Prevalence of factor V Leiden in a Canadian blood donor population

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Abstract • Résumé

Objective: To determine the prevalence of factor V Leiden in a Canadian blood donor population.

Design: Cross-sectional laboratory study.

Setting: Hamilton Centre of the Canadian Red Cross Society.

Participants: Volunteer donors who attended Hamilton Centre blood donor clinics over a 4-day period in August 1994; blood samples from 356 people were evaluable.

Outcome measures: Presence of factor V Leiden.

Results: Factor V Leiden was detected in 19 of the 356 people, for a prevalence rate of 5.3% (95% confidence interval 3.0% to 7.6%). All 19 people were shown to be heterozygous for the mutation.

Conclusion: Factor V Leiden is common in the Canadian population. Its prevalence is similar to that reported in other Western countries. These data are relevant in the clinical management of patients at risk for venous thrombosis and those with recurrent thrombotic disorders.

Objectif : Déterminer la prévalence du facteur V de Leiden dans une population de donneurs de sang du Canada.

Conception : Étude transversale en laboratoire.

Contexte : Centre de Hamilton de la Société canadienne de la Croix-Rouge.

Participants : Donneurs bénévoles qui se sont présentés aux cliniques de sang du Centre de Hamilton pendant une période de 4 jours en août 1994; les échantillons de sang de 356 personnes étaient évaluables.

Mesures des résultats : Présence du facteur V de Leiden.

Résultats : On a détecté le facteur V de Leiden chez 19 des 356 personnes, ce qui donne un taux de prévalence de 5,3 % (intervalle de confiance à 95 %, 3,0 % à 7,6 %). On a démontré que les 19 personnes étaient hétérozygotes quant à la mutation.

Conclusion : Le facteur V de Leiden est répandu dans la population canadienne. Sa prévalence est semblable à celle qui a été signalée dans d'autres pays occidentaux. Ces données sont pertinentes à la gestion clinique de patients à risque de thrombose veineuse et de ceux qui ont des troubles thrombotiques répétitifs.

Until recently a specific molecular defect could be identified in only a minority of patients with venous thromboembolic disease. An inherited abnormality in the circulating levels of functional antithrombin, protein C or protein S (the main inhibitors of the coagulation pathways) could be found in only 5% to 15% of patients with venous thrombosis. Since the discovery of inherited activated protein C (APC) resistance, research findings have indicated prevalence rates of this defect among patients with venous thrombosis ranging from 20% to 60%.[1-3] The only molecular abnormality associated with APC resistance thus far reported is a guanine (G) to adenine (A) substitution at nucleotide 1691 of the gene encoding factor V, predicting an arginine (Arg) to glutamine (Gln) substitution at amino acid 506. This mutation, known as factor V Leiden, is believed to occur in approximately 90% of people with the APC resistance phenotype.[4-7] The bond between amino acid residues 506 and 507 is the location at which activated factor V is cleaved and thereby inactivated by APC. Compared

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with normal activated factor V, activated factor V Leiden is relatively resistant to inactivation by APC, resulting in a hypercoagulable state.[8]

Although APC resistance has been reported to occur in approximately 5% of the European population,[1,3] its prevalence in Canada is unknown. We conducted this study, therefore, to determine the prevalence of factor V Leiden in a healthy Canadian population using blood samples from consecutive volunteer blood donors.

Methods

This unlinked laboratory study was approved by the institutional review board at the McMaster University Medical Centre. Blood samples could not be linked to the original donor; therefore, clinical data could not be obtained on people found to have factor V Leiden, nor could fresh plasma be obtained from them to determine whether they had the APC resistance phenotype.

Over a 4-day period in August 1994, 7 mL of citrated blood, routinely collected for screening of transmissible diseases, was obtained from consecutive volunteer blood donors by personnel at the Hamilton Centre of the Canadian Red Cross Society. After the screening tests were completed, the buffy coats were removed and frozen at –70°C until DNA extraction. Samples were excluded from analysis if the screening test result was positive, the sample clotted or the tube containing the sample broke during frozen storage. DNA was extracted from the buffy coats with the use of the Qlamp Blood Kit (Qiagen, Chatsworth, Calif.).

A 267 base-pair DNA segment including nucleotide 1691 of the factor V gene was amplified using the polymerase chain reaction (PCR), with *Taq* DNA polymerase (Promega, Madison, Wis.) and the primers and conditions described by Bertina and associates.[4] The PCR-amplified product was digested with the restriction enzyme *Mnl*I (New England Biolabs, Beverly, Mass.) at 37°C for 1 hour. The wild-type (normal) allele gives digestion products measuring 163, 67 and 37 base pairs in length. Factor V Leiden abolishes one of two *Mnl*I cleavage sites (present in the wild-type allele), which results in two fragments, 200 and 67 base pairs in length. The digestion products underwent electrophoresis on a 3.5% agarose gel and were then visualized with ethidium bromide staining. All samples found to contain the altered *Mnl*I cleavage site were cloned with the CloneAmp pUC19 System (Life Technologies, Burlington, Ont.) and sequenced according to the dideoxynucleotide chain-termination method described by Sanger and associates.[9] Electrophoresis of these products was performed on an 8% acrylamide/urea gel, which was exposed to x-ray film for visualization.

For allele-specific oligonucleotide hybridization, 5 µL of amplified DNA was denatured at 100°C for 10 minutes and then quench-cooled on ice. The denatured DNA was dot blotted onto 0.2 µm neutral-charge nylon membrane (ICN, Montreal, Que.) with the use of a 96-well filtration manifold apparatus (Schleicher & Schuell, Keene, NH).[10] The following oligonucleotides were used for hybridization: 5′-TGGACAGGCAAGGAATACA-3′ (mutant), and 5′-GTATTCCTCGCCTGTCCA-3′ (wild type). These probes were labelled at the 3′ end with fluorescein–deoxy uridine triphosphate and hybridized to the PCR-amplified sample DNA on the membrane with the use of an enzyme chemiluminescent 3′-oligo-labelling and detection kit (Amersham, Buckinghamshire, England). Stringency washing was performed at 54°C for 15 minutes. Anti-fluorescein–horseradish peroxidase (HRP) conjugate (provided in the kit) was added in order to detect the probe that was base-paired to the target DNA on the membrane. Addition of the detection reagents resulted in HRP-catalysed light production, which was detected on x-ray film.

Results

Samples were obtained from 378 consecutive blood donors; 22 samples were excluded because the screening test results were positive (20 samples), the sample clotted (1 sample) or the tube broke during frozen storage (1 sample).

Of the remaining 356 samples 19 were found to have factor V Leiden, for a prevalence rate of 5.3% (95% confidence interval 3.0% to 7.6%). Electrophoresis of the digestion products revealed that all 19 were heterozygous for the mutation (Fig. 1). People homozygous for factor V Leiden would have been expected to have only fragments of 200 and 67 base pairs. All 19 were confirmed through DNA sequencing to have a G–A muta-

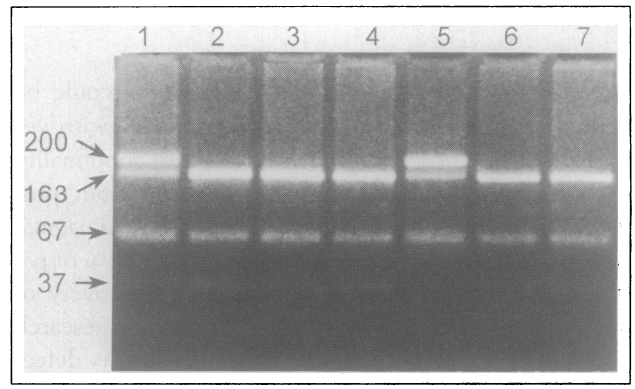

Fig. 1: Electrophoresis of 267 base-pair DNA product amplified using the polymerase chain reaction (PCR), after digestion with restriction enzyme *Mnl*I. People with only the wild-type (normal) allele have fragments of 163, 67 and 37 base pairs (lanes 2, 3, 4, 6 and 7). Heterozygous people with both the wild-type (normal) and factor V Leiden (mutant) alleles have fragments of 200, 163, 67 and 37 base pairs (lanes 1 and 5).

tion at nucleotide 1691 of the factor V gene (Fig. 2). Allele-specific oligonucleotide hybridization, performed on all 356 samples, confirmed the results of the restriction-enzyme digestion.

Discussion

To our knowledge this is the only Canadian prevalence study of factor V Leiden reported to date. The prevalence rate of 5.3% found in the Hamilton blood donor population is within the rates of 2% to 9% reported in other countries (Table 1). The exception appears to be Japan, where the mutation was not found in any of 192 healthy volunteers.[16] The heterozygote frequency in our study predicts a homozygous frequency of factor V Leiden of 0.06% (6 per 10 000), assuming the population is in Hardy–Weinberg equilibrium. Therefore, the absence of homozygous people in our study group is not surprising.

The blood donor population in our study was similar in demographic composition to the Canadian population as a whole, within the age restriction of 17 to 70 years. An independent survey company interviewed 731 donors and concluded that blood donors were a virtual cross-section of Canadian society with respect to educational level, economic sector, age, religion and place of birth (COMPAS Inc., Ottawa: A report to the Canadian Red Cross Society on findings from the Spring 1995 National Donor Survey, 1995). Furthermore, the age, sex and geographic (urban and rural) distribution of donors at the Hamilton Centre was similar to the national average for blood donors (Canadian Red Cross Society: unpublished data). Thus, we believe that the people in our study were representative of the Canadian population as a whole.

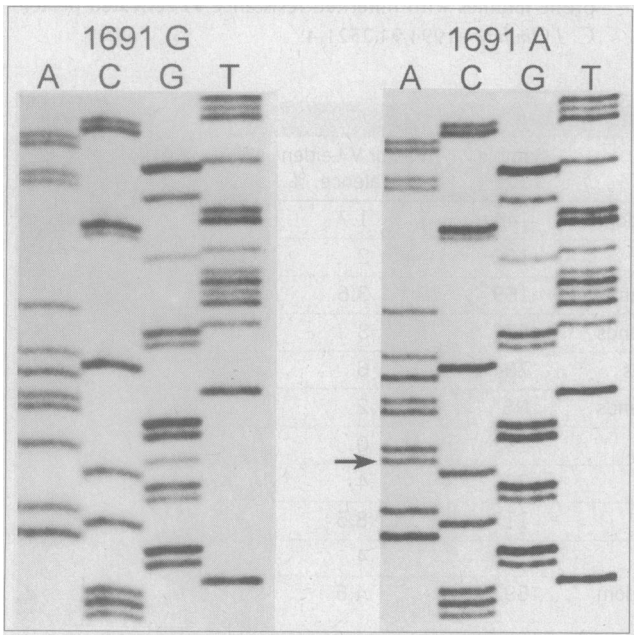

Fig. 2: Autoradiogram after allele-specific oligonucleotide hybridization, showing nucleotide sequence of PCR-amplified DNA fragment containing nucleotide 1691 of the factor V gene in normal (1691 G) and factor V Leiden (1691 A) alleles. Adenine (A) occupies the position of nucleotide 1691 in the mutant allele (arrow); guanine (G) occupies the corresponding nucleotide in the normal allele.

The presence of factor V Leiden is associated with a 5- to 10-fold increase in lifetime risk of venous thrombosis for heterozygous people. In people who are from thrombosis-prone families and have factor V Leiden, this risk has been reported to be even higher.[14,24] None the less, most heterozygous people will never have a venous thromboembolic event, and as a group they appear to have lower rates of thrombosis than heterozygous people with an antithrombin, protein C or protein S deficiency.[25] People homozygous for factor V Leiden are at higher risk of venous thrombosis than heterozygous people, and it has been estimated that the former are 50 to 100 times as likely to have thrombosis as those without the mutation.[24] Although factor V Leiden is a risk factor for venous thrombosis, several groups of investigators have failed to demonstrate an association with arterial thrombosis.[15,20,26–28]

In certain subgroups that are already at increased risk for thrombosis (e.g., women taking oral contraceptives and patients with hereditary protein C deficiency) the added presence of factor V Leiden may have a multiplicative effect on thrombosis risk.[13,29,30] In one study, young women taking oral contraceptives who had factor V Leiden were found to have a 30-fold increase in risk for thrombosis compared with women who had neither risk factor.[13] This far exceeded the increased risk associated with either risk factor alone. The absolute risk of venous thrombosis in people with both risk factors was 28.5 per 10 000 women per year, as compared with 0.8 per 10 000 per year among women with neither risk factor.[13] This means that a woman who inherits factor V Leiden and takes oral contraceptives for 15 years would have a 1 in 23 chance of experiencing a venous thrombotic event during that period. The issue of screening all women for factor V Leiden before they start taking oral contraceptives has been raised;[13,29] however, no recommendations have been published in this regard.

Virtually every practising physician likely has several patients with factor V Leiden. But who should be screened for APC resistance? Screening all patients with venous thrombosis has been suggested,[1] but its utility is unproven. At the very least, it would be reasonable to screen patients with recurrent thrombosis, those with idiopathic thrombosis who are not elderly, those with familial thrombosis and those with thrombosis who are taking oral contraceptives.

Guidelines for the management of inherited thrombotic disorders and recommendations pertaining to

long-term anticoagulation therapy have been published.[25] Dahlbäck and associates[24,31] have made similar recommendations specifically addressing the management of patients with factor V Leiden.[24,31] They suggested that patients with the mutation but no history of thrombosis should be given prophylaxis only in high-risk situations (e.g., major surgery). It has also been recommended that oral contraceptives not be prescribed to these patients.[25,31] Dahlbäck and associates[24,31] have also suggested that venous thrombosis in people heterozygous for factor V Leiden should be managed the same way as other hereditary thrombophilic defects. Thus, long-term anticoagulation therapy should be considered for heterozygous people who have suffered recurrent thrombosis. Homozygous people and heterozygous people with a second genetic defect such as an antithrombin or a protein C or S deficiency should receive aggressive thrombosis prophylaxis and treatment.[24,31] Although these recommendations are based on the available evidence, randomized controlled trials specifically designed to address the management of patients with factor V Leiden have not yet been published. The management of these patients is in the early stages of evolution; however, when a person is found to carry the mutation, counselling should be offered and screening of other family members considered.

Our study indicates that factor V Leiden is common in the Canadian population. Although further studies are required to clarify the management of these people, increased awareness of this genetic risk factor for venous thrombosis is warranted.

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Table 1: Reported prevalence rates of factor V Leiden

Study	Location	Sample size	Factor V Leiden prevalence, %
Beauchamp et al[11]	United Kingdom	144	1.7
Arruda et al[12]	Brazil	100	2
Vandenbroucke et al[13]	The Netherlands	169	3.6
Bertina et al[14]	The Netherlands	472	3
Ridker et al[15]	United States	704	6
Bertina et al[4]	The Netherlands	NS*	2
Takamiya et al[16]	Japan	192	0
Hakala et al[17]	Finland	303	4
Aschka et al[18]	Germany	117	8.5
Van Bockxmeer et al[19]	Australia	126	4
Emmerich et al[20]	United Kingdom and France	692	4.6
März et al[21]	Germany	196	4.3
Catto et al[22]	United Kingdom	208	6.3
Le Ouerrec et al[23]	France	300	2.7
Present study	Hamilton, Ont.	356	5.3

*NS = not stated.

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