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ORIGINAL ARTICLE

Is Inherited Thrombphilia in Pregnancy a Risk Factor for Familial Stroke?

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A R T I C L E I N F O

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A B S T R A C T

Context: Inherited thrombophilia is an abnormality of blood coagulation that increases the risk of thromboembolic disease. During pregnancy, the thrombogenic potential of this inherited disorder is enhanced because of the hypercoagulable state produced by normal pregnancy-associated changes in several coagulation factors.

Aims: The purpose of this research was to find the association of polymorphisms and mutations of coagulation factors.

Settings and Design: This Study is the first comprehensive study of thrombophilia genes in the Iranian population.

Methods and Material: This current research was performed in a group of 70 women referred to the Department of Medical Genetics. due to reproductive failures with history of thrombosis in pregnancy, First degree families have a history of heart attacks and strokes. The clinical data on family history of diabetes, high cholesterol, high blood pressure, a history of recurrent pregnancy loss has been observed.

Results: The most common mutation was PAI-1 with (62.9 %) heterozygote 4G/5G alleles and (14.3%) homozygote 4G/4G alleles. The second and third mutations were MTHFR-C677 T with (44.3%) heterozygote and (8.6%) homozygotes and MTHFR-1298 C with (41.4%) heterozygotes and (12.9%) homozygotes.

Conclusions: These data indicate that polymorphic variation in the prevalence of thrombophilia in women with recurrent miscarriage with our population has had a lot of different European populations. Mutations in this gene in the percentage of individuals who have a family history of stroke or heart rate have been observed.

INTRODUCTION

Inherited thrombophilia is the leading cause of maternal thromboembolism and is associated with an increased risk of recurrent spontaneous abortion (1-2). Thrombophilia is the predisposition to venous thromboembolism and is caused by inherited and acquired factors, alone or in combination(3). With the discovery of APC resistance and the prothrombin gene mutation, more than half of all patients with clinical characteristics of thrombophilia are now diagnosed with an inherited disorder. The hypercoagulable work-up of patients with venous thromboembolism is important, because can influence the duration and management of anticoagulation therapy, as well as affect other decisions regarding life and health

issues. The clinician should be equipped with an algorithm of how to approach a patient with a thromboembolic event, from decisions regarding which thrombophilia tests to order to how the results of these tests affect patient management(3). Protein C (PC) and protein S (PS) are vitamin K-dependent glycoprotein that acts as natural anticoagulants. The proteolytic activation of PC by thrombin occurs on the surface of endothelial cells and involves thrombomodulin and endothelial PC receptor(4). Resistance to activated protein C increases in the second and third trimesters. Protein S activity decreases due to reductions in total protein S antigen, levels and activity of the fibrinolytic inhibitors, thrombin activatable fibrinolytic inhibitor (TAFI), plasminogen activator inhibitor type 1 (PAI-1) and PAI-2 increase. Growing evidence indicates that PC, APC and PS, besides their known anticoagulant properties have multiple actions such as anti-apoptotic and anti-inflammatory activities, regulation of gene expression and stabilization of endothelial barrier protection. This activity appears to be mediated by two key receptors, protease activated receptor (PAR1) and EPCR(4). Cytoprotective signalling induced by APC can be noticed not only in the endothelium but also in many other cell types there in neurons, which may be show potential neuroprotective acute and chronic therapies(4).

To our knowledge, this Study is the first comprehensive study of thrombophilia genes in the Iranian population. The purpose of this study was to investigate the effects of factor V Leiden FVG1691A, FVH1299R, PTH 20210G>A, MTHFR polymorphism 677C>T, MTHFR A1298 C, Factor XIIIV34L, PAI-14G/5G, EPCR A4600G and EPCR G4678 C involved in reproductive failure.

SUBJECTS AND METHODS

Seventy patients with recurrent miscarriage received in Imam Khomini hospital "TUMS "during from "MAY 2011 to MAY 2013 " were investigated in a retrospective study, and the frequency of polymorphic variations was calculated. The recurrent pregnancy loss (RPL), had been trying to achieve successful pregnancy for greater than 1 year without success and known causes of infertility were excluded (semen anomalies, karyotype abnormalities, uterine malformations, etc). The enrolment was performed from "May 2011 until May 2013". The trial was approved by the Ethics Committee of health reproductive Centers. Written informed consent was obtained from all patients before Genetic test. Women aged 18–41 years with recurrent miscarriage with Thrombophilia were assessed for eligibility after referral.

Detection of genotype mutation: All reagents used in these steps provided by CVD Strip Assay kit Manufactured by Vienna lab (CVD Strip assay 4-360).

RESULTS

In this study a significant association between the loss of pregnancy and genetic thrombophilia gene mutations have been reported including factor V Leiden FVG1691A, FVH1299R, PTH 20210G>A, MTHFR polymorphism 677C>T, MTHFR A1298 C, Factor XIIIV34L, PAI-14G/5G, EPCR A4600G and EPCR G4678 C (Table1&2).

Table1: EPCR haplotypes

Haplotypes	Number of mutations							Total (%)
	0	1	2	3	4	5	6	
A1A1	0	0	2	3	3	0	1	9 (12.9)
A1A2	0	1	5	6	8	6	3	29 (41.4)
A2A2	2	1	1	4	4	4	0	16 (22.9)
A2A3	0	1	0	2	6	1	0	10 (14.3)
A3A3	0	0	0	1	1	1	0	3 (4.3)
A1A3	0	0	0	1	0	1	1	3 (4.3)
Total	2	3	8	17	22	13	5	70 (100)

Table 2: Mutation with heterozygote and homozygote

	MTHFR C677 T	MTHFR 1298 C	FV LIEDEN	FVH 1299R	Factor XIII Val34Leu	PTH FactorII	PAI-1 4G/5G	EPCR G4678 C	EPCR 4600G
Normal	33 (47.1)	32 (45.7)	62 (88.6)	35 (50.0)	53 (75.7)	67 (95.7)	16 (22.9)	23 (32.9)	56 (80.0)
Heterozygote	31 (44.3)	29 (41.4)	8 (11.4)	35 (50.0)	15 (21.4)	3 (4.3)	44 (62.9)	35 (50.0)	10 (14.3)
Homozygote	6 (8.6)	9 (12.9)	0 (0.0)	0 (0.0)	2 (2.9)	0 (0.0)	10 (14.3)	12 (17.1)	4 (5.7)
Total	70 (100)	70 (100)	70 (100)	70 (100)	70 (100)	70 (100)	70 (100	70 (100)	70 (100)

In our study the most common mutation was PAI-1 with (62.9%) heterozygote 4G/5G alleles and (14.3%) homozygote 4G/4G allels.The second and third mutations were MTHFR-C677 T with (44.3 %) heterozygote and (8.6%) homozygote and MTHFR-1298 C with (41.4%) heterozygote and (12.9%) homozygote Factor XIII (FXIII) showed the V34L mutation with (21.4%) heterozygote and (2.9%) homozygote & Prothrombin (PTH; Factor II) G20210A was (4.3%).The mutation of FV Leiden G1691A was (11.4%) and FV R2 haplotype (H1299R) was (5.6%).

The clinical data on family history of diabetes, high cholesterol, high blood pressure, a history of recurrent pregnancy loss has been observed.

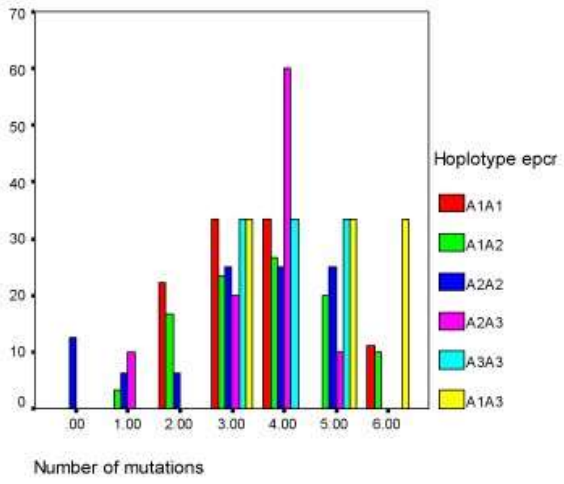
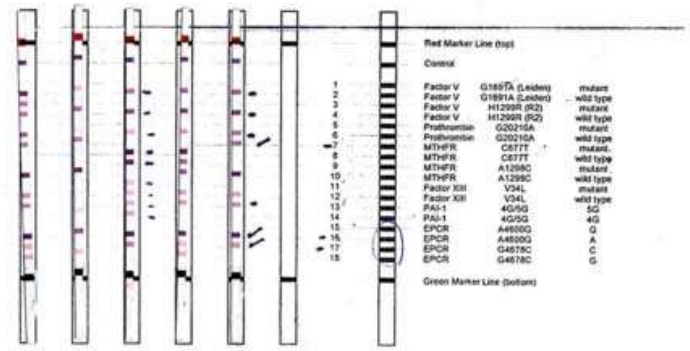


Figure1: Crosslation between Haplotyping EPCR and Number of genes mutation.



factor V Leiden FVG1691A, FVH1299R, PTH ":Homozygote and heterozygote mutations of 20210G>A, MTHFR polymorphism 677C>T, MTHFR A1298 C,Factor XIIIV34L, PAI-14G/5G, EPCR A4600G and EPCR G4678 C involved in reproductive failure.

DISCUSSION

Inherited thrombophilia is the leading cause of maternal thromboembolism and is associated with an increased risk of recurrent spontaneous abortion (1-2).Thrombophilia is the predisposition to venous thromboembolism and is caused by inherited and acquired factors, alone or in combination(3).

The genetic contributions to ischemic stroke is important not only so as to explain, or predict, the minority of cases that occur in the absence of well-established risk factors, such as smoking, hypertension and diabetes, but also to account for wide variability of stroke incidence within individuals who do harbour these common, acquired risk-factors. Moreover, appreciating the biochemical basis for risk-associated genes can motivate novel therapeutic strategies, including pharmacogenomics (15)

Inherited deficiency of coagulation factor XIII: The reduced clotting activity associated with the Leu564 factor XIII variants may be compounded by increased fibrinolysis associated with PAI-1 5G/5G, which potentially increases the likelihood of intracranial hemorrhage (5). The promoter region of the PAI-1 gene has a common single guanine nucleotide insertion/deletion polymorphism that influences transcription of the gene The 5G allele has another repressor protein binding site that is absent from the 4G allele. As a consequence, the 4G allele produces up to 6 times more MRNA in vitro and is associated with higher plasma levels.PAI-1 activity in vivo.2-5 High levels of PAI-1 seem to influence processes of smooth muscle cell proliferation plaque, and matrix remodelling in the direction of promo ting atherothrombosis. Many studies have concluded that inheritance of the 4G/4G genotype confers an increased risk for coronary heart disease. But, results for cerebrovascular disease have been conflicting with that study finding that 4G/4G confers an increased risk of stroke.(6)

Polymorphisms in the MTHFR gene are also associated with an increased risk of spina bifida(7), another common type of neural tube defect. Polymorphisms in the MTHFR gene have also been studied as possible risk factors for a variety of common conditions. These include heart disease, stroke, high blood pressure (hypertension), and high blood pressure during pregnancy (preeclampsia), an eye disorder called glaucoma, psychiatric disorders, and certain types of cancer. The 677C>T polymorphism in the MTHFR

gene has also been suggested as a risk factor for cleft lip and palate, a birth defect in which is a split in the upper lip and an opening in the roof of the mouth. Studies of MTHFR gene variations in people with these disorders have had mixed results, with associations found in this studies but not in others. So, it remains unclear what role changes in the MTHFR gene play in determining the risk of these complex conditions. Hyperhomocyste in aemia has been associated with an increased risk of thrombosis and neural tube defects. Homozygosity for MTHFR polymorphisms (C677T, 1298C) is a relatively common cause of mildly elevated plasma homocysteine levels, but these mutations do not appear to increase the risk of VTE in pregnant or nonpregnant women "http://ghr.nlm.nih.gov/condition/spina-bifida"

The risk of VTE is augmented during pregnancy because of the aforementioned physiological changes in the coagulation system, as well as predisposing physical changes, such as stasis large veins of the lower extremities from uterine compression, decreased mobility, and the delivery process (especially cesarean birth). But, the absolute risk remains low at about 0.1 percent.(8)

Suggest that the Leu564 variants of coagulation factor XIII may be markers for genetic susceptibility to hemorrhagic stroke in women aged <45 years. Hemorrhagic stroke occurs when an artery ruptures and causes blood leakage into the brain parenchyma or the subarachnoid space. Overall, it accounts for ≈20% of stroke victims and is the most common form of stroke among young adults. Data from family studies have suggested the importance of genetic influence in hemorrhagic stroke, particularly subarachnoid hemorrhage (SAH. (5, 9) But, the specific genetic factors are poorly defined. Because intracranial aneurysms are associated with rare inherited connective tissue disorders, most earlier candidate gene studies have focused on structural vessel wall proteins, such collagen, or, in the case of intracerebralhemorrhage (ICH), cerebral amyloid deposition. The events required for a hemorrhagic stroke to become clinically manifest include not only the formation of a weakened or abnormal vessel wall but also vessel rupture and hemorrhage. Inherited deficiency of coagulation factor XIII. The reduced clotting activity associated with the Leu564 factor XIII variants may be compounded by increased fibrinolysis associated with PAI-1 5G/5G, which potentially increases the likelihood of intracranial hemorrhage.(5)

The prothrombin gene mutation is a guanine to adenine transition at nucleotide 20210 in the 3' untranslated region of prothrombin. Heterozygous carriers have 30 percent higher plasma prothrombin levels than (10)

In the Leiden Thrombophilia Study, the prothrombin gene mutation independently conferred a 2.8-fold increased risk of venous thrombosis. The common presence of two thrombophilic defects (eg, factor V Leiden and the prothrombin gene mutation) increases the thrombotic risk three- to fivefold above the risk of a single defect. The prothrombin gene mutation is a risk factor for deep vein thrombosis in pregnant women and for cerebral vein thrombosis, especially in those taking oral contraceptives. It is controversial whether heterozygous carriers have an increase in the rate of recurrent venous thromboembolism.Theprothrombin gene mutation is not a risk factor for cerebrovascular ischemic disease in older patients, but may be a risk factor in younger patients.(2)

thrombophilia Screeningin high-risk situations thrombophilia testing is recommended: Recurrent fetal loss, especially in the 2nd or 3rd trimester, VTE during pregnancy or the postpartum period, Strong family history of VTE in first degree relatives, Asymptomatic, first-degree relatives of a proband with a known Inherited thrombophilia, Maternal Pregnancy-Related Stroke(11).

A stroke, or cerebrovascular accident (CVA), is the rapid loss of brain function due to disturbance in the blood supply to the brain. This can be due to ischemia caused by blockage (thrombosis arterial embolism), or a hemorrhage.(12)

Three quarters of strokes happen after the age of 60 years. Age is the most important no modifiable risk factor of stroke. In patients who suffer a stroke at a younger age than the majority of stroke patients, genetic influences are probably more important than in older patients.(13) Genetic factors are considered to play a role in the etiology of stroke. Stroke is the second most common cause of death and the most common cause of disability in developed countries. (12)Stroke is a multifactorial disease caused by a combination of environmental and genetic factors many epidemiologic studies have documented a significant genetic part in the occurrence of strokes Genes encoding products involved in lipid metabolism, thrombosis, and inflammation are believed to be potential genetic factors for stroke. Although a large group of candidate genes have been studied, most of the epidemiological results are conflicting. Studies of stroke as a monogenic disease have made huge progress.(14)

Stroke is one of the leading causes of death, disability, and health finance cost in both developed and developing world countries. The genetic contributions to ischemic stroke is important not only so as to explain, or predict, the minority of cases that occur in the absence of well-established risk factors, such as smoking, hypertension and diabetes, but also to account for wide variability of stroke incidence within individuals who do harbour these common, acquired risk-factors. Moreover, appreciating the biochemical basis for risk-associated genes can motivate novel therapeutic strategies, including pharmacogenomics (15)

Genetic Risk Factors for Thrombosis in Pregnancy as a risk factor of stroke in relative family.in high-risk situations thrombophilia testing is recommend-

ed: Recurrent fetal loss, especially in the 2nd or 3rd trimester, VTE during pregnancy or the postpartum period, Strong family history of VTE in first degree relatives, Asymptomatic, first-degree relatives of a proband with a known Inherited thrombophilia. These genetic variations test can thus greatly contribute to lowering the individual's CVD risks.

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REFERENCES

1. McNamee K, Dawood F, Farquharson RG. Thrombophilia and early pregnancy loss. Best Pract Res Clin Obstet Gynaecol. Feb;26(1):91-102.
2. Mierla D, Szmal C, Neagos D, Cretu R, Stoian V, Jardan D. Association of Prothrombin (A20210G) and Factor V Leiden (A506G) with Recurrent Pregnancy Loss. Maedica (Buchar). 2012 Sep;7(3):222-6.
3. Perry SL, Ortel TL. Clinical and laboratory evaluation of thrombophilia. Clin Chest Med. 2003 Mar;24(1):153-70.
4. Wypasek E, Undas A. Protein C and protein s deficiency - practical diagnostic issues. Adv Clin Exp Med. 2013 Jul-Aug;22(4):459-67.
5. Reiner AP, Schwartz SM, Frank MB, Longstreth WT, Jr., Hindorff LA, Teramura G, et al. Polymorphisms of coagulation factor XIII subunit A and risk of nonfatal hemorrhagic stroke in young white women. Stroke. 2001 Nov;32(11):2580-6.
6. Festa A, D'Agostino R, Jr., Rich SS, Jenny NS, Tracy RP, Haffner SM. Promoter (4G/5G) plasminogen activator inhibitor-1 genotype and plasminogen activator inhibitor-1 levels in blacks, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Circulation. 2003 May 20;107(19):2422-7.
7. Bassuk AG, Kibar Z. Genetic basis of neural tube defects. Semin Pediatr Neurol. 2009 Sep;16(3):101-10.
8. Practice bulletin no. 138: inherited thrombophilias in pregnancy. Obstet Gynecol. Sep;122(3):706-16.
9. Chen F, Qiao Q, Xu P, Fan B, Chen Z. Effect of Factor XIII-A Val34Leu Polymorphism on Myocardial Infarction Risk: A Meta-Analysis. Clin Appl Thromb Hemost. Sep 15.
10. Granel B, Morange PE, Serratrice J, Ene N, Cremades S, Swiader L, et al. [Heterozygous prothrombin gene mutation G20210A and associated diseases]. Rev Med Interne. 2003 May;24(5):282-7.
11. Anderson FA, Jr., Spencer FA, editors. Risk factors for venous thromboembolism. 2003/06/20 ed2003.
12. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet. 2008 May 10;371(9624):1612-23.
13. Lalouschek W, Endler G, Schillinger M, Hsieh K, Lang W, Cheng S, et al. Candidate genetic risk factors of stroke: results of a multilocus genotyping assay. Clin Chem. 2007 Apr;53(4):600-5.
14. Guo JM, Liu AJ, Su DF. Genetics of stroke. Acta Pharmacol Sin. Sep;31(9):1055-64.
15. Bentley P, Peck G, Smeeth L, Whittaker J, Sharma P. Causal relationship of susceptibility genes to ischemic stroke: comparison to ischemic heart disease and biochemical determinants. PLoS One. 2010;5(2):e9136.
16. Gonsalves WI, Pruthi RK, Patnaik MM. The new oral anticoagulants in clinical practice. Mayo Clin Proc. 2013 May;88(5):495-511.