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Case Report

Digenic Inheritance of PROC and SERPINC1 Mutations Contributes to Multiple Sites Venous Thrombosis

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Abstract

Venous thromboembolism (VTE) represents a worldwide health challenge, impacting millions of people each year. The genesis of venous thrombosis is influenced in part by genetic components. Hereditary thrombosis is described as a genetically determined susceptibility to VTE. In the present study, a male patient was referred to our department presenting with multiple venous thrombosis events in different locations. Given a lack of identifiable risk factors, we aimed to investigate the possible genetic factor underlying venous thrombosis. Whole-exome sequencing was employed to examine genes linked to inherited thrombophilia in the proband. Putative variants were subsequently confirmed through Sanger sequencing within the family. The proband was identified as carrying two genetic mutations. One is the novel c.400G > C (p.E134Q) mutation affecting the final nucleotide of exon 5 in the PROC gene, potentially impacting splicing. The other is a previously reported heterozygous nonsense variant c.1016G > A (p.W339X) in the SERPINC1 gene. The proband inherited the former from her mother and the latter from her father. The presence of digenic inheritance in the patient reflects the complex phenotype of venous thrombosis and demonstrates the significance of an unbiased approach to detect pathogenic variants, especially in patients with a high risk of hereditary thrombosis.

Keywords

- digenic inheritance
- inherited thrombophilia
- PROC gene
- SERPINC1 gene
- whole-exome sequencing

Introduction

Venous thromboembolism (VTE) is a significant health burden globally, resulting in preventable morbidity and mortality. It is estimated that 0.1 to 0.2% of the population is affected by this condition annually.[1] Thrombophilia is a collection of conditions that increase the risk of thromboembolism by inherited or acquired factors. The clinical manifestations are primarily VTE, but some individuals may also experience arterial thrombosis and obstetric complications.[2] Inherited thrombophilia commonly arises from alterations in factor V Leiden, prothrombin G20210A, antithrombin III (ATIII), protein C, and protein S. Acquired risk factors, including immobilization, trauma, and surgical interventions, contribute to an elevated likelihood of venous

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thrombosis.[3] The final diagnosis of inherited thrombophilia relies on genetic sequencing of associated genes.

Whole-exome sequencing (WES) is a next-generation sequencing method that examines the nucleotide sequence of all the protein-coding regions of one's genome.[4] Although exome comprises less than 2% of the human genome, it contains 85% of the known disease-causing variants.[5] WES has been used in a wide range of clinical studies, especially in hereditary diseases and emerges as a promising new diagnostic tool. We previously identified a pathogenic missense variant of PROS1 by utilizing WES.[6] In this study, we described an individual experiencing recurrent thrombosis at various locations, linked to mutations in the SERPINC1 and PROC gene.

Case Presentation

A 57-year-old male patient was referred to our department with swelling and pain in his right upper extremity. By ultrasonography, the axillary and brachial veins were found to be affected by deep vein thrombosis (►Fig. 1A). A subsequent computed tomography scan indicated that the thrombus had extended into the innominate vein (►Fig. 1B). The patient had a history of lower extremity deep venous thrombosis in his 30s and superior mesenteric venous thrombosis in his 40s (►Fig. 1C). The patient's family history did not show any predisposition to venous thrombosis. After the diagnosis of venous thrombosis, the patient was initially given low-molecular weight heparin followed by oral rivaroxaban to continue anticoagulation therapy. As the absence of any apparent susceptibility to venous thrombotic events and the genetic heterogeneity of hereditary thrombosis, an investigation of this patient's genetic background was conducted and WES was chosen to identify potential genetic susceptibility to thrombophilia.

All participants provided written informed consent for the acquisition of venous blood samples, which were subsequently processed utilizing the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) in accordance with established protocols for DNA extraction. WES was carried out as described earlier.[6] The library preparation required a minimum of 200 ng of DNA, followed by exon capture using the Sure Select XT Human All Exon Kit V6, and subsequently sequenced using the Illumina Hiseq 2000 platform with 2×150 bp paired-end reads. Sequenced reads were demultiplexed, and sequencing quality was assessed using the Fast QC tool.

In this study, a standardized methodology was utilized to examine genomic sequences. Initially, sequence reads were aligned to the UCSC hg19 reference genome employing the Burrows Wheeler Aligner with default parameters. Duplicate sequencing reads were handled by utilizing Picard Mark

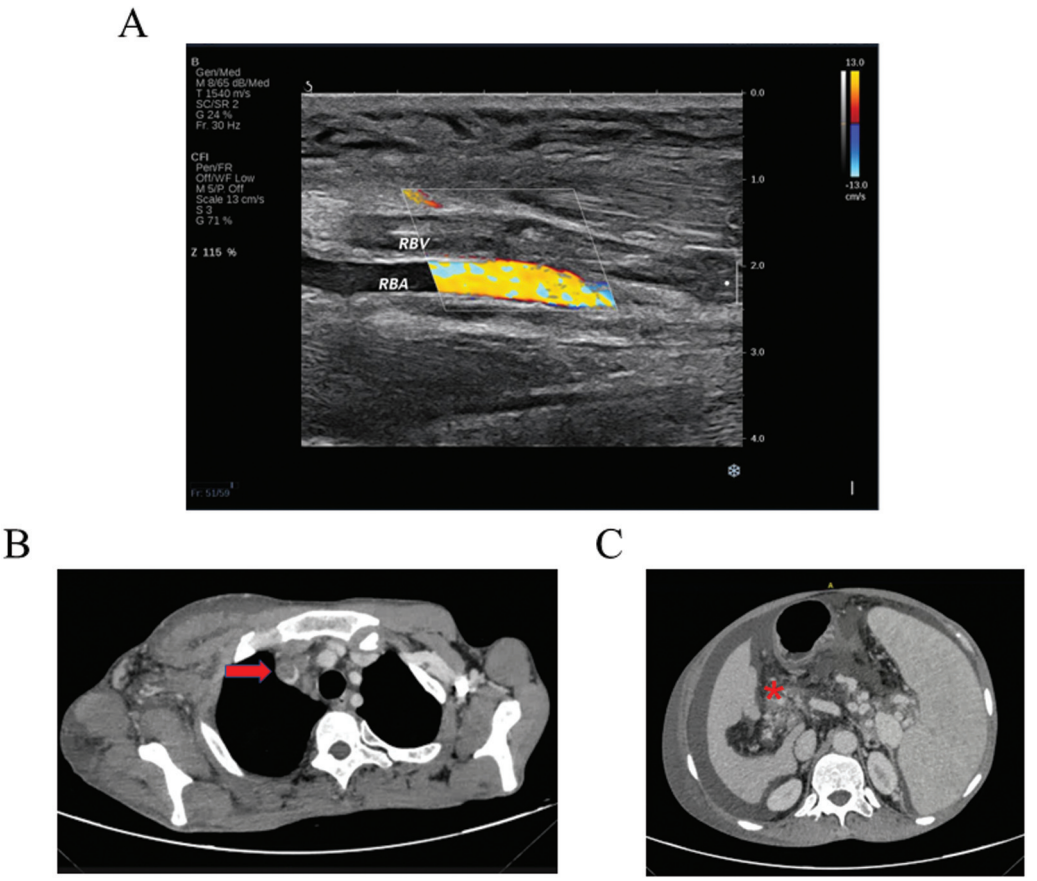

Fig. 1 Imaging findings of the proband. (**A**) Doppler ultrasound showed a solid long hypoechoic mass filling the right axillary and brachial veins. (**B**) Multi-slice computed tomography showed thrombus extending to the innominate vein (red arrow). (**C**) Computed tomography revealed cavernous transformation of portal vein secondary to portal vein thrombosis (red asterisk).

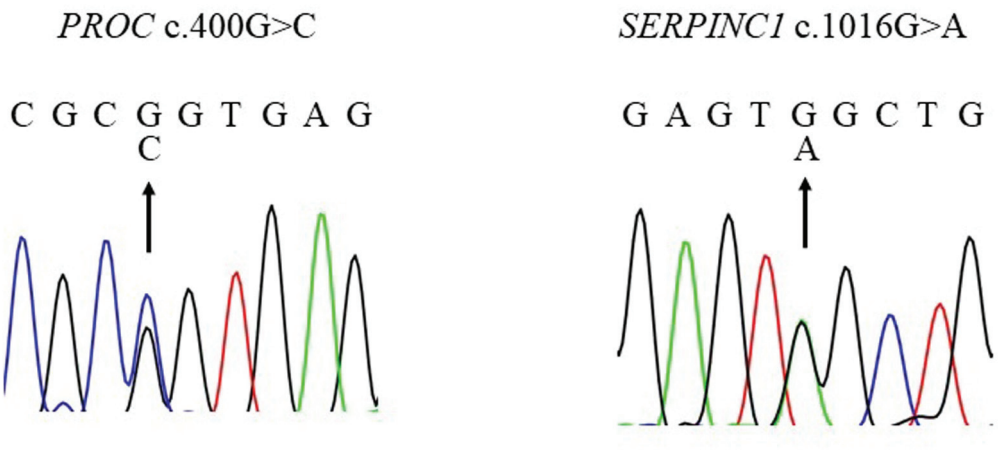

Fig. 2 Digenic variants of PROC and SERPINC1 identified in the proband. The black arrows indicate the genetic mutation sites.

Duplicates. Subsequently, the Genome Analysis Toolkit (GATK) was utilized for local realignment of reads surrounding in-frame insertions and deletions (indels) and recalibration of base quality scores. Variants, encompassing single-nucleotide variants and indels, were detected using the GATK Haplotype Caller and annotated via ANNOVAR. These identified variants were screened against public single-nucleotide polymorphism databases such as dbSNP, 1000G, ESP, and ExAC in order to filter out common variants. Only the variants predicted as deleterious by The PolyPhen2 and SIFT algorithms were included in the analysis. Ultimately, the validation of putative disease-associated variants discovered in family members was achieved through Sanger sequencing.

The results revealed that a high-sequencing coverage of approximately 114× was achieved for each sample, 99.1% of short reads were mapped, and 97% of calls had an average base call quality of Q30 or higher. After eliminating low-quality variants and those with insufficient coverage, confident variants were obtained. Variants exhibiting a minor allele frequency of ≥0.01% were ruled out using population databases, such as 1000 Genomes, ExAC, and ESP, and the analysis centered on missense, frameshift, nonsense, and splice site variants in genes related to the blood coagulation cascade. The final analysis revealed two variants. The first is a heterozygous missense variant (c.400G > C: p.E134Q) in exon 5 of PROC (NM_000312), affecting the last nucleotide of the exon. Analysis using the Splice Site Prediction by Human Splice Finder showed that it resulted in an alteration of the wild type donor site, most probably affecting splicing. There are reports suggesting that apparent missense mutations affecting the last nucleotide of an exon can lead to missplicing.[7] Unfortunately in our study, RNA was unavailable for testing the effects of c.400G > C on splicing, but it seems possible that the variant exerts pathogenic effects either by the amino acid substitution or by an effect on splicing. The second variant is a previously described nonsense variant in exon 5 of SERPINC1 (NM_000488: c.1016G > A: p.W339X), both of which were confirmed through Sanger sequencing (►**Fig. 2**). The same PROC variant was identified in the proband's mother and the same SERPINC1 variant was identified in the proband's father upon sequencing of other family members. Moreover, the proband had reduced protein C and antithrombin (AT) activity levels at 34 and 42%, respectively (►**Table1**). Our findings suggest that the mutations in PROC and SERPINC1 are causative of the proband's multiple venous thrombosis episodes.

Discussion

Thrombophilia constitutes a diverse collection of conditions marked by a propensity for thromboembolism, stemming from an array of inherited or acquired defects in anticoagulant proteins, coagulation factors, fibrin, or the existence of acquired risk factors.[2] The prevalence of genetic risk factors for VTE exhibits considerable variation between Eastern and Western populations. In contrast to the rarity of natural anticoagulant deficiencies, such as AT, protein C, and protein

Table 1 Clinic and genetic characteristics of the family

Family member	Age (y)	SERPINC1 c.1016G > A(p.W339X)	PROC c.400G > C(p.E134Q)	AT:A (%)	AT:Ag (%)	PC:A (%)	PC:Ag (%)	PS:A (%)	PS:Ag (%)	VTE
Patient	57	+	+	42	47	34	39	98	105	+
Father	78	+	–	50	48	106	108	93	99	–
Mother	75	–	+	110	102	45	40	103	94	–

Note: Reference range: AT:A, 80–120%; AT:Ag, 80–120%; PC:A, 70–130%; PC:Ag, 80–120%; PS:A, 55–140%; PS:Ag, 60–150%.
Abbreviations: AT:A, antithrombin activity; AT:Ag, antithrombin antigen; PC:A, protein C activity; PC:Ag, protein C antigen; PS:A, protein S activity; PS:Ag, protein S antigen; VTE, venous thromboembolism.

S, in Western countries, these deficiencies pose significant risk factors in Asian nations.[8] Our study described a pioneer case presented with mutations in both the *SERPINC1* and *PROC* genes, which led to recurrent multisite thrombosis. Notably, the parents of the patient with a single gene mutation did not display a significant history of thrombosis, highlighting the incomplete penetrance of mutated genes.

SERPINC1, located on human chromosome 1q23-25, encodes ATIII, a crucial inhibitor of the coagulation cascade. The gene consists of seven exons and six introns.[9] ATIII binds to the active center serine of thrombin and forms a complex with thrombin, known as the thrombin–AT complex, thereby neutralizing its activity. Heparin enhances AT affinity by changing its conformation through an exposed reactive center loop, leading to at least a 1000-fold increase in AT activity.[10] The Human Gene Mutation Database reports numerous missense/nonsense variants and a small number of small deletions/insertions and splicing mutations in *SERPINC1*. Among the reported *SERPINC1* mutations, four mutations are relatively common in general population: Dublin(p.Val30Glu),[11] CambridgII (p.Ala416Ser),[12] Budapest (p.Leu131Phe),[13] and Basel (p.Pro73Leu).[14] AT deficiency is classified into two types based on their phenotypes: Type I AT deficiency is typified by a proportional reduction in AT antigen and activity, whereas type II AT deficiency is marked by diminished AT activity alongside normal or near-normal AT antigen levels.[15] Nonsense mutations, splicing mutations, and small frame-shifting deletions and insertions result in type I deficiency, while missense mutations or small frame-shifting deletions give rise to type II deficiency. However, some missense mutations also cause type I deficiency due to conformational effects.[16] The c.1016G > A(p.W339X) variant was previously reported in a young female with VTE by Ding et al.[17] This variant is anticipated to culminate in either the total lack of a functional AT protein or the expression of a truncated, nonfunctional protein, which aligns with the type I AT deficiency demonstrated by the proband harboring the associated mutation.

The *PROC* gene, which comprises 9 exons and 8 introns, is located on the 2q13-14 region of the human chromosome.[18] Protein C, a vitamin K-dependent serine protease produced by the liver, plays a crucial role in natural anticoagulation.[19] When it binds to the thrombin–thrombomodulin complex on the vascular endothelium, protein C is converted into activated protein C (APC). In collaboration with protein S, APC deactivates factors Va and VIIIa, ultimately resulting in decreased thrombin and fibrin generation. Furthermore, protein C enhances fibrinolysis by neutralizing circulating plasminogen activator inhibitor and augmenting tissue plasminogen activator activity.[20] A deficiency in protein C can increase the risk of thrombosis, and there are two types of congenital protein C deficiency: Type I, defined by reduced antigenic levels and function, and Type II, characterized by diminished function while maintaining normal antigenic levels. The Human Gene Mutation Database contains more than 390 *PROC* mutations, including missense, nonsense, insertion, deletion, and splicing mutations, with missense mutations accounting for over 65% of all *PROC* mutations.[21] Most missense mutations result in the abnormal folding of synthesized protein C, which accumulates in the endoplasmic reticulum (ER), leading to ER-associated degradation and reduced protein C antigen and function.[22,23] The proband exhibited a heterozygous missense mutation (c.400G > C:p.E134Q) in the PROC gene, which remains unreported in public polymorphism databases and is anticipated to be deleterious according to Polyphen2 and SIFT predictions. In addition, an effect of this variant on splicing is possible, because the mutation is located at the last nucleotide of exon 5 and alters the conserved splice donor sequence. In line with the interpretation standards and guidelines for sequence variants, as well as the joint consensus from the American College of Medical Genetics and the Association of Molecular Genetic, this variant is regarded as "likely pathogenic (II)" and fulfills one strong and one moderate criterion, specifically PS2, PM2, PP1, and PP3.[24]

In digenic inheritance, disease arises due to protein–protein or protein–DNA interaction between two genes or proteins.[25] The Digenic Diseases Database classifies digenic cases into two types based on Schäffer's definition of digenic inheritance. The first type, known as "true inheritance," requires variants at both motifs to cause disease, and neither variant alone is sufficient to produce the phenotype. In this type, it is difficult to determine which gene variant has a greater effect on disease symptoms. The second type is a composite class, where variants in both genes have an "additive" effect, since the variation in the first gene is sufficient to cause the disease, but the variation in the second gene increases the severity of the disease. However, there are exceptions that can complicate the classification of double gene inheritance.[26] This phenomenon has been observed in a variety of diseases with complex genetic mechanisms, such as diabetes,[27] dyslipidemia,[28] pituitary stalk interruption syndrome,[29] and isolated hypogonadotropic hypogonadism/Kallmann syndrome.[30] Under normal circumstances, AT and protein C act as inhibitors in the coagulation cascade, thereby regulating normal blood flow.[31] In our study, the PROC mutation was inherited from the mother and the SERPINC1 mutation was inherited from the father, while the parents had not suffered a thrombotic event. Hematologic examination of the patient reported in this case manifested that the patient's both protein C activity and AT activity were considerably lower than normal levels in healthy individuals, whereas the corresponding parents were only below the normal range of either protein, which could explain why the parents didn't show any obvious symptoms. With regard to hereditary thrombosis, a previous case has been reported in which both SERPINC1 and PROC mutations were found in a patient with severe recurrent thromboembolism, diagnosed as having a type I AT deficiency with decreased protein C.[32] It is obvious that case is similar to our case, both of which meet the definition of composite inheritance. It further demonstrates the advantages of WES for the final diagnosis of hereditary thrombosis.

Conclusions

In summary, we presented a case of an individual who exhibited multiple venous thrombosis events in distinct

locations. WES analysis identified a novel heterozygous missense variant, c.400G > C (p.E134Q), in the PROC gene, which has the potential to impact splicing. Additionally, a previously reported heterozygous nonsense variant, c.1016G > A (p.W339X), was found in the SERPINC1 gene within the individual's genome. WES demonstrated its ability to rapidly identify genetic abnormalities associated with complex inheritance patterns with high accuracy.

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Institutional Review Board, The Second Affiliated Hospital of Nanchang University. After explanation of the possible consequences of the study, written informed consent was obtained from all study participants.

Consent for Publication

The patient provided informed consent for the publication of this study.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article

Authors' Contributions

Recruited and phenotyped the participants: X.G.L., W.W. Z. Performed molecular genetic experiments: X.G.L., W.W. Z. Analyzed the data: X.G.L., JBZ, W.W.Z. Wrote the manuscript: X.G.L., J.B.Z. Revised: F.Z.L., W.Q.M., W.M.Z. All authors read and approved the final manuscript.

Web links and URLs

1. Public single nucleotide polymorphism databases: dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/); 1000G (http://www.1000genomes.org/); ESP. (http://evs.gs.washington.edu/EVS); ExAC (http://exac.broadinstitute.org). Accessed September 15, 2022.
2. A toolkit that offers various utilities for working with high-throughput sequencing (HTS) data in the BAM format: Picard MarkDuplicates (http://sourceforge.net/projects/picard/). Accessed September 15, 2022.
3. Computational algorithms designed to predict the functional impact of amino acid substitutions on proteins: The PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.bii.a-star.edu.sg/) algorithm. Accessed September 15, 2022.
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Conflict of Interest

The authors declare that they have no conflict of interest.

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