Contribution of low density lipoprotein receptor-related protein genotypes to coagulation factor VIII levels in thrombotic women

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The contribution of low density lipoprotein (LDL) receptor-related protein (LRP) to variance of factor VIII (FVIII) levels in plasma was investigated in thrombotic women through analysis of frequent LRP genotypes. The G allele of the LRP -25C/G polymorphism, predicting increased LRP expression, was associated with 15% and 18% mean reductions of FVIII activity and protein levels, respectively. The combination of -25C/G LRP polymorphism with FVIII D1241E and ABO polymorphisms produced a gradient of FVIII levels, thus supporting the notion that several factors, acting in FVIII biosynthesis, post-translational modification and removal from circulation, have additive effects on the variance of FVIII levels in plasma.

Key words: LRP genotypes, FVIII levels, venous thrombosis.

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High levels of coagulation factor VIII (FVIII), potentially determined by a number of genetic and environmental factors, have been consistently associated with an increased risk of arterial[1] and venous thrombosis.[2,3] Findings from cellular and animal models[4] have indicated a molecular interaction between the low density lipoprotein (LDL) receptor-related protein (LRP) and FVIII, which could modulate coagulation.[5] LRP, a multifunctional receptor belonging to the LDL receptor family[6] and abundantly expressed on a wide range of different cell types, participates in the regulation of the distribution phase of FVIII by directing the clotting factor to intracellular pathways of degradation.[7,8] A mouse model of hepatic LRP deficiency showed elevated plasma FVIII levels.[4] The physiological contribution of LRP to FVIII levels is poorly understood in humans, and studies of the association between rare LRP polymorphisms and FVIII coagulant activity have produced conflicting results.[9,10] We hypothesized that frequent LRP genotypes, predicting differential LRP expression,[11,12] together with specific FVIII chromogenic and antigenic assays, could reveal a physiological contribution of LRP to FVIII levels.

Design and Methods

Two hundred women (mean age±SD: 34±9 years) were randomly selected from among unrelated women referred for investigation of thrombophilic states after a single objectively confirmed episode of deep venous thrombosis of a lower limb (with or without pulmonary embolism). Carriers of known thrombophilic defects (antithrombin, protein C or protein S) or mutations (factor V Leiden or prothrombin 20210G/A), as well as patients with lupus anticoagulant, abnormal liver function, overt evidence of autoimmune or neoplastic disease were excluded. None of the patients was pregnant, on oral contraceptives or hormone replacement therapy. Blood was sampled at least 3 months after the thrombotic episode and 3 weeks after withdrawal of any antithrombotic treatment. Factor VIII activity was measured by a chromogenic method using a commercial assay (Coamate Factor VIII, Chromogenix by Instrumentation Laboratory, Milan, Italy), as described elsewhere.[13] An enzyme-linked immunosorbent assay (ELISA) was used to measure FVIII activity (Factor VIII Ag, Diagnostica Stago).

The LRP –25C/G polymorphism (GenBank, accession number Y18524) was detected by polymerase chain reaction (PCR) using a mutagenized (underlined nucleotide) forward primer 5'-CCCTCTCCCCCATCAGCC**G**C-3' and a reverse primer 5'-CCTCCGGCTGCAAAAATGC-3' followed by Hha I restriction analysis (G allele, 21 and 180 bp; C allele, 201 bp). Genotyping for ABO and for FVIII D1241E was performed as previously described.[9,14] The Mann Whitney test was used to calculate differences in FVIII levels and activated protein C (APC) resistance in patients grouped by carriership of the LRP -25G allele. One-way analysis of vari-

Table 1. FVIII levels and APC resistance according to LRP –25C/G genotypes.

Genotypes	n	%	FVIII activity*	FVIII antigen*	APC-sr*
CC	156	78	1.93 (1.83-2.02)	1.38 (1.23-1.52)	0.96 (0.93-0.99)
CG+GG	44	22	1.65 (1.47-1.83)	0.99 (0.73-1.25)	1.04 (0.97-1.11)

*Values are given as mean (95% CI). FVIII chromogenic activity and antigen levels are expressed as IU/mL. APC-sr, APC sensitivity ratio. *Differences between genotype groups, Mann Whitney test p<0.020.*

ance (ANOVA) was used to compare FVIII levels among groups with combined genotypes.

Results and Discussion

The FVIII levels observed in the present study (mean 1.87 IU/mL±0.62) were higher than those reported for other thrombotic cohorts. This difference could not be explained by the presence of overt inflammation. Since all the thrombotic patients in our sample were women, gender, a factor influencing FVIII levels,[2] could have contributed to the observed higher FVIII levels. Patients were characterized for the -25C/G polymorphism of the LRP gene, which creates a new GC box potentially recognized by the transcription factor SP1.[12] The functional role of this polymorphism has been suggested by increased mRNA and protein LRP expression levels in CG heterozygotes.[12]

We found that the CG and GG genotypes, predicting increased LRP expression, were present in the fifth of the patients and were associated with a mean reduction of 0.28 IU/mL (15%) of FVIII activity levels (Table 1). We also observed a parallel effect on protein levels (mean reduction 18%) (Table 1). Only one G-carrier was present in the group of patients with the highest levels of activity (>90th percentile), whereas seven G-carriers were detectable in the group with the lowest levels (<10th percentile). In accordance with the hypothesis of a direct effect of FVIII levels on APC resistance,[15,16] the mean APC sensitivity ratio was higher in G-carriers than in CC homozygotes (Table 1). These results suggest that the increased expression of LRP predicted by the G allele could increase the clearance of FVIII from the circulation. However, von Willebrand factor antigen levels were not available, which prevented an evaluation of the interplay of FVIII with both its carrier and receptor.

The finding that LRP genotypes modulate FVIII levels is an independent indication, in a human model of venous thrombosis, that LRP physiologically interacts with FVIII and that LRP gene variations are biologically plausible genetic determinants of FVIII levels. To test

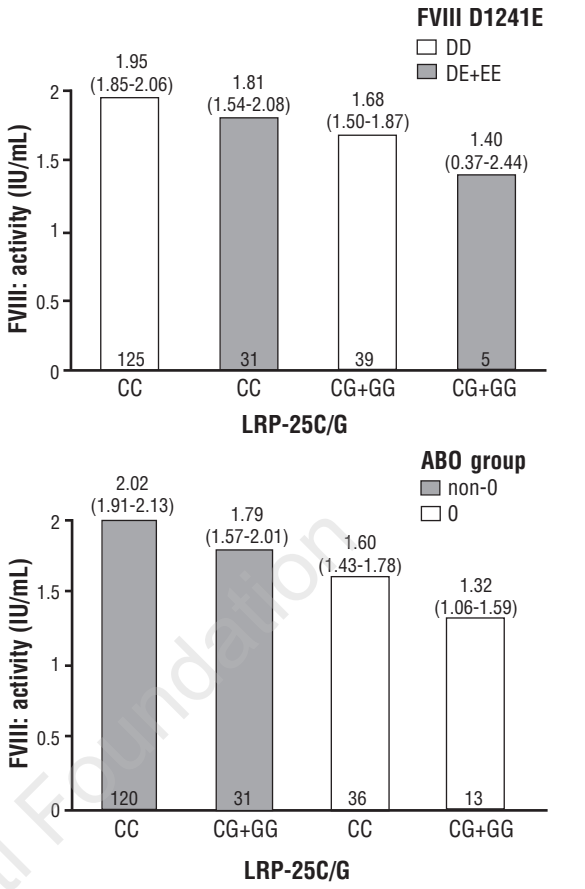

Figure 1. Levels of FVIII activity according to combined LRP –25CG/FVIII D1241E or LRP –25CG/ABO genotypes. Numbers on the bars represent mean FVIII levels (95% CI). The number of subjects in each category is reported at the bottom of the bars. ANOVA (upper panel: F=2.95, *p*=0.034; lower panel: F=8.88, *p*<0.0001).

the hypothesis of additive effects produced by multiple genetic modulators of FVIII levels, patients were also grouped according to other known genetic determinants of FVIII levels, the FVIII D1241E substitution[14] and ABO blood groups.[17,18] A gradient of FVIII levels driven by genotypes was observed (Figure 1), with mean activity levels varying from 1.95 to 1.40 IU/mL for LRP/FVIII, and from 2.02 to 1.32 IU/mL for LRP/ABO combined genotypes. The LRP/ABO genotypes jointly produced the largest mean difference (53%).

Although considering subjects with combinations of all markers led to small genotype groups this approach was explored to gain additional information. We found large mean differences (69%) ranging from 2.05 IU/mL for genotypes predicting higher FVIII levels (LRP -25CC/non O/FVIII DD) to 1.21 IU/mL for those associated with lower levels (LRP -25CG+GG/O/FVIII DE + EE). These data support the notion that several factors, acting in FVIII biosynthesis, post-translational modification and removal of the clotting factor from the circulation, have additive effects on the variance of FVIII levels in plasma.

Taking into account the relatively small contribution of LRP and FVIII genetic components to FVIII levels, large clinical studies are needed to establish the potential increased or decreased thrombotic risk conferred by these genotypes.

FB, GM and FM conceived and designed the study. CL and MC had a major role in the selection of thrombotic women, and performed and analyzed the clinical coagulation laboratory data. BL and MP performed the genotyping in all subjects. All authors critically contributed to the interpretation of the results. GM and FB wrote the paper. FB supervised the whole work and was responsible for the final approval of the version to be published. The authors declare that they have no potential conflicts of interest.

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