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, NDUF5 and ATP5O, may be potential biomarkers for RVTE.

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 anticoagulant 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
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
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^{-27}) 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^{-25}), 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

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.
The GO terms for biological processes of the 22 specific DEGs of RVTE were mainly translational elongation ($P=3.52\times10^{-7}$), negative regulation of the force of heart contraction by chemical signal ($P=0.001$) and cell proliferation ($P=0.002$). In the cellular component category, enriched GO terms were mainly associated with the cytosolic small ribosomal sub-unit ($P=1.56\times10^{-5}$), ribosomal sub-unit ($P=1.84\times10^{-5}$) 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 ($P=2.55\times10^{-6}$), 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 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/GO BPID | 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^{-4} | 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; GO BPID, 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.
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:00022627    | 4.11x10^{-10}| 6         | 36       | Cytosolic small ribosomal sub-unit  |
| GO:00022625    | 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:00022625    | 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:00022627    | 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:0005622     | 7.82x10^{-5} | 19        | 8645     | Intracellular organelle             |
| GO:0005622     | 0.000279     | 20        | 10646    | Intracellular                       |
| Non-overlap    |              |           |          |                                     |
| GO:00022627    | 1.56x10^{-5} | 3         | 36       | Cytosolic small ribosomal sub-unit  |
| GO:00033279    | 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 ($P=0.03$). 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
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).

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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:0003732      | 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      | 4.69x10⁻¹⁰    | 12        | 158      | Structural constituent of ribosome                                    |
| GO:0003732      | 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.
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
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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 present study also identified NDUFA5 and ATP ATP5O as DEGs, which were significantly associated with oxidative phosphorylation. The oxidative stress injury and excitotoxicity 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 excitotoxicity 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.

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