REVISTA BRASILEIRA
DE HEMATOLOGIA
E HEMOTERAPIA

Artigo / Article

Low prevalence of Factor V Leiden and the prothrombin G20210A mutation in a healthy population from the central-south region of Chile

Baixa prevalência do Fator V Leiden e da mutação da protrombina G20210A em uma população sã da região centro-sul do Chile

Iván Palomo[1]
Fabian Segovia[1]
Daniela Parra[2]
Marcelo Alarcón[1]
Edgardo Rojas[1]

*Thrombosis is a result of the interaction between predisposing genetic polymorphisms and acquired risk factors. The two prothrombotic polymorphisms which are most frequently associated with thrombosis are factor V (FV) Leiden and the prothrombin (PT) G20210A mutation. The objective of this work was to investigate the prevalence of both factors in the central-south region of Chile. Determination of the frequency was carried out by means of a genetic analysis of 1200 healthy, non-native individuals. The mutation of FV Leiden was found in 1.25% of the population and the PT G20210A mutation in 1.33%. None of the individuals were homozygosis or had both polymorphisms. The prevalences of FV Leiden and the PT G20210A mutation are less common in the healthy population. Rev. Bras. Hematol. Hemoter. 2009;**31**(3):143-146.*

Key words: *Factor V Leiden; prothrombin G20210A mutation.*

Introduction

The thrombosis constitutes one of the most common causes of morbidity and mortality in western society. It is a multifactor disease whose pathogenesis involves the interaction between acquired and genetic factors.[1,2] Among the last ones, punctual mutations are found in FV gene (G1691A or FV Leiden) and in PT gene (G20210A), which delivers a protein resistant to the activated protein C (APC) and increases PT plasmatic level, respectively, thus constituting the most frequent inherited pre-despondent factors of the thrombosis.[3]

The APC resistance of FV was described by Dahlback, Carlsson & Svensson in 1993;[4] and then, Bertina *et al*.,[5] in 1994 described the genetic alteration, which in 90% of the cases occurs due to the G1691A punctual mutation in FV gene. The FV Leiden is found in the long arm of the chromosome 1 (1q21) and the transmission of the gene is autosomal dominant. This polymorphism constitutes the most common genetic risk factor in patients with venous thrombosis, with 20-30% prevalence. Moreover, it is established that the origin was found 21,000-34,000 years ago in Middle East from where it was spread through Europe, which could explain the dependent frequency of the population, being the Caucasian the one that presents a major prevalence .[3,6] On the other hand, PT G20210A mutation represents the second most frequent prothrombotic polymorphism in humans. Its transmission is also autosomal dominant codified in the short arm of the chromosome 11 (11p11). In 1996, Poort *et al*.[7] described this mutation which has been described in 8-10% of the patients with venous thrombosis, increasing three times the risk of suffering this type of thrombosis. Its origin and distribution have been associated in the Caucasian population the same way as that

[1]*Professor, Department of Clinical Biochemistry and Immunohematology, Faculty of Health Sciences, Universidad de Talca, Talca, Chile.*
[2]*Medical Technologist, Department of Clinical Biochemistry and Immunohematology, Faculty of Health Sciences, Universidad de Talca, Talca, Chile.*

Department of Clinical Biochemistry and Immunohematology, Faculty of Health Sciences, Universidad de Talca, Talca, Chile.

Correspondência: *Iván Palomo*
Departamento de Bioquímica Clínica e Inmunohematología
Facultad de Ciencias de la Salud, Universidad de Talca
Casilla 747, Talca, Chile.
Telefono-Fax: 56-71-200488
E-mail: ipalomo@utalca.cl
Doi: 10.1590/S1516-84842009005000042

of FV Leiden.[8] On the other hand, to be a carrier of both polymorphisms increases the dependent form of the thrombosis risk.[9]

In Chile the existing information regarding these polymorphisms in the healthy population is little. Pereira *et al.*[10] report a prevalence of 3.8% for FV Leiden and Palomo *et al.*,[11] of 1.3% for FV Leiden and 2.5% for the PT G20210A mutation, but both studies include reduced number of individuals, 159 and 160, respectively. Due to the importance of both hereditary thrombophilias as risk factors of venous thrombosis, it seems necessary to know the local prevalence of both polymorphisms in a greater number of healthy individuals.

Patientes and Method

The study included 1200 healthy persons from the center-south region of Chile. The average age was 26±4 years; 52.5% women and 47.5% men. Nobody experienced current or past thromboses, neither had family history of arterial or venous thrombosis. Persons were selected as blood donors or healthy volunteers hailing from center-south cities of Chile, mainly from Maule region. The sampling group is not indigenous, it is made up of people from Picunche ethnia and a low percentage of european people.[12] Each individual signed an agreement where it was mentioned that they accepted to participate in this study. This study was conducted considering the ethical norms and was approved by the bioethical committee of Universidad de Talca.

A blood sample of 4 mL was collected from each person which was ant coagulated with EDTA in a proportion of 1mg/mL.

Genetic analysis from G1691A mutation

The DNA was extracted from leukocytes of a peripheral blood using the standard extraction procedure, non enzymatic.[13] The magnification and the digestion of DNA were carried out according to what was previously described.[14] Briefly, the PCR RFLP (Polymerase Chain Reaction, Restriction Fragment Length Polymorphism) was carried out with the primer 1: 5'- TGCC CAGT GCTTA ACAA GACCA-3' and primer 2: 5'-TGTTATCACACTGGTGCTAA-3'. The magnification was realized in a thermocycler (PRC-100 thermocycler, Mj. Research Inc.). The Mn11 enzyme restriction was used for digestion (New England Biolabs, Beverly, MA). The digested product of PCR was analyzed in agarose gel 3% by ethidium bromide stain.

Genetic analysis of G20210A PT mutation

Nested PCR, a method reported by Poort *et al*. was used.[15] This detects the mutation using a PCR specific allele without the necessity of digestion with an enzyme restriction. Two external primers were used (1 forward: 5'-TCCGCCTGAAGAAGTGGATA-3' and 2 backward: 5'-GAGTGCTCGGACTACCAGCGTGC-3') and two internal primers forward (nested) (3 forward: 5'-TTCCCAATAAAAGT GACTCTCAGCA-3' and 4 forward: 5'-TTCCCA ATAA AAGTGACTCTCAGCG-3'). The two internal primers 3 and 4 differ in the last nucleotide, for example 20210G or A. The fragment of DNA of 270 pb produced by the external primers 1 and 2 serve as a template for the primers 3 or 4 and an internal control of the PCR reaction. By using 3 or 4 primers a fragment of 148 pb is generated. The product was magnified and analyzed per protocol described above.

Results

Fifteen out of 1200 (1.25%) persons presented FV Leiden and 16/1200 (1.33%) presented PT G20210A mutation. The allelic frequency of polymorphism G of FV Leiden was 0.99, and for the polymorphism G of the PT G20210A mutation was 0.99. The studied polymorphisms found in every individual were heterozygous and no cases of double polymorphism were found.

Discussion

Our findings indicate that the prevalence of FV Leiden and PT G20210A mutations in the non-indigenous population from the center-south of Chile are of 1.25 and 1.33%, respectively, without the presence of homozygosity and double polymorphism.

In 1996, Pereira *et al.* described a prevalence of 3.8% of FV Leiden in Chile, this report could be falsely augmented due to the height of subjects included in the study and their ethnic characteristics; probably with a higher proportion of subjects with Caucasian ancestors. In fact, our report includes a number of participants 7.5 times taller, with a selection made at random, considering the whole population of Talca city and with the application of exclusion criteria.

The prevalence of both thrombophilias has been determined in the world, observing a variation which considers whether the population in study is Caucasian, Asian, African, or an ethnic minority.

A meta-analysis on FV Leiden that involved around 90,000 healthy controls showed a frequency of 3% in white population and 0.2% in Asian population.[16] In African population other studies on FV Leiden had shown low frequency (<1.5%).[17-19] In the United States the frequency of FV Leiden in the Caucasian population is between 5.3 and 8.6%, in the Afro-American between 1.2 and 1.4% and in the Asian 0.5%.[20,21] The Australian population includes many ethnic groups among them the Asian population, Caucasian and Middle East, are those who had shown a frequency of 0.3%, 2.8% and 6.2%, respectively.[22] On the other hand, Asian population presented a lower prevalence of this polymorphism than Thai people with 0.5%[22] and Japanese, Chinese, Korean and Mongolian whose prevalence is closed to 0%.[3] It is

interesting to observe that the prevalence of FV Leiden between the healthy population of a region in Turkey is 8.7% of which 1% presents homozygosity[24] and a Lebanese community showed a frequency of 13.8%.[25]

As far as the prevalence of PT G20210A mutation, it is observed a clear dependent distribution of the population. In Australia, ethnic groups of Asian, Caucasian and Middle East origin have shown a frequency of 0.3%, 1%-2.4% and 1.6%, respectively.[21] The same as FV Leiden Asian population presented low prevalence of G 20210A polymorphism, thus in Thai population a prevalence of 0.5% was found,[23] and in Japanese, Chinese, Korean and Mongolian population, a prevalence closed to 0% was found.[3] Also, a Lebanese community showed a frequency of 3.6% of this polymorphism. [25]

The major prevalence of FV Leiden and PT G20210A in the Caucasian population supports the theory that both mutations emerged 21.000 to 34.000 years ago, only in the Caucasian population, after the separation of Mongols and Caucasian.[6,8] The revision of 26 European and neighboring countries showed that the FV Leiden could have expanded from the region of Anatolia, Turkish peninsula,[3,26] to Europe and to other continents later.

In Latin American countries as Colombia, Chile, Mexico and Brazil the prevalence of both polymorphisms are less than observed in caucasic population, both in patients with venous thromboses and in healthy population,[10,11,27,28] the same as in Argentina[29] but with a smaller difference. This information agrees with the studies mentioned above and with our findings. So, world distribution of FV Leiden and PT G20210A is dependent of race and Chilean prevalence of both is low due to a historical low immigration of Caucasian people.

Resumo

*A trombose pode ser o resultado da interação de polimorfismo genético e fatores de riscos adquiridos. Os polimosfirmos pro-trombóticos mais frequentes são fator V (FV) Leiden e a mutação da protrombina (PT) G20210A. O objetivo deste trabalho foi investigar a prevalência de ambos os polimorfismos na região centro-sul do Chile. Foram realizadas análises genéticas (PCR RFLP) de 1.200 pessoas saudáveis, não nativas da região. Foram encontrados 1,25% de mutação do Fator V Leiden e 1,33% da mutação da protrombina G20210A. Não foi detectada homozigose em ambos os polimorfismos. A prevalência de FV Leiden e da mutação G20210A é baixa na população estudada. Rev. Bras. Hematol. Hemoter. 2009;**31**(3):143-146.*

Palavras-chave: *Fator V Leiden; mutação da protrombina G20210A.*

References

1. Heit JA. Risk factors for venous thromboembolism. Clin Chest Med. 2003;24(1):1-12.
2. Luyendyk JP, Tilley RE, Mackman N. Genetic susceptibility to thrombosis. Curr Atheroscler Rep. 2006;8(3):193-7.
3. Bauduer F, Lacombe D. Factor V Leiden, prothrombin 20210A, methylenetetrahydrofolate reductase 677T, and population genetics. Mol Genet Metab. 2005;86(1-2):91-9.
4. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA. 1993; 90(3):1004-8.
5. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, *et al*. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994; 369(6475):64-7.
6. Zivelin A, Griffin JH, Xu X, Pabinger I, Samama M, Conard J, *et al*. A single genetic origin for a common Caucasian risk factor for venous thrombosis. Blood. 1997;89(2):397-402.
7. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996;88(10):3698-703.
8. Zivelin A, Rosenberg N, Faier S, Kornbrot N, Peretz H, Mannhalter C, *et al*. A single genetic origin for the common prothrombotic G20210A polymorphism in the prothrombin gene. Blood. 1998; 92(4):1119-24.
9. De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, *et al*. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med. 1999;341(11): 801-6.
10. Pereira J, Quiroga T, Goycoolea M, Muñoz B, Hidalgo P, Kaltwasser G, *et al*. Activated C protein resistance: laboratory study and prevalence of the defect in the Chilean population. Rev Med Chil. 1996;124(6):663-8.
11. Palomo I, Pereira J, Alarcón M, Pinochet C, Vélez MT, Hidalgo P, *et al*. Factor V Leiden and prothrombin G20210A among Chilean patients with venous and arterial thrombosis. Rev Med Chil. 2005; 133(12):1425-33.
12. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 1991;19(19):5444.
13. Cripe LD, Moore KD, Kane WH. Structure of the gene for human coagulation factor V. Biochemistry. 1992;31(15):3777-85.
14. Poort SR, Bertina RM, Vos HL. Rapid detection of the prothrombin 20210 A variation by allele specific PCR. Thromb Haemost. 1997; 78(3):1157-8.
15. Ye Z, Liu EH, Higgins JP, Keavney BD, Lowe GD, Collins R, *et al*. Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. Lancet. 2006;367 (9511):651-8.
16. Hira B, Pegoraro RJ, Rom L, Moodley J. Absence of Factor V Leiden, thrombomodulin and prothrombin gene variants in black South African women with pre-eclampsia and eclampsia. BJOG. 2003;110(3):327-8.
17. Pottinger P, Sigurdsson F, Berliner N. Detection of the factor V Leiden mutation in a nonselected black population. Blood. 1996;87(5):2091.
18. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet. 1995;346(8983):1133-4.

19. Limdi NA, Beasley TM, Allison DB, Rivers CA, Acton RT. Racial differences in the prevalence of Factor V Leiden mutation among patients on chronic warfarin therapy. Blood Cells Mol Dis. 2006; 37(2):100-6.

20. Ridker PM, Miletich JP, Hennekens CH, Buring JE. Ethnic distribution of factor V Leiden in 4047 men and women. Implications for venous thromboembolism screening. JAMA. 1997;277(16):1305-7.

21. Said JM, Brennecke SP, Moses EK, Walker SP, Borg AJ, Williams JT, *et al*. Ethnic differences in the prevalence of inherited thrombophilic polymorphisms in an asymptomatic Australian prenatal population. Hum Biol. 2006;78(4):403-12.

22. Angchaisuksiri P, Pingsuthiwong S, Aryuchai K, Busabaratana M, Sura T, Atichartakarn V, *et al*. Prevalence of the G1691A mutation in the factor V gene (factor V Leiden) and the G20210A prothrombin gene mutation in the Thai population. Am J Hematol. 2000;65(2):119-22.

23. Kabukcu S, Keskin N, Keskin A, Atalay E. The frequency of factor V Leiden and concomitance of factor V Leiden with prothrombin G20210A mutation and methylene tetrahydrofolate reductase C677T gene mutation in healthy population of Denizli, Aegean region of Turkey. Clin Appl Thromb Hemost. 2007;13(2):166-71.

24. Almawi WY, Keleshian SH, Borgi L, Fawaz NA, Abboud N, Mtiraoui N, *et al*. Varied prevalence of factor V G1691A (Leiden) and prothrombin G20210A single nucleotide polymorphisms among Arabs. J Thromb Thrombolysis. 2005;20(3):163-8.

25. Lucotte G, Mercier G. Population genetics of factor V Leiden in Europe. Blood Cells Mol Dis. 2001;27(2):362-7.

26. Torres JD, Cardona H, Alvarez L, Cardona-Maya W, Castañeda SA, Quintero-Rivera F, *et al*. Inherited thrombophilia is associated with deep vein thrombosis in a Colombian population. Am J Hematol. 2006;81(12):933-7.

27. Ruiz-Argüelles GJ, Garcés-Eisele J, Reyes-Núñez V, Ramírez-Cisneros FJ. Primary thrombophilia in Mexico. II. Factor V G1691A (Leiden), prothrombin G20210A, and methylenetetrahydrofolate reductase C677T polymorphism in thrombophilic Mexican mestizos. Am J Hematol. 2001;66(1):28-31.

28. Genoud V, Castañon M, Annichino-Bizzacchi J, Korin J, Kordich L. Prevalence of three prothrombotic polymorphisms. Factor V G1691A, factor II G20210A and methylenetetrahydrofolate reductase (MTHFR) C 677T in Argentina. On behalf of the Grupo Cooperativo Argentino de Hemostasia y Trombosis. Thromb Res. 2000;100(3):127-31.

Avaliação: Editor e dois revisores externos

Suporte Financeiro: Supported by Cardiovascular Diseases Risk Factors Research Program (PIFRECV; http://pifrecv.utalca.cl), Universidad de Talca, Chile

Recebido: 30/05/2008

Aceito após modificações: 11/04/2009