Thrombophilia is now considered a multi-causal condition, with interplay of acquired genetic risk factors. In order to estimate the frequency of the factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations in the Jordanian population, we screened 200 healthy Jordanian individuals. 40% were females. Mean age was 32.1 years for males and 30.0 years for female participants. A PCR method detected 15.0% factor V Leiden (87% heterozygous, 13% homozygous), 2% prothrombin G20210A (100% heterozygous), and 24% MTHFR C677T (67% heterozygous, 33% homozygous).

We conclude that the prevalence of factor V Leiden and MTHFR C677T is elevated in this population of Jordanians. However the incidence of G20210A is relatively low.

Quantification of these genetic thrombosis risk factors in various populations will contribute to a better understanding of the interaction of genetic and environmental risk factors.

ABBREVIATIONS: APC-R = activated protein C resistance; AS-PCR = allele specific polymerase chain reaction; FVL = factor V Leiden; M = mutant; MTHFR = methylene-tetrahydrofolate reductase; PCR = polymerase chain reaction; W = wild.

INDEX TERMS: factor V Leiden; FVL; methylene-tetrahydrofolate reductase; MTHFR; Prothrombin G20210A; thrombophilia.

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Thrombosis risk factors predispose towards thrombosis but, due to the episodic nature of thrombosis, interaction with other components is required before onset of the clinical disorder. A well-established genetic predisposition to thrombosis is a single point mutation in the gene encoding coagulation factor V (G1691A) leading to factor V Leiden (FVL) which was identified as the molecular basis for the phenotype of activated protein C resistance (APC-R) in the majority of affected individuals.1, 2 This mutation is associated with a five- to ten-fold risk for heterozygotes and 80-fold risk for homozygotes.3

In 1996, Poort found that the genetic variant in the 3′ untranslated region (a G to A transition at position 20210) is associated with elevated plasma prothrombin levels and an almost three-fold increased risk of venous thrombosis.4

Hyperhomocysteinemia is a risk factor in both arterial and venous thrombosis.5 Recently a common mutation causing C677T in MTHFR coding sequence was observed in individuals with reduced specific MTHFR activity, increased thermolability, and elevated homocysteine concentrations in plasma.6 This coding sequence may be a genetic risk factor, although some studies have failed to show any associations.7, 8 Variability in risk ratio among populations studied could be explained by differences in the environmental risk factors or in the genetic makeup of different ethnic origins.

This study aims to establish the frequency of these three genetic mutations in a Jordanian population.

MATERIALS AND METHODS

Blood specimens were collected from 120 male Jordanian soldiers. The mean age was 32.1 years. Eighty specimens were obtained from female volunteer blood donors. The mean age of the female study group was 30.0 years. All of
the individuals assayed were healthy, had no personal or family history of thrombosis, were free of blood coagulation disorders, and none were on any kind of medication.

Blood was collected in 5 mL K<sub>3</sub> EDTA (2mg/mL) evacuated tubes. Genomic DNA was extracted from 300 µL ofuffy coat, using the Wizard Genomic DNA Purification kit (Promega, Madison WI, USA). This assay system was designed so that normal alleles were amplified in one reaction tube (‘W’ = wild) while the mutant (M = mutant) alleles were amplified in a second reaction tube (‘M’) for factor V G1691A, G20210A, and C677T each in a separate tube. To detect the six alleles we used six tubes for each patient. In the W reaction, allele-specific primers for the normal prothrombin 20210G, factor V 1691G, and MTHFR 677C alleles potentially direct the amplification of 340bp, 270bp, and 193bp products, respectively, depending on the allele(s) present in the target template. Alternatively, in the M reaction, allele-specific primers for the normal prothrombin 20210A, factor V 1691A, and MTHFR 677T alleles potentially direct the amplification of 340bp, 270bp, and 193bp products, respectively, depending on the allele(s) present in the target template.

Genotyping
Allele-specific amplification (ASA-PCR) was used to amplify genomic DNA. Analysis was accomplished by subjecting samples to ‘W’ and ‘M’ PCR amplifications for wild and mutant types respectively, using the set of primers used by Hessner.<sup>9</sup>

Procedure
Reactions were performed for prothrombin 20210, Factor V Leiden, and MTHFR mutations separately. FVL ‘W’ and ‘M’ reactions were performed with 180 ng of genomic DNA in 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.18M dNTP, 124 ng normal forward primer, 89 ng normal reverse ‘W’ primer, 106 ng reverse mutant ‘M’ primer, and 1 U of Taq DNA polymerase (Promega-USA).

Prothrombin ‘W’ and ‘M’ reactions were performed with 200 ng of genomic DNA in 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.18 M dNTP, 47 ng normal forward primer, 71 ng normal reverse ‘W’ primer, 67 ng reverse mutant ‘M’ primer, and 1 U of Taq DNA polymerase (Promega-USA). MTHFR ‘W’ and ‘M’ reactions were performed with 200 ng of genomic DNA, 10 mM Tris HClpH 8.3, 50 mM KCl, 0.8mM MgCl<sub>2</sub>, 0.2 M dNTP, 9 ng normal forward primer, 10 ng reverse normal ‘W’ primer, 18 ng reverse mutant ‘M’ primer, and 1 U of Taq DNA polymerase (Promega-USA). Reactions were conducted in a total volume of 25 µL. One cycle consisted of 94 °C for 30 sec, 64 °C for 30 sec, and 72 °C for 30 sec, followed by 34 cycles at 94 °C for 15 sec, 64 °C for 15 sec, and 72 °C for 30 sec in a Perkin Elmer 9600 thermal cycler.

The PCR product was analyzed by electrophoresis through 2% agarose gel and visualized by ethidium bromide staining.

RESULTS
Thirty individuals had factor V Leiden (15%), 4 had the prothrombin G20210A mutation (2%), and 48 had the MTHFR C677T mutation (24%). Two individuals showed positive results for all three mutations. Ten individuals were found to have both the FVL and MTHFR mutations (Table 1).

DISCUSSION
The regional and ethnic frequencies of FVL, prothrombin G20210A, and MTHFR C677T mutations have been studied in some European, Asian, African and African-American, and Arab populations.<sup>10,11</sup> The only factor studied in a Jordanian population has been FVL.<sup>12</sup> The prevalence of factor V Leiden in the general population is variable according to the region and the ethnic group.<sup>13</sup> This study evaluates the prevalence of these three mutations in healthy Jordanian individuals. In agreement with the Awidi study, and other studies in the Middle East, the present study showed a high frequency of FVL (15%).<sup>11,12,14</sup> These results are similar to some European countries, such as Greece and Turkey, but higher than Saudi Arabia and Egypt.<sup>10,13,15,16</sup> The highest prevalence of FVL is usually found among Northern Europeans.<sup>17</sup> It is usually less than 1.5% in Southern Europeans.<sup>18</sup> A high frequency in the Jordanian population might rise from the consanguinous marriages common in this area of the world. Although it has been reported that the prevalence of FVL in non-Europeans was seven times lower than among Europeans, the presence of high frequency of FVL in European Countries

<table>
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<tr>
<th>Factor V G1691A</th>
<th>Prothrombin G20210A</th>
<th>MTHFR C677T</th>
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<tbody>
<tr>
<td>Heterozygous</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Homozygous</td>
<td>2</td>
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<tr>
<td>Normal</td>
<td>85</td>
<td>98</td>
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which are near to the Middle East suggests that the distribution of FVL is not only centered in Europe.\footnote{19}

The prevalence of prothrombin G20210A in this study was 2\%, which parallels closely the figures found by Poort and in the Greek population while it appears very rare among African and Asian populations.\footnote{8,10,20}

The world wide distribution of MTHFR 677 variant is not as thoroughly characterized as factor V 1691A. This study found a prevalence of 24\% (67\% heterozygous, 33\% homozygous) compared to 35.3\%, 44.8\%, and 54.5\% reported among Greek, Italian, and Spanish populations respectively, where the present study is similar to a study done in Netherlands.\footnote{10,19,21} It seems from these figures that Jordanians in our study had a lower prevalence of this disorder than those in the other populations we cited in previous protocols.\footnote{5} These data suggest that there is a high degree of heterogeneity in the distribution of the MTHFR 677T allele.

Further studies in more Middle Eastern populations are required for a better breakdown of thrombophilic risk factors in these study groups. Population based studies on the prevalence of this mutation in additional Middle Eastern groups may contribute to a better understanding of the interaction between genetic, ethnic, and environmental risk factors.

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