A tale of *A1298C* mutation and recurrent pulmonary embolism: rare association

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Abstract

Pulmonary Embolism (PE) and Deep Venous Thrombosis (DVT) represent critical manifestations within the spectrum of Venous Thromboembolic Disease (VTE). The *MTHFR A1298C* gene mutation occurs in approximately 7% to 12% of individuals in North America, Europe, and Australia. It is less prevalent among Hispanic populations, with a frequency of 4% to 5%, and even lower in Chinese and Asian populations, where it ranges from 1-4%. A 50-year-old male with a history of recurrent PE and long-term anticoagulation, despite effective anticoagulation, was not getting relieved. Genetic testing revealed a heterozygous *MTHFR A1298C* mutation which is very rare and elevated serum homocysteine levels, contributing to a hypercoagulable state. Comprehensive evaluation and management are essential to prevent further thromboembolic events in such patients.

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Introduction

Pulmonary Embolism (PE) and Deep Venous Thrombosis (DVT) represent critical manifestations within the spectrum of Venous Thromboembolic Disease (VTE). PE occurs when a thrombus migrates from the venous circulation and becomes lodged in the pulmonary arterial system. The clinical presentation of acute PE varies significantly, ranging from asymptomatic and incidentally discovered cases to severe instances leading to immediate death.[1,2] As the third most common cardiovascular disorder, PE affects up to 5% of the population during their lifetime.[2] Recurrent venous thromboembolism, defined as events occurring after the initial course of adequate antithrombotic treatment, significantly contributes to the disease burden. The annual incidence of PE is 1 per 1000 individuals, escalating with age from 1.4 per 1000 people aged 40-49 to 11.3 per 1000 people aged 80 years or older. Approximately 30% of individuals experience recurrent VTE, resulting in an attack rate of up to 30 per 1000 person-years when considering both incident and recurrent events.[3]

Homocysteine, a sulfur-containing amino acid, is central to two metabolic pathways: remethylation to methionine, which requires folate and vitamin B12 (or betaine in an alternative reaction), and transsulfuration to cystathione, requiring pyridoxal-5'-phosphate.[4] Elevated levels of homocysteine (hyperhomocysteinemia) have been linked to an increased risk of atherosclerosis and, when combined with other thrombophilic risk factors, may contribute to various vascular occlusive pathologies.[5] The enzyme 5,10-Methylenetetrahydrofolate Reductase (MTHFR) plays a crucial role in the remethylation of homocysteine to methionine. MTHFR catalyzes the formation of 5-methyl tetrahydrofolate, the methyl donor in the methionine synthase reaction, which requires cobalamin (vitamin B12) as a cofactor. A homozygous deficiency in MTHFR is rare but is associated with severe hyperhomocysteinemia, developmental delays, neurological abnormalities, and vascular complications.[6]

The MTHFR gene has at least two significant polymorphisms: C677T and A1298C. The 677T allele is linked to reduced enzyme activity, lower serum and red blood cell folate levels, and mildly elevated plasma homocysteine concentrations. Although the A1298C polymorphism also affects MTHFR activity, it does not

cause significant biochemical changes. Normal MTHFR activity is essential for maintaining adequate levels of circulating folate and methionine and preventing homocysteine accumulation. Individuals who are double heterozygous for MTHFR C677T and A1298C polymorphisms exhibit lower enzyme activity compared to those with heterozygosity for either variant alone. The 677TT genotype results in approximately 30% of the enzyme activity seen in the 677CC genotype, while heterozygotes (677CT) retain around 65% of the enzymatic function.[7] This case report highlights the clinical course of a patient with recurrent PE, attributed to a rare heterozygous MTHFR A1298C gene mutation leading to hyperhomocysteinemia. This mutation induced a hypercoagulable state, emphasizing the need for careful monitoring and management of such patients to prevent recurrent thromboembolic events.

Case Report

A 50-year-old male presented with a sudden onset of shortness of breath, diffuse chest pain, and generalized body ache, persisting for the past 10 days. His past medical history includes a diagnosis of Pulmonary Tuberculosis (PTB) 20 years ago, for which he completed a six-month course of Antitubercular Therapy (ATT). In 2019, he experienced a Road Traffic Accident (RTA) that resulted in a femur fracture, which was managed conservatively. The patient has a significant history of recurrent PE, with documented episodes occurring in 2020 and 2021. He has been on long-term anticoagulation therapy with warfarin 5 mg daily since February 2021. He has no history of Hypertension (HTN) or Diabetes Mellitus (DM).

Upon presentation, a CT pulmonary angiography performed in January 2023 revealed multiple filling defects in the pulmonary arteries, including the right upper lobar artery, apical and posterior segmental branches, right lower lobar pulmonary artery, apical and lateral segmental branches, and the right middle lobar pulmonary artery and its medial and lateral segmental branches with tortious pulmonary artery vessels (Figure 1A, 1B) consistent with the diagnosis of PE. The first episode of PE was considered to be because of the RTA he had in 2019 but the cause of recurrent PE was still unknown, hence laboratory investigations were conducted to assess the underlying etiology of his recurrent thromboembolic

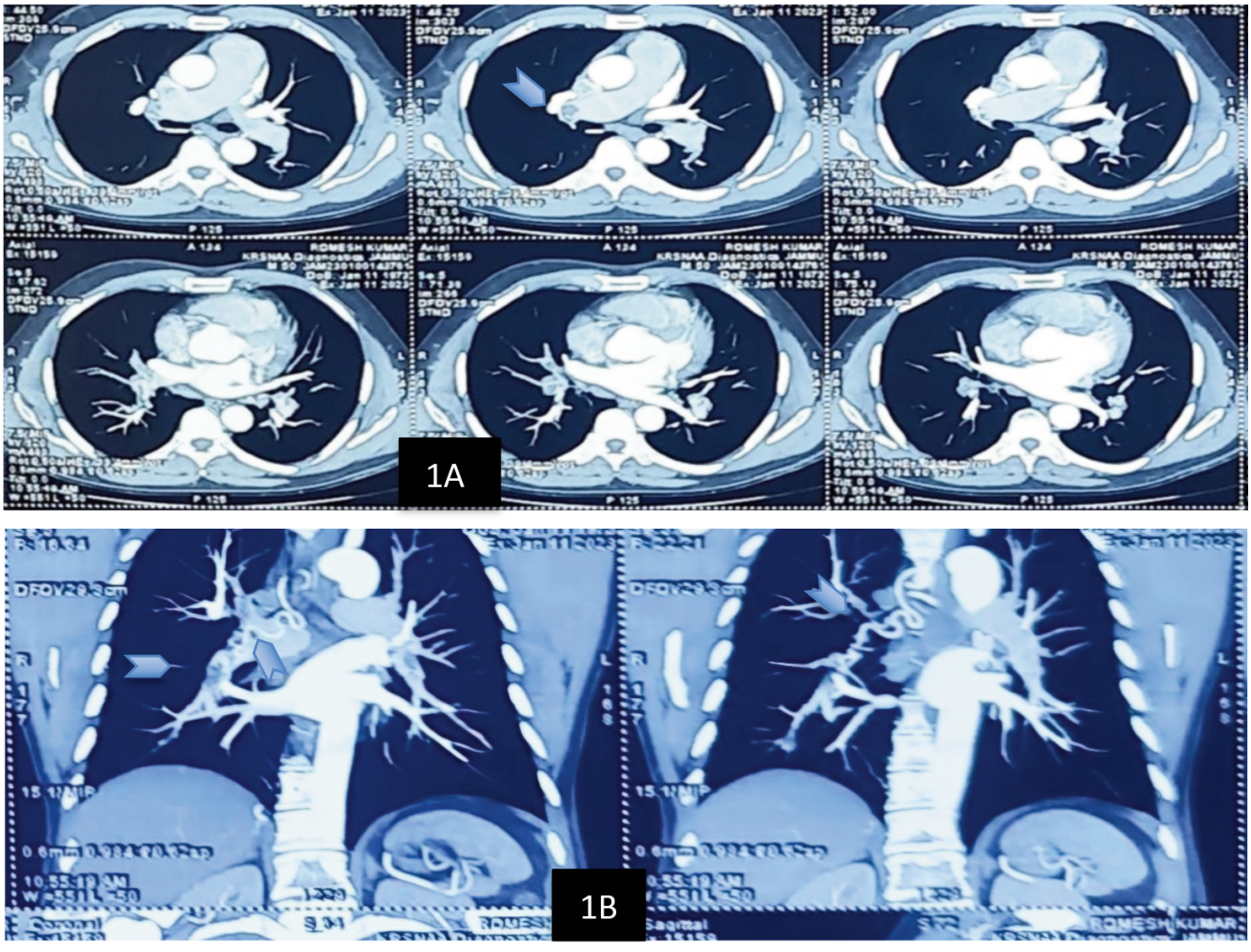


Figure 1. A) Computed Tomography (CT) pulmonary angiography (axial view) showing uptake defect in the right main pulmonary artery marked with an arrow. **B**) CT pulmonary angiography (coronal section) showing uptake defect in the right lower lobe segmental branches of the pulmonary artery marked with an arrow which is causing tortuosity of the right lower lobe segmental and subsegmental pulmonary artery.

events. Genetic testing revealed that the patient did not have Factor V Leiden mutation, or prothrombin gene mutation. The patient's protein C level was 141% (normal range: 70-140%), protein S level was 84% (normal range: 60-140%), and antithrombin level was 97% (normal range: 80-120%). Antiphospholipid Antibodies (APLA) were not detected.

Further laboratory results showed a D-dimer level of 0.65 µg/mL (normal <0.5 µg/mL), indicating ongoing fibrinolysis. His vitamin B12 level was 480 pg/mL, and vitamin B9 (folate) level was 10 pg/mL. His Prothrombin Time (PT) was 34.2 seconds, with an International Normalized Ratio (INR) of 3.19. Notably, the patient's serum homocysteine level was significantly elevated at 40 µmol/L (normal up to 15 µmol/L), indicating hyperhomocysteinemia, hence he was evaluated for MTHFR gene mutation. MTHFR C677C mutation was negative. However, a heterozygous MTHFR A1298C mutation was detected (Figure 2). A Doppler ultrasound of the bilateral lower limbs did not reveal any thrombus, and a 2D echocardiogram showed a left ventricular ejection fraction (LVEF) of 55-60% with no Right Atrium (RA) or Right Ventricle (RV) dilatation. The patient after a complete evaluation was discharged on tab apixaban 5mg twice daily and is called for six monthly evaluations for recurrent PE.

The elevated serum homocysteine levels, likely due to the detected heterozygous MTHFR A1298C mutation, contributed to a hypercoagulable state in this patient, despite adequate anticoagulation with warfarin. This case underscores the importance of considering genetic factors in patients with recurrent PE and highlights the need for comprehensive evaluation and management strategies to prevent further thromboembolic events.

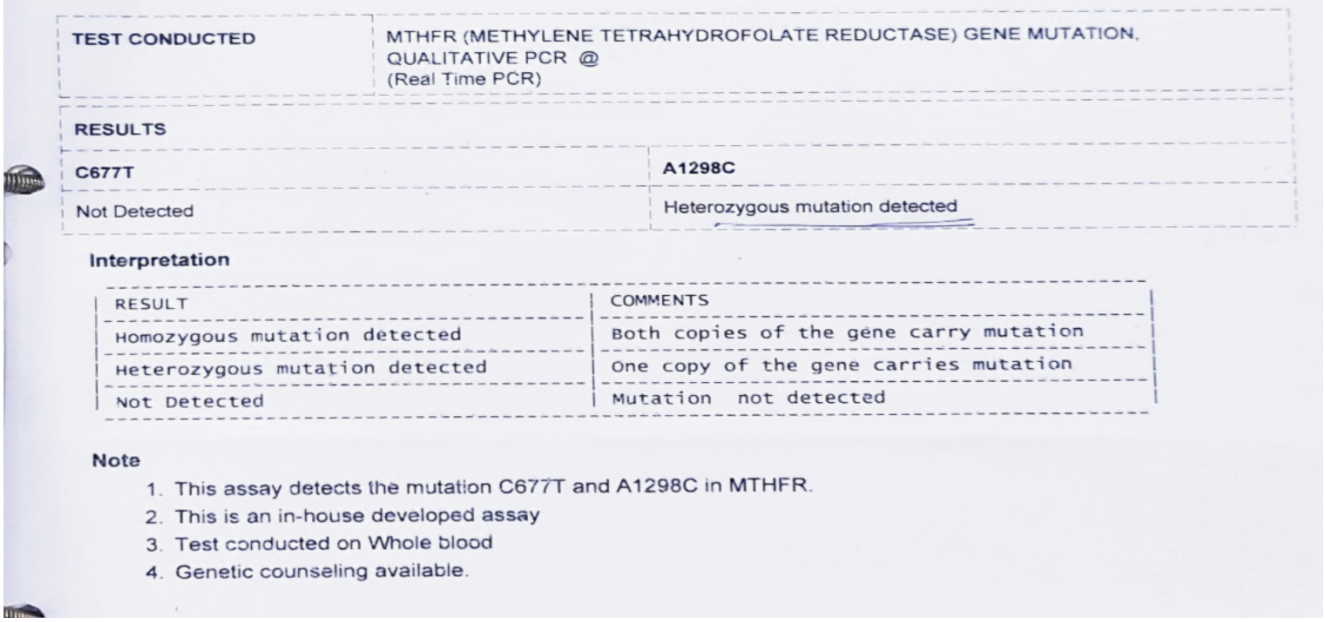
Discussion

This case report highlights the clinical complexity of recurrent PE in a patient with a heterozygous MTHFR A1298C mutation, leading to hyperhomocysteinemia and a resultant hypercoagulable state. The connection between elevated homocysteine levels and cardiovascular risk is well-documented, with Cortese *et al*.[6] noting that homocysteine, a thiol compound derived from methionine, is involved in two main metabolic pathways: the remethylation cycle, requiring folate and vitamin B12, and the transsulfuration pathway, requiring vitamin B6. These pathways illustrate the gene-environment interaction in homocysteine metabolism, where genetic defects or cofactor deficiencies can elevate homocysteine levels, contributing to vascular disease risk.

Cortese *et al*.[6] also discussed a common polymorphism in the gene coding for 5,10-Methylenetetrahydrofolate Reductase (MTHFR) (C677T), which is associated with decreased enzyme activity due to thermolability. Homozygosity for this variant (Val/Val) leads to mild-to-moderate hyperhomocysteinemia, a recognized independent risk factor for atherosclerosis. However, the heterozygous A1298C mutation, as seen in our patient, also affects MTHFR activity and can elevate homocysteine levels, albeit the impact may be less pronounced compared to the C677T variant. The genetic influence of MTHFR polymorphisms on homocysteine levels is modulated by factors such as sex and nutrient intake, particularly folate and vitamin B12.

In agreement with the findings of Eldibany *et al.,*[5] our patient's condition underscores the controversial yet significant association between hyperhomocysteinemia and thromboembolic events. While severe hyperhomocysteinemia is unequivocally linked to atherosclerosis, the effect of mild hyperhomocysteinemia is less clear. Coinheritance of MTHFR defects and other thrombophilic risk factors, such as Factor V Leiden, increases the risk of venous thromboembolism, highlighting the multifactorial nature of thrombotic disorders. In this case, despite the absence of Factor V Leiden and other common thrombophilic mutations, the presence of hyperhomocysteinemia due to the MTHFR A1298C mutation significantly contributed to recurrent PE.

Selhub *et al*.[4] emphasized the critical roles of folate and vitamin B12 in homocysteine metabolism through remethylation to methionine and transsulfuration to cystathionine, with S-adenosylmethionine coordinating these pathways. Disruptions in these metabolic routes can lead to hyperhomocysteinemia, a condition



TEST CONDUCTED	MTHFR (METHYLENE TETRAHYDROFOLATE REDUCTASE) GENE MUTATION, QUALITATIVE PCR @ (Real Time PCR)

RESULTS

C677T	A1298C
Not Detected	Heterozygous mutation detected

Interpretation

RESULT	COMMENTS
Homozygous mutation detected	Both copies of the gene carry mutation
Heterozygous mutation detected	One copy of the gene carries mutation
Not Detected	Mutation not detected

Note

1. This assay detects the mutation C677T and A1298C in MTHFR.
2. This is an in-house developed assay
3. Test conducted on Whole blood
4. Genetic counseling available.

Figure 2. Methylenetetrahydrofolate Reductase (MTHFR) gene mutation, qualitative (real-time) Polymerase Chain Reaction (PCR) report.

increasingly associated with vascular disease risk. This aligns with our patient's elevated homocysteine levels and recurrent thrombotic events, suggesting a disrupted metabolic balance exacerbated by the MTHFR mutation. Moreover, Brustolin *et al.*[7] reviewed the broader implications of hyperhomocysteinemia, linking it to various disorders, including vascular and neurodegenerative diseases, autoimmune disorders, and more. The prevalence of hyperhomocysteinemia in approximately 5% of the general population and its association with genetic variants in homocysteine metabolism, particularly MTHFR polymorphisms, underscore the clinical relevance of these findings. Our patient's elevated homocysteine level of 40 µmol/L, significantly above the normal range, highlights the metabolic dysregulation contributing to his recurrent thromboembolic events. Liu *et al.*[8] found that the MTHFR A1298C and C677T polymorphisms, particularly when both mutations are present, seem to be associated with an increased risk of VTE. The MTHFR Val/Val genotype has been found to exacerbate the rise of plasma Homocysteine (Hcy) in hemodialysis patients.[9] However, no significant effect of MTHFR polymorphism on plasma Hcy levels was observed in End-Stage Renal Disease (ESRD) patients undergoing hemodialysis with high-dose folate supplementation (2 grams per day).[10] Conversely, MTHFR polymorphism is an important predictor of plasma total Hcy and folate levels in kidney transplant recipients.[11] The MTHFR A1298C polymorphism has been associated with various health conditions globally, but its study in the Indian context remains limited. Zhang *et al.*[12] highlighted that this polymorphism is significantly related to schizophrenia and major depression in Asian populations, while Kumar *et al.* noted its association with elevated homocysteine levels, especially in individuals adhering to a vegetarian diet. The Minor Allele Frequency (MAF) of MTHFR A1298C in Kumar's cohort was 0.44, indicating a higher prevalence compared to other populations. Akash *et al.*[13] found that MTHFR A1298C was linked to increased male infertility in both South and North India, although their results were influenced by various extrinsic factors such as nutritional status and lifestyle. Despite these insights, research on MTHFR A1298C polymorphism in India has been relatively sparse, particularly regarding its association with recurrent Pulmonary Thromboembolism (PTE). Existing studies have linked this polymorphism to conditions such as Down syndrome and stroke within the Indian population, but there is a noticeable lack of research on its connection to recurrent PTE.[14,15] This gap in the literature highlights a critical area for future investigation. The association of this polymorphism with elevated homocysteine levels, as reported by Kumar *et al.*,[16] suggests a potential link to hyperhomocysteinemia-related conditions, including PTE. However, without specific studies focusing on this relationship, any such connection remains speculative. To address this gap, there is a pressing need for comprehensive studies across various regions in India to explore the potential impact of MTHFR A1298C polymorphism on recurrent PTE. Such research should consider the diverse genetic, nutritional, and lifestyle factors that characterize the Indian population. Understanding the role of MTHFR A1298C in conditions like recurrent PTE could have significant clinical and public health implications, given the high prevalence of this polymorphism in the Indian population. This would not only fill the existing gaps in the literature but also contribute to developing targeted interventions and management strategies for individuals with this genetic mutation.

Conclusions

The case underscores the importance of considering genetic and metabolic factors in patients with recurrent PE. The heterozygous MTHFR A1298C mutation and resultant hyperhomocysteinemia played a crucial role in this patient's thrombotic risk. Comprehensive management, including genetic counseling and tailored anticoagulation therapy, is essential to prevent recurrent events. This case also highlights the need for further research to elucidate the interactions between genetic polymorphisms, nutrient intake, and thrombotic risk, ultimately improving patient outcomes through personalized medical approaches.

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