Gene microarray analyses for potential biomarkers of single and recurrent venous thromboembolism

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Abstract. Venous thromboembolism is a major cause of morbidity and mortality with a high recurrence rate. The present study aimed to explore the molecular mechanisms and potential biomarkers of single venous thromboembolism (SVTE) and recurrent venous thromboembolism (RVTE). The microarray dataset GSE19151 was downloaded from Gene Expression Omnibus, which contained data from whole blood samples from 63 healthy controls, 32 SVTE and 38 RVTE patients. Differentially expressed genes (DEGs) in the SVTE and RVTE groups compared with those in the controls were identified using the *t*-test, followed by clustering analysis of DEGs and samples. Functional and pathway enrichment analyses were performed for DEGs in patients with RVTE and SVTE, as well as specific DEGs in patients with RVTE. The identified 42 DEGs in RVTE were mainly enriched in biological processes of cellular protein metabolism, gene expression and translational elongation as well as in pathways associated with ribosomes, Parkinson's disease and oxidative phosphorylation. In SVTE, 20 DEGs were identified, which were mainly involved in biological processes of biopolymer biosynthesis, translational elongation and cellular protein metabolism as well as pathways associated with ribosomes and cardiac muscle contraction. In RVTE, 22 specific DEGs were mainly involved in translational elongation, negative regulation of the force of heart contraction by chemical signals, cell proliferation, ribosomal pathways and protein export. The identified DEGs of SVTE, including COX7C and UQCRQ, may be potential biomarkers for SVTE, and the specific DEGs of RVTE, including ADRBK1, NDUFA5 and ATP5O, may be potential biomarkers for RVTE.

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Introduction

Venous thromboembolism (VTE), comprising deep venous thrombosis and pulmonary embolism, is a frequent disease with high morbidity and mortality, which affects 1-2 per 1,000 individuals (1-3). Furthermore, VTE is associated with a significant rate of recurrence in as many as 30% of VTE patients after termination of the standard course of anti-coagulant therapy (4-6). Therefore, it is desired to explore the molecular mechanisms and potential biomarkers that enable clinicians to identify patients at a risk of single VTE (SVTE) or recurrent VTE (RVTE) for prompt clinical diagnosis and early prevention (7).

Kyrle *et al* (8) reported that patients with a high level of factor VIII have an increased risk of RVTE. A study by Comp and Esmon (9) suggested that the levels of protein S may be used in the evaluation of RVTE. Various established and novel biomarkers, including D-dimer, E-selectin, P-selectin, thrombin, inflammatory markers and C-reactive protein, have been investigated for their predictive value in SVTE and RVTE (10-13). However, only a small number of biomarkers, such as D-dimer, associated with a first or recurrent event of VTE were highlighted by these studies, while novel and promising biomarkers, including P-selectin and inflammatory cytokines, are still controversial (1). A study by Lewis *et al* (14) performed a pathway enrichment analysis of differentially expressed genes (DEGs) in samples from patients with SVTE and samples from patients with RVTE and found that insulin-like growth factor receptor 1 and Akt pathways may be useful for distinguishing patients with SVTE from those with RVTE.

The present study identified DEGs in RVTE and SVTE, as well as specific DEGs in RVTE. Functional and pathway enrichment analyses for these DEGs were performed to explore the molecular mechanisms and potential biomarkers of SVTE and RVTE in order to facilitate the diagnosis and clinical therapy management of VTE.

Materials and methods

Affymetrix microarray data. The gene expression profile dataset GSE19151 was obtained from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), which was deposited by Lewis *et al* (14). Microarray data from 133 whole

Table I. Specific differentially expressed genes of recurrent VTE.

Probe ID	Mean of recurrent VTE (R)	Mean of healthy (N)	Log2 (R/N)	P-value	Gene symbol	ENTREZ GENE_ID
201304_at	6.22	4.64	1.58	$2.55x10^{-5}$	NDUFA5	4698
200679_x_at	9.86	8.67	1.19	$4.85x10^{-10}$	HMGB1	3146
201317_s_at	9.49	8.16	1.33	$2.18x10^{-7}$	PSMA2	5683
200977_s_at	9.41	8.16	1.24	$1.39x10^{-5}$	TAX1BP1	8887
201152_s_at	11.86	10.69	1.17	$1.17x10^{-6}$	MBNL1	4154
201163_s_at	8.79	7.54	1.24	$1.19x10^{-12}$	IGFBP7	3490
201593_s_at	9.38	8.26	1.12	$3.27x10^{-10}$	ZC3H15	55854
201401_s_at	8.57	9.69	-1.13	$1.39x10^{-11}$	ADRBK1	156
200834_s_at	12.54	11.54	1.01	$1.97x10^{-11}$	RPS21	6227
201367_s_at	8.91	10.14	-1.24	$2.12x10^{-7}$	ZFP36L2	678
201392_s_at	9.09	10.22	-1.13	$5.39x10^{-10}$	IGF2R	3482
201200_at	10.98	9.82	1.16	$1.72x10^{-11}$	CREG1	8804
200012_x_at	13.08	11.66	1.42	$1.40x10^{-6}$	RPL21	6144
200061_s_at	11.34	9.94	1.40	$4.02x10^{-9}$	RPS24	6229
200741_s_at	13.56	12.34	1.22	$1.28x10^{-11}$	RPS27	6232
200880_at	8.92	7.73	1.19	$1.17x10^{-7}$	DNAJA1	3301
201012_at	10.84	9.29	1.55	$2.33x10^{-9}$	ANXA1	301
201273_s_at	10.76	9.69	1.07	0.000332	SRP9	6726
201332_s_at	8.22	9.35	-1.13	$1.44x10^{-13}$	STAT6	6778
201406_at	10.35	9.03	1.32	$6.59x10^{-5}$	RPL36A	6173
201023_at	9.93	8.76	1.17	0.000185	TAF7	6879
200818_at	10.17	9.16	1.02	$5.51x10^{-5}$	ATP5O	539

VTE, venous thromboembolism; R, recurrent; N, normal.

blood specimens were available, including 63 samples from healthy controls, 32 samples from patients with SVTE (sampled at <three years since their most recent VTE) and 38 samples from subjects with recurrent venous thromboembolism (RVTE) on warfarin. The platform was GPL571 (HG-U133A_2) Affymetrix Human Genome U133A 2.0 Array (Affymetrix, Inc., Santa Clara, CA, USA).

DEG analysis and gene clustering analysis. For the GSE19151 dataset, the Bioconductor software package in R (http://www.bioconductor.org/; version 3.1) was implemented to analyze the 133 blood gene chips (15). Background correction and quartile data normalization were performed using the robust multiarray average algorithm with defaulted parameters in the Affy package (http://www.bioconductor.org/packages/release/bioc/html/affy.html; version 1.46.1) (16). The t-test was used to identify DEGs using the Simpleaffy package (http://www.bioconductor.org/packages/release/bioc/html/simpleaffy.html; version 2.44.0) (17). The DEGs were selected with the cutoff criteria of P<0.05 and |log(fold change)|>1. Hierarchical clustering analysis of the DEGs was performed using the Hclust command in R and the default complete linkage method (18).

Gene ontology (GO) functional and pathway enrichment analyses. The Integrated GEne and PROtein annotation Server (IGEPROS; http://www.biosino.org/iGepros/index.jsp) (19) bioinformatics resources consist of an integrated biological knowledge base and analytic tools aimed at systematically extracting biological information from large gene or protein lists. IGEPROS was used to perform the GO (http://www.geneontology.org/) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/pathway.html) pathway enrichment analyses for the identified DEGs with the threshold of P<0.05. The pathview package in R was utilized to depict the KEGG pathway (20).

Results

DEG selection and hierarchical clustering analysis. A total of 42 DEGs were identified between RVTE and normal whole-blood specimens (RVTE vs. control), including 35 up- and 7 downregulated genes. Subsequently, 20 DEGs between SVTE and normal whole-blood specimens (SVTE vs. control) were identified, including 17 up- and 3 downregulated genes. A total of 22 non-overlapping genes were selected as specific DEGs of RVTE, including 18 up- and 4 downregulated genes (Table I). Hierarchical clustering analysis was performed for the 42 DEGs from the 133 whole blood specimens of patients with SVTE, patients with RVTE and healthy controls. The result of this clustering analysis suggested that these DEGs may have important roles in VTE (Fig. 1).

GO enrichment analysis of DEGs. GO enrichment analysis was performed for 42 DEGs in RVTE, 20 DEGs in SVTE

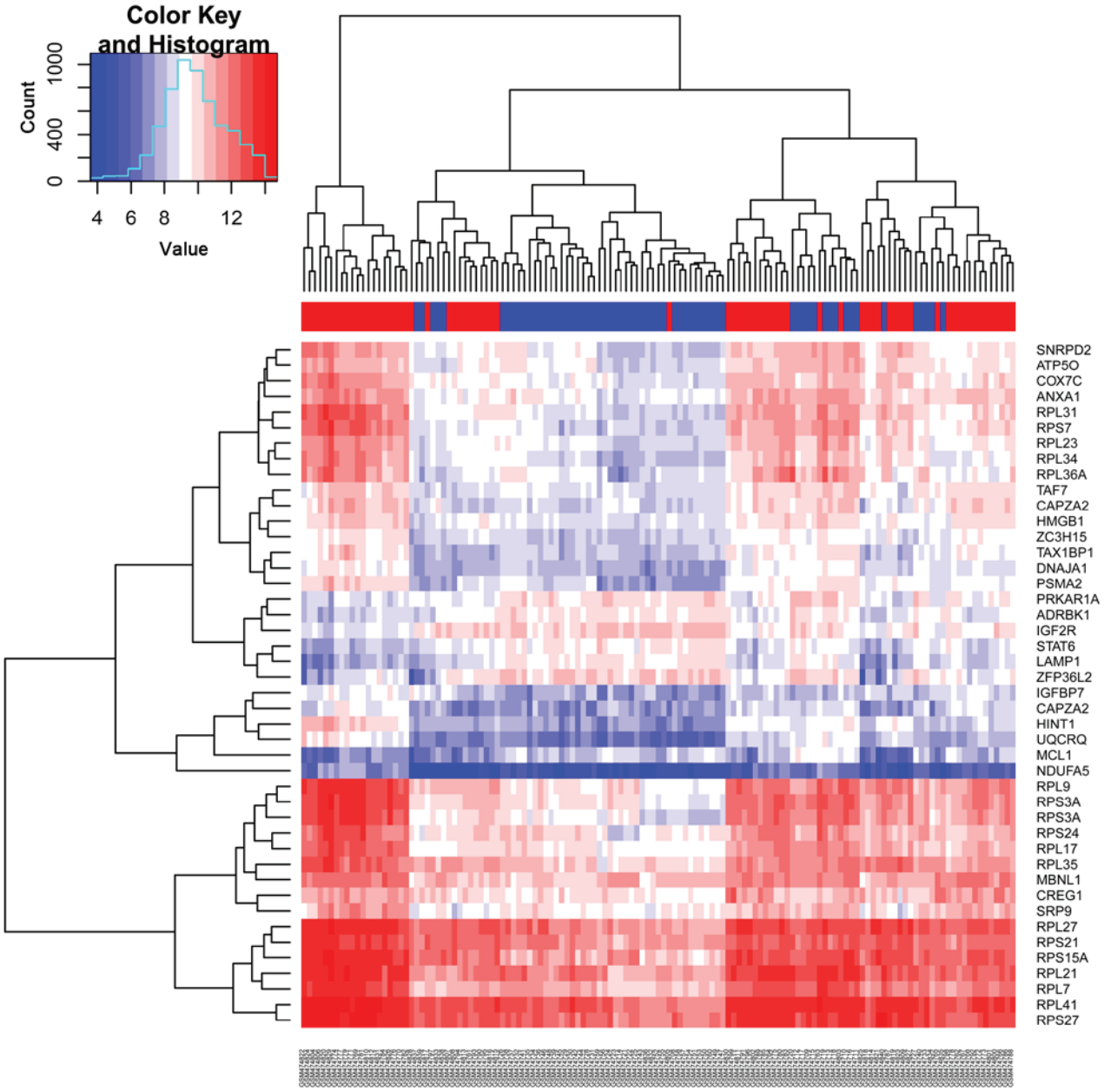

Figure 1. Clustering dendrogram constructed using the Hclust clustering algorithm. Horizontal red and blue bars represent the patient specimens (single or recurrent venous thromboembolism) and healthy specimens, respectively, and vertical axes represent differentially expressed genes. Expression levels are represented by a color key in which bright red represents the lowest levels and bright yellow represents the highest levels, and less saturated shades represent intermediate levels of expression.

and 22 specific DEGs of RVTE. In RVTE, most enriched GO terms of DEGs in biological processes were associated with biopolymer biosynthesis, including cellular protein metabolism ($P=1.12x10^{-8}$), gene expression ($P=1.36x10^{-6}$), translational elongation ($P=9.65x10^{-27}$) and cellular macromolecular biosynthetic processes ($P=8.78x10^{-6}$). In the cellular component category, enriched GO terms were mainly associated with the ribosomal sub-unit ($P=2.03x10^{-18}$), cytosol ($P=2.43x10^{-12}$) and macromolecular complexes ($P=8.61x10^{-11}$). In the molecular function category, GO terms enriched for DEGs in RVTE included structural constituents of ribosomes ($P=9.13x10^{-24}$), insulin-like growth factor binding ($P=0.002$) and beta-adrenergic receptor kinase activity ($P=0.005$) (Tables II-IV).

In SVTE, the most significantly enriched GO terms for DEGs in biological processes included biopolymer biosynthesis ($P=2.35x10^{-5}$), translational elongation ($P=2.35x10^{-5}$), cellular protein metabolism ($P=1.24x10^{-7}$) and rRNA processing ($P=0.006$). The predominantly enriched GO terms of the cellular component category were the cytosol ($P=1.68x10^{-11}$), the cytosolic large ribosomal sub-unit ($P=8.62x10^{-10}$) and macromolecular complexes ($P=4.86x10^{-9}$). The main GO terms of DEGs in SVTE in the molecular function category were structural constituents of ribosomes ($P=4.69x10^{-20}$), RNA binding ($P=2.79x10^{-5}$) and protein channel activity ($P=0.001$). Among the DEGs in SVTE, 12 genes, including RPS3A and RPS7, were involved in translational elongation, 2 genes (UQCRQ and COX7C) were involved in oxidoreductase

Table II. GO functional enrichment analysis of DEGs in patients with RVTE and SVTE, and top 10 specific DEGs of RVTE associated with biological processes.

Category/GOBPID	P-value	Count (n)	Size (n)	Term
RVTE				
GO:0006414	$9.65x10^{-27}$	17	104	Translational elongation
GO:0044267	$1.12x10^{-8}$	22	2361	Cellular protein metabolic process
GO:0010467	$1.36x10^{-6}$	25	3592	Gene expression
GO:0043284	$2.88x10^{-6}$	23	3183	Biopolymer biosynthetic process
GO:0034645	$8.78x10^{-6}$	23	3386	Cellular macromolecule biosynthetic process
GO:0044237	$1.49x10^{-5}$	34	7160	Cellular metabolic process
GO:0009058	0.000114	24	4223	Biosynthetic process
GO:0042274	0.000360	2	10	Ribosomal small subunit biogenesis
GO:0044238	0.001536	31	7368	Primary metabolic process
GO:0006364	0.002059	3	88	rRNA processing
SVTE				
GO:0006414	$7.47x10^{-22}$	12	106	Translational elongation
GO:0044267	$1.24x10^{-7}$	14	2499	Cellular protein metabolic process
GO:0043284	$2.35x10^{-5}$	13	3183	Biopolymer biosynthetic process
GO:0034645	$4.76x10^{-5}$	13	3386	Cellular macromolecule biosynthetic process
GO:0010467	$9.42x10^{-5}$	11	3052	Gene expression
GO:0044237	0.000402	17	7160	Cellular metabolic process
GO:0009058	0.000549	13	4223	Biosynthetic process
GO:0006610	0.004003	1	3	Ribosomal protein import into nucleus
GO:0006364	0.006046	2	88	rRNA processing
GO:0042273	0.010641	1	8	Ribosomal large subunit biogenesis
Non-overlap				
GO:0006414	$3.52x10^{-7}$	5	104	Translational elongation
GO:0000296	0.001547	1	1	Spermine transport
GO:0003108	0.001547	1	1	Negative regulation of the force of heart contraction by chemical signal
GO:0008283	0.002157	6	917	Cell proliferation
GO:0044267	0.002941	9	2361	Cellular protein metabolic process
GO:0006916	0.002998	3	190	Anti-apoptosis
GO:0048523	0.003033	7	1334	Negative regulation of cellular process
GO:0045988	0.003092	1	2	Negative regulation of striated muscle contraction
GO:0045900	0.003092	1	2	Negative regulation of translational elongation
GO:0048295	0.003092	1	2	Positive regulation of isotype switching to IgE isotypes

GO, gene ontology; DEG, differentially expressed genes; RVTE, recurrent venous thromboembolism; SVTE, single venous thromboembolism; non-overlap, non-overlapping DEGs as specific DEGs of RVTE; GOBPID, gene ontology biological process identification number; IgE, immunoglobulin E; Count, DEG numbers enriched in the specific GO term; Size, total gene numbers in the GO database.

activity and 2 genes (HINT1 and LAMP1) were associated with proteolysis (Tables II-IV).

The GO terms for biological processes of the 22 specific DEGs of RVTE were mainly translational elongation ($P=3.52x10^{-7}$), negative regulation of the force of heart contraction by chemical signals (P=0.001) and cell proliferation (P=0.002). In the cellular component category, enriched GO terms were mainly associated with the cytosolic small ribosomal sub-unit ($P=1.56x10^{-5}$), ribosomal sub-unit ($P=1.84x10^{-5}$) and macromolecular complexes (P=0.0008). The specific GO terms of the 22 specific DEGs in RVTE in the molecular function category were mainly associated with structural constituents of ribosomes ($P=2.55x10^{-6}$), insulin-like growth factor binding (P=0.0006), beta-adrenergic receptor kinase activity (P=0.003) and phospholipase A2 inhibitor activity (P=0.004). Two genes (IGF2R and IGFBP7) were shown to be involved in anti-apoptotic signaling, ten genes, including RPL21 and RPS21, were involved in translational elongation and six genes, including NDUFA5, ATP5O and ADRBK1, were associated with the force of heart contraction (Tables II-IV).

Table III. GO functional enrichment analysis of DEGs in patients with RVTE and SVTE, and top 10 specific DEGs of RVTE associated with cellular components.

Category/GOCCID	P-value	Count (n)	Size (n)	Term
RVTE				
GO:0033279	$2.03x10^{-18}$	13	118	Ribosomal sub-unit
GO:0005829	$2.43x10^{-12}$	19	1039	Cytosol
GO:0032991	$8.61x10^{-11}$	25	2482	Macromolecular complex
GO:0022627	$4.11x10^{-10}$	6	36	Cytosolic small ribosomal sub-unit
GO:0022625	$5.80x10^{-10}$	6	38	Cytosolic large ribosomal sub-unit
GO:0043228	$2.18x10^{-9}$	23	2388	Non-membrane-bound organelle
GO:0005737	$1.12x10^{-7}$	35	6946	Cytoplasm
GO:0005840	$1.16x10^{-5}$	4	78	Ribosome
GO:0005622	$5.89x10^{-5}$	39	10646	Intracellular
GO:0005730	0.001773	7	680	Nucleolus
SVTE				
GO:0005829	$1.68x10^{-11}$	13	1039	Cytosol
GO:0022625	$8.62x10^{-10}$	5	38	Cytosolic large ribosomal sub-unit
GO:0032991	$4.86x10^{-9}$	15	2482	Macromolecular complex
GO:0043228	$4.10x10^{-8}$	14	2388	Non-membrane-bound organelle
GO:0044422	$2.04x10^{-6}$	15	3829	Organelle part
GO:0022627	$1.16x10^{-5}$	3	36	Cytosolic small ribosomal sub-unit
GO:0033279	$1.53x10^{-5}$	3	57	Ribosomal sub-unit
GO:0005840	$1.82x10^{-5}$	3	78	Ribosome
GO:0043229	$7.82x10^{-5}$	19	8645	Intracellular organelle
GO:0005622	0.000279	20	10646	Intracellular
Non-overlap				
GO:0022627	$1.56x10^{-5}$	3	36	Cytosolic small ribosomal sub-unit
GO:0033279	$1.84x10^{-5}$	4	118	Ribosomal sub-unit
GO:0032991	0.000837	10	2482	Macromolecular complex
GO:0005737	0.001286	17	6946	Cytoplasm
GO:0008024	0.001373	1	1	Positive transcription elongation factor complex b
GO:0005829	0.002221	6	1039	Cytosol
GO:0005785	0.002744	1	2	Signal recognition particle receptor complex
GO:0043232	0.002811	9	2388	Intracellular non-membrane-bound organelle
GO:0005641	0.006847	1	5	Nuclear envelope lumen
GO:0044422	0.006937	11	3829	Organelle part

GO, gene ontology; DEG, differentially expressed genes; RVTE, recurrent venous thromboembolism; SVTE, single venous thromboembolism; Non-overlap, non-overlapping DEGs as specific DEGs of RVTE; GOCCID, gene ontology cellular component identification number; Count, DEG numbers enriched in the specific GO term; Size, total gene numbers in the GO database.

Pathway enrichment analysis of DEGs. The DEGs identified in the present study were enriched in nine pathways (Table V). The RVTE DEGs were mainly enriched in ribosomal pathways ($P=1.59x10^{-23}$), Parkinson's disease (P=0.007) and oxidative phosphorylation (P=0.008). The SVTE DEGs were enriched in ribosomal pathways ($P=4.58x10^{-19}$) and cardiac muscle contraction (P=0.025). The non-overlapping DEGs were enriched in ribosomal pathways ($P=2.25x10^{-6}$) and protein export (P=0.03).

Metabolic pathway visualization analysis of specific DEGs of RVTE. The metabolic pathways were visualized in schemes depicting the ribosomal pathway (Fig. 2) and the protein export pathway (Fig. 3; http://www.genome.jp/kegg/tool/map_pathway2.html). In the ribosomal pathway, RPL21, RPS21, RPS24 and RPS27 were upregulated and in the protein export pathway, SRP9 was upregulated. The results suggested that these genes may be critical in RVTE and that certain variations in the expression of these genes may lead to an increased risk of recurrence.

Discussion

In recent years, the application of adequate thromboprophylaxis has led to significant progress in the management of

Table IV. GO functional enrichment analysis of DEGs in patients with RVTE and SVTE, and top 10 specific DEGs of RVTE associated with molecular function.

Category/GOMFID	P-value	Count (n)	Size (n)	Term
RVTE				
GO:0003735	$9.13x10^{-24}$	17	158	Structural constituent of ribosome
GO:0003723	$2.87x10^{-6}$	10	646	RNA binding
GO:0005520	0.002031	2	25	Insulin-like growth factor binding
GO:0015266	0.002686	1	1	Protein channel activity
GO:0015077	0.002942	3	107	Monovalent inorganic cation transmembrane transporter activity
GO:0047696	0.005365	1	2	Beta-adrenergic receptor kinase activity
GO:0005010	0.008037	1	3	Insulin-like growth factor receptor activity
GO:0019834	0.008037	1	3	Phospholipase A2 inhibitor activity
GO:0003729	0.008574	2	52	mRNA binding
GO:0005047	0.010702	1	4	Signal recognition particle binding
SVTE				
GO:0003735	$4.69x10^{-20}$	12	158	Structural constituent of ribosome
GO:0003723	$2.79x10^{-5}$	6	630	RNA binding
GO:0015266	0.001245	1	1	Protein channel activity
GO:0003729	0.001876	2	52	mRNA binding
GO:0015077	0.007702	2	107	Monovalent inorganic cation transmembrane transporter activity
GO:0008121	0.009917	1	8	Ubiquinol-cytochrome C reductase activity
GO:0016679	0.009917	1	8	Oxidoreductase activity, acting on diphenols and associated substances as donors
GO:0030552	0.017294	1	14	cAMP binding
GO:0005080	0.018518	1	15	Protein kinase C binding
GO:0008603	0.019741	1	16	cAMP-dependent protein kinase regulator activity
Non-overlap				
GO:0003735	$2.55x10^{-6}$	5	158	Structural constituent of ribosome
GO:0005520	0.000583	2	25	Insulin-like growth factor binding
GO:0047696	0.002881	1	2	Beta-adrenergic receptor kinase activity
GO:0005010	0.004318	1	3	Insulin-like growth factor receptor activity
GO:0019834	0.004318	1	3	Phospholipase A2 inhibitor activity
GO:0005047	0.005753	1	4	Signal recognition particle binding
GO:0035035	0.008618	1	6	Histone acetyltransferase binding
GO:0008312	0.008618	1	6	7S RNA binding
GO:0004703	0.010048	1	7	G-protein coupled receptor kinase activity
GO:0031369	0.011475	1	8	Translation initiation factor binding

GO, gene ontology; DEG, differentially expressed genes; RVTE, recurrent venous thromboembolism; SVTE, single venous thromboembolism; Non-overlap, non-overlapping DEGs as specific DEGs of RVTE; cAMP, cyclic adenosine monophosphate; GOMFID, gene ontology molecular function identification number ; Count, DEG numbers enriched in the specific GO term; Size, total gene numbers in the GO database.

VTE by successfully reducing morbidity and mortality (21). However, to date, methods for effectively preventing and diagnosing SVTE and RVTE have remained controversial (22). The present study used bioinformatics methods to investigate the molecular mechanisms and potential biomarkers of SVTE and RVTE.

In the present study, gene expression profiles of whole blood samples were successfully used to screen for DEGs in specimens from patients with SVTE compared with those in control specimens. With regard to enriched biological processes and pathways for DEGs in SVTE, genes involved in ribosomal pathways, including RPS3A and RPS7, and mitochondrial function, including UQCRQ and COX7C, were indicated to be most consistently affected and modulated. Ribosomal proteins have remained highly conserved during evolution and reflect critical functions in ribosome biogenesis; in addition, several ribosomal proteins were shown to have extra-ribosomal functions in apoptosis, DNA repair and genetic disease (23). A total of 12 DEGs were involved in ribosomal pathways. A paucity of studies have explored the pathogenesis of VTE. It has previously been indicated that the ribosomal-related RP-MDM2-P53 axis may be involved in the molecular pathogenesis of the 5q syndrome, and VTE was reported in 3% of patients with 5q syndrome (24).

Table V. KEGG pathway enrichment analysis of DEGs in patients with RVTE and SVTE, and specific DEGs of RVTE.

Category/KEGGID	P-value	Count (n)	Size (n)	Term
RVTE				
K03010	$1.59x10^{-23}$	17	86	Ribosome
K05012	0.007377	4	133	Parkinson's disease
K00190	0.007772	4	135	Oxidative phosphorylation
K05010	0.017421	4	171	Alzheimer's disease
K05016	0.022594	4	185	Huntington's disease
SVTE				
K03010	$4.58x10^{-19}$	12	86	Ribosome
K04260	0.025106	2	79	Cardiac muscle contraction
Non-overlap				
K03010	$2.25x10^{-6}$	5	86	Ribosome
K03060	0.03007	1	11	Protein export

KEGG, Kyoto Encyclopedia of Genes and Genomes; DEG, differentially expressed genes; RVTE, recurrent venous thromboembolism; SVTE, single venous thromboembolism; Non-overlap, non-overlapping DEGs as specific DEGs of RVTE. KEGGID, KEGG identification number. Count, number of DEGs enriched in the specific GO term; size, number of genes in the GO database.

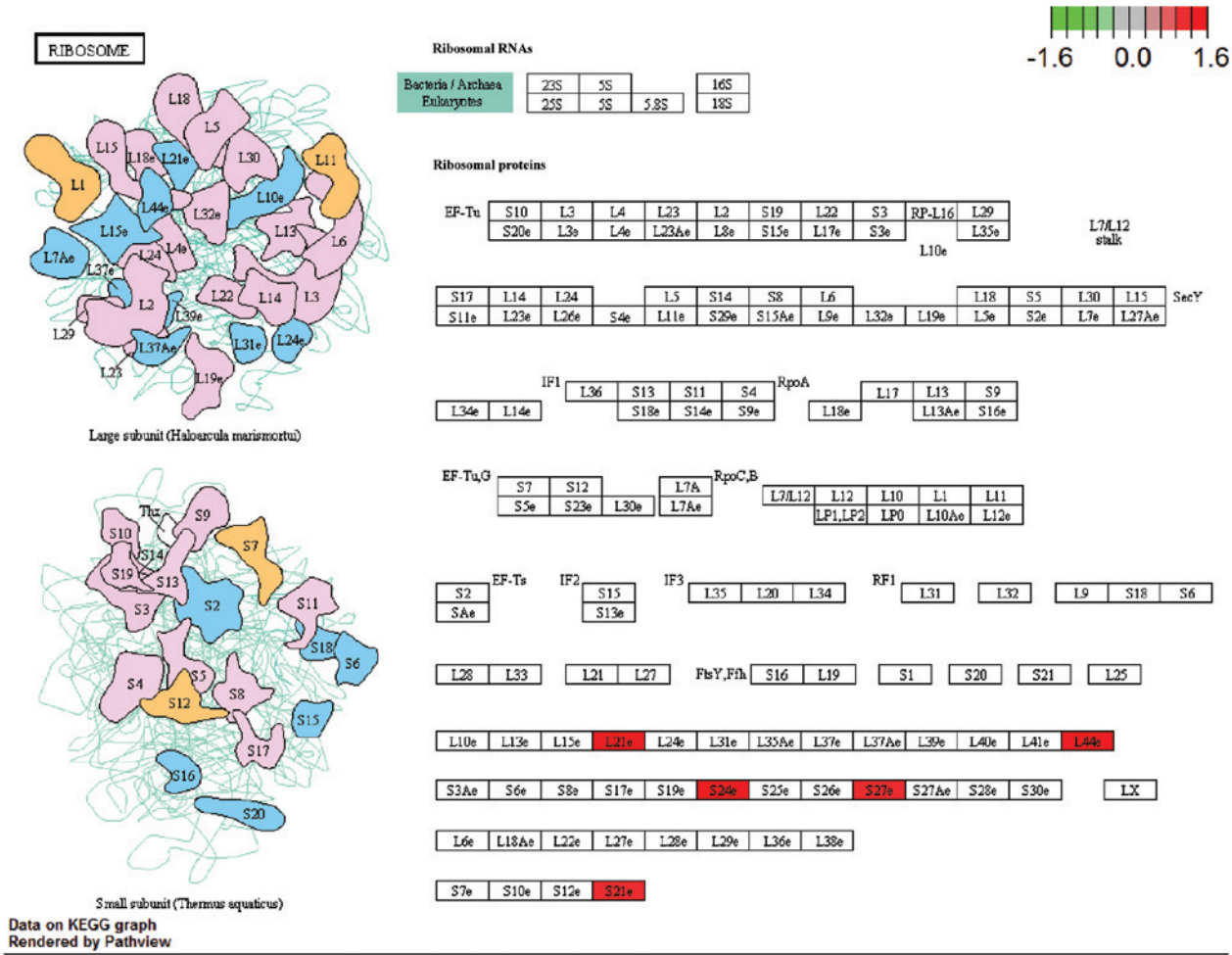

Figure 2. Visualization of the ribosome pathway. Red nodes represent upregulated differentially expressed genes.

COX7C and UQCRQ are constituents of the mitochondrial respiratory chain (25). Mutations of these two genes may increase oxidative stress in coronary artery disease (26). The mortality after VTE is strongly associated with presentation

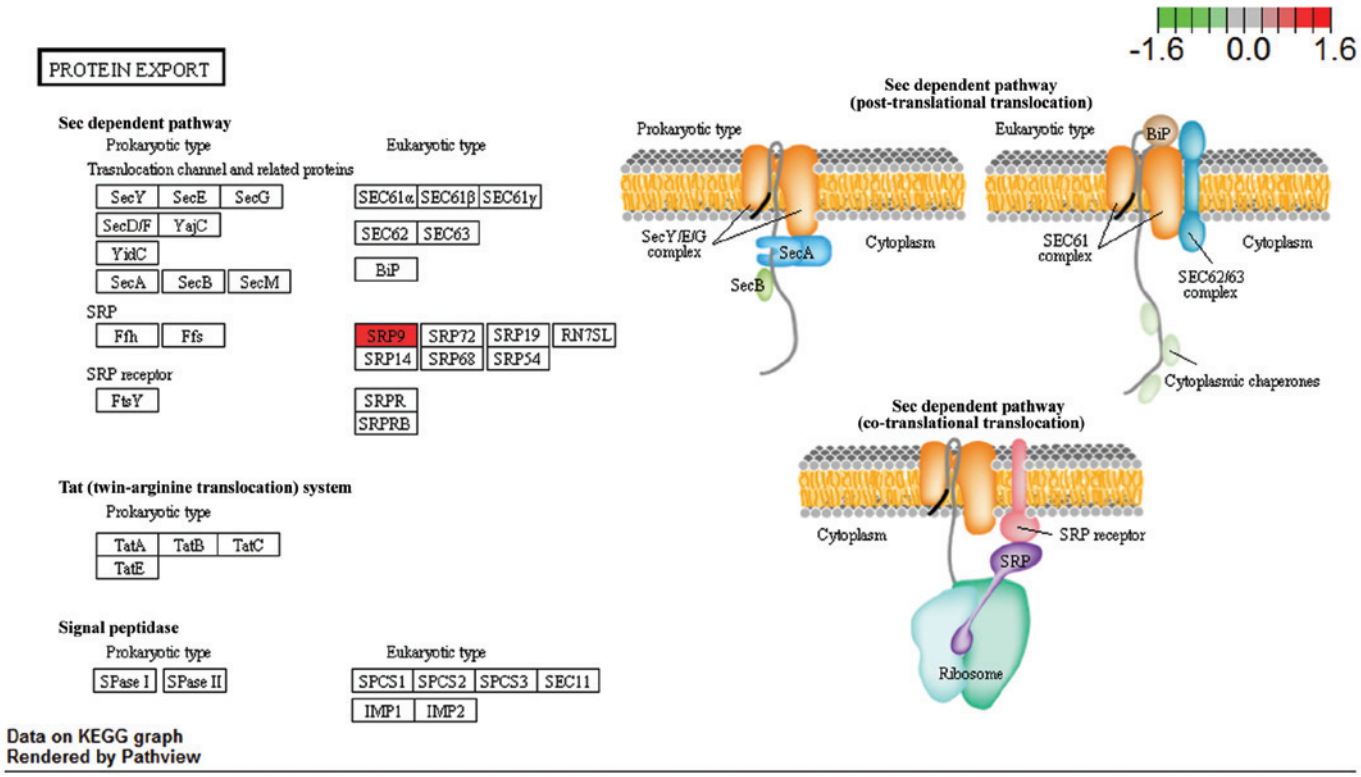

Figure 3. Visualization of the protein export pathway. Red nodes represent upregulated differentially expressed genes.

of underlying cardiovascular disease (27). First-time VTE in numerous patients is idiopathic and challenging to diagnose, while COX7C and UQCRQ may represent novel biomarkers to identify SVTE.

The specific DEGs in RVTE were found to be mainly involved in ribosomal pathways, heart contraction and oxidative phosphorylation. Pathway visualization revealed that RPL21, RPS21, RPS24 and RPS27, which all encode ribosomal proteins, were enriched in ribosomal pathways, while SRP9 was enriched in the protein export pathway. Furthermore, RPL21, RPS21, RPS24 and RPS27 were found to be involved in RVTE through critical ribosome biogenesis or extra-ribosomal functions; of note, the expression of these genes was upregulated in patients with RVTE but unchanged in patients with SVTE. It has been reported that certain diseases, including transient ischemia/reperfusion and pre-eclampsia, are associated with ribosomes (28,29). RPS24 mutation was potentially linked to pathologies of Diamond-Blackfan anemia (30). The above results suggested that RPL21, RPS21, RPS24 and RPS27 may have critical roles in RVTE.

The results of the present study showed that β-adrenergic receptor kinase 1 (ADRBK1) was closely associated with RVTE and involved in heart contraction. Polymorphisms in ADRB2 and LPL genes are known to have central roles in vascular biology (31). A previous study suggested that the use of ADRBK1 as a biomarker significantly improved heart failure therapy (32). Certain studies showed that the ADRBK1/phosphoinositide-3 kinase (PI3K) complex improved cardiac function and that PI3K-dependent phosphorylation of ADRBK1 on Ser670 is responsible for the downregulation of ADRBK1 protein via a proteasome-dependent pathway (33,34). Furthermore, PI3K influences insulin-like growth factor and blood vessel-related factor through G protein beta gamma (35). These results may suggest that ADRBK1 has a critical role in RVTE by serving as a dual effector of the compensatory myocardial diastole and the PI3K response.

The present study also identified NDUFA5 and ATP ATP5O as DEGs, which were significantly associated with oxidative phosphorylation. The oxidative stress injury and exitotoxicity in mitochondria induced by NDUFA5 and ATP5O have been proved to be the cause of a variety of nervous system degenerative diseases, including Parkinson's, Alzheimer's and Huntington's disease (36,37). Free-radical generation and consequent oxidative stress in platelet activation and thrombotic vascular diseases have a distinctive role with the potential injurious effects of homocysteine (38,39). Therefore, NDUFA5 and ATP5O inducing oxidative stress injury and exitotoxicity in mitochondria may also have an impact on RVTE.

In conclusion, the screening performed in the present study identified 42 DEGs in RVTE, including 35 up- and 7 downregulated genes, 20 DEGs in SVTE, including 17 up- and 3 downregulated genes, and 22 specific DEGs in RVTE. Furthermore, functional and pathway enrichment analysis was performed for these identified DEGs. The results indicated that DEGs in SVTE, including COX7C and UQCRQ, may be used as potential biomarkers for SVTE and that specific DEGs in RVTE, including ADRBK1, NDUFA5 and ATP5O, may be considered as potential biomarkers of RVTE. However, experimental studies are required to confirm these results.

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