Genetic Modulation of Coagulation Factor VII Plasma Levels: Contribution of Different Polymorphisms and Gender-related Effects

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Summary

We studied the relationships among different polymorphisms of FVII gene in determining FVII levels, in a sample of 335 male and female Italian volunteers. The hypervariable region 4 (HVR4), the promoter decanucleotide insertion (-323 0/10 bp) and the R353Q polymorphisms of FVII gene were evaluated.

The association of HVR4 or -323 0/10 bp polymorphism with FVII levels differed between gender (Interaction term: p = 0.02 and p = 0.03, respectively), showing stronger effect in males than in females. In males, the R353Q and the HVR4 polymorphisms showed an incremental influence on FVII variance (F = 8.9, p ≤ 0.001 and F = 4.4, p = 0.01, respectively). Moreover, the effects of Q and 10 bp alleles on the reduction of FVII activity levels were significantly potentiated by the presence of H7 allele of HVR4 (Interaction term p = 0.03 for R353Q*HVR4 and p = 0.03 for -323 0/10 bp*HVR4). In conclusion, the effect of FVII polymorphisms on FVII levels was gender dependent and derived from a complex interaction among them. The HVR4 polymorphism seems to add an independent, albeit small, contribution to the regulation of FVII plasma levels.

Introduction

Epidemiological studies have shown that high blood levels of coagulation factor VII (FVII) activity were associated with an increased risk of ischaemic heart disease (IHD) (1-3). Many environmental and biochemical factors influence the plasma levels of FVII; however, they explain only a small part of the variation among individuals. Age, gender, body mass index, insulin resistance, oral contraceptive use and postmenopausal status have been all associated with FVII levels (4). Factors, such as gender, body mass index, insulin resistance, oral contraceptive use and postmenopausal status, have been all associated with FVII levels (4). Dietary fats and blood lipids are important determinants of FVII levels (5-6) although the effect of dietary fats on factor VII levels is strongly dependent on the postprandial or fasting status (7).

Recent studies have demonstrated that genes are involved as well. Common variation of FVII gene can not only directly contribute to FVII levels but also modulate their response to environmental stimuli (8, 10). The human FVII gene spans 13 kilobase pairs and is located on chromosome 13 just 2.8 kilobase pairs 5’ to the factor X gene (11). Green et al. (8) reported a strong association between plasma FVII levels and a common polymorphism in the exon 8 of FVII gene R353Q, leading to a substitution of the arginine residue at position 353 by a glutamine. The R353Q polymorphism may also influence the association of plasma FVII with tryglyceride levels (9). More recently two other common polymorphisms have been described in different portions of the FVII gene, that can be useful in understanding the genetic control of FVII levels and their interaction with environmental factors. These polymorphisms are a decanucleotide insertion at position -323 (-323 0/10 bp) in the promoter of the FVII gene (12) and a tandem repeat unit polymorphism in the hypervariable region 4 (HVR 4) of the intron 7 of FVII gene (13). Both these polymorphisms have been associated with the levels of activity and antigen of FVII (13-14).

We have recently demonstrated in patients with myocardial infarction and family history of CVD, that the allele Q and H7, of R353Q and HVR4 polymorphisms respectively, had a protective effect on the risk of MI (15). The alleles showed an independent effect in reducing the risk and were both associated with low levels of FVII.

To better characterize the effect of HVR4 polymorphism on plasma FVII levels also in relation to the R353Q and -323 0/10 bp polymorphisms and to other factors, we studied a sample of 335 male and female Italian subjects, without history of ischemic vascular disease.

Methods

Study Population

Three-hundred and thirty-five unrelated volunteers (230 males and 105 females, 44 ± 14 years) were recruited in major areas of the Italian territory (North 30%, Center 25%, South 45%). They were all of caucasian origin and resident in their region from at least two generations. Data were collected by ad hoc trained interviewers, using a structured questionnaire which included personal data, family history of thrombosis, cigarette smoking and medical history. Diabetes, hypertension and hyperlipidemia were considered only if the patient was under anti-diabetes, anti-hypertensive or hypolipemic treatment. All questionnaires were checked for reliability and consistency.

Subjects reporting personal history of thrombosis (AMI, stable and unstable angina, stroke and Transient Ischemic Attacks), with defined defects of the hemostatic system, with chronic liver disease or under anticoagulant treatment were excluded.

This work was performed according to the Declaration of Helsinki of 1975 and was approved by the Mario Negri Sud Ethical Committee.
Genetic Analysis

Blood collection for DNA analysis and laboratory tests were performed between 8 and 10 a.m., from subjects who had been fasting overnight and had refrained from smoking for at least 6 h before blood sampling and after 20 min supine rest.

Venous blood was collected from an antecubital vein without stasis into plastic syringes, added to 3.8 % sodium citrate (9:1) in precooled plastic tubes. Plasma was obtained by centrifugation at 2000 g for 20 min at 4 °C and aliquots were frozen at −80 °C until testing.

Genomic DNA was extracted from peripheral blood using standard procedures (16). Enzymatic amplification of DNA was performed by polymerase chain reaction (PCR) using thermostable Taq polymerase (Gibco BRL) according to the manufacturer’s instructions.

Amplification of the HVR4 region in the intron 7 of the FVII gene was modified from Marchetti et al. (17). The sequence of the sense and antisense primers were 5' -AAT GTG ACT TCC ACA CCT CC and 5' -GAT GTC TGT CTT CTG TGT GA, respectively. PCR was performed in a final volume of 25 μl that contained 100 ng genomic DNA, 100 μg/ml of each primer, 100 mM dNTP, 5 % DMSO, 1.5 mM MgCl, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton ×100 and 1.5 U Taq DNA polymerase. Samples were denatured for 2 min at 94 °C and then cycled 32 times through the following steps: 20 s at 92 °C, 20 s, at 57 °C and 40 s at 70 °C.

PCR products were electrophoresed in 2.5% agarose gel and visualized directly with ethidium bromide staining. Three alleles, containing 5 (H5), 6 (H6), 7 (H7) monomer repeats, were detected as 406, 443, 480 bp bands, respectively.

To detect the -323 0/10 bp polymorphism a 214 bp DNA fragment was amplified. Primers 5'-3' were GAGCGGAGTTTTGTTGGCAGCG (upstream) and GGCTGTCGAGGGCCTCTCTC (downstream). PCR was performed in a final volume of 50 μl that contained 100 ng genomic DNA, 100 ng of each primer, 200 mM dNTP, 5 % DMSO, 1.5 mM MgCl, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton ×100 and 1.5 U Taq DNA polymerase. 32 amplification cycles were run at 94 °C for 1 min, 58 °C for 1 min 30 s and 72 °C for 2 min, after a 30 s, at 94 °C prewarming. Restriction enzyme digestion with 10 units of Sty I (Gibco BRL, USA) of the amplified fragments was followed by a run on 2.5% agarose gel. Fragments of 214 bp (0 allele) and 136 +88 bp (10 allele) were detected.

The primers to detect the R353Q polymorphisms were GGGAGACTCCC-CAAAATACAC (upstream) and ACGCCACCTGGGCTTCTCTC (downstream). PCR was performed in the same conditions described for the -323 insertion polymorphism. Restriction enzyme digestion with 5 units of Msp I (Gibco BRL, USA) of the amplified fragments was followed by a run on 2.5% agarose gel. Fragments of 205 bp (A allele) and 272 bp (Q allele) were detected.

Laboratory Measurements

FVII clotting activity was determined by a one-stage clotting assay, using human FVII deficient plasma (Sigma, St. Louis, USA) and recombinant human thromboplastin (Hemolysy, Colnogono Monzese, Italy). Plasma FVII antigen was measured as zymogen FVII by a double antibody ELISA, as described (18): the first antibody was the murine monoclonal 231-7, which preferentially reacts with FVII zymogen in plasma but not FVIIa or PIVKA (Protein Induced by Vitamin K Absence) FVII by western blot and ELISA. The second was a rabbit anti human FVII polyclonal antibody from Diagnostica Stago which reacts with all species of FVII. Cholesterol and triglycerides were measured by automated enzymatic methods (Sigma, St. Louis, USA).

Statistical Analysis

FVII and cholesterol levels were normally distributed, and no heterogeneity of their variances was observed among different genotype groups; values for triglycerides exhibited a log-normal distribution and this variable was natural log-transformed to allow the use of parametric methods; age was treated as non-parametric variable. A chi-squared test was used to compare discrete parameters and to compare genotype distributions for the polymorphisms to those expected if the alleles were in Hardy-Weinberg equilibrium. Allele frequencies were estimated by gene counting. Haplotype frequencies and the coefficients of gametic linkage disequilibrium were calculated by likelihood methods (19).

The interaction between gender and polymorphisms in regulating FVII levels was assessed by multivariate analysis of variance (ANOVA) including the appropriate interaction terms in a model with age and (ln) triglycerides as covariates; subsequent analyses regarding the relation between FVII levels and genotypes were performed by ANOVA, in males and females separately with age and (ln) triglycerides as covariates. Multiple comparisons were performed following Tukey-Kramer approach (20). To assess the independence of the effects of the genotypes and their interaction in modulating FVII levels, multifactorial two-way ANOVA with interaction was used; in view of the infrequency of homozygous for rare Q or 10bp alleles, the genotypes RQ and QQ (0/10bp and 10/10bp) were combined.

The correlations of FVII levels with cholesterol and (ln) triglycerides were analysed by Pearson method. Data for continuous variables are expressed as mean±standard deviation (SD) or mean ± standard error of the mean (SEM); a P value of <0.05 was chosen as the level of significance. All computations were carried out by using the SAS statistical package (21).

Results

Clinical characteristics of the subjects are shown in Table 1. There were more smoker and hyperlipidemic subjects in males than in females; moreover, males showed higher triglyceride and lower FVII coagulant levels than females.

Genotype Frequencies

The allele frequencies at the R353Q, -323 0/10 bp and HVR4 polymorphisms are shown in Table 2. There was no difference in the genotype distribution between males and females and across different Italian geographic areas (data not shown). The genotype distributions at each polymorphism were not different from those predicted by Hardy-Weinberg equilibrium. The data showed strong linkage disequilibrium between R353Q and -323 0/10 bp (standardized disequilibrium statistic
Relationship between Factor VII Polymorphisms and Factor VII Levels

In males, all the three polymorphisms studied were highly significantly associated with both clotting and antigen levels of FVII, in analysis of covariance with age and (ln) triglycerides as covariates (Table 2a). Subjects carrying the Q or 10bp or H7 alleles showed lower FVII activity and antigen levels, in comparison with subjects homozygous for the common alleles; moreover, carriers of H5 allele of HVR4 polymorphism had the highest levels of factor VII. For the HVR4 polymorphism, only the differences between the H7H7 genotype and the other genotypes were statistically significant (Tukey-Kramer approach for multiple comparisons in ANOVA), suggesting a recessive role of the H7 allele in reducing FVII levels. On the contrary, for the R353Q and -323 0/10 bp polymorphisms, the differences in FVII levels among all the three genotypes were statistically significant, suggesting a codominant role of these alleles in reducing FVII levels.

The association of HVR4 or -323 0/10 bp polymorphism with plasma FVII levels differed between gender (Fig. 1). In contrast with the results found in male subjects, the HVR4 polymorphism was not associated with FVII levels in females; this could perhaps be because there are only 8 females with the lowering genotype H7H7. The effect of the 10 bp allele, even though it was still present, was reduced in females. No difference was found in the association between R353Q polymorphism and factor VII levels in respect to male subjects (Table 2b).

The proportion of the variation in plasma factor VII levels that could be explained by the three polymorphisms individually was also different in males and females. In male subjects, the FVII activity variance explained by each polymorphism in a multiple linear regression model including age, (ln) triglycerides and cholesterol as covariates was 26% for the R353Q polymorphism, 22% for the -323 0/10 bp polymorphism and 15% for the HVR4 polymorphism. Similar results were obtained when the variance in FVII antigen distribution was considered: 29% for the R353Q polymorphism, 22% for the -323 0/10 bp polymorphism and 17% for the HVR4 polymorphism. In female subjects, the variance in FVII activity distribution explained by each polymorphism was 12% for the R353Q polymorphism, 8% for the -323 0/10 bp polymorphism and 4% for the HVR4 polymorphism. When FVII antigen distribution was considered, the results obtained were 14% for the R353Q polymorphism, 9% for the -323 0/10 bp polymorphism and 9% for the HVR4 polymorphism.

The relative contribution of the three polymorphisms to FVII activity levels was examined by a multiple analysis of covariance with age and (ln) triglycerides as covariates. In males, the inclusion of R353Q polymorphism in a model in which the -323 0/10 bp and HVR4 genotypes were forced in displayed a significant incremental influence of the model on FVII levels (F = 8.9, p <0.001). The HVR4 polymorphism also showed an incremental influence on FVII variance (F = 4.4, p = 0.01). In contrast, the effect of the -323 0/10 bp polymorphism was no longer significant (F = 1.9, p = 0.2) when it was added to the model.

In females, the inclusion of R353Q polymorphism in a model with -323 0/10 bp and HVR4 genotypes showed a smaller reduction of the total variance (F = 3.7, p = 0.06), in respect to male subgroup analysis; the effect of the introduction of the -323 0/10 bp or HVR4 genotype on FVII levels was no longer significant (F = 0.1, p = 0.8 and F = 0.05, p = 0.9, respectively).

Interaction between Factor VII Polymorphisms

To evaluate a possible interaction between the three polymorphisms in regulating FVII levels, we performed a multivariate analysis of

Table 2 Genotype distribution (number and percentage), rare allele frequencies (with 95% CI) and mean values (±SEM) of FVII activity (FVII:c) (%) and antigen (FVII:ag) (%) levels in Italian subjects grouped by R353Q, -323 0/10 bp and HVR4 genotypes

A) Males

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%)</th>
<th>FVII:c</th>
<th>Statistics</th>
<th>FVII:ag</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/R</td>
<td>160 (69.6)</td>
<td>99±2</td>
<td>F=38.9</td>
<td>103±2</td>
<td>F=34.8</td>
</tr>
<tr>
<td>R/Q</td>
<td>57 (24.8)</td>
<td>83±3</td>
<td>(P&lt;0.0001)</td>
<td>87±3</td>
<td>(P&lt;0.0001)</td>
</tr>
<tr>
<td>Q/Q</td>
<td>13 (5.6)</td>
<td>52±66</td>
<td>66±5</td>
<td>71±8</td>
<td></td>
</tr>
</tbody>
</table>

Rare allele frequency: Q=0.18 (0.15 to 0.22)

B) Females

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%)</th>
<th>FVII:c</th>
<th>Statistics</th>
<th>FVII:ag</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/R</td>
<td>70 (66.6)</td>
<td>103±3</td>
<td>F=7.9</td>
<td>107±3</td>
<td>F=8.8</td>
</tr>
<tr>
<td>R/Q</td>
<td>34 (32.4)</td>
<td>86±4</td>
<td>(P=0.001)</td>
<td>87±4</td>
<td>(P=0.001)</td>
</tr>
<tr>
<td>Q/Q</td>
<td>1 (1.0)</td>
<td>65</td>
<td>82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rare allele frequency: Q=0.17 (0.12 to 0.22)

Fig. 1 Effect of the 10bp or H7 allele on plasma levels of FVII coagulant activity according to gender (mean ± SEM). Interaction terms: -323 0/10 bp*gender, p = 0.03; HVR4*gender, p = 0.02

17% for the HVR4 polymorphism. In female subjects, the variance in FVII activity distribution explained by each polymorphism was 12% for the R353Q polymorphism, 8% for the -323 0/10 bp polymorphism and 4% for the HVR4 polymorphism. When FVII antigen distribution was considered, the results obtained were 17% for the R353Q polymorphism, 14% for the -323 0/10 bp polymorphism and 9% for the HVR4 polymorphism.

The relative contribution of the three polymorphisms to FVII activity levels was examined by a multiple analysis of covariance with age and (ln) triglycerides as covariates. In males, the inclusion of R353Q polymorphism in a model in which the -323 0/10 bp and HVR4 genotypes were forced in displayed a significant incremental influence of the model on FVII levels (F = 8.9, p <0.001). The HVR4 polymorphism also showed an incremental influence on FVII variance (F = 4.4, p = 0.01). In contrast, the effect of the -323 0/10 bp polymorphism was no longer significant (F = 1.9, p = 0.2) when it was added to the model.

In females, the inclusion of R353Q polymorphism in a model with -323 0/10 bp and HVR4 genotypes showed a smaller reduction of the total variance (F = 3.7, p = 0.06), in respect to male subgroup analysis; the effect of the introduction of the -323 0/10 bp or HVR4 genotype on FVII levels was no longer significant (F = 0.1, p = 0.8 and F = 0.05, p = 0.9, respectively).
variance including the three polymorphisms, the terms of interaction between them and age (ln) triglycerides as covariates. For the latter analysis, due to the low number of subjects, we eliminated individuals carrying H5 allele and we combined QQ with RQ and 10 bp/10 bp with 0 bp/10 bp.

The results observed are shown in Fig. 2. In male subjects, we found a significant interaction between R353Q or -323 0/10 bp polymorphism and HVR4 polymorphism in modulating FVII activity levels. In subjects homozygous for H6 allele, the presence of the allele Q or of the allele 10bp was associated with a small, non significant reduction of FVII activity levels [Δ = 12 (%) and Δ = 9 (%)], in comparison with RR or 0bp/0bp genotypes. On the contrary, in subjects homozygous for H7 allele, the effect of the Q or of the 10 bp allele was large [Δ = 39 (%) and Δ = 39 (%)]. Intermediate effects were observed in H6H7 heterozygous subjects [Δ = 18 (%) and Δ = 19 (%)]. The interaction term was significant for the combinations R353Q*HVR4 (p = 0.03) and -323 0/10 bp*HVR4 (p = 0.01), but not for the combination R353Q*-323 0/10 bp (p = 0.2). Concerning the genetic modulation of FVII antigen levels, a similar trend was observed, but the interaction term was significant only for the combination -323 0/10 bp*HVR4 (p = 0.05).

**Gene-environment Interactions**

In both males and females, FVII activity levels were significantly correlated with cholesterol (r = 0.22, p = 0.001) but not with (ln) triglyceride levels (r = 0.12, p = 0.08); similar results were obtained for FVII antigen levels (r = 0.16, p = 0.04 with cholesterol and r = 0.04, p = 0.6 with triglycerides). When analysed by genotypes, the correlation between FVII levels and lipid variables remained unchanged.

**Discussion**

**Factor VII Genotype Distribution**

We evaluated the effect of HVR4 polymorphism on plasma FVII levels in relation to the exonic R353Q and the promoter -323 0/10 bp polymorphisms in a sample of 335 male and female Italian subjects, without history of ischemic vascular disease.

The genotype distributions of the three polymorphisms were similar to those described in Italy by other studies (13, 22). However, they seem to vary across populations with different risk of myocardial infarction. The frequency of the H7, Q and 10 bp alleles ranged between 0.32/0.34, 0.15/0.22 and 0.17/0.25 respectively in Italians and Inuit (13, 15, 22, 23) at low risk of MI and 0.24/0.33, 0.09/0.12 and 0.09/0.14 in North European populations at higher risk (22, 23-25). Since these alleles are associated with low levels of factor VII, their higher frequency in populations at lower risk could support a role of these polymorphisms in the protection from the development of myocardial infarction. This concept is reinforced by our late finding of H7 and Q alleles as protective factors for familial myocardial infarction (15).

**Gender dependent Modulation of Factor VII Levels by Genotype**

The impact of the HVR4 and -323 0/10 bp polymorphisms on FVII levels, both activity and antigen was different in male and female subjects. Indeed in males, all the three polymorphisms were associated with FVII levels; the promoter and the exonic polymorphisms showed effects of equal magnitude, whereas the contribution of the HVR4 polymorphism was considerably lower. In all cases, the effect was more pronounced on FVII antigen levels. On the contrary, in females only the R353Q polymorphism was strongly associated with FVII levels, while the HVR4 polymorphism was not and the effect of the -323 0/10 bp polymorphism was weaker. These findings suggest that hormones or other gender-specific factors could be important in the phenotypic expression of these genetic variants, by interacting with regulatory elements of the gene. The factor VII promoter contains hormone responsive elements that can upregulate the synthesis of FVII to be influenced by such regulation and suggests that linkage disequilibrium with the promoter polymorphism can explain the gender-dependent effect described for R353Q genotype by other reports (26). Experimental studies will be required to clarify their relevance in the regulation of FVII levels by different genotypes in males and females. However, in agreement with our data, an amino acid change is unlikely to be influenced by such regulation and suggests that linkage disequilibrium with the promoter polymorphism can explain the gender-dependent effect described for R353Q genotype by other reports (26).

It is possible that all the polymorphisms are functionally relevant, but the effect on phenotype is regulated by different mechanisms. Some authors reported that the strongest effect on FVII activity was associated with the 0/10 bp genotype (14, 23); indeed, the decanucleotide
insertion in the promoter has been shown, in transfection experiments, to reduce promoter activity by 33% compared to the more common allelic sequence (26). We found, in agreement with other authors (22, 29) that R353Q genotype was the strongest predictor of FVII activity in a population including male and female subjects. The Q allele of R353Q polymorphism was associated with reduced FVII levels also in absence of the decanucleotide promoter insertion (30). Moreover, in a transient transfection assay with FVII cDNA containing the base substitution, the Q allele determined a defective secretion of FVII from the cells, without altering the synthesis of the molecule (30).

Concerning the HVR4 polymorphism, it is conceivable that, being in a non-translated portion of the gene, it is a marker polymorphism in linkage disequilibrium with a functional mutation within the FVII gene, such as the R353Q polymorphism or the ten base pair insertion polymorphism. However, such a DNA locus contains a consensus splice sequence at the 5’ repeat, which, even not translated, could be important in regulating the splicing of nascent RNA (31). According to this hypothesis, Bernardi et al. found that a mutation in the 37 bp repeats allele of the HVR4 polymorphism was the only genetic variation detectable in seven families from the region of Lazio, with definite deficiency of FVII activity (32). Although the contribution of the HVR4 polymorphism is lower than that of the other two, it also adds an independent contribution to FVII levels, supporting the suggestion that the HVR4 variation may cause small differences in splicing efficiency (22). Furthermore, we found that the H7 allele of HVR4 polymorphism can potentiate the effect of the rare alleles of the R353Q, or the -323 0/10 bp polymorphism in reducing the levels of FVII. Finally, the H7 allele is independently and significantly associated with a reduction in the risk of familial myocardial infarction (15). All these findings support a functional role of the intronic polymorphism and suggest the relevance of polymorphic clusters more than single variations in the regulation of FVII levels.

Polymorphic clusters have been already shown to play a role in the distribution of protein levels among individuals. This is the case of the HLA-DRB1 genes, where polymorphisms in the regulatory region affect the level of cell surface protein expression conditioning the extent of T cell activation (33). The presence of different haplotypes has been also associated with predisposition for disease. Polymorphisms in the apo A1-III gene cluster or in the protein C gene have been associated with predisposition to atherosclerosis and thrombosis when individuals are carriers of some haplotypes (34, 35).

Factor VII Gene-environment Interactions

A genotype-specific correlation between FVII and triglycerides has been described; however, it has not been completely elucidated. Some studies reported that the correlation of FVII levels with triglycerides was stronger in carriers of the R allele of the R353Q polymorphism (9, 14, 36). A study in Indian adults reported the same finding, but with an opposite effect: the correlation was stronger in Q allele-carriers (28). Other studies, conducted in subjects in fasting conditions, did not find the association at all (22, 29, 37, 38). Our results, obtained from subjects in fasting conditions, also do not show any influence of the genotypes. It is possible that, together with the different ethnic origin of the population studied, the inclusion of females in the sample and the fasting or non-fasting status could account for the differences among these studies.

In conclusion, the contribution of genetic variation in FVII gene on FVII levels seem to derive from complex interactions between polymorphisms in different sites of the FVII gene and could depend on their interaction with other biological factors such as the hormonal status. Further experimental studies, on the effect of the simultaneous presence of different genotype combinations in response to different stimuli, will help to better clarify these correlations.

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Appendix

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Barletta General Hospital (AM Messina), Bologna “Sant’Orsola” Hospital (G Palareti), Cagliari University Hospital (F Marongiu), Milano “Sacco” Hospital (E Rossi), Napoli “Federico II” University Hospital (A Siani), Napoli 2nd University Hospital (D De Lucia), Pescara General Hospital (T Bonfini), San Giovanni Rotondo, Casa Sollievo della Sofferenza (A Vilella), Verano, Fondazione S. Maugeri (F Soffiantino).

References


33. Singal DP, Qui X. Polymorphism in both X and Y box motifs controls level of expression of HLA-DRB1 genes. Immunogenetics 1996; 43: 50-6.


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