Iranians of whom 52 were females and 109 were males. The median age was 35, the youngest being 18 and the oldest 66 years of age. They represented individuals from all the Iranian regions who live in Tehran, the capital. For the factor V Leiden mutation 9 heterozygotes were found (frequency of 5.5%), with an allele frequency of 2.7% and for the G20210A mutation 5 heterozygotes were found (frequency of 3.1%), with an allele frequency of 1.5%. The frequency of factor V Leiden in Iran lies between those of Turkey in the west border of the country (9.8-10.3%) (11, 12) and those of Pakistan and India in the east (0-4.3%) (1, 3). Azerbaijan in the northwestern border of Iran has an heterozygous frequency as high as 14% (13) while Saudi Arabia, being southeast to Iran though not a neighbor, has an heterozygous frequency of 2.5% (4). In Iran, the prothrombin mutation has a frequency of 3.1%, comparable to that of Southern Europe (3%) (7). Since no similar data for this mutation are available for neighboring countries like Turkey, Azerbaijan, Pakistan and Saudi Arabia, it is very difficult to establish whether or not there is a west-to-east decreasing frequency of the mutation as seen for factor V Leiden. A relatively high frequency of the factor V Leiden mutation in Iranians and a frequency of the prothrombin mutation similar to that found in Europe strengthen the notion of European migration to the East toward Iran thousands of years ago.

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Received July 6, 1999 Accepted September 14, 1999

Thromb Haemost 2000; 83: 352-4

Comparison of the Risk of Pulmonary Embolism and Deep Vein Thrombosis in the Presence of Factor V Leiden or Prothrombin G20210A

Dear Sir,

Recently, we have read in your publication an interesting paper of Turkstra et al. that focuses on a key question as to what could be the role of the factor V Leiden (FVL) specifically in the pulmonary embolism (PE) (1). Previous papers have suggested a lower prevalence of FVL mutation among patients with PE as opposed to those with a history of venous thrombosis (VT). This apparent anomaly was first described by Desmaurais et al. using the APC-resistance method (2). Recently, several groups have established the risk of PE, using the reference method (DNA analysis), as only a half or less than the estimated for VT (3-5). In the report of Martinelli et al. the results were more unequivocal; among the FVL carriers, the estimated risk for isolated PE was 1.7 (CI 95% = 0.2-10) vs. a risk of 10 (CI 95% = 5-15) for VT (4). In accordance with previous reports, Turkstra et al. hypothesised if the
Letters to the Editor

structure of the thrombus in these patients could be more rigid and stable (1, 3-5). Whereas the relative benign outcome of FVL mutation, we can understand better some previous reports that described a similar prevalence among centenarians and young people (6), or excluded this mutation as a cause of premature death (7-10).

Analysing our series of patients we achieved similar conclusions. Moreover, we aimed to verify if the clinical presentation of the FII G20210A carriers showed an equivalent bias.

We studied 264 consecutive thromboembolic events observed in our hospital in 246 patients (mean age = 57 years, range 17 to 93, 50% females). They had been diagnosed of deep venous thrombosis (DVT), pulmonary embolism (PE) or both, using objective methods as contrast venography (n = 198), colour coded Doppler exam (n = 38), lung scan (n = 148) or computerised tomography (in three cases of axillary or mesenteric thrombotic locations). The PE was diagnosed when a high probability lung scan was obtained or when a medium-high probability lung scan was associated to a positive venography or Doppler exam. The venography was considered the gold-standard method for the diagnosis of DVT and used whenever it was technically possible and the patient consent was obtained. Otherwise the ultrasonographic study was done. When a patient diagnosed of DVT showed symptoms or signs of PE, a lung scan was performed to establish or rule out the diagnosis of DVT+PE.

The non-matched control group (thrombosis free) included 320 subjects (mean age = 57 years, range 25 to 90, 46% females) from the same geographic area and ethnic origin. We determined the FVL and FII G20210A mutations in both populations using the standard PCR techniques.

Among the patients, the prevalences were divided in three groups according to clinical presentation as: isolated DVT, DVT+PE or apparently primary pulmonary embolism. Three patients with DVT that recurred as PE and one PE that recurred as DVT were also included on the DVT+PE group. Another fourteen recurrent cases didn't change of diagnostic group.

The FVL was demonstrated in 13% of patients and 3.1% of controls (p <0.0001) with an odds ratio of 4.6 (CI 95% = 2.2-9.6). For FII G20210A the prevalence was 10.2% among the patients group and 4.1% in the controls (p <0.01), with a estimated risk of 2.7 (CI 95% = 1.30-5.3).

Considering separately the DVT group, the prevalence of FVL increased to 17.6% (26/148) and the risk reached 6.6 whereas for the prothrombin mutation both, prevalence (9.5%) and odds ratio (2.5) remained similar to the whole group (Table).

In contrast among the PE group (primary or DVT associated) (n = 98) only six patients were FVL(+) (6.1%) (p = 0.2) versus 11 that showed the FII 20210A allele (11.2%) (p = 0.01). The estimated risk attributed to the FVL dropped to 2.0 (CI 95% = 0.7-5.7) being three times inferior than DVT and lower (but not significantly) than the risk attributed to the prothrombin mutation (3.0, CI 95% = 1.3-6.9) (see Table).

Since our conclusions are based on a reduced number of cases, the confidence intervals for the risk estimations are wide and overlapped. Nevertheless our results suggest that FVL could be a similar or maybe a less important risk factor than FII 20210A with respect to the PE. Our findings about FVL seem even more conclusive that some previously reported (3, 5) and are close to those published by Martinelli et al. (4). The specific implications of the prothrombin mutation in pulmonary embolism remain unclear, although a significant role has been suggested (11). If extended studies can confirm these preliminary results, we should formulate that FVL is a more relevant risk factor than prothrombin mutation (as it has been considered) for VT but not for PE.

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References


Table

<table>
<thead>
<tr>
<th></th>
<th>Episodes / Patients</th>
<th>FVL (n=32)</th>
<th>FII G20210A (n=25)</th>
<th>FVL (-) FII G20210A (-)</th>
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<tbody>
<tr>
<td>OR (95% CI)</td>
<td>P (Fisher)</td>
<td>OR (95% CI)</td>
<td>P (Fisher)</td>
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<tr>
<td>PE</td>
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<td>3 (3.8)</td>
<td>1.3 (0.3-4.7)</td>
<td>0.7</td>
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<tr>
<td>DVT+PE</td>
<td>267 / 213</td>
<td>3 (14.3)**</td>
<td>5.2 (1.3-20.4)</td>
<td>0.04</td>
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<tr>
<td>All PE</td>
<td>105 / 98</td>
<td>6 (6.1)**</td>
<td>2.0 (0.7-5.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>DVT</td>
<td>159 / 148</td>
<td>28 (17.6)**</td>
<td>6.6 (3.1-14.1)</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

* Includes three patients with DVT that recurred as PE and one PE that recurred as DVT
** Includes two double heterozygous with DVT+PE
*** Includes two double heterozygous with DVT

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Dear Sir,

The factor V (G1691A) mutation (factor V Leiden) and the prothrombin (factor II) (G20210A) mutation are common genetic risk factors associated with venous thrombosis (1, 2). Genotyping for these risk factors has become routine clinical diagnostic practice. A variety of methods is available for this purpose, most of which involve separate polymerase chain reaction (PCR) amplification and mutation detection by restriction fragment analysis for factor V and/or prothrombin. Since factor V Leiden and prothrombin (G20210A) genotyping is requested by restriction fragment analysis for factor V and/or prothrombin. Since factor V Leiden and prothrombin (G20210A) genotyping is requested simultaneously as part of a thrombophilia risk factor screen for most patients, combining the analyses of the two mutations in a single procedure is more efficient. Therefore, several methods based on multiplex PCR have now been described to allow double mutation detection (3-7). These approaches use techniques such as PCR-mediated site-directed mutagenesis (3), restriction fragment analysis (4), heteroduplex analysis (5), single-strand conformation polymorphism (SSCP) analysis (6) or allele-specific PCR (7). Although inclusion of additional risk factors in the genotyping procedure has been described for some of these methods, their mutation detection strategies usually involve multiple banding patterns and will not easily be expanded to include more risk factors. Moreover, they require considerable manual intervention, which renders these procedures relatively inefficient and error-prone.

We have evaluated a new assay for the automated simultaneous detection of the factor V Leiden and prothrombin (G20210A) variants which can easily be adapted to include an array of related risk factors. First, relevant parts of both gene fragments are amplified by multiplex PCR. Second, mutation detection is performed with the use of a reverse hybridisation line probe assay (LiPA™) (8, 9) which can be performed manually or using the commercially available Auto-LiPA™ instrument (Innogenetics NV, Gent, Belgium). Ninety-nine individuals were genotyped for factor V Leiden and prothrombin (G20210A) by conventional separate PCR amplification and restriction digestion methods (1, 2), as well as by the multiplex PCR and LiPA™ method. Genomic DNA was isolated from EDTA anticoagulated patient blood with the use of QIAamp columns (QIAGEN GmbH, Hilden, Germany). For the conventional procedure, a 220 bp fragment of exon 10 of the factor V gene and a 345 bp fragment of the 3’ untranslated region of the prothrombin gene were specifically amplified in separate reactions. Primers used for the factor V gene were essentially as described by Bertina and coworkers (1), with a modification in the reverse primer (forward: 5’-TGCCCAGTGCTTAACAAAGCCA-3’; reverse: 5’-CTTGAAGGAAATGGCCCATTA-3’). The primers for the prothrombin gene fragment were: 5’-TCTAGAAACAGTTGCTGCCG-3’ (forward) and 5’-ATAGCAGGTGGAGCATTGAAGC-3’ (reverse), as described by Poort and coworkers (2). The same DNA samples were then analysed with the research prototype kit INNO-LiPA™ factor V/I (Innogenetics NV, Gent, Belgium). A multiplex PCR was performed to simultaneously amplify a 138 bp fragment of exon 10 of the factor V gene and a 115 bp fragment of the 3’ untranslated region of the prothrombin gene, using biotin-labelled primers. The multiplex PCR has been carefully optimized to ensure sensitive and specific amplification of the relevant gene fragments. It is important to note that if PCR primers for other targets are to be added in future, the efficacy of simultaneous amplification needs to be established for the PCR-LiPA™ system to be successful. The LiPA™ comprises sequence-specific oligonucleotide probes immobilised as parallel lines on nitrocellulose strips. The amplified biotinylated DNA was hybridised with probes on the strips which were subsequently incubated with alkaline phosphatase-labelled streptavidin to generate a purple precipitate in a reaction with the chromogenic substrate BCIP/NTB (bromochloroindolylphosphate and nitroblue...