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ORIGINAL RESEARCH

# Prevalence, Genetic Background, and Clinical Phenotype of Congenital Thrombophilia in Chronic Thromboembolic Pulmonary Hypertension

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## ABSTRACT

**BACKGROUND** The role of congenital thrombophilia in chronic thromboembolic pulmonary hypertension (CTEPH) remains unresolved.

**OBJECTIVES** The purpose of this study was to investigate the prevalence, genetic background, and clinical phenotype of congenital thrombophilia in CTEPH.

**METHODS** In total, 367 patients with CTEPH from May 2013 to December 2020 were consecutively enrolled in this cross-sectional study in FuWai Hospital and Peking Union Medical College Hospital in China. The primary outcome was the occurrence of congenital thrombophilia diagnosed through tests for congenital anticoagulants activity (including protein C, protein S, and antithrombin III), factor V Leiden and prothrombin G20210A sequence variants. Next-generation sequencing was conducted for patients with congenital thrombophilia. Clinical phenotype was compared between patients with and without thrombophilia.

**RESULTS** A total of 36 (9.8%; 95% CI: 6.8%-12.9%) patients were diagnosed as congenital thrombophilia, including 13 protein C deficiency (3.5%; 95% CI: 1.6%-5.4%), 19 protein S deficiency (5.2%; 95% CI: 2.9%-7.5%), and 4 antithrombin III deficiency (1.1%; 95% CI: 0%-2.2%). No factor V Leiden or prothrombin G20210A sequence variants were identified. Genotype for patients with thrombophilia revealed that 10 (76.9%) protein C deficiency patients were PROC sequence variant carriers, 4 (21.1%) protein S deficiency were PROS1 sequence variant carriers, and 2 (50.0%) antithrombin III deficiency were SERPINC1 sequence variant carriers. In the logistic regression model, male sex (OR: 3.24; 95% CI: 1.43-7.31) and proximal lesion in pulmonary arteries (OR: 4.10; 95% CI: 1.91-8.85) had significant differences between the congenital thrombophilia and nonthrombophilia group in CTEPH patients.

**CONCLUSIONS** Congenital thrombophilia was not rare. Male sex and proximal lesion in pulmonary arteries might be the specific clinical phenotype for CTEPH patients with congenital thrombophilia. (JACC: Asia 2022;2:247–255) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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**ABBREVIATIONS AND ACRONYMS**

**AT III** = antithrombin III

**CTEPH** = chronic thromboembolic pulmonary hypertension

**PCD** = protein C deficiency

**PSD** = protein S deficiency

Chronic thromboembolic pulmonary hypertension (CTEPH) is a complex and life-threatening disease, with pathological characteristics of organized thromboemboli and persistent obstruction in pulmonary arteries.[1,2] CTEPH is considered to be a rare long-term complication of acute pulmonary embolism, with reported incidence of 0.4%-6.2%.[3,4]

Previous studies have shown that a history of recurrent or unprovoked pulmonary embolism is a risk factor for developing CTEPH. However, as an important risk factor for venous thromboembolism and its recurrences,[5] the relationship between congenital thrombophilia and CTEPH has not been confirmed yet.[6] Elevated clotting factor VIII levels[7] and positive lupus anticoagulant[8] are considered as risk factors for CTEPH, and the prevalence of fibrinogen abnormalities also increases in CTEPH patients.[9,10] But for more solid risk factors for venous thromboembolism, including protein C deficiency (PCD), protein S deficiency (PSD), antithrombin III (AT III) deficiency, factor V Leiden, and prothrombin G20210A sequence variants, there is a lack of correlation with CTEPH.[11-13] The prevalence of thrombophilia in CTEPH patients was comparable with normal control subjects. However, these studies are mainly based on small population groups, especially for PCD, PSD, and AT III deficiency, in which <50 patients completed anticoagulant activity testing. In addition, all evidence was from a Caucasian population, and the prevalence of congenital thrombophilia in venous thromboembolism had a huge ethnic diversity.[14] Considering the ethnic diversity and small size of the reported cohorts, the validity of these studies was limited, and it is inconclusive to infer the true prevalence of congenital thrombophilia for CTEPH.

Therefore, the aim of our study was to investigate the prevalence, genetic background, and clinical phenotype of major congenital thrombophilia (PCD, PSD, AT III deficiency, factor V Leiden, and prothrombin G20210A sequence variants) in a large patient population with CTEPH.

## METHODS

**STUDY DESIGN AND PATIENTS.** From May 2013 to December 2020, all patients diagnosed as CTEPH in FuWai Hospital and Peking Union Medical College Hospital were consecutively enrolled in this cross-sectional study. For the diagnosis of CTEPH,[15] 3 criteria had to be satisfied: 1) at least 3 months of effective anticoagulation, including warfarin or new oral anticoagulant drugs (Rivaroxaban or Dabigatran); 2) typical imaging characteristics of CTEPH assessed by computed tomography pulmonary angiography and/or direct pulmonary angiography; 3) confirmed precapillary pulmonary hypertension, defined as mean pulmonary artery pressure ≥25 mm Hg and pulmonary arterial wedge pressure ≤15 mm Hg. Patients unavailable for congenital anticoagulants activity test and genetic test for factor V Leiden and prothrombin G20210A sequence variant were excluded for analysis. The institutional review board of FuWai Hospital and Peking Union Medical College Hospital approved the study protocol, and each patient provided written informed consent.

The primary outcome was the occurrence of congenital thrombophilia diagnosed through the tests for congenital anticoagulants activity (including protein C, protein S, antithrombin III), factor V Leiden, and prothrombin G20210A sequence variants. Demographics, history of venous thromboembolism and recurrent venous thromboembolism, hemodynamic parameters, and other clinical parameters, including New York Heart Association functional class and N-terminal fragment of pro-brain natriuretic peptide, were collected and compared between patients with and without thrombophilia. Also, distribution of pulmonary artery lesions was described as previously reported.[8] Level I and II are considered as proximal lesions, while level III and IV are considered distal

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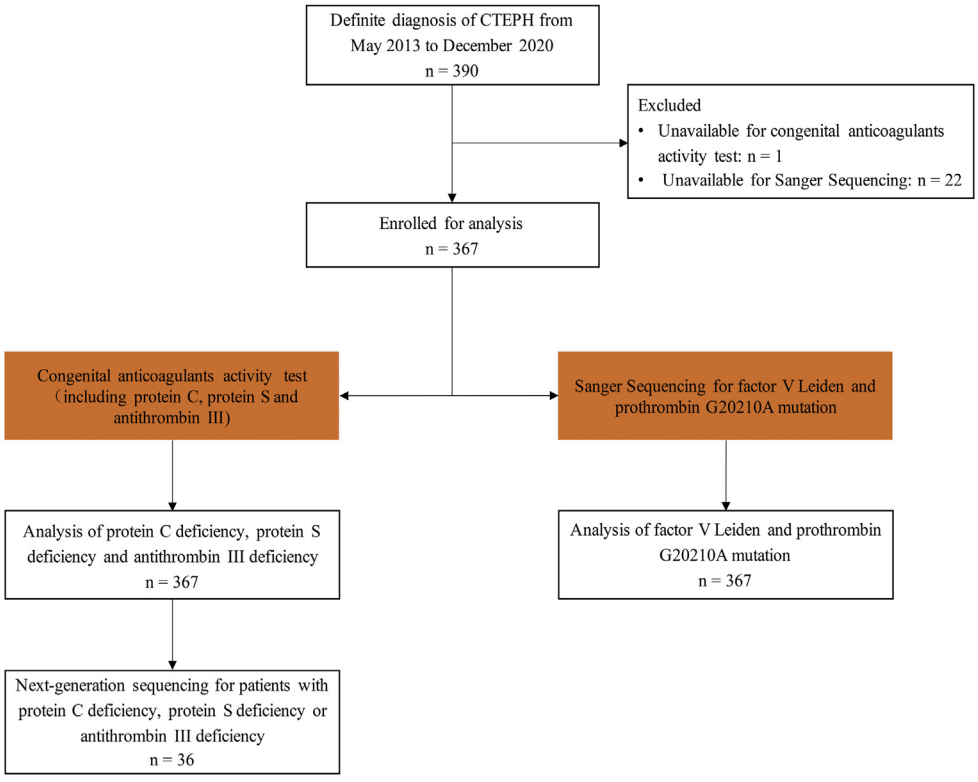
**FIGURE 1** Flow Chart




A total of 367 patients were enrolled for analysis. Of these, 36 patients diagnosed as congenital thrombophilia received next-generation sequencing. CTEPH = chronic thromboembolic pulmonary hypertension.

lesions. The final classification was according to the more proximal lesions in either left or right pulmonary arteries.

**CONGENITAL ANTICOAGULANT ACTIVITY TESTS AND NEXT-GENERATION SEQUENCING FOR PATIENTS WITH CONGENITAL ANTICOAGULANT DEFICIENCY.** Anticoagulation therapy with warfarin was replaced by novel oral anticoagulants at least 2 weeks before the anticoagulant activity test, which did not influence the activity of plasma anticoagulants. Congenital anticoagulant deficiency was defined by reduced anticoagulant activity below 2 SDs, precluding acquired factors, such as the application of warfarin and heparin, autoimmune diseases, malignancy, and pregnancy. The tests include screening for protein C activity (HemosIL Protein C, Instrumentation Laboratory Co), protein S activity (ProS, Instrumentation Laboratory Co, in FuWai Hospital; HemosIL Protein S Activity, Instrumentation Laboratory Co, in Peking Union Medical College Hospital), and antithrombin III activity (HemosIL Liquid Antithrombin, Instrumentation Laboratory Co). Protein C and antithrombin III were analyzed with chromogenic substrate assays, whereas protein S was determined with a clotting assay using ACL TOP 700 (Instrumentation Laboratory Co). The reference ranges were determined according to our laboratory data, and the tests were repeated twice at an interval of at least 1 week. At least 1 test was performed 1 month after a new venous thromboembolism event.[16,17]

All patients diagnosed as congenital anticoagulant deficiency would be genotyped through next-generation sequencing. The possible pathogenic variants would be identified in the reported thrombophilia-related gene, including PROC, PROS1, C4BPA, and SERPINC1. The detailed methods of next-generation sequencing and sequence variant analysis are shown in the Supplemental Methods.

**FACTOR V LEIDEN AND PROTHROMBIN G20210A SEQUENCE VARIANT TESTS.** Patients with a homozygous or a heterozygous sequence variant of factor V

**TABLE 1 Characteristics of CTEPH Patients With and Without Thrombophilia**

| | Overall CTEPH (n = 367) | With Thrombophilia (n = 36) | Without Thrombophilia (n = 331) | *P* Value[a] |
|---|---|---|---|---|
| Demographics | | | | |
| Age, y | 54.0 ± 14.9 | 50.8 ± 15.7 | 54.4 ± 14.8 | 0.174 |
| Male | 200 (54.5) | 28 (77.8) | 172 (52.0) | 0.003 |
| Clinical parameters | | | | |
| VTE history | 282 (76.8) | 35 (97.2) | 247 (74.6) | 0.002 |
| Age of first VTE event | 50.3 ± 15.4 | 47.6 ± 15.6 | 50.7 ± 15.7 | 0.299 |
| Recurrent VTE history | 29 (7.9) | 6 (16.7) | 23 (6.9) | 0.084 |
| NYHA functional class | | | | 0.923 |
| I/II | 140 (38.1) | 14 (38.9) | 126 (38.1) | |
| III/IV | 227 (61.9) | 22 (61.1) | 205 (61.9) | |
| NT-proBNP, pg/mL | 1,969 ± 2,316 | 2,419 ± 2,371 | 1,921 ± 2,305 | 0.222 |
| Hemodynamic parameters | | | | |
| RAP, mm Hg | 8.0 ± 4.7 | 8.0 ± 4.3 | 8.1 ± 4.7 | 0.911 |
| Mean PAP, mm Hg | 49.7 ± 12.4 | 46.1 ± 13.4 | 50.1 ± 12.2 | 0.066 |
| PAWP, mm Hg | 10.0 ± 2.9 | 9.1 ± 2.9 | 10.1 ± 2.9 | 0.055 |
| Cardiac index, L/min/m$^2$ | 2.5 ± 0.6 | 2.6 ± 0.6 | 2.5 ± 0.7 | 0.339 |
| PVR, WU | 9.4 ± 4.5 | 7.9 ± 3.9 | 9.6 ± 4.6 | 0.038 |
| $SaO_2$, % | 89.9 ± 5.2 | 90.6 ± 3.7 | 89.9 ± 5.3 | 0.461 |
| $SvO_2$, % | 62.6 ± 8.2 | 64.1 ± 7.0 | 62.4 ± 8.2 | 0.240 |
| Pulmonary artery lesions[b] | | | | <0.001 |
| Level I and II | 148 (41.1) | 25 (71.4) | 123 (37.8) | |
| Level III and IV | 212 (58.9) | 10 (28.6) | 202 (62.2) | |
| Treatment | | | | |
| PEA | 95 (25.9) | 12 (33.3) | 83 (25.1) | 0.283 |
| BPA | 169 (46.0) | 17 (47.2) | 152 (45.9) | 0.882 |

Values are mean ± SD or n (%). [a]The *P* value compares the differences between patients with and without anticoagulant deficiency. [b]Total 360 patients were examined with pulmonary angiography, with 35 patients with thrombophilia and 325 patients without thrombophilia.

BPA = balloon pulmonary angioplasty; CTEPH = chronic thromboembolic pulmonary hypertension; NT-proBNP = N-terminal fragment of pro-brain natriuretic peptide; NYHA = New York Heart Association; PAP = pulmonary artery pressure; PAWP = pulmonary artery wedge pressure; PEA = pulmonary endarterectomy; PVR = pulmonary vascular resistance; RAP = right atrial pressure; $SaO_2$ = arterial oxygen saturation; $SvO_2$ = mixed venous oxygen saturation; VTE = venous thromboembolism.

Leiden or prothrombin G20210A were diagnosed as having congenital thrombophilia.[18,19] Genomic DNA was extracted from patients' peripheral blood through salting out. The genetic test was performed by Sanger sequencing. The results of sequencing were analyzed by 2 experienced technicians (T-Y.L.). The primers and experimental conditions of the PCR are shown in the Supplemental Methods.

**STATISTICAL ANALYSIS.** Categorical variables were summarized using numbers (percentage) and compared using chi-square test or Fisher exact test. Continuous variables were summarized as mean ± SD and compared using unpaired Student's *t*-test. Logistic regression models were used to identify the risk factors of the primary outcome by estimating ORs of having congenital thrombophilia and 95% CIs. Univariate logistic regression models were estimated for the following factors: age; gender; venous thromboembolism and recurrent venous thromboembolism history; New York Heart Association functional class; N-terminal fragment of pro-brain natriuretic peptide; hemodynamics characteristics; and distribution of pulmonary artery lesions. A *P* value <0.05 was considered statistically significant. The statistical analyses were performed using SPSS version 23.0 (SPSS Inc).

## RESULTS

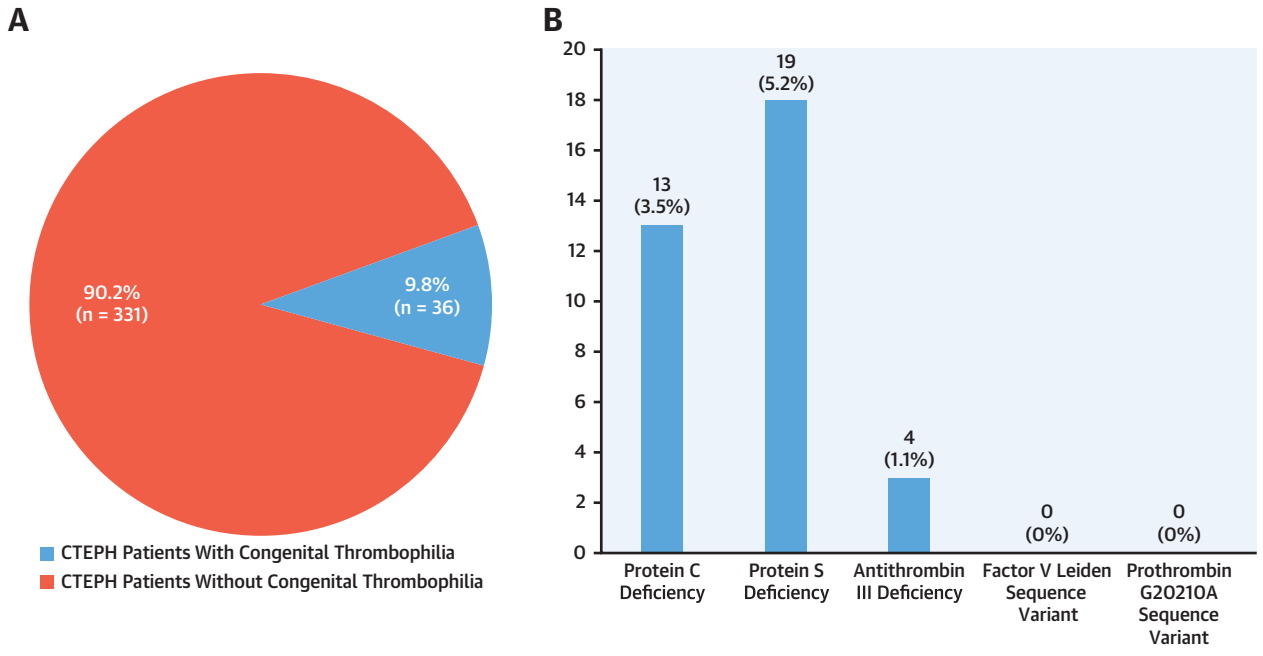
**STUDY PATIENTS.** Between May 2013 and December 2020, a total of 390 patients were diagnosed with CTEPH. Among them, 1 patient was unavailable for congenital anticoagulant activity test, and 22 patients were unavailable for genetic tests of factor V Leiden and prothrombin G20210A sequence variant, contributing to the 367 patients enrolled for analysis (Figure 1).

The clinical phenotypes of enrolled patients are shown in Table 1. The mean age was 54.0 ± 14.9 years, with slightly more male patients (54.5%). A total of 282 (76.8%) patients had a venous thromboembolism history, and 29 (7.9%) had recurrent thromboembolism events. These patients showed severely compromised hemodynamic parameters and impaired cardiac function.

**PREVALENCE OF CONGENITAL THROMBOPHILIA IN CTEPH.** Among the 367 CTEPH patients enrolled, a total of 36 (9.8%; 95% CI: 6.8%-12.9%) patients met the diagnostic criteria for congenital thrombophilia, including 13 PCD (3.5%; 95% CI: 1.6%-5.4%), 19 PSD (5.2%; 95% CI: 1.6%-5.4%) and 4 AT III deficiency (1.1%; 95% CI: 0%-2.2%) (Central Illustration). No carrier of factor V Leiden or prothrombin G20210A sequence variant was found.

**GENETIC BACKGROUND OF PATIENTS WITH CONGENITAL ANTICOAGULANT DEFICIENCY.** All 36 patients with congenital anticoagulant deficiency were genotyped with either whole-exome sequencing or whole-genome sequencing. Patients were screened for rare deleterious variants in the reported thrombophilia gene. The deleterious sequence variants of PROC were confirmed in 76.9% (10 of 13) PCD patients, including 9 heterozygotes and 1 homozygote. For PSD patients, rare deleterious variants of PROS1 or another PSD-causing gene like C4BPA were detected, and only 4 of 19 (21.1%) patients had rare deleterious variants identified in PROS1. All of them were heterozygote carriers. In the 4 patients with AT III deficiency sequenced, 2 (50.0%) had a heterozygote deleterious sequence variant in SERPINC1. Detailed information of gene sequence variants was shown in Table 2.

**CENTRAL ILLUSTRATION** **Prevalence of Congenital Thrombophilia in Patients With Chronic Thromboembolic Pulmonary Hypertension**




Lian T-Y, et al. JACC: Asia. 2022;2(3):247–255.

**(A)** Prevalence of overall congenital thrombophilia in chronic thromboembolic pulmonary hypertension patients. **(B)** Distribution of protein C deficiency, protein S deficiency, antithrombin III deficiency, factor V Leiden and prothrombin G20210A sequence variants in chronic thromboembolic pulmonary hypertension patients.

**COMPARISONS OF CLINICAL PHENOTYPES IN CTEPH PATIENTS WITH AND WITHOUT CONGENITAL THROMBOPHILIA.** Phenotypes of CTEPH patients between patients with and without congenital thrombophilia were compared (Table 1). There was no statistically significant difference in the age at diagnosis between the 2 groups (50.8 ± 15.7 years vs 54.4 ± 14.8 years; $P = 0.174$). However, thrombophilia occurred more frequently among male patients (77.8% vs 52.0%; $P = 0.003$). Additionally, patients with thrombophilia more frequently had a history of venous thromboembolism (97.2% vs 74.6%; $P = 0.002$) and showed less compromised hemodynamics with significantly lower pulmonary vascular resistance (7.9 ± 3.9 WU vs 9.6 ± 4.6 WU; $P = 0.038$). According to the results of pulmonary artery imaging classification, patients with thrombophilia had significantly more proximal lesions (level I and II) in pulmonary arteries (71.4% vs 37.8%; $P < 0.001$).

The results from the univariate regression analyses are presented in Table 3. In total, 5 factors reached statistical significance in univariate logistic model, including gender (OR: 3.24; 95% CI: 1.43-7.31; $P = 0.005$), venous thromboembolism history (OR: 11.90; 95% CI: 1.61-88.23; $P = 0.015$), recurrent venous thromboembolism history (OR: 2.68; 95% CI: 1.01-7.09; $P = 0.047$), pulmonary vascular resistance (OR: 0.90; 95% CI: 0.82-0.99; $P = 0.038$), and proximal lesion in pulmonary arteries (OR: 4.10; 95% CI: 1.91-8.85; $P < 0.001$).

## DISCUSSION

In this relatively large cohort with standardized diagnosis of both congenital thrombophilia and CTEPH, we revealed that the prevalence of congenital thrombophilia in CTEPH was 9.8%. Next-generation sequencing for patients with congenital thrombophilia reported that only one-half of them can be explained by known genetic defects. Gender, venous thromboembolism history, recurrent venous thromboembolism history, pulmonary vascular resistance, and proximal lesion in pulmonary arteries has a statistically significant difference between congenital thrombophilia and nondeficiency group in CTEPH.

**PREVALENCE OF CONGENITAL THROMBOPHILIA.** In the present study, we found that 9.8% of CTEPH patients experienced congenital thrombophilia. No

**TABLE 2 Detailed Information of Sequence Variants in Patients With Congenital Anticoagulants Deficiency**

| ID | Thrombophilia Type | Gene | Sequence Variant[a] | GnomAD_ALL[b] | GnomAD_EAS[b] | Reported Previously | FATHMM[c] |
|---|---|---|---|---|---|---|---|
| 1 | Protein C deficiency | PROC | c.C1032G:p.Y344X | Absent | Absent | – | NA |
| 2 | Protein C deficiency | PROC | c.G325C:p.G109R | Absent | Absent | – | D |
| 3 | Protein C deficiency | PROC | c.C118T:p.R40C | 0.000025 | Absent | – | D |
| 4 | Protein C deficiency | PROC | c.400+5G>A | Absent | Absent | Reitsma et al[31] | NA |
| 5 | Protein C deficiency | PROC | c.G632A:p.R211Q | Absent | Absent | Poort et al[32] | D |
| 6[d] | Protein C deficiency | PROC | c.C1010T:p.T337I | 0.000004 | Absent | Wu et al[33] | D |
| 7 | Protein C deficiency | PROC | c.C118T:p.R40C | 0.000025 | Absent | – | D |
| 8 | Protein C deficiency | PROC | c.570delG:p.M190fs | Absent | Absent | – | NA |
| 9 | Protein C deficiency | PROC | c.G664A:p.D222N | Absent | Absent | – | D |
| 10 | Protein C deficiency | PROC | c.G1218A:p.M406I | 0.000012 | 0.000174 | Miyata et al[34] | D |
| 11 | Protein S deficiency | PROS1 | c.G1424A:p.C475Y | Absent | Absent | – | D |
| 12 | Protein S deficiency | PROS1 | c.T1915G:p.C639G | Absent | Absent | Bustorff et al[35] | D |
| 13 | Protein S deficiency | PROS1 | c.C301T:p.R101C | 0.000025 | Absent | Boinot et al[36] | D |
| 14 | Protein S deficiency | PROS1 | c.74dupA:p.N25fs | Absent | Absent | Zhang et al[37] | NA |
| 15 | Antithrombin III deficiency | SERPINC1 | c.C856T:p.Q286X | Absent | Absent | – | NA |
| 16 | Antithrombin III deficiency | SERPINC1 | c.G951C:p.L317F | Absent | Absent | – | D |

[a]Abbreviations are in accordance with nomenclature guidelines as recommended by the Human Genome Variation Society (http://varnomen.hgvs.org). The letter "c." is used to indicate coding DNA, where nucleotide 1 is the A of the ATG translation initiation codon. The letter "p." is used to indicate change at the protein level. [b]The minor allele frequency in Genome Aggregation Database (GnomAD) (http://gnomad.broadinstitute.org/) (ALL), and in the East Asian cohort of GnomAD (EAS). [c]FATHMM was used for detrimental mutant decision of missense mutant. The letter "D" meant the mutants are likely detrimental. The stopgain, frameshift deletion, and mutant in splicing region did not have this score. [d]This patient is a homozygous carrier of sequence variant PROC c.C1010T.

patient with factor V Leiden or prothrombin G20210A sequence variant was detected. This prevalence is higher than the reported prevalence of congenital thrombophilia in the general Chinese Han population (4.93%),[20] and is similar to the prevalence in pulmonary embolism patients (7.1%).[21] According to previous studies, the relationship between congenital thrombophilia and CTEPH remained unclear. Multiple studies showed that in CTEPH patients, the prevalence of common congenital thrombophilia was comparable to the normal population or idiopathic pulmonary hypertension patients.[6,11-13] However, the cohort of CTEPH patients in these studies was small, especially for those who completed the test for anticoagulant protein activity, which were lower than 50 patients. So, the validity of those small cohorts was very limited. Moreover, according to a literature review,[14] the prevalence of congenital thrombophilia in venous thromboembolism has a huge ethnic diversity. Asian venous thromboembolism patients have a higher prevalence of PCD, PSD, and AT III deficiency, but lower prevalence of factor V Leiden and prothrombin G20210A sequence variants compared with Caucasian patients. Lian et al[21] reported that the prevalence of factor V Leiden and prothrombin G20210A sequence variants was 0.2% in Chinese pulmonary embolism patients, and Pepe et al[22] reported the factor V Leiden sequence variant was also found only in 1 (0.2%) venous thromboembolism patient from non-European populations. This is consistent with the conclusion we obtained in CTEPH patients. However, some large clinical trials about CTEPH have reported different conclusions. In CTEPH patients from Europe,[23] the prevalence of protein S deficiency, protein C deficiency, and antithrombin III deficiency were 9.6%, 8.9%, and 0.7%, whereas 7.7% of patients carried factor V Leiden sequence variants and 3.5% prothrombin gene sequence variants. In Japanese CTEPH patients,[24] the prevalence of protein C deficiency is 2.6% and protein S deficiency is 2.3%. Whether in Caucasian and Mongolian races or in Chinese Han people and Japanese, the significant discrepancy still exists on the prevalence of congenital thrombophilia and their subtype.

**GENETIC BACKGROUND OF CONGENITAL THROMBOPHILIA.** Since they were first recognized as inherited diseases in the 1980s,[25,26] AT III deficiency, PCD, and PSD were all considered to be autosomal dominant disorders. The pathogenic gene for AT III deficiency is SERPINC1 coding antithrombin, which has more than 250 reported detrimental sequence variants.[27] The pathogenic sequence variant of PCD is concentrated in the PROC, and more than 360 sequence variants have been reported.[28] The common pathogenic gene of PSD is PROS1, with more than 200 reported sequence variants, mainly missense sequence variants and small In/Del.[29] The gene C4BPA, which encodes the complement C4b-binding protein that binds to protein S, is believed to be related to a part of PSD patients without PROS1 sequence variants. However, these known sequence variants associated with congenital anticoagulants

deficiency could only explain 10%-70% of anticoagulant deficiency patients.[29] Therefore, we genotyped CTEPH patients diagnosed with congenital anticoagulant deficiency via next-generation sequencing and screened the reported pathogenic genes PROC, PROS1, SERPINC1, and C4BPA. Considering that all patients with thrombophilia have no family history of venous thromboembolism, we did not genotype their family members. The results revealed that <50% of patients had deleterious variants in these genes, and the proportions were especially low in patients with PSD and AT III deficiency. In addition to the known pathogenic genes, our study indicated that there would be other gene sequence variants decreasing the anticoagulant activity by affecting transcription, expression, or interaction with anticoagulant proteins. For patients who did not have pathogenic sequence variants in known genes, further bioinformatics analysis and a larger cohort of CTEPH patients for validation should be performed in future.

**CLINICAL PHENOTYPE OF CONGENITAL THROMBOPHILIA.** In our study, the proportion of male CTEPH patients with congenital thrombophilia was higher compared with the nondeficiency group, which was consistent with the result in patients with pulmonary embolism, as reported previously.[21] However, in the previous study with venous thromboembolism patients,[17,21] the thrombophilia group was usually younger, which was not consistent with our results in CTEPH patients. This difference might be subject to the complicated mechanism for the development of CTEPH from pulmonary embolism, and further investigations are needed. Previous history of venous thromboembolism was observed to be more frequent in patients with congenital thrombophilia. However, considering the wide CIs (OR: 11.90; 95% CI: 1.61-88.23), the data of venous thromboembolism history may not follow a normal distribution. There was a similar deficiency for pulmonary vascular resistance (95% CI: 0.82-0.99). The role of venous thromboembolism history and pulmonary vascular resistance would need to be verified in another large independent cohort. The results of pulmonary artery lesions classification for patients with or without thrombophilia were also unexpected. Patients with thrombophilia have more proximal lesions. This interesting finding might be due to the development of larger thrombi in the deep veins that terminate their transit in the proximal branches of the pulmonary arteries. It has been reported that there is a marked preference of pulmonary embolism for the right lung.[30] This result is similar to the conclusion of our previous study on antiphospholipid syndrome-positive CTEPH patients, another acquired thrombophilia.[8] However, this needs to be further elucidated.

**TABLE 3 Univariate Logistic Regression Analyses of Congenital Thrombophilia**

| | OR (95% CI) | *P* Value |
|---|---|---|
| Demographic characteristics | | |
| Age, y | 0.99 (0.96-1.01) | 0.175 |
| Gender, male vs female | 3.24 (1.43-7.31) | 0.005 |
| Clinical parameters | | |
| VTE history | 11.90 (1.61-88.23) | 0.015 |
| Recurrent VTE history | 2.68 (1.01-7.09) | 0.047 |
| NYHA functional class, III/IV vs I/II | 0.97 (0.48-1.96) | 0.923 |
| NT-proBNP, pg/mL | 1.00 (0.99-1.00) | 0.234 |
| Hemodynamic parameters | | |
| RAP, mm Hg | 1.00 (0.92-1.07) | 0.911 |
| Mean PAP, mm Hg | 0.97 (0.95-1.00) | 0.067 |
| PAWP, mm Hg | 0.89 (0.79-1.00) | 0.056 |
| Cardiac index, L/min/m$^2$ | 1.28 (0.77-2.14) | 0.339 |
| PVR, WU | 0.90 (0.82-0.99) | 0.038 |
| $SaO_2$, % | 1.03 (0.95-1.11) | 0.459 |
| $SvO_2$, % | 1.03 (0.98-1.08) | 0.240 |
| Pulmonary artery lesions, levels I and II vs levels III and IV[a] | 4.10 (1.91-8.85) | <0.001 |

Odds ratio and *P* value were calculated from logistic model analyses of congenital thrombophilia.
[a]Total 360 patients were examined with pulmonary angiography, with 35 patients with thrombophilia and 325 patients without thrombophilia.
Abbreviations as in Table 1.

**STUDY LIMITATIONS.** First, although we consecutively recruited patients from 2 referral pulmonary hypertension centers in China to enhance the representativeness of the sample, selection bias is still inevitable. Second, this is an exploratory observational study. Although we included more than 10 potential factors and performed logistic regression analysis, the results may be subject to possible confounding factors, false positive errors, and measurement bias. Third, for genetic testing, the results were limited to the disadvantage of next-generation sequencing. The large fragment deletion could not be detected accurately.

## CONCLUSIONS

The prevalence of congenital thrombophilia in CTEPH is 9.8%. Only one-half of them can be explained by known genetic defects. Male sex and proximal lesion in pulmonary arteries might be the specific clinical phenotype for CTEPH patients with congenital thrombophilia.

## FUNDING SUPPORT AND AUTHOR DISCLOSURES

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## PERSPECTIVES

**COMPETENCY IN MEDICAL KNOWLEDGE:** In this relatively large cohort with standardized diagnosis of both congenital thrombophilia and CTEPH, we confirmed that congenital thrombophilia was not rare and may be associated with male sex and proximal lesion in pulmonary arteries in CTEPH.

**TRANSLATIONAL OUTLOOK:** Our findings may provide strong evidence in the association of congenital thrombophilia with CTEPH. However, further studies focusing on the underlying mechanisms of patients with congenital thrombophilia developing CTEPH are still needed.

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**KEY WORDS** antithrombin III deficiency, chronic thromboembolic pulmonary hypertension, congenital thrombophilia, genotype, protein C deficiency, protein S deficiency

**APPENDIX** For an expanded Methods section, please see the online version of this paper.