Molecular analysis of prothrombotic gene variants in venous thrombotic diseases. Different risk factors in different sex and clinical forms.

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Research article

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Abstract

Background The role of prothrombotic gene variants as risk factors for venous thrombotic diseases is controversial and the ordering of tests for thrombophilia in the clinical context is scarcely respective of evidence-based data. **Methods** We studied FVL, FVR2, FII G20210A, MTHFR C677T and A1298C, beta-fibrinogen -455 G>A, FXIII V34L, HPA-1 L33P variants and PAI-1 4G/5G alleles in: 343 patients with deep vein thrombosis (DVT), 164 with pulmonary embolism (PE), 126 with superficial vein thrombosis (SVT), 118 with portal vein thrombosis (PVT), 75 with cerebral vein thrombosis (CVT), 119 with retinal vein thrombosis (RVT) in comparison with 430 subjects from the general population (GP) of the same geographical area (Southern Italy). **Results** About 40% of patients with DVT, PE and SVT have a prothrombotic predisposition represented by FVL, FVR2 and FII G20210A variants (significantly more frequent than in the general population), significantly higher in PE males. While, in patients with PVT and CVT only FII G20210A variant is more frequent particularly in females. Finally, RVT is poorly related to prothrombotic risk factors, confirming that local vascular and other factors have a pivotal role in its pathogenesis. **Conclusions** Our data indicate that only FVL, FVR2 and FII G20210A are related to vein thrombotic diseases while all the other gene variants, frequently ordered in the clinical context, do not have a role as risk factors. Furthermore, the evidence of a sex difference for some variants, once confirmed in larger populations, may help to promote sex-specific prevention of such diseases.

Background

Venous thromboembolism (VTE) includes deep vein thrombosis (DVT) and pulmonary embolism (PE) that frequently appears as a complication of DVT. More recently, thrombophlebitis was classified as superficial vein thrombosis (SVT), and was recognized as a high-risk condition to generate PE in turn [1]. For other rarer entities with unusual thrombosis locations, like cerebral [2], portal [3] and retinal vein [4], it is discussed if the pathogenesis and risk factors are the same of VTE [5]. Finally, VTE is the third more frequent cause of cardiovascular diseases and death and represents a relevant medical and social problem for the high occurrence and for the severity of the phenotype in a percentage of cases [6,7]. Such diseases may be prevented and effectively treated; therefore, the search of risk factors is a major objective of biomedical research [8].

Venous thromboembolism is a multifactorial condition which results from the combination of multiple and synergic predisposing factors, acquired and inherited ones. Among them, hypercoagulability states, now termed “thrombophilia”, play a pivotal role in the pathogenesis of VTE. Some variants found in genes involved in haemostasis and its inhibition display a procoagulant effect [5].

The most known prothrombotic variants are: Factor V Leiden (FVL, R506Q variant) that causes a resistance of FV to protein C inhibition; the G20210A variant of prothrombin that enhances the synthesis and the activity of Factor II (FII) and the C677T variant of methylene-tetrahydrofolate reductase (MTHFR) that impairs the activity of the enzyme causing an increase of serum homocysteine. In addition, other gene variants have been studied in patients with various forms of venous thrombosis, like FV H1299R

(FVR2), MTHFR A1298C, Factor XIII (FXIII) V34L, Human Platelet Antigen (HPA)-1 L33P, beta-fibrinogen -455G>A, and Plasminogen Activator Inhibitor (PAI)-1 4G/5G variants [5, 9].

Discordant results were reported so far on the role of such variants as risk factors for venous thrombosis, mainly because: i) heterogeneous criteria have been used so far to select the patients; ii) most studies pooled male and female patients and women are frequently underrepresented [10] thus obscuring eventual sex differences [7, 11]; iii) some studies compared the frequency of prothrombotic gene variants in patients and controls of different geographical areas. Finally, as regards the clinical context, the ordering of tests for thrombophilia is strongly heterogeneous [12] and scarcerly respective of guidelines [13].

The aim of this study was to assess the allele and genotype frequency of nine prothrombotic gene variants in different forms of venous thrombosis in comparison to a large group of subjects from the general population of Campania region (Southern Italy) and to compare the frequency of such variants in patients of different sex and age.

Results

General population. The allele and the genotype frequencies of each gene variant are displayed in Tables 1A and 2 (column GP). No differences were observed between males and female neither in the allele or the genotype frequency (Table 1B, column GP).

Deep vein thrombosis, pulmonary embolism, superficial vein thrombosis. For each of these three diseases, the allele frequency of FVL, FVR2 and FII G20210A variants was significantly higher in DVT, PE and SVT patients with respect to subjects from the GP. For all the other variants we did not find any significant difference (Table 1A). This result was mirrored by a different distribution of the genotype frequency of FVL, FVR2 and FII G20210A variants between the groups of patients with DVT, with PE and with SVT and the general population (Table 2). In particular, each of the three variants determined a significantly increase in the odds of disease in subjects bearing the specific variant (Table 3).

Finally, when assessing sex differences in the variants-diseases association, a significant interaction was observed for the FVL variant that causes almost a six-fold increase in the odds of PE disease in male patients while failed to be a significant predictor in females (Table 3). Moreover, the allele frequencies of FVL and FVR2 were higher in males than in females with DVT, PE or SVT, although significantly only for PE subjects (Table 1B). No significant interaction effect was observed with age (first episode before or after 50 years old) in DVT, PE and in SVT patients for each of the variants (Table 3).

Portal venous thrombosis. The allele (Table 1A) and the genotype (Table 2) frequency of the FVL and of the FII G20210A variants was significantly different between the group of patients with PVT and the GP, leading to a significant increase in the odds of PVT associated to these two variants (Table 4A). For all the other variants no differences were observed between patients with PVT and the GP. The analysis of

sex and age differences did not reveal any significant interaction effect (Table 4A), even if a higher (but not significant) frequency of FII G20210A was found in female patients (Table 1B).

Cerebral venous thrombosis. As shown in Table 1A and Table 2, only the allele and the genotype frequency of the FII G20210A variant was significantly different in the patients with CVT respect to GP, leading to a significant increase in the odds of CVT associated to this variant (Table 4B). For all the other variants, no significant differences were observed between patients with CVT and the GP. No significant interaction effects with sex and age were observed for all the variants (Table 4B), only the frequency of FII G20210A variant was higher (although not significantly) in females (Table 1B).

Retinal venous thrombosis. For none variant the allele (Table 1A) or the genotype (Table 2) frequency was significantly different between patients with RVT and the GP. Similarly, no significant interaction effects with sex and age were observed for these variants (data not shown).

Discussion And Conclusions

This study firstly compared the allele and the genotype frequency for nine prothrombotic gene variants between patients with various forms of venous thrombosis and a large group of control subjects from the same geographical area.

Pulmonary embolism, DVT and SVT share a genetic predisposition represented by FVL, FVR2 and FII G20210A gene variants, whose frequency is significantly higher as compared to the GP. These data match with the current literature [5, 18-20]. Interestingly, the allele frequency of FVL and FVR2 resulted higher in males than in females with PE, DVT and SVT, although significantly only for PE subjects (while in the general population no sex difference was identified for all the variants examined). A sex difference in the allelic frequency of FVL and FII G20210A variants was previously found by our group in females with young AMI and for MTHFR C677T mutation in males with AMI [11]. Differently, most previous studies did not find (or did not assess) a sex difference for prothrombotic variants in patients with venous or arterial thrombotic disorders [21]. Only Farajzadeh M, et al [20] in an Iranian population with VTE, found a significantly higher frequency of the FII G20210A and PAI-1 4G allele in females. The sex difference in the allele frequency of prothrombotic variants in patients with venous thrombosis, once confirmed on larger populations, may impact on the strategies for care and prevention of these diseases, considering that the well known sex difference in the epidemiology of thrombotic diseases is due to mostly still unknown factors [7]. Moreover, the frequency of all other prothrombotic gene variants was not significantly different between patients with VTE and the GP. For some gene variants our data are in agreement with previous evidences: two studies excluded beta-fibrinogen -455 A>G [22, 23] and the FXIII V34L [24] as risk factors for VTE. Similarly, FXIII V34L, beta-fibrinogen -455, HPA-1, MTHFR C677T and A1298C variants and PAI-1 4G/5G alleles did not demonstrate a relationship with VTE [25]. While, differently from our study, MTHFR A1298C and C677T variants were found as risk factors for VTE [26]; and another study [27] revealed that only the MTHFR A1298C but not the C677T MTHFR variant was a

risk factor for VTE. A significant increase of the PAI-1 4G allele frequency was found only in VTE patients that had also the FVL [28]. We did not confirm such data (data not shown).

In patients with portal vein thrombosis, FVL and FII G20210A variants resulted more frequent as compared to the GP in agreement to a previous study [4], while Parik et al. [29] excluded a major role of prothrombotic gene variants in portal thrombosis. The major limit of our study is that all cases with portal vein thrombosis had chronic liver disease and it is known that inflammation, fibrosis and alterations of local circulation are the main pathogenic basis of portal hypertension in such patients [3]. Interestingly, the frequency of FII G20210A is higher (although not significantly) in our females with PVT.

In patients with cerebral venous thrombosis we found only a significantly higher frequency of the FII G20210A variant respect to the GP, and such data match with two studies [2, 30]. While, our data exclude a role of MTHFR and FVL variants that in previous studies had been reported to be associated to a higher risk for the CVT [30, 31]. Interestingly, also in CVT (as we observed in PVT) the frequency of FII G20210A is higher in females than in males. Cerebral venous thrombosis is typical of young subjects and is about three times more frequent in females [2], and our results confirm this data supporting a role of hormones in its pathogenesis in addition to local risk factors [32]. However, a role of the FII G20210A, particularly in females with CVT, seems to be considered and confirmed in larger populations.

Finally, prothrombotic gene variants have a scarce role as risk factors for retinal vein thrombosis. It is well known that RVT has peculiar local risk factors different from those that act in other forms of VTE, while the potential role of thrombophilia (including FVL and G20210A) seems to be marginal [33]. Similarly, Janssen [4] found that FVL and G20210A and other prothrombotic gene variants have a minor role in the pathogenesis of RVT, being atherosclerosis the main cause [33, 34]. Thus, routine testing for prothrombotic variants must be considered not advisable in patients with RVT [33].

From all these observations, it may be concluded that prothrombotic gene variants have a relevant role in DVT, PE and SVT: about 40% of patients show a prothrombotic predisposition represented by at least one among FVL, FVR2 or FII G20210A variants. Such predisposition is significantly more pronounced in males confirming that sex-related risk factors act in VTE. All the other prothrombotic variants do not have a role, despite the ordering of tests for thrombophilia is strongly heterogeneous and still scarcely respective of evidence-based data. While, portal and cerebral vein thrombosis seem to be less related to prothrombotic gene variants, and only FII G20210A seems to act as a risk factor, particularly in females, again confirming the existence of sex-related risk factors. Finally, retinal vein thrombosis is poorly related to prothrombotic risk factors, confirming the view that local vascular factors have a pivotal role in its pathogenesis. The evidence of a sex related difference in some prothrombotic variants is a relevant result that, once confirmed in larger populations, may help to address prevention of such diseases in a sex-specific way.

Conclusions: Our data indicate that only FVL, FVR2 and FII G20210A are related to vein thrombotic diseases while all the other gene variants, frequently ordered in the clinical context, do not have a role as risk factors. Finally, this work suggests that improving the appropriateness of laboratory test requests

means a better benefit for the patient and a reduction of unnecessary costs for the National Health System.

Materials And Methods

Patients

The study was approved by the Ethical Committee of the University Federico II, Naples, Italy (protocol n. 380/18) and was conducted in accordance with the Helsinki Declaration. Before the analysis, the data were anonymized. Our laboratory acts as the reference lab for molecular diagnostics in Campania Region (Southern Italy, about 5 million of inhabitants). During the last twelve years (i.e., 2007-2018), we received several thousands of requests for molecular analysis of thrombophilia for different diseases. We now retrospectively analyzed the results obtained in patients referred as affected by various forms of venous thrombotic diseases in comparison to a group of subjects from the general population (GP) of the same geographical area (Campania region, Southern Italy).

For each subject, we recorded all anagraphical and clinical data and verified that the diagnosis of each disease had been performed according to current guidelines [14-16]. Patients with doubtful diagnosis were excluded from the study. In addition, in cases of more patients belonging to the same family, we considered only the first case temporally analyzed. In details, our study population included: i) 430 subjects from the GP that at the anamnesis did not refer episodes of venous thrombosis (median age: 43 years; range: 5-85 years; 265 females); ii) 343 patients that experienced at least an episode of DVT (median age: 53 years; range: 10-92 years; 172 females); iii) 164 patients that experienced at least an episode of PE (median age: 56 years; range: 12-93 years; 101 females); iv) 126 patients that experienced at least an episode of SVT (median age: 53 years; range: 16-79 years; 81 females); v) 118 patients that experienced at least an episode of PVT as a complication of chronic liver disease (median age: 54 years; range: 6-79 years; 52 females); vi) 75 patients that experienced at least an episode of CVT (median age: 40 years; range: 1-76 years; 54 females); vii) 119 patients that experienced at least an episode of RVT (median age: 55 years; range: 5-88 years; 65 females). The informed consent was obtained to anonymously use all samples for research purposes.

DNA extraction

A blood sample was collected by venipuncture using the Vacutainer system in EDTA tubes. DNA was extracted from leukocytes using a commercial automated procedure (Roche, Italy). The DNA was spectrophotometrically quantified (also to verify the purity) and analyzed by real time PCR (Roche, Italy) for: FVL (R506Q variant); FVR2 (H1299R variant); FII, G20210A; MTHFR, C677T and A1298C; beta-fibrinogen, -455 G>A; FXIII, V34L; HPA-1, L33P variants and PAI-1 4G/5G alleles.

Statistical analysis

Both allele and genotype frequencies were reported as absolute numbers and percentages. Differences among groups were accordingly assessed using the Chi-Square test. Moreover, in order to quantify the effect of each variant on the disease risk, univariate Odds Ratios (ORs) with the corresponding 95% Confidence Intervals (95% CIs) were computed. Multivariable logistic regression models were built to assess whether age and sex could act as effect modifiers of the variant-disease association. In case of a significant interaction, ORs were computed separately for each sex and for each age group. Statistical analyses were performed with the statistical platform [17].

List Of Abbreviations

VTE: venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism; SVT: superficial vein thrombosis; CVT: cerebral vein thrombosis; PVT: portal vein thrombosis; RVT: retinal vein thrombosis; FVL: Factor V Leiden, R506Q variant; FII: Factor II; MTHFR: methylene-tetrahydrofolate reductase; FVR2: Factor V, H1299R variant; FXIII: Factor XIII; HPA: Human Platelet Antigen; PAI: Plasminogen Activator Inhibitor; OR: Odd's ratio.

Declarations

Ethics approval and consent to participate: this study was approved by the Ethical Committee of the University Federico II, Naples, Italy (protocol n. 380/18) and was conducted in accordance with the 1964 Helsinki Declaration and its later amendments. A written informed consent was obtained from all enrolled individuals to anonymously use all samples for research purposes

Availability of data and materials: all relevant data are available within the article.

Competing interest: all authors declare that they have no conflicts of interest.

Authors' contributions: All authors contributed to the study conception and design. Material preparation and analysis were performed by GC, RL and AE. Clinical data and samples collection were performed by AMDL and GC. Statistical analysis was performed by DB. Data collection, data interpretation and critical revision were performed by FA, FZ and GC. The first draft of the manuscript was written by GC and MC. All authors read and approved the final manuscript.

References

1. Milio G, Siragusa S, Minà C, Amato C, Corrado E, Grimaudo S, et al (2008) Superficial venous thrombosis: prevalence of common genetic risk factors and their role on spreading to deep veins. *Thromb Res* 123:194-199. <https://doi.org/10.1016/j.thromres.2008.01.013>
2. Green M, Styles T, Russell T, Sada C, Jallow E, Stewart J, et al (2018) Non-genetic and genetic risk factors for adult cerebral venous thrombosis. *Thromb Res* 169:15-22. <https://doi.org/10.1016/j.thromres.2018.07.005>

3. Fernandez M (2015) Molecular pathophysiology of portal hypertension. *Hepatology* 61:1406-1415. <https://doi.org/10.1002/hep.27343>
4. Janssen MC, den Heijer M, Cruysberg JR, Wollersheim H, Bredie SJ (2005) Retinal vein occlusion: a form of venous thrombosis or a complication of atherosclerosis? A meta-analysis of thrombophilic factors. *Thromb Haemost* 93:1021-1026. <https://doi.org/10.1160/TH04-11-0768>
5. Hotoleanu C (2017) Genetic risk factors in venous thromboembolism. *Adv Exp Med Biol* 906:253-272. https://doi.org/10.1007/5584_2016_120
6. Oger E (2000) Incidence of venous thromboembolism: a community-based study in Western France. EPI-GETBP study group. Groupe d'étude de la thrombose de Bretagne occidentale. *Thromb Haemost* 83:657-660. <https://doi.org/10.1055/s-0037-1613887>
7. Speed V, Roberts LN, Patel JP, Arya R (2018) Venous thromboembolism and women's health. *Br J Haematol* 183:346-363. <https://doi.org/10.1111/bjh.15608>
8. Favaloro EJ (2019) Danger of false negative (exclusion) or false positive (diagnosis) for 'congenital thrombophilia' in the age of anticoagulants. *Clin Chem Lab Med* 57:873-882. <https://doi.org/10.1515/cclm-2018-1041>
9. Liguori R, Quaranta S, Di Fiore R, Elce A, Castaldo G, Amato F (2014) A novel polymorphism in the PAI-1 gene promoter enhances gene expression. A novel pro-thrombotic risk factor? *Thromb Res* 134:1229-1233. <https://doi.org/10.1016/j.thromres.2014.09.021>
10. Schaefer BM, Caracciolo V, Frishman WH, Charney P (2003) Gender, ethnicity, and genes in cardiovascular disease. Part 2: implications for pharmacotherapy. *Heart Dis* 5:202-214. <https://doi.org/10.1097/01.HDX.0000074437.07268.00>
11. Tomaiuolo R, Bellia C, Caruso A, Di Fiore R, Quaranta S, Noto D, et al (2012) Prothrombotic gene variants as risk factors of acute myocardial infarction in young women. *J Transl Med* 10:235. <https://doi.org/10.1186/1479-5876-10-235>
12. Lippi G. (2014) Thrombophilia testing. Useful or hype? *Clin Chem Lab Med* 52:467-469. <https://doi.org/10.1515/cclm-2013-0561>
13. Carraro P, European communities confederation of CCLM working group on guidelines for investigation of disease (2003) Guidelines for the laboratory investigation of inherited thrombophilias. Recommendations for the first level clinical laboratories. *Clin Chem Lab Med* 41:382-391. <https://doi.org/10.1515/CCLM.2003.059>
14. Tait C, Baglin T, Watson H, Laffan M, Makris M, Perry D, et al (2012) Guidelines on the investigation and management of venous thrombosis at unusual sites. *Br J Haematol* 159:28-38. <https://doi.org/10.1111/j.1365-2141.2012.09249.x>
15. Konstantinides SV (2014) 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J* 35:3145-3146. <https://doi.org/10.1093/eurheartj/ehu393>
16. Tritschler T, Kraaijpoel N, Le Gal G, Wells PS (2018) Venous thromboembolism: advances in diagnosis and treatment. *JAMA* 320:1583-1594. <https://doi.org/10.1001/jama.2018.14346>

17. R Core Team (2018) R: A language and environment for statistical computing foundation for statistical computing, Vienna, Austria. Available at <https://www.r-project.org/>
18. Alhenc-Gelas M, Nicaud V, Gandrille S, van Dreden P, Amiral J, Aubry ML, et al (1999) The factor V gene A4070G mutation and the risk of venous thrombosis. *Thromb Haemost* 81:193-197. <https://doi.org/10.1055/s-0037-1614441>
19. Rosendaal FR, Reitsma PH (2009) Genetics of venous thrombosis. *J Thromb Haemost* 7 Suppl 1:301-304. <https://doi.org/10.1111/j.1538-7836.2009.03394.x>
20. Farajzadeh M, Bargahi N, Poursadegh Zonouzi A, Farajzadeh D, Pouladi N (2014) Polymorphisms in thrombophilic genes are associated with deep venous thromboembolism in an Iranian population. *Meta Gene* 2:505-513. <https://doi.org/10.1016/j.mgene.2014.06.004>
21. Roach RE, Cannegieter SC, Lijfering WM (2014) Different risks in men and women for first and recurrent venous thrombosis: the role of genes and environment. *J Thromb Haemost* 12:1593-1600. <https://doi.org/10.1111/jth.12678>
22. Renner W, Cichocki L, Forjanics A, Koppel H, Gasser R, Pilger E (2002) G-455A polymorphism of the fibrinogen beta gene and deep vein thrombosis. *Eur J Clin Invest* 32:755-758. <https://doi.org/10.1046/j.1365-2362.2002.01070.x>
23. Camilleri RS, Cohen H (2005) No association between pulmonary embolism or deep vein thrombosis and the -455G/A beta-fibrinogen gene polymorphism. *Blood Coagul Fibrinolysis* 16:193-198.
24. Corral J, González-Conejero R, Iniesta JA, Rivera J, Martínez C, Vicente V (2000) The FXIII Val34Leu polymorphism in venous and arterial thromboembolism. *Haematologica* 85:293-297.
25. Bezgin T, Kaymaz C, Akbal Ö, Yilmaz F, Tokgöz HC, Özdemir N (2018) Thrombophilic gene mutations in relation to different manifestations of venous thromboembolism: a single tertiary center study. *Clin Appl Thromb Hemost* 24:100-106. <https://doi.org/10.1177/1076029616672585>
26. Liu F, Silva D, Malone MV, Seetharaman K (2017) MTHFR A1298C and C677T polymorphisms are associated with increased risk of venous thromboembolism: a retrospective chart review study. *Acta Haematol* 138:208-215. <https://doi.org/10.1159/000480447>
27. Lupi-Herrera E, Soto-López ME, Lugo-Dimas AJ, Núñez-Martínez ME, Gamboa R, Huesca-Gómez C, et al (2019) Polymorphisms C677T and A1298C of MTHFR gene: homocysteine levels and prothrombotic biomarkers in coronary and pulmonary thromboembolic disease. *Clin Appl Thromb Hemost* 25:1076029618780344. <https://doi.org/10.1177/1076029618780344>
28. Wang J, Wang C, Chen N, Shu C, Guo X, He Y, et al (2014) Association between the plasminogen activator inhibitor-1 4G/5G polymorphism and risk of venous thromboembolism: a meta-analysis. *Thromb Res* 134:1241-1248. <https://doi.org/10.1016/j.thromres.2014.09.035>
29. Parikh S, Shah R, Kapoor P (2010) Portal vein thrombosis. *Am J Med* 123:111-119. <https://doi.org/10.1016/j.amjmed.2009.05.023>
30. Marjot T, Yadav S, Hasan N, Bentley P, Sharma P (2011) Genes associated with adult cerebral venous thrombosis. *Stroke* 42:913-918. <https://doi.org/10.1161/STROKEAHA.110.602672>

31. Lauw MN, Barco S, Coutinho JM, Middeldorp S (2013) Cerebral venous thrombosis and thrombophilia: a systematic review and meta-analysis. *Semin Thromb Hemost* 39:913-927. <https://doi.org/10.1055/s-0033-1357504>
32. Lussana F, Dentali F, Ageno W, Kamphuisen PW (2007) Venous thrombosis at unusual sites and the role of thrombophilia. *Semin Thromb Hemost* 33:582-587. <https://doi.org/10.1055/s-2007-985754>
33. Stem MS, Talwar N, Comer GM, Stein JD (2013) A longitudinal analysis of risk factors associated with central retinal vein occlusion. *Ophthalmology* 120:362-370. <https://doi.org/10.1016/j.ophtha.2012.07.080>
34. Giannaki K, Politou M, Rouvas A, Merkouri E, Travlou A, Theodosiadis P, Gialeraki A (2013) Retinal vein occlusion: genetic predisposition and systemic risk factors. *Blood Coagul Fibrinolysis* 24:279-283. <https://doi.org/10.1097/MBC.0b013e32835bfda1>

Tables

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