

Factor V Leiden, factor V Cambridge, factor II GA20210, and methylenetetrahydrofolate reductase in cerebral venous and sinus thrombosis: A case-control study

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Background: Factor V G1691A (FV Leiden), FII GA20210, and methylenetetrahydrofolate reductase (MTHFR) C677T mutations are the most common genetic risk factors for thromboembolism in the Western countries. However, there is rare data in Iran about cerebral venous and sinus thrombosis (CVST) patients. The aim of this study was to evaluate the frequency of common genetic thrombophilic factors in CVST patients. **Materials and Methods:** Forty consequently CVST patients from two University Hospital in Isfahan University of Medical Sciences aged more than 15 years from January 2009 to January 2011 were recruited. In parallel, 51 healthy subjects with the same age and race from similar population selected as controls. FV Leiden, FII GA20210, MTHFR C677T, and FV Cambridge gene mutations by polymerase chain reaction technique were evaluated in case and control groups. **Results:** FV Leiden, FII GA20210, and FV Cambridge gene mutations had very low prevalence in both case (5%, 2%, 0%) and control (2.5%, 0%, 0%) and were not found any significant difference between groups. MTHFR C677T mutations was in 22 (55%) of patients in case group and 18 (35.5%) of control group ($P = 0.09$). **Conclusion:** This study showed that the prevalence of FV Leiden, FII GA20210, and FV Cambridge were low. Laboratory investigations of these mutations as a routine test for all patients with CVST may not be cost benefit.

Key words: Cerebral venous and sinus thrombosis, factor II GA20210, factor V Cambridge, factor V Leiden, methylenetetrahydrofolate reductase

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INTRODUCTION

Venous thrombosis is the third most common cardiovascular affliction after ischemic heart disease and stroke.^[1] Cerebral venous and sinus thrombosis (CVST) is a rare presentation of venous thrombosis,^[2] however, that was found to be the second common cause of stroke in young women,^[3] and associated with substantial mortality and morbidity.^[4] Various

risk factors can be identified in 70-80% of patients, such as local infections (middle ear or facial skin infections), thrombophilic states (factor V [FV] Leiden [G1691A] gene mutation; methylenetetrahydrofolate reductase [MTHFR] C677T; deficit of antithrombin III, protein C, and protein S; FII GA20210 mutation; hyperhomocysteinemia with or without elevated factor VIII levels; and antiphospholipid syndrome), systemic inflammatory diseases (for example Behcet's disease), medication treatment (contraception), pregnancy, and the puerperium,^[5-7] and in about 20% no risk factor is

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identified.^[2] FV G1691A (Leiden), FII GA20210, and MTHFR C677T mutations are the most common genetic risk factors for thromboembolism in Western countries^[8,9] however, there is rare data about the association of these mutation and CVST in Iran. Therefore, the aim of this study is to evaluate the frequency of common genetic thrombophilic factors in CVST and to offer practical suggestion for doing laboratory investigations in CVST patients.

MATERIALS AND METHODS

Patients and control subjects

Forty CVST patients from two University Hospital in Isfahan University of Medical Sciences aged more than 15 years from January 2009 to January 2011 were recruited consequently. Patients were diagnosed according to accepted criteria with magnetic resonance imaging and venography by the absence of normal hypointense flow void in the involved sinuses and if needed angiography. In parallel 51 healthy subjects with age- and sex-matched from the same race (Iranian) selected as controls.

Blood sample collection and coagulation tests

After obtaining written informed consent for genetic analysis, 10 ml of peripheral blood was collected into ethylenediaminetetraacetic acid and deoxyribonucleic acid (DNA) analyzed for FV G1691A (Leiden), FII GA20210, MTHFR C677T, and FV Cambridge gene mutations (Arg306Thr). Genetic test was done for all case and control, however, other following known etiologic factor was evaluated in CVST patients; antithrombin activity (amgdolytic assay); protein C activity; protein S antigen, anticardiolipin and antiphospholipid antibodies, and fasting homocysteine by enzyme-linked immunosorbent assay method and lupus anticoagulants by sensible partial thromboplastin time and Russell's viper venom time test. For antiphospholipid and anticardiolipin the positive result was higher than 10 GPL and for homocysteine the level higher than 14 was considered as hyperhomocysteinemia. For Antithrombin activity; protein C activity and protein S level, the blood sample was taken 6 months later than the acute phase and after finishing anticoagulant therapy.

Deoxyribonucleic acid extraction and analysis

Total genomic DNA was extracted from peripheral blood leucocytes by the salting-out procedure. DNA fragment of the FV Leiden was amplified and digested with Mnl I and Nla III (New England Biolabs). In the allele-specific polymerase chain reaction (PCR), the presence of the FV G1691A (Leiden) mutation was identified by using two primers: Sense primer FV1-TGCCCA GTG CTT AAC AGA CCA and antisense primer FV2A-TCT CTT GAA GGAAAT GCC CCA TTA, to prime for fragment 1 (F1); or FV2B-AAG GAC AAA AGT ACC TGT ATT CCA, to prime for F2.

PCR was performed using 2U of Taq polymerase enzyme (Promega) and 1.5 mmol/L of magnesium chloride (MgCl₂), 500 ng of DNA, and 25 pmol of primers, respectively. The amplification conditions comprise of a denaturation step of 1 cycle at 95°C for 5 min followed by 1 cycle of denaturation (95°C, 1 min), annealing (60°C, 1 min), and 35 cycle of extension (72°C, 1 min). For optimized amplification of F2 dimethyl sulfoxide was added.

Products were resolved on 2% agarose gel and examined after staining with ethidium bromide. The same protocol was used for other mutation but primers and restriction enzyme used for FV Cambridge, MTHFR C677T, FII GA20210 were 5' TGT CTT TCT GTC CTA AC 3' and 5' TCT TGA ACC TTT GCC CA 3', BstNI; 5' TGA AGG AGA AGG TGT CTG CGG GA 3' and 5' AGG ACG GTG CGG TGA GAG TG 3', Hinf I; 5' TCT AGA AAC AGT TGC CTG GC 3' and 5' ATA GCA CTG GGA GCA TTG AAG C 3', HindIII, respectively. Also, we add 50 mmol/L of Tris-HCL (PH9), 50 mmol/L of KCL, and 1% Triton X-100 only for MTHFR amplification.

Review

Additionally, we performed a narrative review of all studies that evaluated FV Leiden, FII GA20210, MTHFR C677T, and FV Cambridge gene in CVST and venous thromboembolic disease. Key words were CVST, deep vein thrombosis, venous thromboembolic disease, and FV Leiden, FII GA20210, MTHFR C677T, and FV Cambridge gene mutations. We searched Medline-PubMed, ISI, Scopus, and Cochrane databases up to 2014. We searched references lists from articles identified by search as well as a key review article to identify additional articles. We identified case-control, case series, and case report.

Statistical analysis

The results were analyzed by SPSS for Windows version 18 (SPSS Inc., Chicago, IL, USA). The variables were compared between patients and control groups by Student's *t*-test or with Mann-Whitney U test or Fisher's exact test, as appropriate. We also calculated the crude odds ratio (OR) and 95% confidence intervals. $P < 0.05$ were considered as significant. The results were reported as the mean \pm standard deviation (SD).

RESULTS

Table 1 shows clinical features of patients. The most frequent symptom was a headache (92.5%), and the most frequent sign was pupil edema. Mean age of CVST and controls was 33.45 (SD: 10.55) and 30.75 (SD: 9.01), respectively ($P = 0.19$); also, sex distributions between two groups have not any significant difference ($P = 0.085$). Venous infarction occurred in 28 (70%) patients and hemorrhagic infarct in

14 (35%). Frequency of etiologic factors showed in Table 2. The prevalence of hyperhomocysteinemia was significantly higher in patients than controls (OR: 2.732 [1.041-7.194]) ($P = 0.033$). FV G1691A (Leiden), FII GA20210, and FV Cambridge gene mutations (Arg306Thr) mutant had very low prevalence in both case and control and no significant difference found between two groups [Table 3]. MTHFR C677T mutation had not significant differences between groups ($P = 0.09$).

DISCUSSIONS

In this study we found low prevalence and nonsignificant differences of genomic mutations of FV G1691A (Leiden), FII GA20210, MTHFR C677T, and FV Cambridge gene (Arg306Thr) in case and control groups in our population in central area of Iran. However, MTFHR mutations had more prevalent in CVST group with near significant differences, but other mutations had very low prevalence in both groups without significant differences.

The mutation in FV Leiden G1691A and the prothrombin gene G20210A are the two most prevalent identified risk factor of inherited thrombophilia. Table 4 showed the studies and the correlations of genomic mutations of FV G1691A (Leiden), FII GA20210, MTHFR C677T, and FV Cambridge gene (Arg306Thr) with CVST in different regions of the world. The frequency of FV 1691A in patients with CVT varied from 3.7% to 25%, for prothrombin 20210A was found to be from 0% to 20% and for MTHFR 677TT frequency was reported from 0 to 36%.^[13] However, two systematic meta-analysis showed that the summary OR for developing CVST was almost 2-3 in FV Leiden carrier, 5.5-9 in FII GA20210 carrier, and 2-4 in MTHFR C677T carrier.^[69-71]

There are few studies investigating its association with CVST in the Middle East. Rahimi *et al.* study showed that in the Western population of Iran FV Leiden (16.7 %) but not FII GA20210 and MTHFR C677T mutation as a risk factor for CVST.^[33] Otrock *et al.* study also showed 32.1% CVST patients in Lebanon had FV Leiden mutation.^[43] The result of this study regarding to MTHFR C677T and FII GA20210 mutation was in accordance with Rahimi *et al.* study.^[33] With regard to Table 4, it appears that in Iran the prevalence of FV G1691A (Leiden), FII GA20210, and FV Cambridge gene (Arg306Thr) much lower than the Western countries.

Race ethnic differences may account for the heterogeneous distribution of inherited thrombophilia. From the West to the East the frequency of FII G20120A mutation and FV Leiden mutation and correlation with CVST become less. In North America and Europe, this frequency and its correlation with CVST was high and in the Middle East is moderate and in Asia is low. Therefore, it appears higher

Table 1: Clinical feature of the CVST patients at admission

Symptom and sign	CVST (n = 40) (%)
Headache	37 (92.5)
Pupil edema	33 (82.5)
Weakness	20 (50)
Loss of consciousness	18 (45)
Cognitive impairment	17 (42.5)
Seizure	9 (22.5)
Meningeal sign	5 (12.5)
Coma	2 (5)

CVST = Cerebral venous and sinus thrombosis

Table 2: Frequency of etiologic factors in CVST patients

Etiologic factors	n (%)
Hypercoagulable state	
Oral contraceptives	25 (62.5)
Anticardiolipin antibodies IgM	11 (27.5)
Anticardiolipin antibodies IgG	6 (15)
Antiphospholipid antibodies IgG	8 (20)
Antiphospholipid antibodies IgM	6 (15)
Nonanticardiolipin antibodies IgM	2 (5)
Nonanticardiolipin antibodies IgG	2 (5)
Lupus anticoagulants	0 (0)
ANA	1 (2.5)
ANCA	1 (2.5)
Hyperhomocysteinemia	10 (25)
Protein S deficiency	0 (0)
Protein C deficiency	0 (0)
Antithrombin III deficiency	0 (0)
Puerperium and pregnancy	4 (10)
Surgery	1 (2.5)
Infection disease	0 (0)
Trauma	0 (0)
Malignancy	0 (0)
Unknown etiology	10 (25)

CVST = Cerebral venous and sinus thrombosis; ANCA = Antineutrophil cytoplasmic antibodies; ANA = Antinuclear antibodies

Table 3: Frequency of factor V G1691A (FV Leiden), FII GA20210, MTHFR C677T and factor V Cambridge gene mutations in case and control groups

Mutation	Patient's n (%)	Controls n (%)	OR (95% CI)	P
Factor V Leiden G1691A	2 (5)	1 (2)	0.380 (0.33-4.384)	0.83
Factor II G20210A	1 (2.5)	0 (0)	0	0.903
MTHFR C677T	22 (55)	18 (35.5)	0.446 (0.191-1.041)	0.096
Heterozygous	18 (56.3)	14 (43.8)		0.16
Homozygous	4 (50)	4 (50)		
Factor V Cambridge	0 (0)	0 (0)	0	

MTHFR = Methylene tetrahydrofolate reductase; OR = Odds ratio; CI = Confidence interval

frequency of FII G20120A and FV Leiden mutations in white Caucasian in the North and South America, moderate frequency in Caucasian in the Middle East and lower frequency in Asian and African (references).

Table 4: Summary of studies involving factor V Leiden, factor V Cambridge, factor II GA20210 and MTHFR in cerebral venous and sinus thrombosis

Location of study	Authors and years of publish	Type of mutation	Number of case (%)	Number of control (%)	Correlation to type of venous thromboembolism		Type of study	
					CVST	DVT		
Brasil	Gadelh, <i>et al.</i> , 2005 ^[10]		26	217			Case-control	
		G20210A	6 (23)	3 (1)	Yes			
		Factor V Leiden	2 (8)	3 (1)	No			
	Milano, <i>et al.</i> , 2003 ^[11]		1				Case report	
		G20210A			Yes			
	Rodrigues, <i>et al.</i> , 2004 ^[12]		42	134			Case-control	
		G20210A	7 (16.7)	1 (0.7)	Yes			
	Voetsch, <i>et al.</i> , 2000 ^[13]		14	225			Case-control	
		G20210A	2 (14)	5 (2.5)	Yes			
		Factor V Leiden	1 (7.1)	13 (5.8)	No			
	Camargo, <i>et al.</i> , 2005 ^[14]		50				Case series	
		G20210A	8.70					
	Belgium	Simons, <i>et al.</i> , 2000 ^[15]		2				Case report
			G20210A			Yes		
Canada	Eikelboom, <i>et al.</i> , 1999 ^[16]		1				Case report	
		G20210A			Yes			
China	Zheng, <i>et al.</i> , 2000 ^[17]		145	122			Case-control	
		G20210A	0 (0)	0 (0)	No	No		
	Yanqing, <i>et al.</i> , 2003 ^[18]		364	140			Case-control	
		Factor V Leiden	12 (3.2)	7 (5)	No	No		
France	Zuber, <i>et al.</i> , 1996 ^[20]		19	57			Case-control	
		Factor V Leiden	4 (21)	1 (2)	Yes			
Germany	Biousse, <i>et al.</i> , 1998 ^[21]		35				Case series	
		G20210A	2 (5.7)					
	Benbih, <i>et al.</i> , 2008 ^[22]		1				Case report	
Factor V Leiden				Yes				
Germany	Weih, <i>et al.</i> , 1998 ^[23]		12	187			Case-control	
		Factor V Leiden	3 (25)	1 (0.53)				
	Lüdemann, <i>et al.</i> , 1998 ^[24]		55	272			Case-control	
		Factor V Leiden	8 (14.5)	17 (6.25)	Yes			
	Reuner, <i>et al.</i> , 1998 ^[25]		45	354			Case-control	
		G20210A	4 (8.9)	8 (2.3)	Yes			
	Weih, <i>et al.</i> , 1998 ^[26]		1				Case report	
		G20210A			Yes			
	Weih, <i>et al.</i> , 2000 ^[27]		33				Case series	
		G20210A	4 (12)		Yes			
Heckmann, <i>et al.</i> , 2001 ^[28]		3				Case report		
	Factor V Leiden	8 (24)		Yes				
Meenakshi-Sundaram, <i>et al.</i> , 2005 ^[29]		1				Case report		
	Factor Leiden			Yes				
Lichy, <i>et al.</i> , 2006 ^[30]		77	203			Case-control		
	G20210A	8 (10.4)	5 (2.5)	Yes				
		Factor V Leiden	22 (28.6)	15 (7.4)	No			

(Continued)

Table 4: (Continued)

Location of study	Authors and years of publish	Type of mutation	Number of case (%)	Number of control (%)	Correlation to type of venous thromboembolism		Type of study
					CVST	DVT	
Greece	Selvi, <i>et al.</i> , 2009 ^[31]	Factor V Leiden	1		Yes		Case report
India	Biswas A, <i>et al.</i> , 2008 ^[32]	Factor V Leiden	155	120			Case-control
		Factor V Cambridge	16 (10.3)	1(0.8)	No		
Iran	Rahimi, <i>et al.</i> , 2010 ^[33]	Factor V Leiden	0 (0)	0(0)			Case-control
		G20210A	24	100	No		
		MTHFR	4 (16.7)	2 (2)	Yes		
Italy	Martinelli, <i>et al.</i> , 1996 ^[34]	Factor V Leiden	14 (58.3)	44 (44)	Yes		Case-control
		G20210A	25	75			
	Martinelli, <i>et al.</i> , 1998 ^[35]	Factor V Leiden	5 (20)	2 (2.7)	Yes		Case-control
		G20210A	120	120			
	Madonna, <i>et al.</i> , 2000 ^[36]	Factor V Leiden	22 (38)	3 (3)	Yes	Yes	Case-control
		G20210A	21 (34)	3 (3)	Yes	Yes	
		MTHFR	10	259			
	Rigamonti, <i>et al.</i> , 2002 ^[37]	Factor V Leiden	5 (50)	16 (6.3)		Yes	Case-control
		MTHFR	1 (10)	15 (5.8)			
	Boncoraglio, <i>et al.</i> , 2004 ^[38]	MTHFR	3 (33.3)	45 (17.4)			Case report
G20210A		3			Yes	Case-control	
Factor V Leiden		26	100	No		Original article	
MTHFR		3 (11/5)	3 (3)	Yes			
Homocystein		0 (0)	3 (3)	No			
Tufano, <i>et al.</i> , 2014 ^[39]	Factor V Leiden	7 (27)	25 (25)	No		Case-control	
	G20210A	10 (38/5)	13 (13)	No			
Ventura, <i>et al.</i> , 200 ^[40]	Factor V Leiden	56	184			Case-control	
	G20210A	59 (5.4)	198 (8.2)	Yes			
	Homocystein	71 (29.1)	193 (5.5)	Yes			
Colaizzo, <i>et al.</i> , 2007 ^[41]	Factor V Leiden	30	40			Case-control	
	G20210A	9 (30)	1 (2.5)	Yes			
	MTHFR	13 (43/3)	4 (10)	Yes			
Lebanon	Uthman, <i>et al.</i> , 2004 ^[42]	Factor V Leiden	184	286			Case-report
		MTHFR	21 (11.4)	9 (3.1)	Yes	Yes	
Mexico	Cantu, <i>et al.</i> , 2004 ^[44]	Factor V Leiden	15 (8.1)	10 (3.5)	No	Yes	Case-report
		MTHFR	1		Yes		
		G20210A	1		Yes		
Poland	Kurkowska-Jastrzebska, <i>et al.</i> , 2003 ^[45]	Factor V Leiden	16				Case series
		MTHFR	0 (0)		No		
Portugal	Verdelh, <i>et al.</i> , 2001 ^[46]	Factor V Leiden	5 (31.2)		Yes		Case-control
		MTHFR	8 (50)		Yes		
		G20210A	45	90			
Spain	Sánchez Del Rio, <i>et al.</i> , 1996 ^[47]	Factor V Leiden	10 (22)	9 (10)	Yes		Case-report
		MTHFR	1		Yes		
Spain	Alvarez, <i>et al.</i> , 1999 ^[48]	Factor V Leiden	1				Case-report
		G20210A	1		Yes		
		Factor V Leiden	64	103			
		G20210A	2 (2.7)	3 (3)	Yes	No	Case-control
		Factor V Leiden	9 (14/1)	1 (1)	Yes	Yes	

(Continued)

Table 4: (Continued)

Location of study	Authors and years of publish	Type of mutation	Number of case (%)	Number of control (%)	Correlation to type of venous thromboembolism		Type of study
					CVST	DVT	
	Mira, <i>et al.</i> , 2000 ^[49]	G20210A Factor V Leiden	36 6 (17) 7 (19)				Case series
	Mira, <i>et al.</i> , 2002 ^[50]	Factor V Leiden	1		Yes		Case report
	Ortín, <i>et al.</i> , 2004 ^[51]	G20210A	1		Yes		Case report
	Madroño-Vuelta, <i>et al.</i> , 2004 ^[52]	G20210A	1		Yes		Case report
	Romero, <i>et al.</i> , 2007 ^[53]	G20210A Factor V Leiden MTHFR	15 2 (13/3) 4 (26.6) 6 (40)	15 1 (6.6) 8.30 8 (15)	Yes Yes No		Case-control
Sweden	Barba <i>et al.</i> , 2008 ^[54]	Cambridge II	1		Yes		Case report
Switzerland	Wilder-Smith, <i>et al.</i> , 1997 ^[55]	Factor V Leiden	1		Yes		Case report
Tunisia	Salem-Berrabah, <i>et al.</i> , 2011 ^[56]	Factor V Leiden	1		Yes		Case report
Turkey	Altinisik, <i>et al.</i> , 2008 ^[57]	G20210A Factor V Leiden	50 2 (4) 3 (6)	25 2 (8) 0 (0)	No No	No No	Case-control
	Yakaryilmaz, <i>et al.</i> , 2009 ^[58]	G20210A Factor V Leiden	1		Yes Yes		Case report
UK USA	Ozkurt, <i>et al.</i> , 2011 ^[59]	Factor V Leiden MTHFR	1		Yes		Case report
	Kellett, <i>et al.</i> , 1998 ^[60]	G20210A	1		Yes		Case report
	Hillier, <i>et al.</i> , 1998 ^[61]	G20210A Factor V Leiden MTHFR	15 0 (0) 2 (14) 4 (28)	300 4 (1) 24 (8) 136 (45)			Case-control
	Hourihane, <i>et al.</i> , 1997 ^[62]	Factor V Leiden	1		Yes		Case report
	Dulli, <i>et al.</i> , 1996 ^[63]	Factor V Leiden	3		Yes		Case report
	Liu, <i>et al.</i> , 2000 ^[64]	G20210A Factor V Leiden	1		Yes Yes		Case report
	Maag, <i>et al.</i> , 2003 ^[65]	Factor V Leiden	1		Yes		Case report
	Stephan, <i>et al.</i> , 2003 ^[66]	Factor V Leiden	1		Yes		Case report
	Porres-Aguilar, <i>et al.</i> , 2007 ^[67]	G20210A	1		Yes		Case report
	Kanaan, <i>et al.</i> , 2008 ^[68]	MTHFR	1		Yes		Case report
	Mahadeo, <i>et al.</i> , 2010 ^[69]		1				Case report

MTHFR = Methylenetetrahydrofolate reductase; CVST = Cerebral vein and sinus thrombosis; DVT = Deep vein thrombosis

Only one case report study in the Western countries showing correlation FV Cambridge and CVST,^[54] however, in this study we did not find this mutation in our sample of Iranian population.

This study showed that the prevalence of FV Leiden, FII GA20210, and FV Cambridge were low in our case and control groups. Laboratory investigations of these mutations as a routine test for all patients with CVST may not be cost benefit although the low sample size may be a limitation factor.

On the other hand with regard to high prevalence of CVST and venous thromboembolism in Iran, the Middle East and the East Asia^[72-74] maybe other genomic thrombophilic risk factors is responsible for venous thrombosis, therefore it appears the role of other new mutations in the future should be investigated.

Most studies similar to this study showed a high prevalence of oral contraceptives pills (OCPs) as a risk factor in CVST.^[70,75-79] However, the coexistence of OCPs and some prothrombotic states such as FV Leiden, GA20210, and hyperhomocysteinemia lead to magnify the risk of CVST in women.^[79]

CONCLUSION

The findings of this study indicated that the prevalence of FV Leiden, FII GA20210, and FV Cambridge were low. So, laboratory investigations of these mutations as a routine test for all patients with CVST may not be cost beneficial. Further studies with larger sample size and investigation of all thrombophilic risk factors is recommended.

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Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTION

MS conceived and designed the study, recruited samples and contributed to discussion and revision of the manuscript, MS analyzed the data and wrote the manuscript. All authors discussed the results and reviewed and edited the manuscript.

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