Prevalence of 1691G>A *FV* mutation in Poland compared with that in other Central, Eastern and South-Eastern European countries

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Abstract

The 1691G>A *FV* variant has been described as a common genetic risk factor in venous thromboembolism. The purpose of this study was to provide a further frequency value for 1691G>A *FV* in Poland and to collate summary data from Central (Poland, Czech, Slovakia), Eastern (Russia, Belarus, Ukraine) and South-Eastern (Slovenia, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Macedonia, Bulgaria) European countries. For this purpose in 2007 the 1691G>A *FV* variant was analyzed by polymerase chain reaction-restriction fragment length polymorphism from DNA collected in 2005-2006. We studied 650 subjects: 400 newborns and 250 older individuals (mean age 46.1 y) from Poland and compared results with reports from other countries, as well as with the frequency trend of 845G>A *HFE* across South-Eastern European countries using centroid cities. From our 1691G>A *FV* study we identified 626 GG homozygotes, 23 GA heterozygotes, and 1 AA homozygote (n = 650), giving an A allele frequency of 1.9%, and a summed frequency value for Poland of 2.0% (n = 1588); the frequency in Central European countries was 3.9% (n = 4559), mostly due to the high value in the Czech Republic: 5.1% (n = 2819); the South-Eastern European countries had 2.5% (n = 2410). Among the Eastern European countries the 1691G>A *FV* allele frequency was 1.9% (n=791), between the South-Eastern and Eastern European countries there was no significant difference (p=0.17). We confirm that the 1691G>A *FV* allele frequency in Poland, as well as other countries compared, is significantly lower than that in Czech.

KEY WORDS: Factor V, FV Leiden, centroids

INTRODUCTION

Synthesized in the liver, blood coagulation Factor V (FV) is a multidomain glycoprotein encoded by a gene consisting of 25 exons, located on chromosome 1q23. The 1691G>A *FV* transition in exon 10 of factor 5 causes an arginine to glutamine substitution (R506Q) known as Factor V Leiden. This genetic disorder is characterized by poor anticoagulant response to activated protein C and is the most common risk factor for thromboembolic disease [1]. Major clinical observations are that the presence of 1691G>A *FV* increases risk of deep vein thrombosis [2-4] and is also associated with a increased relative risk for pregnancy loss and possibly other obstetric complications [5,6].

The frequency of the 1691G>A *FV* allele varies worldwide and differences are observed between geographic locations and ethnic populations: The 1691G>A *FV* allele is very rare or non-existent in Asia (0.6%) and some regions of Africa (0.0%) [7-9]. On the other hand, Settin et al. [10] has described the prevalence of the mutant allele at the level of 10.2% in Egypt. In Poland, the prevalence of the variant 1691G>A *FV* has been previously given by several researchers [11-15]. One objective of this research was to give a larger sample size with a 650 subjects from the West Pomeranian province of Poland. This value is then compared to previous groups of Poles and values from other countries. Note that the population now inhabiting the region of West Pomerania resulted from extensive mixing of Polish peoples from all regions of Poland after the Second World War and therefore can provide a representative sample for the whole of Poland [16, 17]. A second objective was to present summary data: to our knowledge summary data for 1691G>A *FV* from Central (Poland, Czech, Slovakia), Eastern (Russia, Belarus, Ukraine) and South-Eastern (Slovenia, Croatia, Bosnia and Herze-

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govina, Serbia, Montenegro, Macedonia, Bulgaria) European countries has not been presented before. The third aim of this study was to provide summed frequency values for 1691G>A *FV* in these countries, gathered from studies using similar methods ie. by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

MATERIAL AND METHODS

Samples

The experimental study was performed (in 2007) on a group of 650 Polish individuals, divided into two subgroups: 400 newborns (187 female and 213 male) - and 250 older subjects (mean age 46.1 y, range: 2-87 y, 169 female and 81 male) - all inhabitants of the West Pomeranian province of Poland. The older subjects, of Polish origin, were consecutive healthy visitors to the analytical laboratory VITA at Darlowo, Poland, and no exclusion criteria were used. The sub-group of newborns has been described previously [14]. All neonates were of Polish origin, with Polish grandparents, and informed consent was obtained from all parents. The Ethical Committee of the Pomeranian Medical University approved the protocol of the study (BN- 001/57/05).

Procedure

For identification of the NM_000130.4:c.1691G>A *F5* alteration (here designated as 1691G>A *FV*) we used PCR-RFLP. Genomic DNA was extracted from 100 µL of umbilical blood (for newborns) or full blood (for older subjects), using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). For a 10 µl-PCR, ~20 ng of genomic DNA was used. The PCR mixture contained 10x buffer (pH 8.3, 1.5 mM $MgCl_2$), 0.2 mM each of the deoxynucleoide triphosphates, 0.5 U Polymerase *Taq* (MBI Fermentas, Lithuania) and 4 pmol each of the forward and reverse primers as designed by Gandrille et al. [18]. Primers were synthesized by TIB MOLBIOL, Poznan, Poland. PCRs were performed in a Mastercycler Gradient device (Eppendorf, Hamburg, Germany) with the following temperature profile: initial denaturation at 94˚C for 5 min; 35 cycles of 20 s at 94˚C, 40 s at 56˚C and 40 s at 72˚C; and final extension step at 72˚C for 7 min. Amplification was followed by digestion of a 241 bp product with Hind III restriction enzyme (5'-A↓AGCTT-3') (MBI Fermentas, Lithuania) for 16 hours at 37˚C. The PCR digestion products were separated in 3 % agarose gels, stained with ethidinum bromide and recorded with a DS-34 Polaroid Instant Camera (Polaroid, Germany) using UV light (Transilluminator 4000, Stratagene, La Jolla, CA, USA). Hind III digestion yields fragments: 241 bp. (homozygote GG); 241, 209 and 32 bp. (heterozygote GA); 209 and 32 bp. (homozygote AA). Genotypes of GA and AA subjects were also confirmed by DNA sequencing (3100-Avant Genetic Analyzer, Applied Biosystems Hitachi, Foster City, CA, USA). For summary trend data of 1691G>A *FV* in South-Eastern European countries including Turkey the values are plotted against latitude of centroid cities (from BRRG - Buero fuer Raumforschung, Raumplanung und Geoinformation, Oldenburg, Germany: http://www.brrg.de/database.php?language= en&cId=0&dId=47) with latitude derived from Google maps (Google Inc, USA; http://www.mapcrow.info): Centroid cities "represent the political, administrative and cultural centre of the region". Graphical materials were developed using Designworks software (GSP Ltd, London, UK) and Microsoft Office (Microsoft, Redmond, WA, USA).

Statistical analysis

Fifty-five statistical comparisons (using z-tests) were made between all pairs of summary prevalence values for all countries studied. With Bonferroni correction a critical *p*-value of (0.05/55 = 0.0009) was used to define statistical significance. Z-tests and correlation coefficients (tested by linear regression) were calculated using Statistica (data analysis software system, version 8.0, StatSoft, Inc. 2007, www.statsoft.com).

RESULTS

The frequency of the 1691G>A *FV* allele in the study group (n = 650) proved to be 1.9%. We identified 626 GG homozygotes, 23 GA heterozygotes, and 1 AA homozygote, conforming to the expected Hardy-Weinberg equilibrium. This gives a summed frequency value for Poland of 2.0% (n = 1588). The average frequency of the 1691G>A *FV* allele in Central, Eastern and South-Eastern European countries was 3.2 % and varied from 0.6% (Belarus) to 5.1% (Czech Republic). The frequency of 1691G>A *FV* from our results (Table 1) and summary data from other countries (Table 2) are shown, and a map showing the summed frequencies for 1691G>A *FV* is shown in Figure 1. In Central European countries the frequency of 1691G>A *FV* was 3.9% (n = 4559) and varied from 5.1% in the Czech Republic through 2.1% in Poland (including our study) to 1.3% in Slovakia [11-13, 15, 19, 20]. The Czech Republic value was found to be significantly different from that in Poland, Russia, Ukraine, Slovenia, Croatia and Serbia/Montenegro, us-

TABLE 1. Allele frequencies for 1691G>A *FV* in present study.

Population	Group	Number of individuals	Frequency of 1691A *FV* allele (%)
Poland	Present study - newborns	400	2.3
	Present study - older individuals (mean age 46 y.o)	250	1.4
	Present study - whole group	650	1.9

TABLE 2. Allele frequencies for 1691G>A *FV* in Central, Eastern and South-Eastern European countries.

Country	Reference	Number of individuals		Frequency of 1691A *FV* allele (%)	
		per study	sum per country	per study	per country (weighted mean)
Poland	*Our study*	*650*	1588	*1.9*	2.0
	Herrmann FH et al, 1997 [11]	200		2.5	
	Łopaciuk S et al., 2001 [12]	238		2.1	
	Seremak-Mrozikiewicz A et al, 2010 [15]	400		1.8	
	Nizankowska-Mogilnicka E et al, 2003 [13]	100		1.5	
Czech Republic	Procházka M et al, 2003 [19]	2371	2819	5.4	5.1
	Paseka J et al, 2000 [20]	448		3.3	
Slovakia	Hudecek J et al, 2003 [21]	152	152	1.3	1.3
Slovenia	Meglic L et al, 2003 [22]	56	526	2.9	2.5
	Petrovic D et al, 2003 [23]	115		2.2	
	Petrovic D et al, 2001 [24]	132		2.3	
	Bedencic M et al, 2008 [25]	223		3.2	
Croatia	Coen D et al, 2001 [26]	155	749	2.0	1.6
	Jukic I et al, 2009 [27]	200		1.8	
	Cikes V et al, 2004 [28]	168		1.2	
	Alfirevic Z et al, 2010 [29]	106		1.4	
	Eterović D et al, 2007 [30]	120		1.3	
Bosnia and Herzegovina	No data found	No data		No data	
Serbia/*Serbia and Montenegro	Kovac M et al, 2010 [31]	128	499	0.8	2.2
	Mikovic D et al, 2000 [32]	50		2.0	
	* Djordjevic V, et al, 2004 [33]	120		2.9	
	Salatić I et al, 2011 [34]	71		2.8	
	Djordjevic V et al, 2003 [35]	130		2.7	
Macedonia	Arsov T et al, 2006 [36]	130	130	3.5	3.5
Bulgaria	Boyanovsky B et al, 2001 [37]	100	506	4.5	3.6
	Kovacheva K et al, 2007 [38]	80		3.1	
	Ivanov P et al, 2007 [39]	49		3.1	
	Ivanov P et al, 2008 [40]	98		3.6	
	Ivanov P et al, 2009 [41]	79		3.2	
	Ivanov PD et al, 2009 [42]	100		3.5	
Russia	Baranovskaya S et al, 1998 [43]	483	539	1.4	2.4
	Avdonin PV et al, 2006 [44]	56		1.8	
Ukraine	Tatarsky P et al, 2010 [45]	172	172	0.9	0.9
Belarus	Lipay NV et al, 2007 [46]	80	80	0.6	0.6

*Weighted average

ing two-proportion z-tests for comparisons between all countries studied except Turkey (with Bonferroni correction, $p < 0.001$, Table 3). In Eastern European countries the frequency of this mutant allele was 1.9% (n = 791) and varied from 2.4% in Russia through 0.9% in Ukraine to 0.6% in Belarus [43, 45, 46]. The prevalence of the 1691G>A *FV* allele in South-Eastern European countries was 2.5% (n=2410) varying from 3.6% in Bulgaria to 1.6% in Croatia. Unfortunately no data were found for Bosnia and Herzegovina, despite an extensive search. The 1691G>A *FV* variant follows a roughly increasing trend from West to East (Figure 2).

DISCUSSION

In the countries examined, which are predominantly inhabited by Slavic peoples, the allele frequencies for 1691G>A *FV* provide a mosaic (Figure 1). In our study group the frequency of the 1691G>A *FV* allele was consistent with the previous summed frequency value for Poland (2.0%) [11-13,15] and is similar to that in France (2.2%) [47], Switzerland [48] and the Netherlands [49-50] (each 2.2%) as well as to that in Serbia (2.2%) [31-35] and Russia (2.4%) [43, 44]. This value is, however, significantly different from that in the adjacent Czech Republic. To our knowledge we give the first summary study of the frequency distribution of the 1691G>A *FV* allele in Central, Eastern and South-Eastern European countries. A total of 7760 control individuals originating from 11 countries in provide the value for the frequency (3.2%) of this mutated al-

TABLE 3. *p*-values from two-proportion z-tests between 1691A *FV* frequency values of Central, Eastern and South-Eastern European countries. Significant differences, after Bonferroni correction (critical $p = 0.05/55 = 0.0009$), are shown in bold.

Country	*p*-value									
	Macedonia	Bulgaria	Russia	Poland	Belarus	Ukraine	Slovakia	Slovenia	Croatia	Serbia/ Montenegro
Czech Republic	0.2162	0.0413	0.0001	<0.0001	0.0098	0.0004	0.0028	0.0003	<0.0001	0.0001
Macedonia	-	0.9347	0.2932	0.0854	0.0573	0.0221	0.0769	0.3478	0.0285	0.2060
Bulgaria	-	-	0.1069	0.0037	0.0448	0.0101	0.0414	0.1456	0.0013	0.0617
Russia	-	-	-	0.4288	0.1445	0.0864	0.2437	0.8813	0.1459	0.7615
Poland	-	-	-	-	0.2095	0.1608	0.3978	0.3297	0.3464	0.6972
Belarus	-	-	-	-	-	0.7257	0.4837	0.1310	0.3231	0.1773
Ukraine	-	-	-	-	-	-	0.6242	0.0728	0.3305	0.1245
Slovakia	-	-	-	-	-	-	-	0.2121	0.6994	0.3252
Slovenia	-	-	-	-	-	-	-	-	0.1075	0.6543
Croatia	-	-	-	-	-	-	-	-	-	0.2745
Serbia/Montenegro	-	-	-	-	-	-	-	-	-	-

FIGURE 1. Allele frequencies (bold), number of subjects (not bold) for 1691G>A *FV* in Central, Eastern and South-Eastern countries (summed frequencies from references in Table 2).

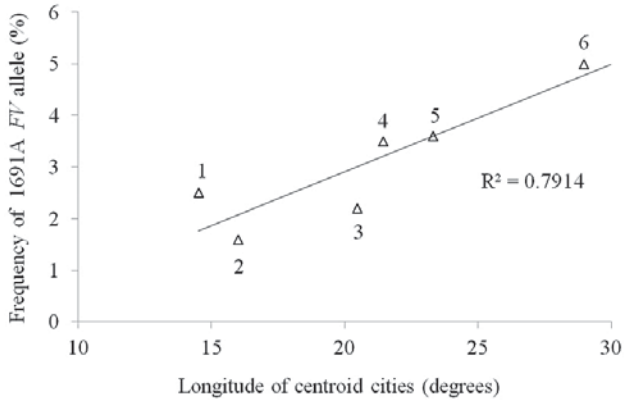

FIGURE 2. Trend in frequency of 1691G>A *FV* in South-Eastern Europe. Centroid cities: 1, Ljubljana (Slovenia); 2, Zagreb (Croatia); 3, Belgrade (Serbia and Montenegro); 4, Skopje (Macedonia); 5, Sofia (Bulgaria); 6, Istanbul (Turkey)

lele in these countries. However, if the Czech value is removed the summed frequency for the 10 remaining countries is 2.2%. These percentages indicate that future genetic counseling will be of some benefit in this region. The reasons for this mosaic in 1691G>A *FV* frequencies is not known. However, population movements have contributed to ethnic groups, cultures and consequently inheritance mixing, both throughout thousands of years of prehistory as well as in recent documented history. In the Southern Slavs there is a rough upward trend for 1691G>A *FV* in a southeasterly direction from Croatia (1.6%) and Serbia (2.2%) to Macedonia (3.5%) and Bulgaria (3.6%) (Figure 2). In neighboring Turkey this trend continues in a southeasterly direction as the frequency is even higher, at 5.0% [51-57]. This trend opposes that for 845G>A *HFE* [58]. It would be interesting to fill the gaps in the data for 1691G>A *FV* in Bosnia and Herzegovina, and it would be interesting to examine the prevalence of 1691G>A *FV* in the Western Slavic group in Germany (the Sorbs).

CONCLUSIONS

The frequency of the 1691G>A *FV* allele in Poland, summed from our study and previous studies, 2.0%, was similar to that in most countries studied, and similar to the summed frequency value for all Central, Eastern and South-Eastern countries (with the value for the Czech Republic removed), ie. 2.2%. The values for Poland, Russia, Ukraine, Slovenia, Croatia and Serbia/Montenegro were significantly different from that in the adjacent Czech Republic. In the South-Eastern European countries there is a rough upward trend for 1691G>A *FV* in a southeasterly direction, which opposes that for 845G>A *HFE* (Figure 2 [58]).

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DECLARATION OF INTEREST

The authors state there is no conflict of interest.

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