affect protein function, we focused on the four SNP variants corresponding with four DEGs in the VTE group. These were HNMT (chr2: 138759649 C > T, rs11558538, adjusted P-value = 1.2 × 10^{-9}), POLL (chr10: 103340056 G > A, rs3730477, adjusted P-value = 5.12 × 10^{-4}), ZNF292 (chr6: 87925827 A > G, rs9362415, adjusted P-value = 2.95 × 10^{-8}), and DPCD (chr10: 103361088 C > T, rs7874, adjusted P-value = 4.36 × 10^{-5}). The adjusted P-value of HNMT was the lowest in this study. Functional analysis showed that most disease-causing DEGs were involved in anemia, sickle cell, pulmonary thromboembolisms, heparin-induced thrombocytopenia, thrombophilia, and so on, as shown by Metascape functional analysis (Fig. 4). As summarized in Table 3, we found that HNMT was expressed in heparin-induced thrombocytopenia, dermatitis, and atopic cases, conditions that may have strong impacts on VTE.
| Gene      | Chr | SNP        | Mut_type | Mutation | Location | Position | Func.ref | Exonicfunc.ref | 1 KG         | ExAC_AL    |
|-----------|-----|------------|----------|----------|----------|----------|----------|----------------|--------------|------------|
| HNMT      | ch2 | rs11558538 | SNP      | C/T      | ontarget | 138759649| exonic   | nonsynonymous SNV | 0.0595048   | 0.1017     |
| USP14     | chr18 | rs56806027 | SNP      | T/A      | flank150 | 204815   | intronic |               | 0.68111     |            |
| SERPING1  | chr18 | rs17072114 | SNP      | T/C      | flank150 | 61584817 | intronic |               | 0.171326    |            |
| UBXN4     | chr2 | rs80198954 | SNP      | C/A      | ontarget | 136511842| exonic   | synonymous SNV  | 0.0373403   | 0.019      |
| HNMT      | ch2 | rs74265494 | SNP      | G/A      | ontarget | 136511886| intronic |               | 0.0397364   | 0.0191     |
| USP14     | chr18 | rs372143998| InDel    | GT/G     | flank150 | 136527319| intronic |               | 0.2468      |            |
| SERPING1  | chr18 | rs200613240| InDel    | T/TA     | flank150 | 136529987| intronic |               |              |            |
| USP14     | chr18 | rs78878675 | SNP      | G/A      | ontarget | 136530157| intronic |               | 0.0397364   | 0.019      |
| USP14     | chr18 | rs78339162 | SNP      | A/G      | flank150 | 136539993| intronic |               | 0.0397364   |            |
| ZNF271P   | chr18 | rs12965288 | SNP      | C/A      | ontarget | 32888090 | ncRNA_exonic |               | 0.249401    |            |
| SELP      | chr1  | rs34841246 | SNP      | C/A      | ontarget | 32888546 | ncRNA_exonic |               | 0.220847    |            |
| SELP      | chr1  | rs86800287 | InDel    | AT/A     | ontarget | 176913216| intronic |               | 0.431909    |            |
| POLL      | chr10 | rs1055364  | SNP      | C/A      | ontarget | 103338730| UTR3     |               | 0.163339    |            |
| POLL      | chr10 | rs1055362  | SNP      | A/G      | ontarget | 103338733| UTR3     |               | 0.163339    |            |
| SELP      | chr1  | rs3730477  | SNP      | G/A      | ontarget | 13340056 | exonic   | nonsynonymous SNV | 0.0998403   | 0.1678     |
| SELP      | chr1  | rs3730476  | SNP      | A/G      | ontarget | 13340144 | exonic   | nonsynonymous SNV | 0.163139    | 0.2306     |
| SELP      | chr1  | rs3730475  | SNP      | A/G      | ontarget | 13340179 | intronic |               | 0.163339    | 0.232      |
| SELP      | chr1  | rs3730474  | SNP      | T/C      | flank150 | 13340235 | intronic |               | 0.163139    |            |
| SELP      | chr1  | rs3730465  | SNP      | A/G      | flank150 | 13343533 | intronic |               | 0.164936    |            |
| SELP      | chr1  | rs3730462  | InDel    | CTGTTG/C | ontarget | 13345941 | intronic |               | 0.162939    | 0.2281     |
| ASTN1     | chr1 | rs86800287 | InDel    | AT/A     | ontarget | 176913216| intronic |               | 0.431909    |            |
| UGGT1     | chr2 | rs35069237 | InDel    | GT/G     | ontarget | 128949841| UTR3     |               | 0.338858    |            |
| NFATC1    | chr18 | rs8096658  | SNP      | C/G      | flank150 | 77156537 | intronic |               | 0.328275    |            |
| MTHFR     | chr1  | rs11121832 | SNP      | T/C      | flank150 | 11860120 | intronic |               | 0.759185    |            |
| FCR1B     | chr1  | rs827371   | SNP      | T/C      | flank150 | 12093566 | intronic |               |              |            |
| MAP3K8    | chr10 | rs3034     | SNP      | G/A      | flank150 | 30749895 | UTR3     |               | 0.882987    |            |
| MGMT      | chr10 | rs278288   | SNP      | T/G      | flank150 | 13126532 | upstream |               | 1           |            |
| MGMT      | chr10 | rs55973415 | SNP      | G/A      | flank150 | 131557750| intronic |               | 0.2143      |            |
| ZNF2929   | chr6  | rs563101504| InDel    | GACACAC/G| ontarget | 87925827 | intronic |               |              |            |
Table 3

Functional enrichment analysis of disease-causing DEGs using Metascape

| GO          | Description                      | Log10 P Count Genes |
|-------------|----------------------------------|---------------------|
| C0002895    | Anemia Sickle cell               | −5.9 6 FOS|GYPA|MTHFR|SELP|AHSP|UGGT1 |
| C0524702    | Pulmonary thromboembolisms       | −5.5 3 MTHFR|SELP|USP14 |
| C0002875    | Cooley’s anemia                  | −5.2 4 GYPA|MTHFR|AHSP|UGGT1 |
| C0272285    | Heparin-induced thrombocytopenia | −5.1 3 FCGR1B|HNMT|SELP |
| C0004135    | Ataxia telangiectasia            | −4.8 5 FOS|GYPA|MGMT|MTHFR|NFATC1 |
| C0268138    | Xeroderma pigmentosum            | −4.5 3 MGMT|MTHFR|UGGT1 |
| C0011615    | Dermatitis, Atopic               | −4.5 6 ASTN1|FOS|HNMT|MGMT|MTHFR|SELP |
| C0008626    | Congenital-chromosomal disease   | −4.5 6 FCGR1B|FOS|MGMT|MTHFR|NFATC1|SELP |
| C0278996    | Malignant chromosomal disease    | −4.4 6 FCGR1B|FOS|MGMT|MTHFR|NFATC1|SELP |
| C3887461    | Head and neck carcinoma          | −4.4 6 FCGR1B|FOS|MGMT|MTHFR|NFATC1|SELP |
| C0014170    | Endometrial neoplasms            | −4.2 4 MAP3K8|FOS|MGMT|MTHFR |
| C0947751    | Vascular inflammations           | −3.9 4 SERPING1|MAP3K8|FOS|SELP |
| C1704436    | Peripheral arterial diseases     | −3.9 4 MAP3K8|FOS|MTHFR|SELP |
| C0011884    | Diabetic retinopathy             | −3.7 5 SERPING1|MAP3K8|FOS|SELP|MTHFR |
| C0024814    | Marinesco-Sjogren syndrome       | −3.7 3 MAP3K8|MGMT|MTHFR |
| C0333516    | Tumor necrosis                   | −3.7 4 FOS|MGMT|MTHFR|SELP |
| C3469521    | Fanconi anemia                   | −3.6 4 GYPA|MGMT|MTHFR|SELP |
| C4551686    | Malignant neoplasm of soft tissue| −3.6 5 MAP3K8|FOS|MGMT|MTHFR|NFATC1 |
| C0015625    | Fanconi anemia                   | −3.5 4 GYPA|MGMT|MTHFR|SELP |
| C0398623    | Thrombophilia                    | −3.5 3 SERPING1|MTHFR|SELP |

3.5 Protein Structure and Characterization of Missense HNMT Mutations
The Thr105ile (rs11558538) polymorphism in the HNMT gene (ch2: 138759649 C > T, rs11558538, adjusted P-value = 1.2 x 10^-9) was the biggest difference identified in a gene, and should result in nonsense-mediated decay and loss function of this protein. The 3D location is shown in Fig. 5A and Fig. 5B. The variant was positioned in the α-helix, where its side chain hydroxyl formed two hydrogen bonds with a backbone oxygen after mutation, causing a marked decrease in the levels of both HNMT enzymatic activity and immunoreactive protein[26, 27]. HNMT is an enzyme that has been implicated in neurotransmission by inactivating histamine in the central nervous system[28]. However, histamine increases vascular permeability through the histamine H1 receptor to activate nerve endings, relaxing vascular smooth muscle[29].

3.6 Molecular Docking

The drug–target interactions for HNMT were predicted using DGIdb, and the results are presented in Table 4, providing a theoretical therapeutic mechanism for VTE prevention. Six drugs targeting HNMT have been predicted for VTE, including Amodiaquine, Harmaline, Aspirin, Metoprine, Dabigatran, and Diphenhydramine.

Molecular docking analysis was attempted to assess the potential noncovalent binding of HNMT with these small molecules drugs. In general, a lower binding energy indicated a stronger binding between HNMT and a compound. Table 4 shows the six drugs that best interfaced with HNMT. To visualize these docking results, the 3D interaction diagrams of HNMT and their corresponding best-matched drugs were drawn, as shown in Fig. 6. These drugs, such as Aspirin and Dabigatran, have been utilized to recanalize vessels and prevent thrombi growth clinically in VTE patients[30–32]. The 3D interaction diagram of Aspirin at the active site of HNMT revealed that this interaction was stable through forming hydrogen bonds with the key residues Lys55 and Lys135 (Fig. 6B). Aspirin is commonly administered to inhibit platelet aggregation and prevent thrombus formation[33]. Additionally, three hydrogen bonds formed with residues Phe9, Tyr15, and Ser91 contributed to stabilizing the interaction between Dabigatran and HNMT. Dabigatran has been approved for use in orthopedic surgery, venous thromboprophylaxis, acute VTE treatment, and extended prevention of recurrent VTE[34]. Our data had shown that HNMT can potentially become a new target for VTE treatment.

| Gene  | Drug      | Sources     | PMIDs       | Binding Energy (kcal. mol⁻¹) | Binding Residues |
|-------|-----------|-------------|-------------|-----------------------------|-----------------|
| HNMT  | Amodiaquine | DrugBank;   | 6789797     | -2.48                       | GLN197; LYS55; ASP203 |
| HNMT  | Aspirin   | DrugBank    | 19178400    | -3.24                       | LYS55; LYS135 |
| HNMT  | Harmaline | PharmGKB    | 1530666     | -5.07                       | GLU28          |
| HNMT  | Metoprine | TTD; DTC    | 10592235    | -3.65                       | GLN192; ASP194 |
| HNMT  | Dabigatran| TTD         | -           | -4                          | PHE9;Tyr15;SER91|
| HNMT  | Diphenhydramine | DrugBank; | 23896426    | -4.69                       | ASP194        |

**Table 4**

Candidate drugs targeted HNMT

**Discussion**

In the present study, transcriptomics and proteomics technology were used to explore the potential pathways and pathogenic mutations of VTE occurrence. High-throughput pharmacology and molecular docking may allow for the investigation of novel biomarkers for detecting this complex diseases[35].

We first studied VTE by downloading transcriptome-wide expression data from the GEO database and a total of 232 DEGs were identified. Results of GO analysis of the gene enrichment in these datasets showed that the VTE-associated DEGs were significantly enriched in RNA catabolic process. Wang, HX found previously that RPL9, RPL35, and RPS7 were hub genes in the PPI network of GSE13985, which was used to identify potential markers of atherosclerosis development[36]. Interestingly, a study from Mi, YH reported that the major risk factors for atherothrombotic disease were also significantly associated with VTE, which contributes to the explanation of why atherosclerosis is an independent risk factor for VTE[37]. KEGG pathway analysis revealed that the DEGs related to VTE were mainly enriched in the ribosome pathway. Recent evidence has suggested that the ribosome affects the translation of platelets, platelet aggregation, and resultant thrombus formation[38, 39]. It may be reasonable for us to then hypothesize that ribosomal proteins might have crucial functions in VTE development. However, there is no direct evidence that RNA catabolic processes and the ribosome pathway are directly involved in VTE.

To verify the above results, whole-exome sequencing (WES) was performed to detect the pathogenic mutations of DEGs. POLL encodes the novel DNA polymerase lambda and the mutation of POLL (rs3730477) encoded R438W Pol λ leading to genomic instability and mutagenesis in cells[40]. DPCD (rs7874) is named from an uncharacterized genomic region surrounding POLL. DPCD is a novel gene in primary ciliary dyskinesia and severe cases can induce pulmonary embolism[41]. ZNF292 (rs9362415) acts as a transcription factor and plays an important role in DNA recognition and apoptosis regulation[42]. However, little is known about the role of DNA related functions in VTE. Notably, we discovered that HNMT (rs11558538) polymorphism was the greatest differentially expressed factor in this study. As is well-known, HNMT is implicated in neurotransmission by inactivating histamine, and histamine has been argued to relax vascular smooth muscle[29]. From a protein structure analysis, we found that the Thr105ile mutation results in hydrogen bonds in the structure...
of HNMT being disrupted, resulting in loss-of-function mutations[43]. The 3D structure diagram of HNMT showed quite different in protein conformations between the Thr105 and Ile105 variants. Furthermore, a list of drugs targeting HNMT with potential therapeutic efficacy against VTE were selected, most notably Aspirin and Dabigatran. As a consequence, we inferred that the high expression of mutated HNMT acted on vascular smooth muscle and may further promote vasoconstriction and thrombosis through RNA catabolic process and the ribosome. However, the mechanisms of these genes and drugs in VTE are still unclear. In future work, we hope to verify our conclusions experimentally to elucidate the effects of Thr105Ile (rs11558538) in HNMT for VTE.

Conclusions
The current study was designed to investigate potential DEGs and genetic variant of DEGs in VTE (Fig. 7). RNA catabolic process and ribosomes pathway identified through integrated bioinformatic analysis of GSE48000 and GSE19151 datasets may play crucial roles in the development of VTE. Additionally, Thr105Ile (rs11558538) polymorphism in HNMT was identified as a risk factor for VTE in the mechanism of damage and dysfunctional to the vascular endothelial cell and vascular smooth muscle. In the future, more in-depth investigation about the mechanism of these candidate genes is warranted for VTE.

Abbreviations
VTE: venous thromboembolism; DEGs: Differentially expressed genes; GEO: Gene Expression Omnibus; DEGs: Differentially expressed genes; WES: Whole-exome Sequencing; KEGG: Kyoto Encyclopedia of Genes and Genomes; WES: whole-exome sequencing; HNMT: Histamine N-Methyltransferase; PCA: Principal component analysis; BPs: Biological Processes; MFs: Molecular Functions; CCs: cellular components; SNPs: Single-nucleotide Polymorphisms; INDELs: insertion-deletions.

Declarations
Acknowledgments
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Authors’ contributions
JD were responsible for acquisition of data, analysis and interpretation of data. YZ and YC critically revised the work. YC were responsible for the conception and design of the study and final approval of the version to be submitted. The manuscript was written by JD. All authors read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Ethics approval and consent to participate
All patients participating in this study were informed, signed informed consents and voluntarily participated, and this study was approved by the ethics committee of the Shanghai Institute of Planned Parenthood Research and ethical committees of participating hospitals.

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no competing interests.

Author details
NHC Key Lab of Reproduction Regulation (Shanghai Institute for Biomedical and Pharmaceutical Technologies), Fudan University, Shanghai, 200032, China.

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Figures

Figure 1

Pearson’s correlation test and PCA on GSE48000 and GSE19151 data. (A) Pearson’s correlation test for GSE48000. The color reflects the intensity of the correlation. (B) PCA of samples from the GSE48000 dataset. Principal component 1 values for VTE samples are plotted on the X axis, and principal component 2 values for control samples are plotted on the Y axis. The closer the distance between the two groups, the smaller the differences between the two groups. (C) Pearson’s correlation test for GSE19151. The color reflects the intensity of the correlation. (D) PCA of samples from the GSE19151 dataset.
Figure 2

Volcano plot of VTE DEGs. Red, upregulated DEGs with log2FC>1 and adjusted P-value < 0.05. Green, downregulated DEGs with log2FC<1 and adjusted P-value < 0.05. (A) Volcano plot illustrating the DEGs of the GSE48000 dataset. (B) Volcano plot illustrating the DEGs of the GSE19151 dataset.

Figure 3
Bubble map for GO and KEGG pathway analyses of DEGs. P-value < 0.05 was considered statistically significant. (A) Biological processes, (B) molecular function, (C) cell component, (D) KEGG pathways, (E) heatmap of enriched terms across DEGs, colored by P-values, via Metascape.

Figure 4
Heatmap of enriched terms across disease-causing DEGs, via Metascape.
Figure 5

3D structure of HNMT. (A) Diagram of the HNMT structure depicting the location of Thr105. (B) Diagram of the HNMT structure depicting the location of the Ile105 mutation.
Figure 6

The 3D structure diagram of the drugs and HNMT with the active sites. (A) Structures of the pocket of binding between HNMT and Amodique. (B) Structures of the binding between HNMT and Aspirin. (C) Structures of the binding between HNMT and Harmaline. (D) Structures of the binding between HNMT and Metropine. (E) Structures of the binding between HNMT and Dabigatran. (F) Structures of the binding between HNMT and Diphenhydramine.

Figure 7

Summary diagram regarding the genetic variant of DEGs in VTE.

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