Scientific Research Publishing

Neuroscience & Medicine, 2017, 8, 68-76
http://www.scirp.org/journal/nm
ISSN Online: 2158-2947
ISSN Print: 2158-2912

Common Prothrombotic Gene Mutations in Cerebral Venous Sinus Thrombosis in North-West of Iran

Elyar Sadeghi-Hokmabadi[1], Ebrahim Sakhinia[2*], Mehdi Farhoudi[1*], Somayyeh Hasaneh[1], Morteza Ghojazadeh[3], Mohammad Amin Farzi[1], Sasan Andalib[4], Masoumeh Nemati[2], Omid Abbaszadeh[1], Reza Rikhtegar[1], Reshad Mirnour[1]

[1]Neurosciences Research Center, Department of Neurology, Imam-Reza Hospital, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Connective Tissue Research Center, Department of Medical Genetics, Faculty of Medicine and Tabriz Genetic Analysis Centre (TGAC), Tabriz University of Medical Sciences, Tabriz, Iran
[3]Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Department of Neurosurgery, Poursina Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
Email: *farhoudi_m@tbzmed.ac.ir, *esakhinia@yahoo.co.uk

How to cite this paper: Sadeghi-Hokmabadi, E., Sakhinia, E., Farhoudi, M., Hasaneh, S., Ghojazadeh, M., Farzi, M.A., Andalib, S., Nemati, M., Abbaszadeh, O., Rikhtegar, R. and Mirnour, R. (2017) Common Prothrombotic Gene Mutations in Cerebral Venous Sinus Thrombosis in North-West of Iran. *Neuroscience & Medicine*, **8**, 68-76.
https://doi.org/10.4236/nm.2017.84009

Received: September 11, 2017
Accepted: December 1, 2017
Published: December 4, 2017

Abstract

Objective: Cerebral venous sinus thrombosis (CVST) is a life-threatening cerebrovascular disease which has high prevalence and mortality rate in Iran. Thrombophilia caused by gene mutation is a common cause of CVST. The present study aimed at assessing the prevalence of thrombophilic gene mutations in Iranian CVST patients and then comparing it with normal population. ***Materials and methods***: In a case-control study, polymerase chain reaction-restriction fragment length polymorphism (PCR_RFLP) and amplification-refractory mutation system (ARMS-PCR) were carried out to detect common thrombophilic mutations in 70 CVST patients. Next, it was compared with 82 sex- and age-matched healthy controls. ***Results***: Factor-V-Leiden, Factor-V-Leiden HR2, Factor prothrombin II, MTHFR (667C/T) and MTHFR (1298A/C) prevalence were significantly high in cases of CVST as compared to the controls (P values: 0.012, 0.019, 0.007 and 0.036, respectively). However, there was no significant difference between the two groups in plasminogen activator inhibitor (PAI), angiotensin-converting enzyme (ACE), beta-fibrinogen (FGB), Factor VIII, Factor XIII, and tissue plasminogen activator (tPA) mutations. ***Conclusion***: The findings of the present study suggest that Factor V-Leiden, Factor-V-Leiden HR2, prothrombin II (G20210A), and MTHFR (667C/T & 1298A/C) mutations are more frequent in CVST. Detection of these mutations may help clinicians to decide on the duration of treatment and referral to genetic counseling for valuable prevention.

Keywords

Gene, Mutation, Cerebral Venous Sinus Thrombosis, Thrombophilia

1. Introduction

CVST is a relatively rare life-threatening cerebrovascular disease that specifically affects young and middle-aged women. The prevalence of CVST is 5 per million, which constitutes 0.5% of total strokes [1] [2] with a mortality rate of below 10% [1]. The prevalence of CVST in Iran has been demonstrated to be 12.3 per million, and it has a higher mortality rate compared to western countries [3]. The higher prevalence and mortality rate of CVST in Iranian patients necessitates further investigation of its etiology [3].

Several risk factors have been reported for thrombosis in CVST patients recently [4]. Besides the acquired risk factors of thrombosis, 10% - 15% of CVST cases may show a form of hereditary thrombophilia [4]. On the other hand, people with a genetic background predisposed for thrombosis should avoid taking oral contraceptives (OCP) and hormone replacement therapy (HRT), especially when there is a history of venous thrombosis [5]. Furthermore, these mutations may cause thrombophilia and increase the risk of CVST during pregnancy and the postpartum period [6].

Anticoagulation therapy is recommended for patients with homozygous gene mutations causing long-time thrombophilia. Thus, it is important to determine the genetic risk factors of thrombosis so as to deduce the duration of treatment [7].

Owing to the high incidence of CVST and its mortality rate in Iran, and also due to lack of similar studies determining the inherited predisposing factors of CVST in northwest Iran, we conducted this study to assess the prevalence of thrombophilic gene mutations in Iranian CVST patients and compare it with normal population.

2. Materials and Methods

2.1. Epidemiologic Considerations

In a case-control study conducted from October 2014 to December 2015, all patients (n = 70) with a definite diagnosis of CVST, who were referred to Imam-Reza and Razi hospitals, had been enrolled. These two tertiary hospitals are the main referral destinations for neurological diseases in northwest Iran. The cases were diagnosed based on clinical manifestations and MRI and MRV findings. As for the control group, 82 healthy controls (with the same ethnicity and origin) with no family history of venous or arterial thrombosis were selected and matched for age and gender. Common gene mutations involved in thrombophilia (as described below) were assessed in all the participants.

2.2. Genotyping Procedure

Blood samples (2 ml) were taken from the patients' right brachial vein using a 2-ml sterile disposable syringe and a 22-guage sterile disposable needle without using any local anesthetic, while DNA samples were extracted using the salting-out method. Appropriate forward and reverse primers (Table 1) were designed, and PCR conditions were optimized. A standard PCR protocol was used based on our previous study and Mutations were assessed using the PCR-RFLP and ARMS-PCR techniques [8]. The RFLP analysis was carried out using restriction enzymes (Table 2).

Table 1. Forward and reverse primers, temperature and size of amplified fragments.

	The nucleotide sequence of primer	Annealing temperature	size of amplified fragment
MTHFR C677T	F-TGAAGGAGAAGGTGTCTGCGGGA	59˚C	198 bp
	R-AGGACGGTGCGGTGAGAGTG	58˚C	
MTHFR A1298C	F-CTTTGGGGAGCTGAAGGACTACTAC	59˚C	163 bp
	R-CACTTTGTGACCATTCCGGTTTG	55˚C	
FV G1691A	F-CATGAGAGACATCGCCTCTG	54˚C	147 bp
	R-GACCTAACATGTTCTAGCCAGAAG	56˚C	
FII G20210A	F-TCTAGAAACAGTTGCCTGGC	52˚C	345 bp
	R-ATAGCACTGGGAGCATTGAAGC	55˚C	

a. Sample of a Table footnote (Table footnote is dispensable).

Table 2. Restriction enzymes used for each SNP.

Mutation	Restriction enzyme	Cutting position
MTHFR C677T	Hin fI	G ANTC CTNA G
MTHFR 1298	MboII	GAAGA(N)8 CTTCT(N)7
V G1691AF	HindIII	A AGCTT TTCGA A
FII G20210A	MnlI	CCTC(N)7 GGAG(N)6

2.3. Ethical Considerations

Written informed consent was obtained from each participant. This research was approved by the ethics committee of the Tabriz University of Medical Sciences (approval number: 94.3 - 6.2).

2.4. Statistical Analysis

The descriptive data was described as frequency–percentage and mean ± SD. The quantitative variables were compared using the independent sample t test, while the categorical (qualitative) variables were compared by chi-square test or Fisher's exact test. SPSS version 17 was used for data analysis. P-value < 0.05 was considered as statistically significant.

3. Results

The mean age of the CVST group was 37.3 ± 12.2, while the same for the control group was 33.8 ± 10.1 years (P = 0.058). There were 56 women and 14 men in the CVST group compared to 55 women and 27 men in the control group (P = 0.073). Figure 1 shows PCR analysis of extracted DNA samples.

The frequency of gene mutations is listed in Table 3. As shown, the prevalence of Factor-V-Leiden, Factor-V-Leiden HR2, Factor prothrombin II,

Table 3. Prevalence of gene mutations in each group.

Gene	CVST Group, N (%) N = 70	Control group, N (%) N = 82	P Value
Factor-V-Leiden (H)[a]	8	1	0.012
Factor-V-Leiden HR2(H)	24	15	0.019
Factor-V-Leiden HR2(M)[b]	2	0	0.019
Prothrombin II (G20210A)(H)	7	0	0.004
MTHFR (667C/T)(H)	20	41	0.007
MTHFR (667C/T)(M)	8	2	0.007
MTHFR (1298A/C)(H)	32	48	0.036
MTHFR (1298A/C)(M)	15	6	0.036
Plasminogen activator inhibitor (PAI)(H)	36	48	0.602
Plasminogen activator inhibitor (PAI)(M)	15	13	0.602
Angiotensin-converting enzyme (ACE)(H)	40	44	0.808
Angiotensin-converting enzyme (ACE)(M)	18	25	0.808
Beta-fibrinogen (FGB)(H)	31	35	0.953
Beta-fibrinogen (FGB)(M)	3	3	0.953
Factor VIII(H)	26	33	0.110
Factor VIII(M)	1	7	0.110
Factor XIII(H)	13	18	0.558
Factor XIII(M)	0	1	0.558
Tissue Plasminogen Activator (tPA)(H)	37	22	0.000
Tissue Plasminogen Activator (tPA)(M)	6	29	0.000
Glycoprotein1a(H)	36	35	0.109
Glycoprotein 1a(M)	6	17	0.109

a. heterozygote, b: mutant (homozygote).

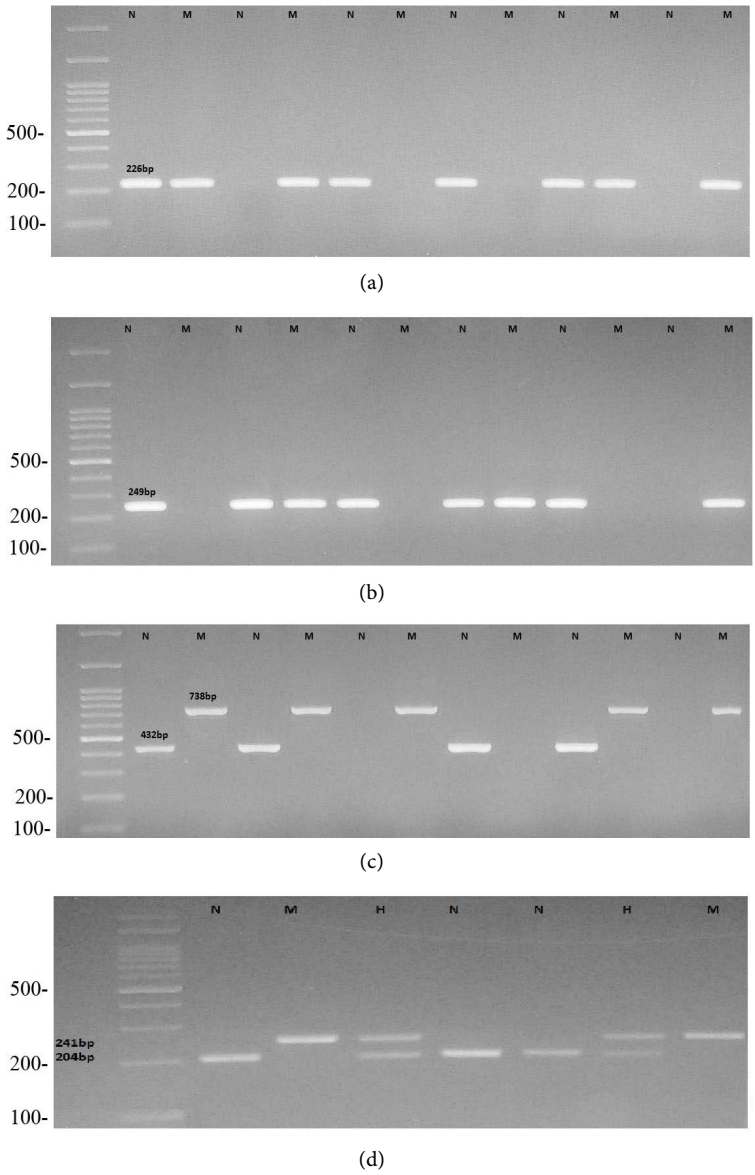

Figure 1. Polymerase Chain Reaction analysis of extracted DNA samples. (a): MTHFR (667C/T) mutation; (b) Factor-V-Leiden mutation; (c) Tissue Plasminogen Activator (tPA) mutation, (with ARMS-PCR technique); (d) MTHFR (1298A/C) mutation with PCR- RFLP technique). N: Normal, M: Mutant.

MTHFR (667C/T), and MTHFR (1298A/C) mutations were significantly higher in the CVST group (P values: 0.012, 0.019, 0.007 and 0.036, respectively).

4. Discussion

There is a growing research interest in genes associated with coagulation factors and their inhibitors. About 30% - 40% of patients with CVST had a hereditary background of thrombophilia [4]. The results of this study showed that the frequency of Factor V-Leiden, Factor V-Leiden HR2, prothrombin II (G20210A), and MTHFR (667C/T & 1298A/C) mutations were significantly higher in CVST

patients, but there was no significant difference in the frequencies of PAI, ACE, FGB, Factor-VII, Factor-XIII, and tPA mutation between the two groups.

4.1. Factor V-Leiden Mutation

Factor V-Leiden mutation, which shows an irregular geographical dispersion, is the most common hereditary risk factor of venous thrombosis [1]. This mutation is not present in Africans, Americans, Asians, and native Australians. However, almost 5% of white Americans, Canadians, and north Europeans as well as 15% of people of Sweden and Cyprus carry this mutation [9]. In Iran, the prevalence of Factor V-Leiden mutation is approximately 3.3% among thrombophilic patients, even though its prevalence among the general population is still unknown [9] [10]. Zuber *et al.* studied the Factor V Leiden mutation in French patients with CVST and showed the heterozygous form of this mutation in four (21%) among 19 patients compared to one (1.75%) among the 57 controls [11]. In another Spanish study, the heterozygous Factor V Leiden mutation was found in one (6%) patient after assessing 16 patients with CVST [12].

We found Factor V Leiden mutation in eight (11.4%) patients and one (1.2%) control. Furthermore, Factor V Leiden-HR2 was detected in 26 patients (37.1%) and 15 controls (18.3%) respectively. It was significantly high in patients with CVST compared to controls.

4.2. Prothrombin II (G20210A)

Zivelin *et al.* studied prothrombin II (G20210A) polymorphism in 1,670 Jews of various ethnic groups, and its frequencies in European Ashkenazi Jews, North African Jews (Sefadric), Iranian Jews, and Ethiopian Jews were 6.7%, 5.5%, 2%, and 0%, respectively [13].

Martinelli *et al.* reported that 20% of their Italian CVST patients and Sánchez–Martín *et al.* reported that 12% of their Spanish CVST patients had prothrombin II (G20210A) mutation [14]. We found prothrombin II (G20210A) mutation in 10% of our patients and in none of the subjects in the control group. Ashjazadeh *et al.* reported that the prevalence of prothrombin II (G20210A) mutation was not significantly higher in CVST patients in southern Iran with predominant Fars ethnicity [1]. Our findings were inconsistent perhaps because of different ethnic groups and different geographical areas were studied.

4.3. MTHFR Mutation

MTHFR mutation increases the blood homocysteine level, leading to a susceptibility to thrombosis. This mutation was seen in 5% - 15% of different races [13] [15] [16]. Salomon *et al.* studied patients with idiopathic venous thromboembolism (VTE) and reported that the rate of MTHFR-C677T mutation was higher in VTE patients compared to healthy subjects (22.8% vs. 14.3%) [17]. The rate of MTHFR-C677T mutation in CVST patients has been reported to be about 12.5% - 14.9% [12] [18]. In this study, the rate of MTHFR-C677T mutation was 11.4%

in CVST patients compared to 4.2% in the control group. These results are similar to other studies.

4.4. OCP Use

It was earlier observed that OCP users are at increased risk for CVST [19] [20] [21] and this risk increases if there is concomitant mutation in the prothrombin II (G20210A) gene [14]. In this study, out of 41 women with CVST who were not pregnant or in the postpartum state, 30 (73%) were taking OCP, but the proportion of prothrombin II (G20210A) mutation was not higher in OCP users compared to non-OCP users (13% vs. 9%, P = 0.59) in our study, which may be due to the small sample size.

4.5. Limitations

This study has some limitations. First, this was an observational study, and the associations reported in this article do not necessarily clarify causes. Second, the sample size was relatively small and we recommend complementary studies based on a larger sample size.

5. Conclusion

Altogether the evidence found in the present study indicates that some of hereditary thrombophilias are more common in CVST. Therefore, detection of these mutations may help clinicians decide on planning the duration of the treatment and referral to genetic counseling for valuable prevention. However, further investigations with larger samples and various ethnic and geographical distributions are suggested to confirm the role of inherited thrombophilia in CVST.

Compliance with Ethical Standards

Funding: This study was funded by Neuroscience Research Center (NSRC), Tabriz University of Medical Science.

Conflict of Interest

The authors declare that they have no conflict of interests regarding to this publication.

Acknowledgements

None.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Inform consent was obtained from all partici-

pants.

References

[1] Ashjazadeh, N., Poursadeghfard, M. and Farjadian, S. (2012) Factor V G1691A and Prothrombin G20210A Gene Polymorphisms among Iranian Patients with Cerebral Venous Thrombosis. *Neurology Asia*, **17**, 199-203.

[2] Shindo, A., Wada, H., Ishikawa, H., Ito, A., Asahi, M., Ii, Y., Ikejiri, M. and Tomimoto, H. (2014) Clinical Features and Underlying Causes of Cerebral Venous Thrombosis in Japanese Patients. *International Journal of Hematology*, **99**, 437-440. https://doi.org/10.1007/s12185-014-1550-x

[3] Borhani Haghighi, A., Ashjazadeh, N., Safari, A. and Cruz-Flores, S. (2012) Cerebral Venous Thrombosis in Iran: Cumulative Data, Shortcomings and Future Directions. *Iranian Red Crescent Medical Journal*, **14**, 805-810. https://doi.org/10.5812/ircmj.3728

[4] Sarraf, P., Ghaffarpoor, M., Poormahmoodian, H., Harrirchian, H. and Hashemi, H. (2009) Cerebral Vein Thrombosis: Screening of Acquired and Hereditary Thrombophilic Risk Factors. *Acta Medica Iranica*, **47**, 197-202.

[5] Joffe, H. and Goldhaber, S. (2002) Laboratory Thrombophilias and Venous Thromboembolism. *Vascular Medicine*, **7**, 93. https://doi.org/10.1191/1358863x02vm426ra

[6] Uzar, E., Ekici, F., Acar, A., Yucel, Y., Bakir, S., Tekbas, G., Oncel, O. and Tasdemir, N. (2012) Cerebral Venous Sinus Thrombosis: An Analyses of 47 Patients. *European Review for Medical and Pharmacological Sciences,* **16**, 1499-1505.

[7] Agoston-Coldea, L., Rusu, L., Bobar, C., Rusu, M., Mocan, T. and Procopciuc, L. (2008) Recurrent Thrombembolic Risk in Patients with Multiple Thrombophilic Disorders. *Romanian Journal of Internal Medicine*, **46**, 261-266.

[8] Poursadegh Zonouzi, A., Chaparzadeh, N., Asghari Estiar, M., *et al.* (2002) Methylenetetrahydrofolate Reductase C677T and A1298C Mutations in Women with Recurrent Spontaneous Abortions in the Northwest of Iran. *ISRN Obstetrics and Gynecology*, **2012**, Article ID: 9454861.

[9] Paolo, S., Bernd, J., Paolo, P., Daniela, T., Philip, W., Brono, G., *et al.* (2002) Effect of MnII on Factor V. *British Journal of Heamtology*, **33**, 198-202.

[10] Lucotte, G. and Mercier, G. (2001) Population Genetics of Factor V Leiden in Europe. *Blood Cells, Molecules and Diseases*, **27**, 362-367. https://doi.org/10.1006/bcmd.2001.0388

[11] Zuber, M., Toulon, P., Marnet, L. and Mas, J.L. (1996) Factor V Leiden Mutation in Cerebral Venous Thrombosis. *Stroke*, **27**, 1721-1723. https://doi.org/10.1161/01.STR.27.10.1721

[12] Sánchez-Martín, B., Grasa, J.M., Latorre, A., Montañés, M.A., García-Erce, J.A., Torres, M., Calvo, M.T. and Giralt, M. (2002) Acquired and Inherited Hypercoagulability among Patients with Cerebral Venous Thrombosis. *Neurologia*, **17**, 405-409.

[13] Zivelin, A., Rosenberg, N., Faier, Sh., Kornbrot, N., Peretz, H., Mannhalter, C., *et al.* (1998) A Single Genetic Origin for the Common Prothrombin G20210A Polymorphism in the Prothrombin Gene. *Blood*, **92**, 1119-1124.

[14] Martinelli, I., Sacchi, E., Landi, G., Taioli, E., Duca, F. and Mannucci, P.M. (1998) High Risk of Cerebral-Vein Thrombosis in Carriers of Prothrombin Gene Mutation and in Users of Oral Contraceptives. *The New England Journal of Medicine*, **338**, 1793-1797. https://doi.org/10.1056/NEJM199806183382502

[15] Meinardi, J.R., Middeldorp, S., de Kam, P.J., Koopman, M.M., van Pampus, E.C., Hamulyák, K., Prins, M.H., Büller, H.R. and van der Meer, J. (2001) Risk of Venous Thromboembolism in Carriers of Factor V Leiden with a Concomitant Inherited Thrombophilic Defect: A Retrospective Analysis. *Blood Coagulation & Fibrinolysis*, **12**, 713-720. https://doi.org/10.1097/00001721-200112000-00014

[16] Almawi, W.Y., Tamim, H., Kreidy, R., Timson, G., Rahal, E., Nabulsi, M., Finan, R.R. and Irani-Hakime, N. (2005) A Case Control Study on the Contribution of Factor V-Leiden, Prothrombin G20210A, and MTHFR C677T Mutations to the Genetic Susceptibility of Deep Venous Thrombosis. *Journal of Thrombosis and Thrombolysis*, **19**, 189-196. https://doi.org/10.1007/s11239-005-1313-x

[17] Salomon, O., Steinberg, D.M., Zivelin, A., Gitel, S., Dardik, R., Rosenberg, N., Berliner, S., Inbal, A., Many, A., Lubetsky, A., Varon, D., Martinowitz, U. and Seligsohn, U. (1999) Single and Combined Prothrombotic Factors in Patients with Idiopathic Venous Thromboembolism: Prevalence and Risk Assessment. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **19**, 511-518. https://doi.org/10.1161/01.ATV.19.3.511

[18] Ghosh, K., Shetty, S., Madkaikar, M., Pawar, A., Nair, S., Khare, A., Pathare, A., Jijina, F. and Mohanty, D. (2001) Venous Thromboembolism in Young Patients from Western India: A Study. *Clinical and Applied Thrombosis/Hemostasis*, **7**, 158-165. https://doi.org/10.1177/107602960100700214

[19] Farhoudi, M., Ayromlou, H., Sharifipour, E., Charsouei, S., Sadeghihokmabadi, E., Ahmadi, M. and Afrough, A. (2012) Does Low Dose Contraceptive Pills Increase Stroke Rate? A Cross Sectional Study in North-West Iran. *Pakistan Journal of Medical Sciences*, **28**, 501-505.

[20] Mohammadian, R., Sohrabi, B., Mansourizadeh, R., Mohammadian, F., Nazempour, A., Farhoudi, M., Pashapour, A., Taher Aghdam, A.A., Hashemzadeh, A. and Pourkakrodi, M. (2012) Treatment of Progressive Cerebral Sinuses Thrombosis with Local Thrombolysis. *Interventional Neuroradiology*, **18**, 89-96. https://doi.org/10.1177/159101991201800112

[21] Janghorbani, M., Zare, M., Saadatnia, M., Mousavi, S.A., Mojarrad, M. and Asgari, E. (2008) Cerebral Vein and Dural Sinus Thrombosis in Adults in Isfahan, Iran: Frequency and Seasonal Variation. *Acta Neurologica Scandinavica*, **117**, 117-121.