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The Prevalence of Factor V R506Q Mutation-Leiden Among Apparently Healthy Lebanese

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Resistance to activated protein C (APC) degradation caused by a specific point mutation in the factor V (FV) gene, FV:R506Q or FV-Leiden, which replaces Arg506 with Gln at the APC cleavage site within the FV gene, is the most prevalent inherited caused of venous thrombosis. Recent reports suggested that the prevalence of FV-Leiden is high among Caucasians, and very low among non-Caucasians, thereby suggesting that FV-Leiden has originated as a single event in a primary focus. Insofar as FV-Leiden is associated with increased risk of thromboembolic diseases, coupled with its selective worldwide distribution, the aim of this study was to determine the prevalence of FV-Leiden in Lebanon and compare it with those of other countries of Caucasian and non-Caucasian origin. FV-Leiden was determined by PCR, followed by hybridization with specific wild-type and mutant DNA probes. By screening 174 healthy Lebanese subjects, 25 were shown to carry the FV-Leiden mutation, giving an allele frequency of 7.4% and an overall prevalence rate of 14.4%. Of the 25 FV-Leiden carriers, 24 were in the heterozygous state while one was in the homozygous states. No statistical difference in the FV-Leiden prevalence was noted with respect to age, gender, or sect. In addition to Lebanon, which had the highest prevalence rate reported thus far (14.4%), a high prevalence of FV-Leiden was reported for Syria (13.6%), Greece–Cyprus (13.4%), and Jordan (12.3%), an indication that the Eastern Mediterranean is the primary focus of FV-Leiden mutation. The high prevalence of FV-Leiden in Lebanon suggests that screening for this mutation must be considered for those with a family history, and/or those with additional risk factors for venous thrombosis. Am. J. Hematol. 65:45–49, 2000. © 2000 Wiley-Liss, Inc.

Key words: factor V Leiden; coagulation; epidemiology; thrombosis

INTRODUCTION

Factor V (FV) is a single-chain pro-cofactor that acts in concert with other plasma factors, in regulating the blood coagulation [1]. Upon its proteolysis by factor Xa and/or α-thrombin, factor Va is yielded which consists of non-covalently associated heavy (M_r 105 kDa) and light (M_r 74 kDa) chains [2] which, in turn, is cleaved by activated protein C (APC) at Arg506, followed by cleavage at Arg306 and Arg679 [1,3]. Accordingly, loss of Arg506 significantly reduces the rate of factor Va proteolysis [1], thus diminishing overall anti-coagulant activity and accelerating the pathogenesis of thrombosis [4].

Factor V mutation-Leiden (FV-Leiden) is a specific point mutation (G1691 to A; Arg506–Gln) in the FV gene [3,5], which renders the factor Va (FV R506Q) resistant to APC degradation [1,5]. The defective APC anti-coagulant system in turn precipitates increased thrombin generation, enhanced blood coagulation, and heightened risk of venous thromboembolism (VTE), particularly in young adults [6]. Varied prevalence rates (0–14%) for FV-Leiden allele were reported in healthy individuals [7,8], which increased in patients with idiopathic thromboembolism (20–60%) [8,9], thus rating FV-Leiden as the largest inherited predisposing as well as additional risk factor for VTE [4,10], pulmonary embolism [11], and to a lesser extent coronary artery disease

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(CAD; ref. 12), although the significance of the latter remains to be established [4,13].

Studies on its prevalence revealed ethnic and geographical distribution of FV-Leiden. Varied (2–14%) frequency was reported for Caucasians [14,15], FV-Leiden was virtually absent among non-Caucasians [16–19]. This suggested that FV-Leiden mutation must have originated from a single focus, spreading to other world regions by migration of mutation-carrying individuals [20]. Insofar as FV-Leiden is the most common inherited predisposing factor for VTE and disorders of poor anticoagulation [21], coupled with its selective distribution [7,19], the prevalence of FV-Leiden was determined for apparently healthy Lebanese subjects. Values obtained were compared to those of other countries and world regions, in particular Eastern Mediterranean countries, where high prevalence of FV-Leiden was seen.

MATERIALS AND METHODS

Study Group

Healthy Lebanese individuals ($N = 174$) of both sexes, aged 4–69 years, from the five provinces of Lebanon representing the two major sectarian groups were recruited after signing a consent form indicating their acceptance to participate in the study. All institutional ethics requirements were met. EDTA-anticoagulated blood (5-ml sample) was obtained from each participant, and was processed shortly thereafter.

DNA Extraction

Peripheral blood mononuclear cells (PBMC) were extracted from the venous blood by Hypaque-Ficoll (SG 1.076; Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation, washed, and pelleted in a DNase-free sterile microfuge tube. Total genomic DNA was isolated by the phenol–chloroform method, as is standard.

PCR Analysis

A typical PCR mixture comprised genomic DNA (50–200 ng), $MgCl_2$ (1.5 mM), forward and reverse oligonucleotide primers (DiaSorin, Saluggia, Italy), dNTP mixture (at 10 mM final concentration; Pharmacia), and Taq DNA polymerase (2.5 U/reaction tube; Amersham, Buckinghamshire, UK). PCR was carried on a Perkin-Elmer Cetus 9600 thermal cycler according to manufacturer's (Sorin) instructions. Patients were included in the study after signing a consent form. Amplified PCR products were then hybridized with wild-type ("G") and mutant ("A") DNA probes that contained guanine and adenine at position 1691, respectively; detection was by DNA enzyme immunoassay (DEIA). The presence or absence of FV-Leiden was interpreted based on G/A hybridization as follows: FV-Leiden non-carriers, +/−, FV-Leiden heterozygote carriers, +/+; and FV-Leiden homozygote carriers, −/+.

TABLE I. Profile of Study Population

	Total	Males	Females
Number	174	84	90
Religion			
Moslems	65	39	26
Christians	109	45	64
Regional Distribution			
Greater Beirut	59	25	34
Mount Lebanon	48	26	22
South Lebanon	35	19	16
Others	32	14	18
Age			
Mean ± SD (years)	32.5 ± 10.9	31.7 ± 10.3	33.3 ± 11.5
Range (years)	4–69	12–68	34–78

Statistical Analysis

Statistical analysis was performed on SPSS statistics software. Data were expressed as percentages of the mean or as frequency of the allele. Student's *t*-test and Pearson χ-square test were used to assess inter-group significance.

RESULTS

Prevalence of FV-Leiden in Lebanon

The prevalence of FV-Leiden was determined for 174 healthy Lebanese subjects, mean age 32.5 ± 10.9. The subjects were of both sexes (84 males vs 90 females), representing the two major religions (65 Moslems and 109 Christians), and from different provinces of Lebanon (Table I). At the time of specimen collection, all participants did not have any past or present blood coagulation disorders (including venous thrombosis), or reported a family history of venous thrombosis. None of the subjects was on anti-coagulant, anti-hypertensive, or other medication.

FV-Leiden was present in 25 out of 174 participants, which gave an allele frequency of 0.074 and an overall carrier frequency of 14.4% (Table II). The mean age of FV-Leiden carriers was similar to that of FV-Leiden non-carriers (32.6 ± 11.0 and 32.1 ± 10.6, respectively, $P = 0.84$). No difference in FV-Leiden frequency was seen with respect to gender (10/84 and 15/90 for males vs females, respectively, $P = 0.37$) or to sect (8/65 and 17/109 for Moslems vs Christians, $P = 0.55$). Although not statistically significant, heterogeneity in FV-Leiden distribution was noted among different regions in Lebanon, exemplified by its high prevalence in Mount Lebanon and its low prevalence in South Lebanon (8/48 and 3/35, respectively). Of the 25 FV-Leiden carriers, 24 were in the heterozygous state and one was in the homozygous state, resulting in a calculated allele frequency

TABLE II. Distribution of Factor V Mutation-Leiden

Group	Number	Heterozyg.	Homozyg.	Allele freq.	SE
Total	174	24	1	0.074	0.020
By sex					
Males	84	9	1	0.065	0.027
Females	90	15	0	0.083	0.029
By religion					
Moslems	65	8	0	0.062	0.030
Christians	105	16	1	0.083	0.026
By region					
Greater Beirut	59	8	0	0.068	0.033
Mount Lebanon	48	8	0	0.083	0.040
South Lebanon	35	3	0	0.043	0.024
Others	32	5	1	0.109	0.055

of 0.074 (Table II). The single FV-Leiden homozygous carrier was a 61-year-old male who did not show any sign of thrombosis or reported a family history of thrombotic disorders at the time of specimen collection. In any event, his physician was notified as to test results. Furthermore, the observed homozygote to heterozygote ratio was consistent with the Hardy–Weinberg equilibrium ($p = 0.862$, $pq = 0.138$, and $q = 0.006$).

World Distribution of FV-Leiden

In view of its high prevalence among healthy Lebanese subjects, we compared FV-Leiden prevalence in Lebanon with that of other countries and world regions. Lebanon had the highest FV-Leiden prevalence rate of 14.2%, which was closely followed by the eastern Mediterranean countries: Syria (13.6%), Greece-Cyprus (13.4%) [7], and Jordan (12.3%) [15]. High FV-Leiden prevalence was also seen in Turkey [22,23], Argentina [19], UK (whites) [7], Australia [24], USA (Caucasians) [25], and Spain [26], countries with a predominant Caucasian population. In contrast, FV-Leiden was virtually absent from countries with predominant non-Caucasian population, including Senegal [7], Japan [27], China, and Greenland Inuits [28] (Table III).

TABLE III. World Distribution of Factor V Mutation-Leiden

Country	Number	Frequency	Percentage	Reference
Lebanon	**174**	**25**	**14.4**	
Syria	500	68	13.6	A. Nehme'
Greece-Cyprus	187	25	13.4	7
Jordan	400	49	12.3	15
Sweden	288	32	11.1	14
U.K., whites	237	21	8.9	7
Turkey	81	6	7.41	22, 23
Germany	814	58	7.12	17
Argentina	215	11	5.12	19
U.S.A.	4047	150	3.71	7, 25
Australia	500	18	3.60	24
Spain	150	5	3.33	26
Venezuela	126	2	1.60	19
India (Punjab)	150	2	1.33	7, 19
Saudi Arabia	55	0	0.00	7
Japan	382	0	0.00	27
China	93	0	0.00	28
Senegal	96	0	0.00	7

DISCUSSION

Insofar as factor V regulates blood coagulation, and in view of the association of FV-Leiden mutation with thromboembolitic disorders, we assessed the prevalence of FV-Leiden among healthy Lebanese, being cautious as to avoid epidemiologic bias. Both Christians and Moslems are present to varying extents in the five provinces of Lebanon, thus the study sample was intended as representative of the Lebanese at large. Analysis of the FV-Leiden mutation was performed by hybridization with wild type and mutant DNA probes thereby avoiding false negatives/positives, potentially associated with restriction analysis. Results obtained were compared it to those established for other countries and world regions.

It was evident that the prevalence of FV-Leiden in Lebanon of 14.4% is the highest reported thus far. Whereas its prevalence was comparable among males and females, and between Lebanese Moslems and Christians, regional differences in FV-Leiden prevalence were noted in our study population. This was apparent in the (relatively) low frequency in South Lebanon (8.6%) and the high frequency in Mount Lebanon (16.7%). This suggested differential demographic distribution of FV-Leiden in Lebanon, although a more detailed study including a larger sample size will be required to confirm, or alternatively rule out, this conclusion. With the exception of a lone case of homozygote carrier, all FV-Leiden carriers were in the heterozygous state, in agreement with the Hardy–Weinberg equilibrium (1.019).

Data compiled for eastern Mediterranean and other countries revealed that high FV-Leiden prevalence were obtained in the following neighboring countries: Syria (13.6%; A. Nehme', personal communication), Cyprus (13.4%) [7], Jordan (12.3%) [15], and Turkey (7.3%) [22,23] (Table III). This confined the origin and the primary focus of the FV-Leiden mutation in the Eastern Mediterranean basin. As listed in Table III, high prevalence of FV-Leiden was seen in countries with a predominant Caucasian population. These included Sweden [29], Germany [17], and The Netherlands [30]. High FV-Leiden prevalence was also reported in countries associated with significant migration of Lebanese/Syrians and Greeks. These included the United Kingdom [7], Argentina [19], Australia [24], and Spain [26].

Taken together with data obtained for other countries, our results support the notion of a single point origin of the FV-Leiden as was suggested by Zivelin, who suggested that FV-Leiden may have arisen 21,000–34,000

years ago as a single event [20], spreading to different world regions by the migration of mutation-carrying individuals. In support of this notion were the findings that FV-Leiden was nearly absent in Senegal [7], Korea [31], Japan [27], and Ethiopia [32] and among Greenland Inuits [28] and African-Americans [25]. Collectively, this was in perfect agreement with previous findings pertaining to the preferential prevalence of FV-Leiden among Caucasians, as was suggested [18,25].

FV-Leiden is the largest inherited risk factor of venous thrombosis [21]. However, the pathogenesis of venous thrombosis is multi-factorial [33]. Accordingly the high prevalence rate of FV-Leiden among Lebanese VTE patients (69%) is a consequence of the high prevalence rate among healthy individuals as reported here (Almawi et al., unpublished data). Thus, while our results do not support random and indiscriminate screening for FV-Leiden, its high prevalence among apparently healthy individuals (and VTE patients) in Lebanon recommend screening for FV-Leiden in relatives of FV-Leiden carriers, in individuals with family history of venous thrombosis, and in high-risk situations, including pregnancy (34), use of oral contraceptives [35,36], and surgery.

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