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Factor IX and deep vein thrombosis

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Thrombosis has been described as *hemostasis in the wrong place*.[1] Fibrin is a major component of thrombi and anticoagulant drugs which reduce thrombin formation are effective in both prevention and treatment. So, increased circulating levels of coagulation factors, and their functional genotypes, appear prime candidates for mechanisms of both arterial and venous thrombosis. Recent reviews of the epidemiological literature have concluded that testing of these hypotheses, while showing some promising results, still has a long way to go.[2,3]

At present, increased levels of the von Willebrand factor: factor VIII complex show the most consistent associations with both venous and arterial thrombosis, in both case-control and prospective studies.[2-6] For the moment, the causality of these associations cannot be tested, because there are no drug or other interventions which selectively reduce levels of von Willebrand factor (VWF) or factor VIII. However, Mendelian randomization studies support their causal roles in venous and arterial thrombosis. Non O-blood group is associated with increased risk of both venous and arterial thrombosis[7] which is most likely due to mean 25-30% higher circulating levels of VWF: factor VIII complex, due to the expression of non-O proteoglycans on VWF which reduce its clearance from the circulation by the reticulo-endothelial system. Hemophiliacs, who have low levels of Factor VIII (or IX), have a low risk of arterial thrombosis such as coronary heart disease (CHD).[8] A study of CHD risk in hemophilia carriers in the Netherlands[8] suggested that this lower risk is dose-dependent, being intermediate in hemophilia carriers, who have on average about half the circulating levels of Factor VIII (or IX) compared to non-carrier females.

These reports suggest that levels of factors VIII and IX (which have critical roles in hemostasis, as shown by the severe clinical bleeding manifestations of hemophilias A and B) may also play important roles in venous and arterial thrombosis. While factor VIII is an important cofactor in hemostasis, factor IXa is the activated enzyme which plays a key role in maintenance of thrombin (and hence fibrin) formation within the intrinsic system of coagulation. Clinical, epidemiological, and pharmacological studies support the hypothesis that increased factor IX levels play a role in thrombogenesis.[9]

There are two approaches to test this hypothesis. First, development of anticoagulant drugs which selectively reduce factor IX levels,[9] and randomized trials of

these in thrombosis prevention. Second, identification of functional genotypes which alter either factor IX levels, or factor IX function and epidemiological studies of their associations with risk of venous or arterial thrombosis: Mendelian randomization studies.[10]

In this edition of Haematologica, Bezemer and colleagues[11] report a study of the Mendelian randomization approach. In a previous study of almost 20,000 potentially functional single-nucleotide polymorphisms (SNPs) for association with deep vein thrombosis (DVT), they identified an A>G sequence variant in the gene encoding factor IX (rs 6048, *F9 Malmö*).[12] The G allele (frequency 0.32) was associated with a 15-43% decreased risk compared with the A allele; but has not been reported to be associated with hemophilia B. In their currently reported study, they investigated whether this association could be explained by other factor IX variants; or by effects of factor IX Malmö on factor IX antigen levels, activated factor IX, or endogenous thrombin potential which is influenced by factor IX.

Bezemer *et al.* report that, among other SNPs investigated, only one (rs 422187) was strongly linked to factor IX Malmö (r^2=0.94), and was similarly associated with DVT. No other SNP (or haplotype) tested was more strongly associated. Factor IX antigen level, factor IX activation peptide level, and endogenous thrombin potential did not differ between factor IX Malmö genotypes. However, factor IX antigen levels were confirmed as an association with DVT risk.[13,14]

So, what does this study tell us about the hypothesized role of factor IX in venous thrombosis? First, the previously-reported association between factor IX levels, both activity[13] and antigen,[14] has been confirmed in a larger study which adjusted for the known associations of factor IX with age, sex, oral contraceptive use, and other vitamin K-dependent coagulation factors (II, VII and X).[15,16] This study, therefore, provides important confirmation of an association between factor IX levels and risk of venous thrombosis.

Second, investigation of 15 tag SNPs that served as surrogates for other SNPs in the region, and 14 additional candidate SNPs, showed that only the rs 422187 SNP showed a similar association with DVT (and a very strong association with factor IX Malmö). This study, therefore, refines the association of factor IX Malmö as a potentially functional genotype for further study of the association of factor IX with thrombosis.

Third, investigation of the associations between factor IX Malmö and three potential mechanisms for thrombosis (factor IX antigen levels, factor IX activation peptide levels, and endogenous thrombin potential) showed no significant associations. However, the authors, appropriately, discuss that their study cannot exclude effects of the factor IX Malmö genotype on these phenotypes. In addition, it is important to recognise that factor IX activation peptide levels, while recently associated with risk of arterial thrombosis,[17] have not yet been associated with risk of DVT in epidemiological studies. Likewise, endogenous thrombin potential has not yet been associated with risk of venous or arterial thrombosis in epidemiological studies.

In contrast, it would be interesting to perform further studies of the association of factor IX Malmö with factor IX activity, which has been associated not only with increased risk of venous thrombosis,[9,13] but also with a lesser clinical bleeding risk than factor VIII deficiency at comparable plasma levels,[18] possibly due to a stronger effect on hemostasis and thrombosis.

What then should be the future direction of research on factor IX and thrombosis? First, further epidemiological studies should be performed on functional phenotypes (factor IX activity, antigen, activation peptides, and related activities such as thrombin generation *in vitro*) and risk of both venous and arterial thrombosis. Second, such studies should include potential genotypes of interest: the report of Bezemer *et al.*[11] establishes a case for both factor IX Malmö and the rs 422187 mutation in such studies.

Third, and perhaps most important, epidemiologists, geneticists, and coagulationists should recognize that genotypes for coagulation factors may be associated with risk of thrombotic disease, but not with circulating levels of these factors. In addition to the current report of Bezemer and colleagues for factor IX,[11] a recent report by Jood *et al.*[19] shows a dissociation between functional genotypes for fibrinogen, plasma fibrinogen levels, and risk of thrombosis. Functional genotypes for β-fibrinogen were associated with fibrinogen levels, but not with risk of stroke. In contrast, functional genotypes for α- or γ- fibrinogen were not associated with fibrinogen levels, but were associated with risk of stroke; possibly because they affect not fibrinogen levels, but rather fibrin structure. Together with the report of Bezemer *et al.*,[11] this study suggests that genetic epidemiological studies need to progress from simply assay of levels of intermediate phenotypes (e.g. coagulation factors) to inclusion of assay functions.

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