

Full Length Research Paper

Giant aortic arch thrombus, methylenetetrahydrofolate reductase (MTHFR) A1298C heterozygous gene mutation, smoking and hormonal replacement therapy

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We report the case of a mobile aortic arch thrombus possibly induced by the combination of postmenopausal hormonal replacement therapy (HRT) and cigarette smoking in a woman with methylenetetrahydrofolate reductase (MTHFR) A1298C mutation. No other cause for her illness could be identified despite an extensive laboratory work-up for thrombophilic state. Surgical exploration showed the floating aortic arch thrombus attached on a histologically normal aortic wall. At an 8-year follow-up, she remained free of recurrence after discontinuation of HRT and counseling to quit smoking. The probable synergistic impact of tobacco smoking as an additional risk factor for thrombophilic events in women with MTHFR variant and using HRT has yet to be determined. Previous studies and case reports focusing on MTHFR variation and the incidence of thrombotic events have provided conflicting evidence of an association. With the understanding that this case does not yet ascribe cause-and-effect relationship between MTHFR variant and clot formation, important public health concerns are raised. The prevalence of MTHFR A1298C genotype is population-specific, implying that permissive gene-environment interactions other than genetic mutation alone may also be relevant in establishing a clinically overt disease. Causality remains to be proven in prospective evaluation across diverse geographic areas taking into account interactions with dietary and other life-style risk factors. Furthermore, in such genetically predisposed patients, future genome-wide association studies to identify loci variants that determine the overall susceptibility to thrombosis may prove helpful to derive preventive interventions.

Key words: Aortic arch thrombus, hormone replacement therapy, methylenetetrahydrofolate reductase (MTHFR) A1298C gene mutation, smoking.

INTRODUCTION

Thrombus formation in aortic arch is a devastating condition. Herein, we present such a case that was associated with methylenetetrahydrofolate reductase (MTHFR) A1298C genetic variant and normal homocysteine level. Does heterozygous MTHFR A1298C

allelic gene mutation without hyperhomocystenemia increase arterial thrombophilia? The answer to this question remains a subject of debate. The etiology of increased tendency to clotting is thought to be a multigene disorder (Seligsohn and Zivelin, 1997; Ehrenforth et al., 2004; Sacher, 1999), making this genotype variant as a cause of thrombus formation difficult to ascertain. Furthermore, such genetic variation is ethnic and population specific. For example, the overall prevalence of this allelic variant in population-based

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Figure 1. Transesophageal echocardiography long-axis view of the aortic arch showing large mobile thrombus along aortic wall.

United States (US) survey was 28.4% (Chang et al., 2009). In that study, the incidences across racial categories were 31.1, 17.9 and 18.8%, in White, Black, and Mexican American, respectively. In contrast, a study reported by Saltanpour et al. (2011) found the MTHFR allele in more than 38% of Iranian subjects irrespective of the presence of venous thrombosis.

The most common missense mutation identified in the MTHFR gene is the C to T substitution (C677T). Deficiency in this gene, an autosomal recessive disorder, leads to a reduced enzymatic function with a mild hyperhomocysteinemia and coronary artery diseases. Hyperhomocysteinemia has an important role in inducing hypercoagulability state on the venous system in the general population (Den Heijer et al., 1996). However, arterial clot formation in the less common heterozygous allelic mutation MTHFR A1298C has thus far not been reported. In this report, the role of the gene-environmental interaction for vascular damage in case of MTHFR mutation is also highlighted.

METHOD

Case presentation

A 51-year-old female presented with multiple recent bilateral cere-

bellar infarcts and found to have heterozygous methylenetetrahydrofolate reductase MTHFR A1298C. For three months prior to admission, she had been on oral daily postmenopausal HRT for symptoms control. Each tablet contains norethindrone acetate, ethinyl estradiol (1 mg/ 5 mcg). There was no personal or family history of venous or arterial thrombotic disease or coronary artery disease, hypertension, diabetes mellitus or hyperlipidemia, and she has never been on oral contraceptives.

The patient had no history of weight loss, trauma, and no clinical manifestations of inflammatory bowel disease, malignancy, cutaneous ulcers or nodules, tuberculosis, syphilis or vasculitis. She had 13 pack-years history of cigarette smoking and did not drink alcohol or use illicit drugs.

Transthoracic and transesophageal echocardiography demonstrated a large soft mobile echogenic mass with an irregular shape visualized in the aortic arch with absence of intramural hematoma (Figure 1). The heart rhythm, ventricular function and the cardiac valves were normal and no intracardiac source of emboli was identified. With the risk of embolization, and absence of angina symptoms, coronary catheterization was thought to be unwise and therefore was not performed. Computed tomography scans of the head, abdomen and pelvis did not reveal evidence of malignancy. The carotid ultrasound was unrevealing. Results of laboratory evaluation showed normal platelets count and no abnormalities of coagulation factor II, or V, and normal levels of protein C, protein S, and antithrombin III; tests for complement, anticardiolipin antibodies and lupus anticoagulant also yielded negative results. The erythrocyte sedimentation rate and the thyrotropin level were normal. Subsequent screening for thrombophilic state showed heterozygous mutation in gene encoding for 5, 10-methylenetetra-

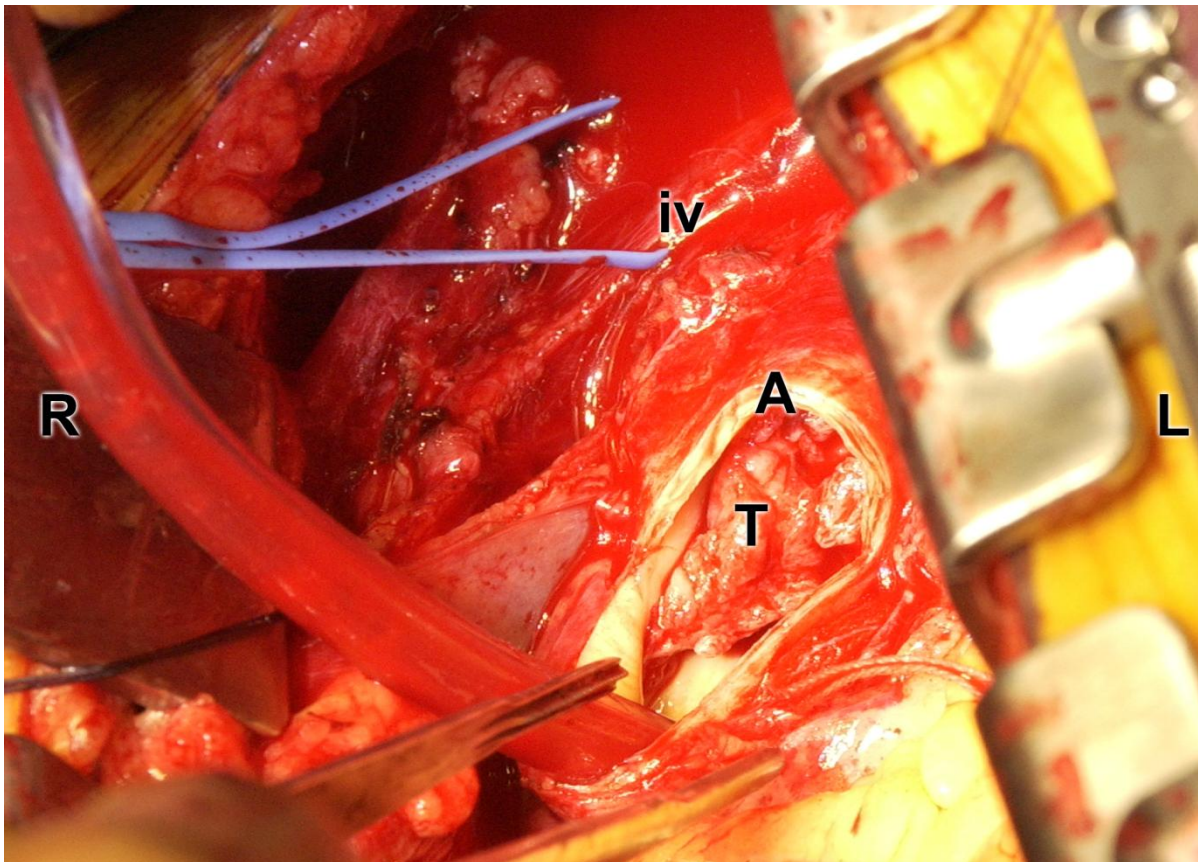


Figure 2. Intraoperative image of the thrombus in the aortic arch. iv, innominate vein; T, thrombus; A, opened aortic arch; R/L, right and left side of the patient).

hydrofolate reductase (MTHFR) A1298C without hyperhomocysteinemia.

Thrombolysis of the clot could not be undertaken because of risk of partial lysis or dislodgment of the thrombus, therefore urgent surgical intervention was indicated to prevent fatal thromboembolic events. Exploration via a midsternotomy revealed a friable thrombotic mass measuring 3.5 × 4.0 cm, localized in the aortic arch and prolapsing outward into the distal aorta beyond the innominate artery (Figure 2).

Excision of a small button of the aortic wall surrounding the thrombus and local patch graft repair were performed. Histopathologic examination revealed a thrombus attached to a normal aortic wall with the absence of protruding atherosclerotic plaque ulcerations or malignancy at the site of insertion of the thrombus (Figure 3).

RESULTS

The patient was discharged home 6 days postoperatively after an uneventful recovery on a regimen of aspirin and folate after discontinuation of HRT and advised smoking cessation. The role of long-term anticoagulant therapy in the treatment of idiopathic arterial thrombosis is controversial, but antiplatelet agents have been shown to be effective in the prevention and treatment of arterial

thrombosis (Guidelines for the Primary Prevention of Stroke, 2011; Baigent et al., 2002). The patient continues to do well at 8 years of follow-up and she reported no further episodes of cerebral infarcts. After that last visit to her internist, the patient moved out of state and was lost to follow-up.

DISCUSSION

The mechanism underlying aortic thrombus formation is complex and likely multifactorial. Aortic atheroma (plaque thickness ≥ 4 mm, ulcerated or with mobile component, is an important non-cardiac source of peripheral or cerebral emboli (Aldons, 2000). There is a lack of data showing direct association between MTHFR A1298C and arterial thrombus formation. Although heterozygous mutation in the gene encoding MTHFR have been identified in this patient, it remains uncertain whether this genetic polymorphism without hyperhomocysteinemia can cause thromboembolic events in the arterial system (Spiroski et al., 2008; Spiroski et al., 2008; Trabetti, 2008; Schwahn and Rozen, 2001; Contractor et al., 2011; Domagala et al., 2002; Kim and Becker, 2003).

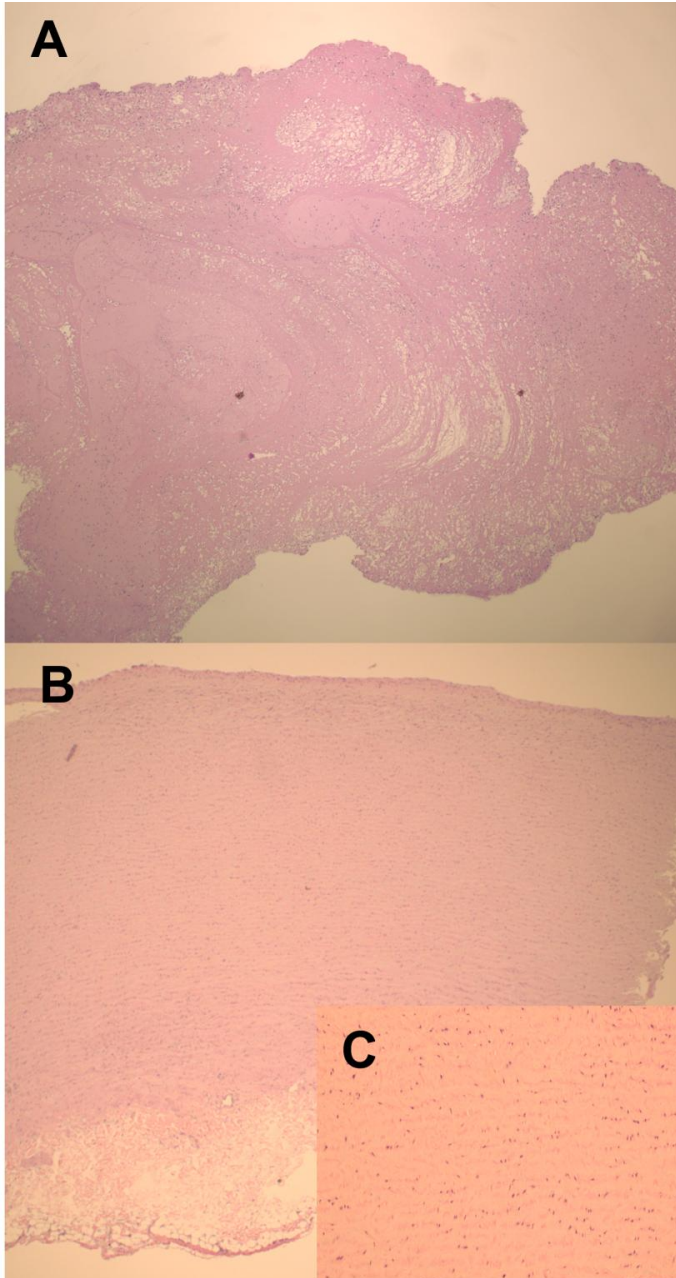


Figure 3. On microscopical examination (hematoxylin and eosin) the aortic wall has no underlying atheromatous plaque. The aortic luminal thrombus (A) was focally adherent to the aortic wall (B). Magnification of the aortic wall (inset C) showed minimal mucinous degeneration without atherosclerotic plaque or other abnormality.

Since this patient had no other cause for her clotting disorder that could be ascertained, a synergistic effect of smoking, estrogen intake, along with her genetic profile may have contributed to her arterial thrombotic event through a loss of endothelial protection, enhanced activity of thromboxane A_2 , and initial platelet activation (Leone, 2007; Pretorius et al., 2010; Khullar and Maa, 2012; Herrington and Howard, 2003; Petitti, 2012).

Role of HRT and smoking in arterial thrombosis

There are indirect estimates of postmenopausal women smokers on HRT in the US general population. According to data drawn from national information sources (Third National Health and Nutrition Examination Survey, conducted in the US between 1988 and 1994), an estimated 37% of postmenopausal women took HRT pills for 1 to 5 years (Women-Health Facts, 2012).

Interestingly, the prevalence of smoking in the US has decreased; however, an estimated 17.4% women continued to smoke in 2007 (Women-Health Facts, 2012). With such a large number of women smokers using HRT, a minute increase in prothrombotic states brought about by other frequent risk factors for thrombophilia such as overweight or obesity, inflammation, malnutrition, malignancy and factor V Leiden, will affect many (Nelson et al., 2012; Cushman et al., 2004; Miller et al., 2002). Multiple studies have shown a moderate increased risk for arterial thrombosis (stroke/myocardial infarction) due to HRT intake (Slooter et al., 2005; Lidegaard et al., 2012; Hannaford, 2000). Together, the data suggest that HRT increases the risk of thrombophilia. This conclusion is congruent with the recommendation by the Agency for Healthcare Research and Quality (US) which does not recommend long-term use of HRT for the same reason (Nelson et al., 2012; Miller et al., 2002; Rossouw et al., 2002).

The MTHFR mutation affects genomic methylation through an interaction with folate (Friso et al., 2002). Consequently, it interacts with multiple other factors. These factors include the genetic make-up of individual patients, geographic regions, ethnicity, associated prothrombotic or inflammatory states, dietary habits, and multiple lifestyle factors and nutritional supplementation (Zheng et al., 2000; Zhao et al., 2011; Gürsoy et al., 2011). Lidegaard et al. (2012) have reported an increased risk by a factor of 1.5 to 2 among users of oral estrogen-progestin. This risk in arterial thrombotic events could be minimized by abstinence from smoking (Hannaford, 2000).

Is MTHFR polymorphism without hypercystenemia associated with idiopathic thrombosis?

In the setting of a population-specific but prevalent MTHFR A1298C polymorphism, the effect of combining smoking and HRT raises important public health concerns in the generation of venous as well as arterial thrombi. As mentioned earlier, this conclusion is difficult to prove, mainly because of unforeseen confounding factors that influence final phenotype or so called "phenotype modifiers" (Girirajan et al., 2012; Girirajan and Eichler, 2010; Dipple and McCabe, 2000). In our case, homocysteine level was normal. However, low dietary intakes of folate and riboflavin, vitamins B_{12}/B_6 , all have been implicated to playing a role in plasma level of

homocysteine (Domagala et al., 2002; Kanth, 2011; Dawson and Waters, 1994).

Impact and prevalence of MTHFR genotype on thrombotic diathesis

Our patient was white of European heritage, and after discontinuation of both smoking and HRT intake, there were no additional reported neurological vascular events up to 8 years after discharge.

Considered in isolation, the risk of increased clotting in MTHFR mutation is still equivocal (Schwahn and Rozen, 2001; Gürsoy et al., 2011; Dölek et al., 2007). Noteworthy, based on data derived from case/control reports, genetic susceptibility to thrombosis and the prevalence of MTHFR may vary in different ethnic populations worldwide (Hannaford, 2000; Dipple and McCabe, 2000; Dawson and Waters, 1994; Gürsoy et al., 2011). A Macedonian case-control study suggested that the prevalence of C677T and A1298C genotypes are connected with increased homocysteinemia level among patients with deep venous thrombosis (DVT) (Domagala et al., 2002). In that study, Domagala et al. (2002) observed a 15% incidence of MTHFR variant in healthy Polish cohort.

According to recent literature, A1298C mutation was equally distributed in the Turkish patient group with DVT compared with the control group (Dölek et al., 2007). Similarly, the report by Solomon et al. (2001), as well as the report by Zetterberg et al. (2002) found no increase in DVT or vascular disease. A 2003 meta-analysis review and a recent study comparing patients with venous thromboembolism and healthy subjects also failed to demonstrate such a link (Domagala et al., 2002; Kim and Becker, 2003). Therefore on the basis of this case and other data, MTHFR A1298C polymorphism alone may not be sufficient to confer clinically overt thrombophilia. There must exist other permissive environmental factors leading to increase clotting propensity. Exposure to smoking and hormone replacement therapy each elicits integrated risk of increased thromboembolic events in parallel to genetically-controlled hypercoagulable response by such factor as the enzyme MTHFR. Moreover, other lifestyle factors and nutrients in the diet have been shown to interact with that enzyme. For example the findings by Huang et al. (2011) in a cross-sectional study further support that notion. In that study, of Puerto-Rican men and women residing in the Boston metropolitan area, subjects with MTHFR A→C displayed significant interactions with alcohol intake, smoking and physical activity in determining plasma homocysteine level. There have also been reports confirming interactions between genetic MTHFR variant and the risk for esophageal cancer in former moderate and heavy drinkers or smokers in the Chinese population (Zhao et al., 2011).

Similarly, recent Medline search to identify association

between MTHFR mutation and arterial circulatory events has suggested that the individual propensity for those events is due to other systemic mechanisms (Kim and Becker, 2003).

Finally and consistent with this view, association between MTHFR mutation and cerebral infarction and DVT has also been reported in the Chinese population by Zheng et al. (2000). In these cohorts of patients, the prevalence of the 677 C→T allele in normal control subjects was 30.7%, similar to that in Caucasians and Japanese.

Taken together, these data show that the prevalence of MTHFR varies among different populations and that in addition to the traditional risk factors (such as tobacco use, hypertension, dyslipidemia, HRT intake, diet and sedentary life-style) complex strong gene-gene and gene-environmental interactions form significant effects on the incidence of overt thrombotic events (Schwahn and Rozen, 2001).

What does this case inform us?

The patient's findings imply the need in maintaining a wide-ranging view of carefully assessing the genetic causes of arterial thrombotic events especially when the reason is not identified in women who are smokers and on HRT. This case also suggests that gene-environment interactions in genetic disorder confer rather very different clinical manifestations among populations that may be relevant in this and other genetic disorders.

Conclusions

The data supporting the relationship between MTHFR mutation and inclination to thrombosis are conflicting. Available clinical and epidemiologic evidence show that there is broad ethnic and regional variability in the clinical response to MTHFR polymorphism. If a link exists between smoking and HRT that predisposes female patients to arterial thrombosis in the setting of this allelic gene mutation that will certainly raise important matter in clinical practice and in public health. Specifically, is it necessary to endorse genetic testing for such mutation in thromboembolic events for risk stratification and therapeutic decisions? Perhaps, the answer to this question remains a matter of individual judgment. There may be specific subgroups of women with certain predisposition to traditional thrombotic risks who are more likely to benefit from a genetic testing for MTHFR before prescribing HRT, even if the use of HRT is intended for the short-term. Future studies of genome-wide sequencing and the interactions with environmental elements-in different geographic areas-may clarify the susceptibility to thrombophilia and may yield results that foster causal relationship. Special attention should be

given to higher thrombotic risk groups to intervene and modify risk factors.

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