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CASE REPORT

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The c.1243T>C mutation in the *PROC* gene is linked with inherited protein C deficiency and severe purpura fulminans

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Key Clinical Message

Purpura fulminans is a severe coagulation disorder that often leads to death in neonates. Mutations in the protein C (PROC) gene can cause protein C deficiency, leading to this disorder. This study aimed to investigate a family with a history of coagulopathies, particularly those related to protein C deficiency. The primary objective was to identify any genetic mutations in the PROC gene responsible for the coagulopathies. The study focused on a male neonate with purpura fulminans who ultimately died at 2 months of age. The patient had low protein C activity levels (6%). The entire PROC gene of the patient and his family was analyzed using next-generation sequencing to identify any genetic mutations. Segregation analysis was conducted to determine if the mutation followed an autosomal dominant inheritance pattern. In silico analysis was also conducted to evaluate the pathogenicity of the identified mutation. Analysis revealed a novel homozygous c.1243T>G variant PROC gene. The mutation resulted in a Phe415Val substitution. The mutation was found in at least three generations of the family. Carrier family members had lower protein C activity levels than wild-type homozygotes. Additionally, the mutation may account for the observed reduction in protein C enzyme activity.

KEYWORDS

next-generation sequencing, *PROC* gene, protein C deficiency, purpura fulminans, venous thromboembolism

1 | INTRODUCTION

Protein C plays a pivotal role in the intricate process of the coagulation cascade. Disorders stemming from insufficiency of this vital factor include hereditary thrombophilia, disseminated intravascular coagulation (DIC), Deep vein thrombosis (DVT), neonatal purpura fulminans (NPF), and hemorrhagic skin necrosis.[1] NPF is a rare clinical condition caused by thrombosis of the dermal microvasculature. This potentially fatal disorder is associated with

perivascular hemorrhage and DIC and typically manifests during the neonatal period.[2] NPF is characterized by the sudden appearance of symmetrical, tender, ecchymotic skin lesions.[3,4] The therapeutic approach to NPF primarily revolves around addressing the underlying cause and correcting any coagulation abnormalities. Supportive treatment measures, including the administration of antibiotics, volume expansion, and tissue oxygenation, are also beneficial.

Inherited Protein C deficiency follows an autosomal recessive (MIM: 612304) and autosomal dominant (MIM: 176860) pattern of inheritance, resulting from mutations in the *PROC* gene.[5] The autosomal dominant form of the disease occurs in approximately 1 in 500 live births.[6,7] Heterozygous carriers of the disease typically remain asymptomatic until older ages and they become highly susceptible to the development of venous thromboembolism (VTE).[4] The severe autosomal recessive type of Protein C deficiency is an infrequent occurrence, with an incidence rate of 1 in 4 million.[8] This variant has been linked to the development of systemic infections, NPF, DIC, and necrosis, which may result in the amputation of the affected limbs.[9,10]

The PROC gene, encompassing 9 exons, is located on chromosome 2q14.3. Prenatal diagnosis through gene analysis may aid in the prevention of protein C deficiency. Despite various studies exploring the genotype–phenotype correlation in individuals with purpura fulminans, there is still a dearth of knowledge in different populations. This case report aims to shed light on another cause of hereditary protein C deficiency and severe purpura fulminans, identifying the missense homozygous point mutation of c.1243T>G in the *PROC* gene.

2 | CASE REPORT

2.1 | Case presentation

The studied neonate was delivered from a 30-year-old mother following an uneventful pregnancy that lasted for 39 weeks and 2 days. Upon birth, the neonate's head circumference, height, and birth weight were measured to be 34 cm, 51 cm, and 3020 g, respectively. The neonate exhibited normal tone and activity at birth, but subsequently presented with symptoms of jaundice and ecchymosis a few hours after delivery. Ecchymosis was observed in the lateral region of the right thigh as well as two parts of the lower abdomen (as depicted in Figure 1A). After the emergence of ecchymosis, bruised necrotic lesions developed at the affected sites on the fourth day. Additionally, during the ophthalmic examination, posterior synechiae formation was observed. A complete blood count test on

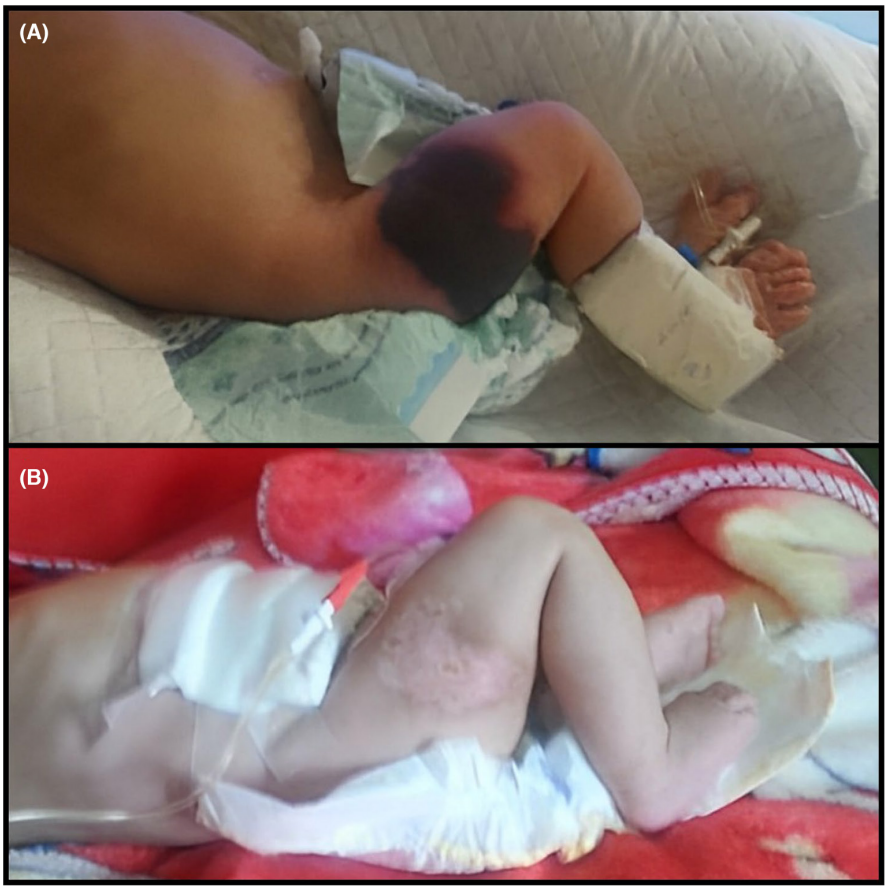

FIGURE 1 (A) Appearance of ecchymosis on the lateral surface of the right thigh, (B) disappearance of the ecchymosis 4 weeks after treatment with fresh frozen plasma.

the first day revealed that the platelet count was 83,000/μL. Following the onset of the symptoms, the neonate was transferred to the neonatal intensive care unit and received phototherapy. Platelet transfusion was subsequently administered once, alongside regular administration of fresh frozen plasma (FFP) 20 mL/kg. every 12 h from the first day of birth and broad-spectrum antibiotic prophylaxis. The treatment resulted in the resolution of ecchymosis (Figure 1B).

To investigate the underlying cause of the ecchymosis, thrombophilia profile tests were conducted 24 h after discontinuing FFP transfusion. Coagulation factors, including proteins C, S, and antithrombin, were measured. Given the low level of Protein C activity (6%) observed, it was suspected that the neonate was suffering from protein C deficiency.

Doppler ultrasound of the lower limbs was conducted, which showed open CFU, SFU, and POUP veins with no evidence of thrombosis. Brain ultrasounds were performed twice a week throughout hospitalization and were all within normal range. Additionally, abdominal and pelvic ultrasounds as well as chest X-rays were conducted and all results were normal. Furthermore, a neonatal cardiac assessment was conducted and yielded normal results.

The parents of the patient were related to each other both as first cousins and through a more distant cousin relationship, where one parent is a first cousin of the other parent's parent. The mother had a prior medical history of gestational diabetes mellitus and mild thrombocytopenia. She also had a history of spontaneous abortion as well as the loss of another infant at 14 days of age with petechial, purpura, and coagulopathy. However, neither case of the spontaneous abortion nor the loss of the infant resulted in a definitive diagnosis. Additionally, it was reported that both grandparents of the patient had a history of VTE during their later years. Given these circumstances, genetic counseling has been requested for the family. The family pedigree is presented in Figure 2.

Although the patient received timely treatment after prompt diagnosis, they unfortunately passed away 2 months later as a result of multiple organ failure. However, genetic counseling was conducted to mitigate the risk of recurrence in future pregnancies, and the subsequent child born to the family was healthy, possessing a heterozygous mutation in the *PROC* gene.

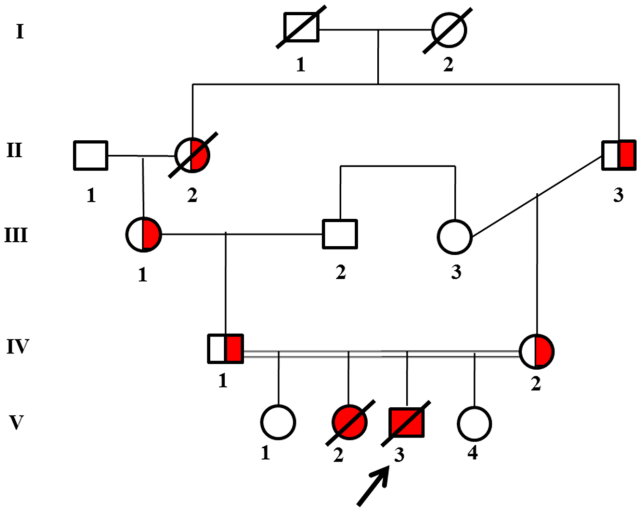

FIGURE 2 The pedigree of the family is comprised of three generations, squares and circles indicate females and males, respectively. The half-colored shapes represent the c.1243T>G mutation carriers, and the color-filled shapes represent people with protein c deficiency. The arrow appoints the proband. The c.1243T>G mutations in the *PROC* gene are co-segregating with the disease in this family. Individuals II-2 and II-3 showed VTE. Individuals V-2 and V-3 suffered from purpura fulminans.

2.2 | DNA extraction and sequencing

Peripheral blood leukocyte samples collected in ethylenediaminetetraacetic acid tubes from the proband and their relatives were used for DNA extraction via the standard salting-out method. The *PROC* gene was analyzed using a commercially available targeted next-generation sequencing (NGS) panel for hereditary Protein C deficiency from BGI, Hong Kong. The NGS panel examined all exons of the target gene with a sensitivity of greater than 99%. This method enabled the simultaneous detection of point mutations, small insertions/deletions, and duplications (<20 bp).[11]

To identify potentially disease-causing variants, a filtering pipeline was established. Given the rarity of mutations causing protein C deficiency, only variants with a frequency below 0.01 were selected.[12]

2.3 | Validation by Sanger sequencing

Direct Sanger sequencing using primers flanking the exon 9 of the *PROC* gene was used to validate the c.1243T>G mutation. Allele-specific detection was performed using designed primers for the identified mutation. Primer sequences for this were designed by using primer3 plus software (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The forward primer (5′-CTACCACAGCA GCCGAGAGAAGG-3′) and reveres primer (5′-AGGGA TGGAAGGACAGACAGCA-3′) produced a target fragment with a length of 458 bp. PCR products were sequenced on a HITACHI ABI Prism 3130xl Genetic Analyzer.

2.4 | Bioinformatics tools

PhastCons and PhyloP programs, developed by Cold Spring Harbor Laboratory in the USA, were employed to assess the evolutionary conservation at the mutation site. PhastCons calculates the degree of conservation in a multiple alignment, assigning scores ranging from 0 (less conserved) to 1 (highly conserved) to each nucleotide. On the other hand, PhyloP evaluates the conservation at individual columns independently, disregarding the neighboring effects, and provides scores ranging from −14 to +6 for each studied nucleotide.

The PredictProtein database (https://predictprotein.org) was utilized to identify the highly conserved amino acids within Protein C. The PredictProtein tool provides a numerical score between 1 and 9 to indicate the level of conservation for each amino acid.

The computational tools SIFT, Polyphen2, and Mutation Taster were employed to assess the potential pathogenicity of the c.1243T>G mutation.[13] Additionally, SNAP2 was employed to evaluate the potential impact of the mutation on the functional properties of Protein C. SNAP2 scores the predicted functional effect of amino acid substitution from values greater than 50, for strong effect, and lesser than −50 for neutral/no effect.[14]

A 3D modeling was conducted using the web-based interactive tools SWISS-MODEL[15] and MOLEonline.[16] Additionally, I-mutant 2.0 was utilized to evaluate the impact of the mutation on the conformational changes and structural stability of protein C.[17]

2.5 | Sequencing and laboratory findings

DNA sequencing analysis revealed that the parents and the pedigree two grandparents of the proband were carriers of a heterozygous c.1243T>G point mutation in exon 9 of the *PROC* gene. The proband, on the other hand, was found to have a homozygous c.1243T>G mutation at the same position, resulting in a novel *PROC* gene mutation that caused a substitution of one amino acid (phenylalanine to valine; Phe415Val). Figure 3 shows the sequencing results.

Examination of Proteins C and S activity levels in the members of this family revealed that the proband (V-3) with homozygous c.1243T>G mutation in the *PROC* gene had only 6% Protein C activity. However, the father and mother of the proband, who were carriers of this mutation, had Protein C activity levels of 86% and 51.5%, respectively. The Protein S activity levels in all of the family members were mostly within the normal range.

It should also be noted that the II-3 and III-1 grandparents, both of whom carried the c.1243T>G mutation, developed venous thromboembolism (VTE) in their elderly age.

2.6 | Bioinformatic findings

The c.1243T>C point mutation identified in the present study has not been previously reported in the literature

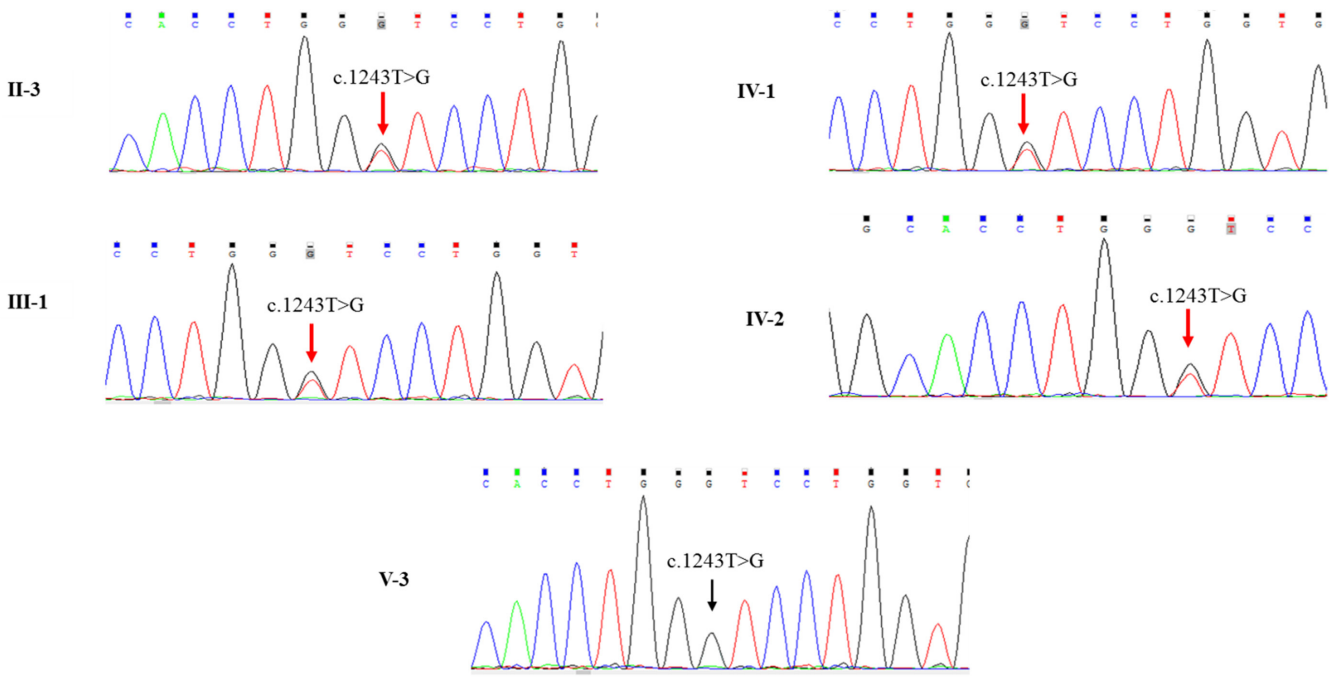

FIGURE 3 Chromatograms showing nucleotide sequences of *PROC* in the regions of c.1243T>G mutation found in the family. Red and black arrows indicate the heterozygous and homozygote nucleotide substitution, respectively.

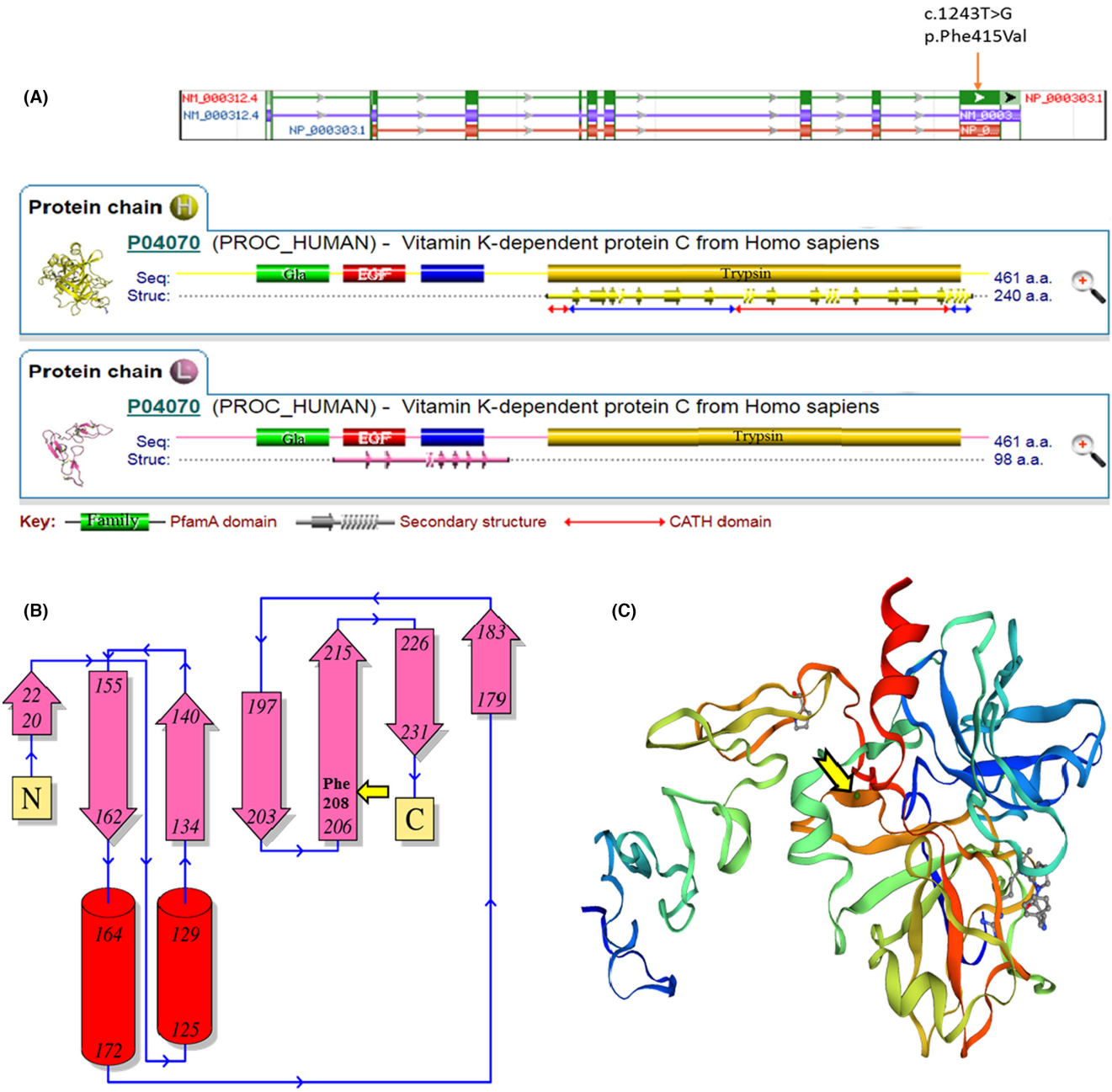

FIGURE 4 (A) Schematic structure of human *PROC* gene and its encoded protein domains. The c.1243T>G is located in the Trypsin domain that is coded by the exon 9. This protein consists of two subunits of light and heavy chains, each of which is produced by this gene. (B) The heavy chain consists of 2 structural domains. Pink arrows and red cylinders represent strand and helix structures, respectively. The p.P415V mutation (yellow arrow) in domain 1 shown in the image is located in the strand section. (C) A prediction of structural alternation resulted from *PROC* p.P415V mutation (yellow arrow) designed by SWISS-MODEL and the Pictorial database of 3D structures in the Protein Data Bank.

(as indicated by www.exac.broadinstitute.org). This novel mutation is located within the last exon (exon 9) of the *PROC* gene, which is a highly conserved region of the protein. PhastCons and PhyloP gave protection scores of 1 and 4.804, respectively, to this mutation.

Based on the findings from the PredictProtein database, it was observed that the amino acid sequence 413–427, specifically at Phe415, has the highest protection score (ranging from 7 to 9) compared to other amino acids in the protein C sequence. Following the Human Gene Mutation Database (www.hgmd.cf.ac), numerous articles have reported single-base mutations within this region of the PROC gene, which accounts for approximately 75% of all mutations within the gene.[18]

Through a comparative analysis, SIFT, Polyphen2, and Mutation Taster were found to classify the c.1243T>C

mutation within the *PROC* gene as being in the "Deleterious" category. These findings are in harmony with previous studies that highlighted the importance of the Trypsin protease region encoded by the exon 9 for the functional activity of Protein C.[1] The functional impact of the c.1243T>C mutation was further evaluated using the computational tool SNAP2, which revealed a score of +45 for the substitution of phenylalanine with valine at amino acid 415 residue, which is notably near the high score border.

2.7 | Structural-based analysis and modeling

Protein C is composed of two subunits, namely light and heavy chains. The heavy chain comprises 240 amino acids and contains two principal structural domains, with the p.Phe415Va mutation occurring within the first domain (Figure 4A). Figure 4B,C visually illustrates the affected site and demonstrates the structural alteration induced by the p.Phe415Val mutation. The protein structure was retrieved from the Protein Data Bank using the accession code 3f6u.[19] In silico analysis revealed that the c.1243T>C mutation resulted in a decrease in the structural stability of Protein C.

Collectively, in silico findings, clinical symptoms, laboratory findings, and family history demonstrate that the c.1243 T > C mutation is indeed pathogenic and capable of causing purpura fulminans in infants and VTE in the elderly. Identifying suspected carriers and using prenatal diagnosis and preimplantation genetic diagnosis can prevent the birth of patients such coagulation disorder.

3 | DISCUSSION

The novel c.1243T>C mutation in the PROC gene, as described in this case study, is considered pathogenic based on the American College of Medical Genetics and Genomics (ACMG) criteria. Several factors support its classification as pathogenic, including its rarity, segregation in an affected family, the impact on protein function (a missense mutation), and its association with a severe clinical phenotype, purpura fulminans, characteristic of homozygous protein C deficiency. This classification enhances our understanding of the genetic basis of protein C deficiency and its clinical manifestations.[20]

In addition to the c.1243T>C mutation, other pathogenic mutations in the PROC gene have been identified, contributing to the genetic heterogeneity of protein C deficiency. Notable examples include the c.1198G>A (p.Gly400Ser) mutation, associated with autosomal recessive protein C deficiency and severe clinical presentations, as well as the p.Ala178Pro variant, which results in type I activity deficiency.[18,21,22] These findings highlight the functional diversity of pathogenic mutations in the PROC gene and their impact on disease severity.

The study of PROC gene mutations is of paramount importance for clinical diagnosis and management, enabling early recognition and treatment, particularly in severe cases to prevent life-threatening complications. It also facilitates genetic counseling for affected families, allowing them to make informed decisions about reproductive choices and potential genetic risk to offspring. Additionally, research on PROC gene mutations can lead to advancements in therapeutic interventions, such as protein C replacement or anticoagulants, which can be life-saving for affected individuals. Moreover, the association between specific mutations and clinical outcomes aids in risk assessment for complications like venous thromboembolism in individuals with protein C deficiency. Overall, the study of PROC gene mutations not only enhances our understanding but also has significant implications for clinical practice, genetic counseling, and research aimed at improving patient outcomes.

4 | CONCLUSION

The c.1243T>G mutation in the *PROC* gene is a newly identified point mutation that results in the structural instability of protein C, reducing its activity. This mutation has been shown to cause purpura fulminans in infants and VTE in the elderly. Therefore, it may be crucial to screen parents with a history suggestive of these coagulation disorders for this mutation. More studies are required to establish the prevalence and importance of this particular mutation of Protein C in blood disorders.

AUTHOR CONTRIBUTIONS

Seyed Mohammad Kazem Nourbakhsh: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision. **mohammad bahadoram:** Conceptualization; data curation; investigation; methodology. **Ali Rashidi-Nezhad:** Formal analysis; resources; supervision; validation. **Laleh Habibi:** Data curation; investigation; resources; software; validation. **Fatemeh Mansouri:** Supervision; validation; visualization; writing – original draft. **Esma'il Akade:** Validation; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

There are no conflicting interests between the parties involved in this research article.

DATA AVAILABILITY STATEMENT

All datasets on which the conclusions of the paper rely (excluding the identity information of the participants) are available to editors, reviewers, and readers from the corresponding author.

ETHICS STATEMENT

The local ethics committee at Tehran University of Medical Sciences approved this study, and informed consent according to the Declaration of Helsinki was obtained from all participants.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

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REFERENCES

1. Zhang H, Bi X, Su Z, Tu X, Wang L, Shen B. A novel compound heterozygous mutations in protein C gene causing neonatal purpura fulminans. *Blood Coagul Fibrinolysis*. 2018;29(2):216-219.
2. Kazi GI, Siddiqui E, Tabassum S, Afzal B, Khan IQ. Neonatal purpura fulminans, a rare genetic disorder due to protein C deficiency: a case report. *J Pak Med Assoc*. 2018;68(3):463-465.
3. Chalmers E, Cooper P, Forman K, et al. Purpura fulminans: recognition, diagnosis and management. *Arch Dis Child*. 2011;96(11):1066-1071.
4. Marlar RA, Neumann A, eds. *Neonatal Purpura Fulminans due to Homozygous Protein C or Protein S Deficiencies*. 1990. Seminars in thrombosis and hemostasis. Copyright© 1990 by Thieme Medical Publishers Inc.
5. Millar DS, Johansen B, Berntorp E, et al. Molecular genetic analysis of severe protein C deficiency. *Hum Genet*. 2000;106(6):646-653.
6. Li X, Zhuang Y, Kang L, Ju X. Genotypic and phenotypic character of Chinese neonates with congenital protein C deficiency: a case report and literature review. *Thromb J*. 2019;17:19.
7. Fisher CL, Greengard JS, Griffin JH. Models of the serine protease domain of the human antithrombotic plasma factor activated protein C and its zymogen. *Protein Sci*. 1994;3(4):588-599.
8. Kizilocak H, Ozdemir N, Dikme G, Koc B, Celkan T. Homozygous protein C deficiency presenting as neonatal purpura fulminans: management with fresh frozen plasma, low molecular weight heparin and protein C concentrate. *J Thromb Thrombolysis*. 2018;45(2):315-318.
9. Su K, Zhang H, Fang W, et al. Protein C deficiency (a novel mutation: ala291Thr) with systemic lupus erythematosus leads to the deep vein thrombosis. *Blood Coagul Fibrinolysis*. 2018;29(8):714-719.
10. Frye KL, McMaster BC, Tomecsek KJ. Purpura in the emergency room: two cases of sepsis with cutaneous manifestations. *Cureus*. 2019;11(6):e4976.
11. Liu Y, Wei X, Kong X, et al. Targeted next-generation sequencing for clinical diagnosis of 561 Mendelian diseases. *PloS One*. 2015;10(8):e0133636.
12. Kiezun A, Stitziel NO, Sunyaev SR. Computational and statistical approaches to analyzing variants identified by exome sequencing. *Genome Biol*. 2011;12(9):227.
13. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;7(8):575-576.
14. Hecht M, Bromberg Y, Rost B. Better prediction of functional effects for sequence variants. *BMC Genomics*. 2015;16(8):1-12.
15. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb viewer: an environment for comparative protein modeling. *Electrophoresis*. 1997;18(15):2714-2723.
16. Berka K, Hanák O, Sehnal D, et al. MOLE online 2.0: interactive web-based analysis of biomacromolecular channels. *Nucleic Acids Res*. 2012;40(W1):W222-W227.
17. Capriotti E, Fariselli P, Casadio R. I-Mutant2. 0: predicting stability changes upon mutation from the protein sequence or structure. *Nucleic Acids Res*. 2005;33(suppl_2):W306-W310.
18. Al Harbi MS, El-Hattab AW. Protein C deficiency caused by a novel mutation in the PROC gene in an infant with delayed onset purpura fulminans. *Case Rep Dermatol Med*. 2017;2017:8915608.
19. Schmidt AE, Padmanabhan K, Underwood MC, Bode W, Mather T, Bajaj SP. Thermodynamic linkage between the S1 site, the Na^+ site, and the Ca^{2+} site in the protease domain of human activated protein C (APC): sodium ion in the APC crystal structure is coordinated to four carbonyl groups from two separate loops. *J Biol Chem*. 2002;277(32):28987-28995.
20. Masson E, Zou WB, Génin E, et al. Expanding ACMG variant classification guidelines into a general framework. *Hum Genomics*. 2022;16(1):31.
21. Alotaibi MD, Albakri AS, Alsulaiman SM. Pediatric retinal detachment in homozygous protein C deficiency: genetic and phenotypic description of a single family. *Ophthalmic Surg Lasers Imaging Retina*. 2022;53(5):293-296.
22. Yue Y, Liu S, Han X, et al. Pathogenic variants of PROC gene caused type I activity deficiency in a familial Chinese venous thrombosis. *J Cell Mol Med*. 2019;23(10):7099-7104.

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