## Compound heterozygous protein C deficiency with pulmonary embolism caused by a novel PROC gene mutation

### Case report and literature review

Zhaorui Zhang, MD\(^a\), Zhen Yang, MD\(^a\), Mei Chen, MD\(^b\), Yuzhu Li, MD, PhD\(^c\)\(^*\)

### Abstract

**Rationale:** Protein C is an anticoagulation agent, and protein C deficiency results in vascular thrombosis disease. Hereditary protein C deficiency is a risk factor for pulmonary embolism in adults. Pathogenic variants of the Protein C, Inactivator Of Coagulation Factors Va And VIIIa (PROC) gene which encodes protein C have been identified as a cause of protein C deficiency.

**Patient concerns:** We describe a patient with a novel mutation in the PROC gene who was diagnosed with pulmonary embolism in a Chinese family.

**Diagnosis:** According to the results of the pulmonary computed tomography angiography (CTA) and the level of blood protein C, the patient was diagnosed with pulmonary embolism caused by protein C deficiency.

**Interventions:** Whole-exome sequencing (WES) was performed for the molecular analysis.

**Outcome:** The results of patient's deoxyribonucleic acid revealed a heterozygous mutation (c.237 + 5G > A) in intron 3 of the PROC gene. His father also harbored the same mutation in the PROC gene. We also reviewed the protein C deficiencies caused by PROC gene mutations in cases.

**Lessons:** A novel mutation in intron 3 of PROC gene has not been previously reported in patients with pulmonary embolism caused by protein C deficiency. After anticoagulation therapy, the patient recovered, and CT showed resolution of the thrombosis. Pulmonary embolism may be caused by protein C deficiency and the rare compound heterozygous mutation in intron 3 of the PROC gene could cause protein C deficiency via impairment of the secretory activity of protein C.

**Abbreviation:** PC = protein C.

**Keywords:** compound heterozygous mutation, protein C deficiency, pulmonary embolism

### 1. Introduction

Pulmonary embolism (PE) is responsible for more than 100,000 cardiovascular disease-related deaths annually in the United States and is the third leading cause of cardiovascular mortality.[1] It has been estimated that only 7% of the patients with PE who died were correctly diagnosed before death.[2] Genetic abnormalities in proteins involved in the coagulation pathway leading to hypercoagulability have been found to be the cause of thrombophilia disease. Factor V Leiden (FV Leiden) and prothrombin G20210A gene mutations are highly prevalent in the Caucasian population. In contrast, in the Asian population, protein C (PC) and protein S (PS) deficiencies have a higher prevalence.[3] PC is a vitamin K-dependent plasma zymogen that is synthesized mainly in the liver and plays an important role in anticoagulation and fibrinolysis by inactivating the blood coagulation factors Va and VIIIa.[4] Protein C is encoded by the Protein C, Inactivator Of Coagulation Factors Va And VIIIa (PROC) gene on chromosome 2q13–q14 and is composed of 9 exons and 8 introns.[5] Several studies have reported the spectrum of PROC mutation, and at least 300 mutations have been reported to date.

Here, we reported a patient diagnosed with pulmonary thromboembolism associated with protein C deficiency caused by a novel PROC gene mutation that has not been previously reported before.

---

*Correspondence: Yuzhu Li, Department of Respiration, The Hainan Hospital of PLA General Hospital, Hainan Province, People's Republic of China.*

Received: 2 June 2022 / Accepted: 7 September 2022

http://dx.doi.org/10.1097/MD.0000000000031221
2. Method

2.1. Ethical approval and consent for publication

The PROC gene mutation analysis was performed in accordance with the ethical committee of the PLA General Hospital. Peripheral blood samples were also obtained from his parents after obtaining consent for deoxyribonucleic acid analysis.

2.2. Detection of mutation

We use whole-exome sequencing (WES) in panel to determine the possible mutations. If possible pathogenic mutations were found, his parents mutations wound be identified.

2.3. Review of literature

We searched PubMed for PROC gene mutations and protein C deficiency in English. Articles that lacked genetic analysis and basic patient information were excluded, and 26 studies were included in our study.[6–31]

3. Case report

A 23-year-old man was admitted to Hainan Hospital with headache for 24 days and hemoptysis for 4 days in 2018-3-12. The patient had headaches since February 2018 and hemoptysis since 2018-3-8. The patient was previously healthy and had a negative medical history. Magnetic resonance imaging of the brain was normal, and ultrasonography of both cephalic veins showed thrombosis.

Physical examination revealed a temperature of 37.1 °C, pulse of 88 beats/minute, respiratory rate of 20 breaths/minute, and blood pressure of 130/80 mm Hg. The patient's state of consciousness was poor. Crackles were heard in both lungs. His heart rate was normal, and no murmur was heard in the auscultation area of each valve. The abdomen was soft with no tenderness or rebound pain, and the liver and spleen ribs were not observed. Muscle strength was normal, and meningeal stimulation was negative.

Laboratory tests showed the following results: white blood cell (WBC), 9.48 × 10^9/L (normal range: 3.5–10^10^9/L), neutrophils: 0.747 (normal range: 0.4–0.75), platelets: 507 × 10^9/L (normal range:100–30010^9/L), C-reactive protein 13.15 mg/dL (normal range:0–8 mg/L), interleukin-6 34.82 pg/mL (0.373–0.463 pg/mL), procalcitonin 0.040 ng/mL (normal range: <0.5 ng/mL), D-dimer: 3654 ng/mL (normal range <500 ng/mL), international normalized ratio (INR):1.46 (normal range: 0.8–1.5), prothrombin time, 15.7 s (11–13 s), fibrinogen level: 7.16 g/L (normal range: 2–4 g/L). We further performed computed tomography angiography (CTA) of the pulmonary artery, which revealed an embolism in the middle segment of the left pulmonary artery (Fig. 1).

For the patients, a young male adult with no history of illness, we further tested the anticoagulation factors, protein C, and protein; functional protein S (PS) activity of 112.4% (normal range: 60%–130%) and PC activity of 44.6% (normal range: normal range: 70%–140%) were measured using the StaClot (Diagnostic Stago Inc, Parsippany, NJ) PS and PC activity assay, which indicated a diagnosis of PC deficiency.

Molecular analysis was performed by targeting inherited diseases presenting as vasculitis or coagulopathy using next-generation sequencing. A heterozygous pathogenic variant located on intron 3 of the PROC gene (c.237 + 5G > A(2q14|NM-000312)) were identified (Fig. 2). His father was also a carrier of the c.237 + 5G > A mutation, while his mother was normal without a mutation in the PROC gene. Therefore, we deduced that the mutation was inherited from her father (Fig. 3).

The patient was treated with low-molecular-weight heparin (LMWH) anticoagulation at 14,000 IU per day (100 IU/kg) based on a weight of 70kg. One month after treatment with LMWH, hemoptysis and chest pain were relieved. Subsequently, rivaroxaban (20 mg per day) was administered. The patient was discharged after taking rivaroxaban for 15 days without any adverse effects and continued taking rivaroxaban out of hospital. The symptoms of headache and hemoptysis disappeared, and reexamination of CTA of the pulmonary artery showed that the embolism had disappeared (Fig. 4). We followed up the patient monthly for 1 year, and no symptoms of thrombosis occurred.

4. Discussion and Conclusions

PC plays an important roles in anticoagulation and fibrinolysis and is often associated with pulmonary embolism. The incidence of heterozygous protein C deficiency is approximately 0.14% to 0.50% of the general population,[32] and homozygote and compound heterozygote for PROC gene mutation are rare disorders (prevalence approximately 1 per 200,000–400,000 individuals). There have been only been a few case reports of pulmonary embolism caused by PROC gene mutation.[14,33] In this case, we reported the case of an adult male with pulmonary embolism with a novel mutation in the PROC gene that had not been previously reported before. We further tested the genetic condition of the patients. His father had the same mutation;
however, he did not show any evidence of deep venous thrombosis or pulmonary embolism. Therefore, we deduced that the intron 3 mutation (c.237+5G > A) in the PROC gene may be an autosomal recessive mutation, and patients with this mutation may or may not present with protein C deficiency.

More than 300 mutations that disrupt protein C levels have been identified. Our article reviewed 26 cases showing that protein C deficiency with a PROC mutation is rare (Table 1). These cases include 15 infants most of whom had homozygous mutation with poor prognosis. The symptoms of them often present as severe purpura fulminans.\cite{8,11,12,17,20-22,27,28,31}

These PROC mutations are located in exons 4 to 9 and intron 8. Exons 7 and 9 were most frequent involved, most of the mutations were point mutations, 5 cases showed a deletion mutation of the PROC gene,\cite{8,9,15,17,21} one case showed a frameshift mutation of duplicated of c246_247.\cite{24} The mutation in 20 cases were mutation in exons, which can directly change the expression of proteins. For example, changes in c.1152C > G leads to p.N384K (replacement of asparagine by lysine) and c.1207G > T leads to p.G403W (glycine by tryptophan).\cite{10}

Changes in the homozygous missense mutation, c.1198G > A leads to the replacement of Gly with Ser in the 400 amino acid.\cite{11} However, not all cases had exon mutations; 1 case reported that a patient suffered from deep venous thrombosis at the age of 26 years, and genetic analysis showed a transition at nucleotide 7054 in intron 7 (7054G to A). The 7054 mutation G to A caused the amplified exon 8 fragment to cause the R87H mutation\cite{19} and led to protein C deficiency and deep venous thrombosis. Interestingly, our case also had

**Figure 2.** The patient with compound heterozygous mutations (c.237+5G > A).

**Figure 3.** Family genetic analysis of the patient and his parents.

**Figure 4.** CTA of the pulmonary artery. CTA = computed tomography angiography.
a mutation of introns in the PROC gene, resulting in protein C deficiency. This suggests that introns may not be nonsense gene sequences; they may affect the expression of upstream and downstream exons. However, the exact mechanism of intron 3 mutation (c.237 + 5G > A) of the PROC gene requires further study to reveal its function.

In conclusion, we identified a novel heterozygous mutation in the PROC gene in a patient with protein C deficiency, which has not been previously reported. The patient was treated with LMWH and rivaroxaban for anticoagulation, with good progress. This article provide a more complete gene mapping with PROC gene in Chinese population. We hope that our report will further assist other clinicians in diagnosing and treating this rare disease.

Acknowledgements
The authors gratefully acknowledge the patient and his family for allowing us to share their information.

Author contributions
Zhaorui Zhang participated in the writing of the paper. Yuzhu Li participated in the study design of the work. Zhen Yang participated in the primary data acquisition and clinical analysis. Mei Chen participated in the genetic analysis of patients.

Conceptualization: Zhaorui Zhang, Zhen Yang, Yuzhu Li.

Data curation: Zhaorui Zhang, Zhen Yang, Mei Chen, Yuzhu Li.

Formal analysis: Zhen Yang, Yuzhu Li.

Investigation: Zhen Yang, Yuzhu Li.

Methodology: Zhen Yang, Yuzhu Li.

Writing – original draft: Zhaorui Zhang.

Writing – review & editing: Zhaorui Zhang.

References
[1] Martinez Licha CR, McCurdy CM, Maldonado SM, et al. Current management of acute pulmonary embolism. Ann Thorac Cardiovasc Surg. 2020;26:65–71.
[2] Zhang R, Kobayashi T, Pugliese S, et al. Interventional therapies in acute pulmonary embolism. Interv Cardiol Clin. 2020;9:229–41.
[3] Anghiasusksiri P. Venous thromboembolism in Asia – an unrecognised and under-treated problem. Thromb Haemost. 2011;106:585–90.
[4] Reda S, Ruhl H, Wirkowski J, et al. PC deficiency testing: thrombin-thrombomodulin as PC activator and aptamer-based enzyme capturing increase diagnostic accuracy. Front Cardiovasc Med. 2021;8:755281.
[5] Cooper PC, Hill M, Maclean RM. The phenotypic and genetic assessment of protein C deficiency. Int J Lab Hematol. 2012;34:336–46.
[6] Deng MY, Liu ZX, Huang HF, et al. Two novel compound heterozygous mutations associated with types I and II protein C deficiency with unusual phenotypes. Thromb Res. 2016;145:93–9.
[7] Kim HJ, Kim DK, Koh KC, et al. Severe protein C deficiency from compound heterozygous mutations in the PROC gene in two Korean adult patients. Thromb Res. 2008;123:412–7.

Table 1
Genetic features of patients with PROC.

| Case | Age | Gender | Variant in PROC | Positions |
|------|-----|--------|-----------------|----------|
| 1†   | 22  | Male   | c.237 + 5G > A  | Intron 3 |
| 2†   | 38  | Male   | c.580C > T      | Exon 7   |
|      |     |        | c.970G > A      | Exon 9   |
|      |     |        | c.820G > T      | Exon 9   |
|      |     |        | c.898G > C      | Exon 9   |
| 3†   | 27  | Male   | G-to-C codon 297| Exon 9   |
|      | 51  | Male   | C-to-T codon 210| Exon 9   |
| 4†   | 7   | NM     | T > C Promoter -1504| NM   |
|      | Birth|NM    | c.340_346delinsATGCC| NM |
|      | Birth|NM    | c.829G > A      | NM      |
| 5†   | 9   | Male   | c.577_579delAAAG| Exon 7   |
| 6†   | 45  | Male   | Heterozygous    | NM      |
|      |     |        | c.1152 A > C    | NM      |
|      |     |        | c.1207 G > T    | NM      |
| 7††  | 8   | Male   | c.1198G > A     | Exon 7   |
| 8††  | 10  | NM     | 6246 G > A      | Exon 7   |
|      | 3   | NM     | Homozygous,3156, del C| Exon 5 |
| 9††  | 40  | Male   | c.1015G > A     | Exon 9   |
|      |     |        | c.577-579delAAAG| Exon 7   |
| 10†† | 40  | Male   | Homozygous c.796 + 3A > T| Intron 8|
| 11†† | 6   | Male   | Homozygous, deletion GCGGGGAGGCT between nucleotides 7173–7182| Exon 8 |
| 12†† | 2   | Female| Heterozygous c.949C > T| Exon 9   |
| 13†† | 19  | Male   | 7054G > A       | Exon 7   |
| 14†† | 26  | Male   | 335 GAC > TAC,  | Exon 4   |
|      | 16  | Female| c.574_576delAAAG| Exon 7   |
| 15†† | 10  | Female| Homozygous c.346G > T| NM    |
| 16†† | 3   | Female| Heterozygous c.1015G > A| Exon 9 |
|      | 20††| Male   | c246_247dupCT   | NM      |
| 21†† | 28  | Male   | g.7271G > A     | Exon 5   |
| 22†† | 63  | Male   | c8516T          | Exon 9   |
| 23†† | 10  | Male   | Homozygous T903C | NM      |
| 24†† | 6   | Female| Homozygous c.1048A > T codon 350| NM R|
|      |     |        | Heterozygous 715_724delGGGCGATGC| Exon 8 |
|      |     |        | Homozygous (A-G)-12| NM    |
|      |     |        | (C > T) codon 253| exon 8  |

NM = not mentioned in the article, PROC = Protein C, Inactivator Of Coagulation Factors Va And VIl a.

* Case one is from this study.
[8] Barg AA, Dardik R, Levin C, et al. Severe protein C deficiency due to novel biallelic variants in PROC and their phenotype correlation. Acta Haematol. 2021;144:327–31.

[9] Wang V, Vo KH, Mahajerin A. Qualitative protein C deficiency due to PROC c.577_579delAAG mutation not detected by chromogenic assays: a case of intractable cerebral sinovenous thrombosis. Pediatr Blood Cancer. 2019;66:e27443.

[10] 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.

[11] Pai N, Shetty S, Ghosh K. Protein C (PROC) gene mutations in two Indian families with purpura fulminans. Ann Hematol. 2010;89:833–6.

[12] Xie W, Liu Z, Chen B. Protein C deficiency resulting from two mutations in PROC presenting with recurrent venous thromboembolism. J Vasc Surg Cases Innov Tech. 2017;3:254–6.

[13] Al-Hamed MH, AlBatniji F, AlDakheel GA, et al. Molecular characterization of novel splice site mutation causing protein C deficiency. Blood Coagul Fibrinolysis. 2016;27:585–8.

[14] Sun L, Li X, Li Q, et al. Multiple arterial and venous thromboembolism in a male patient with hereditary protein C deficiency: a case report. Medicine (Baltim). 2021;100:e25575.

[15] Devi RU, Bharathi SM, Kawankar N. A novel protein C mutation caus- ing neonatal purpura fulminans. Indian Pediatr. 2016;53:1019–21.

[16] Hong J, Ahn SY, Lee YJ, et al. Updated recommendations for the treatment of venous thromboembolism. Blood Res. 2021;56:6–16.

[17] Ding Q, Shen W, Ye X, et al. Clinical and genetic features of protein C deficiency in 23 unrelated Chinese patients. Blood Cells Mol Dis. 2013;50:53–8.

[18] Ichiyama M, Ohga S, Ochiumi M, et al. Fetal hydrocephalus and neonatal stroke as the first presentation of protein C deficiency. Brain Dev. 2016;38:253–6.

[19] Martin G, Thomas MA, Wei XC, et al. Diffuse intracerebral hemorrhage in an infant with a novel homozygous variant leading to severe protein C deficiency. J Pediatr Hematol Oncol. 2021;43:e763–5.

[20] Ishimura M, Saito M, Ohga S, et al. Fulminant sepsis/meningitis due to Haemophilus influenzae in a protein C-deficient heterozygote treated with activated protein C therapy. Eur J Pediatr. 2009;168:673–7.

[21] Park HJ, Song KS, Nah BM, et al. Homozygous type I protein C deficiency in neonatal purpura fulminans with a novel frameshift deletion of 10 base pairs in exon 8 of PROC gene. J Thromb Haemost. 2005;3:593–5.

[22] Yuan X, Li C, Chen X, et al. A study of congenital protein C deficiency with infancy onset of CADASIL in a Chinese baby. J Pediatr Hematol Oncol. 2019;41:e210–5.