# Interactions between genotype and phenotype in bleeding and thrombosis

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The hemostatic balance is the result of an equilibrium between procoagulant and anticoagulant factors that interact with each other to ensure hemostasis at sites of vascular injury. Abnormalities of hemostatic factors due to defects in the corresponding genes can result in a tendency to hemorrhage or thrombosis. In the last few decades, the progressive identification of the mechanisms underlying coagulation disorders led to the awareness that clinical phenotypes are only rarely the result of single gene defects but are more often influenced by multiple factors. Interactions between different genes or between genes and other acquired factors may account for the phenotypic variability of most coagulation disorders, by improving or worsening their clinical manifestations.[1] Examples of interactions between genotype and phenotype in both hemorrhagic and thrombotic disorders are given in this article.

## Gene-gene interactions

### *Aggravation of clinical phenotype*

Several studies provide evidence that an increased thrombotic risk is conferred by the coexistence of more than one prothrombotic gene mutations (Table 1). Gandrille *et al.*[a] found that the gain-of-function factor V Leiden was much more prevalent in patients with protein C deficiency who developed clinical symptoms of venous thromboembolism (VTE) than in healthy controls. Similarly, Koeleman *et al.*[b] found that approximately two thirds of family members heterozygous for both protein C deficiency and factor V Leiden developed VTE, contrasting with one-third among those heterozygous only for protein C deficiency. The same authors also found higher rates of VTE in the presence of the combination of factor V Leiden and protein S deficiency.[c] Brenner *et al.*[d] studied the large family of a patient homozygous for protein C deficiency and found that approximately one-fifth of them were double heterozygotes for factor V Leiden and the T298M protein C gene mutation, and that VTE occurred in approximately half of those heterozygous for both mutations but in none of those carrying one mutation only. Zoller *et al.*[e] found that in families with protein S deficiency the rate of thrombotic events was much higher among individuals who also carried the factor V Leiden mutation than in those with single defects. Van Boven *et al.*[f] demonstrated that nearly all patients with both antithrombin deficiency and factor V Leiden developed thrombosis, whereas those carrying either defect were less frequently symptomatic. The median age of the first thrombotic episode in individuals with combined defects was 10 years younger than the age of onset of carriers of one defect only. A number of studies have shown that the prothrombin G20210A mutation is quite common in thrombophilic families with factor V Leiden, and that the risk of VTE is much higher in individuals with combined defects.[g-n] There is less information on the effect of gene-gene interactions on the risk of arterial thrombosis than on that of VTE. Nevertheless, Butt *et al.*,[n] observed that the prevalence of combined carriership of prothrombin G20210A and the Leu34 polymorphic allele of the A subunit of factor XIII was 12-fold higher among patients with myocardial infarction than among controls.

It is clear from these data (Table 1) that the co-existence of gain-of-function prothrombotic mutations has an aggravating effect on the risk of VTE, the age of onset and severity of clinical symptoms. Thus, the identification of combined prothrombotic mutations in an individual may have therapeutic implications for the decision concerning the duration of anticoagulant therapy. The Seventh Conference of the American College of Chest Physicians (ACCP) on antithrombotic and thrombolytic therapy recommended a longer duration of this therapy (12 months *vs.* 6 months) for patients with a first episode of VTE and two or more thrombophilic mutations than for those with a single defect.[2]

Besides these examples of aggravation of the thrombotic tendency, gene-gene interactions may cause an aggravation of hemorrhagic disorders (Table 2).[o-x] A typical example is the so-called dominant-negative effect observed in some patients heterozygous for type 1 von Willebrand's disease, who show an autosomal dominant pattern of inheritance with high penetrance and expressivity of the disease.[o] This dominant phenotype, characterized by von Willebrand factor levels much lower than expected for a heterozygous defect of the gene, is associated with missense mutations, such as the substitution of a cysteine in the D3 domain (Cys386Arg), which decreases the production of von Willebrand factor by the normal allele.[o] Similarly, a study of a large number of patients with inherited factor VII deficiency demonstrated that the co-expression of factor VII Ala244Val mutation and the Arg353Gln polymorphism had an additive effect on the reduction of factor VII secretion, lowering plasma levels and increasing the risk of bleeding.[p] Another example of aggravation of the bleeding phenotype was given by Kravtsov *et al.*[q] who reported that two mutations in the gene encoding the factor XI catalytic domain (Gly400Val and Trp569Ser) caused severe factor XI deficiency by exerting a dominant-negative effect, due to the fact that non-secretable mutant factor XI traps wild-type polypeptides within the cells through heterodimer formation. Finally, an increasing bleeding tendency was also observed by Di Paola *et al.*[r] in patients with type 1

**Table 1. Main studies on the aggravation of the risk of thrombosis due to gene-gene interactions.**

| First* author | Number of patients | Gene mutations/ protein defects | Results |
|---|---|---|---|
| Gandrille[a] | 113 | FVL; PC def. | FV Leiden was detected in 14% of 113 symptomatic PC deficient patients and in only 1% of 104 healthy controls |
| Koeleman[b] | 48 | FVL; PC def. | VTE was detected in 73% of double heterozygotes and in 31% of individuals heterozygous for only PC deficiency |
| Koeleman[c] | 16 | FVL; PS def. | Thrombosis was detected in 80% of double heterozygotes and in 50% of heterozygotes for PS or FV Leiden only |
| Brenner[d] | 46 | FVL; T298M PC | VTE was detected in 45% of double heterozygotes and in 0% of individuals heterozygous for a single mutation |
| Zöller[e] | 18 | FVL; PS def. | VTE was present in 72% of individuals with combined defects and in 19% of individuals with single gene defects |
| van Boven[f] | 47 | FVL; AT def. | VTE was present in 92% of individuals with combined defects and in 19% of individuals with single gene defects |
| Makris[g] | 101 | FVL; PC, PS, AT def.; PT G20210A | The mean number of VTE episodes was 3.7 in subjects with combined defects and 1.9 in those with single defects |
| Tosetto[h] | 448 | FVL; MTHFR C677T; PT G20201A | PT G20210A heterozygotes were more prevalent among symptomatic carriers of factor V Leiden than among asymptomatic carriers |
| Ehrenforth[i] | 352 | FVL; PT G20201A mutation | Symptomatic FV Leiden carriers had a 3-fold increased frequency of PT G20210A compared to asymptomatic relatives |
| Tirado[j] | 287 | PC, PS, AT def.; FV Leiden | Individuals with combined defects were at increased risk of thrombosis |
| Ferraresi[k] | 132 | FVL; PT G20210A | Double heterozygotes had an earlier onset of VTE (22 years) compared with individuals with single defects (30.5 years) |
| De Stefano[l] | 624 | FVL; PT G20210A | Carriers of FV Leiden and PT G20210A had a 2.6-fold increased risk of recurrent VTE compared to carriers of FV Leiden alone |
| Salomon[m] | 162 | FVL; PC, PS, AT def.; MTHFR C677T; PT G20201A | The presence of more than one gain-of-function mutation was associated with an increased risk of VTE |
| Butt[n] | 500 | FVL; PT G20210A, FXIII-A Leu34 | Combined carrier status of PT G20210A and FXIII-A Leu34 is a risk factor for myocardial infarction |

**References are listed in the online Appendix. FVL, factor V Leiden; PC, protein C; PS, protein S; AT, antithrombin; PT, prothrombin; FXIII, factor XIII; MTHFR: methylenetetrahydrofolate reductase; def, deficiency.*

von Willebrand's disease, due to the concomitant prevalence of the 807C mutant allele, which is associated with a low density of the platelet $\alpha_2\beta_1$ collagen receptor.

### Improvement of clinical phenotype

The mechanisms of discordance between genotype and phenotype in blood coagulation disorders also include interactions resulting in an improvement of the bleeding tendency (Table 2). For example, Castoldi *et al.*[s] found that the co-inheritance of factor V Leiden enhanced thrombin generation and was associated with a milder bleeding tendency in patients homozygous for the factor VII Lazio mutation, whereas patients without the gain-of-function prothrombotic mutation were severe bleeders. Furthermore, a number of studies suggested that gain-of-function mutations improve the clinical phenotype of hemophilia. Nichols *et al.*[t] studied the factor V Leiden mutation in two sets of hemophiliacs with identical factor VIII gene mutations but different disease severity and suggested that co-inheritance of factor V Leiden conferred a clinical benefit. Escuriola Ettinghausen *et al.*[u] screened previously untreated children with hemophilia A for prothrombotic risk factors and found that those carrying gain-of-function gene mutations had their first symptomatic hemorrhage later in life than non-carriers. Moreover, Nowak-Göttl *et al.*,[v] demonstrated that the clinical phenotype of children with severe hemophilia A is influenced by the co-inheritance of prothrombotic genetic factors. The molecular mechanism by which factor V Leiden attenuates the hemophilia phenotype seems to be related to an increase in thrombin generation by reducing thrombin down-regulation through the activated protein C pathway.[3] However, in contrast to the aforementioned findings, other groups failed to observe less bleeding in hemophiliacs carrying factor V Leiden. Indeed, Arbini *et al.*[w] and Arruda *et al.*[x] failed to detect differences in the frequency of bleeding episodes or the response to therapy between severe hemophiliacs with or without the mutation. In an attempt to sum up and reconcile these discordant findings, van Dijk *et al.*[4] reviewed all the articles published between 1963 and 2003 on the contribution of thrombophilic factors to clinical phenotype of subjects with hemophilia and concluded that the presence of factor V Leiden does indeed decrease the clinical severity of severe hemophilia.

## Gene-environment interactions

### Aggravation of clinical phenotype

Interactions between acquired and genetic factors affect the risk of VTE.[1] Factor V Leiden represents a model for the study of these interactions because, given its high prevalence in the general population, combinations with other hereditary or acquired VTE risk factors are relatively common. Normal individuals with blood groups A, B and AB have a higher thrombotic tendency than those with blood group O, perhaps because their plasma levels of factor VIII are higher. In this issue of the journal Minano *et al.*[5] demonstrate that the risk of VTE increases when individuals with non-O blood groups also carry factor V Leiden and prothrombin G20210A mutations. A common acquired risk factor

**Table 2.** Main studies on the aggravation or improvement of inherited bleeding disorders due to gene-gene interactions.

| *First* author* | *Number of patients* | *Gene mutations/protein defects* | *Results* |
|---|---|---|---|
| Eikenboom[o] | 3 | Cys386Arg in D3 domain of the VWF gene | Decreased production of VWF by the normal allele (dominant negative effect) |
| Fromovich-Amit[p] | 61 | Ala244Val, Arg353Gln in the FVII gene | Reduction of FVII secretion, leading to decreased FVII plasma levels |
| Kravtsov[q] | 2 | Gly400Val, Trp569Ser in the FXI gene | Reduction of FXI secretion, leading to decreased FXI plasma levels |
| Di Paola[r] | 148 | 807C polymorphism in the $\alpha_2$ gene | Low density of the platelet $\alpha_2\beta_1$ collagen receptor in type 1 VWD |
| Castoldi[s] | 7 | FV Leiden; FVII Lazio mutation | Milder bleeding tendency in patients homozygous for the FVII Lazio mutation |
| Nichols[t] | NR | FV Leiden; FVIII gene mutations | Moderation of severe hemophilia A phenotype by FV Leiden |
| Escuriola Ettingshausen[u] | 124 | FVL; PC, PS, AT def.; PT G20210A | Delayed onset of hemorrhagic symptoms in hemophilia A children carrying prothrombotic risk factors |
| Nowak-Göttl[v] | 135 | FVL; PC, PS, AT def.; PT G20210A; MTHFR C677T; lp(a) | Moderation of severe hemophilia A phenotype by the co-inheritance of prothrombotic risk factors |
| Arbini[w] | 21 | FVL, PC, PS, AT def. | FVL was detected in only 1/21 of patients with severe hemophilia A or B and milder clinical phenotype |
| Arruda[x] | 113 | FVL | No differences in bleeding frequency between severe hemophilic patients with (3/113) or without FVL |

** References are listed in the online Appendix; VWF, von Willebrand factor; FVII, factor VII; FXI, factor XI; def, deficiency; VWD, von Willebrand's disease; FV, factor V; NR, not reported; lp(a), lipoprotein(a)*

for VTE is the use of oral contraceptives, and various studies have shown a synergism with factor V Leiden. For instance, Vanderbroucke *et al.*[6] calculated that heterozygous women using oral contraceptives had a 35-fold increased risk of VTE , a multiplication of the risks found for users of oral contraceptives (5-fold) and carriers of factor V Leiden (7-fold). In homozygotes for factor V Leiden, oral contraceptive usage is associated with a several hundred-fold increased risk of VTE. The third generation pills are worse in this respect (50-fold increased risk of thrombosis) than the second generation ones.[7] A markedly increased risk of VTE was also seen in oral contraceptive users with concomitant protein C, protein S and antithrombin deficiencies or carriership of the prothrombin G20210A mutation.[8] In postmenopausal women with factor V Leiden who used hormone replacement therapy Rosendaal *et al.*[9] found a 15-fold increased risk of venous thrombosis. Although these genetic and environmental risk factors interact with each other with a multiplicative effect, the molecular mechanism of the interaction is still unknown. Of note, however, is the demonstration that estrogen use induces *per se* an acquired resistance to activated protein C and also enhances resistance due to factor V Leiden.[10] Knowledge of the additional risk associated with carriership of factor V Leiden might help to prevent thrombosis in risk situations through the more aggressive adoption of prophylactic measures.

Another paradigmatic example of an interaction between genetic and environmental factors is the regulation of plasma levels of homocysteine. Indeed, genetic factors (C677T mutation in the *MTHFR* gene associated with a thermolabile variant with decreased enzyme activity) and nutritional factors (inadequate intake of folate and vitamin $B_{12}$) are important interrelated determinants in the production of increased homocysteine. Accordingly, the hereditary metabolic disorder hyperhomocysteinemia will manifest itself mainly in individuals with poor nutritional intake of these vitamins.[11] A number of studies have also analyzed the relationship between hyperhomocysteinemia and gain-of-function prothrombotic mutations.[12-14] For example, Ridker *et al.*[12] found that individuals with hyperhomocysteinemia and factor V Leiden had a 20-fold higher risk of developing idiopathic VTE than that subjects with neither abnormality (relative risk 21.8; 95% confidence interval, 3.9-12.2, $p$=0.0004). De Stefano *et al.* estimated that the risk in carriers of both hyperhomocysteinemia and factor V Leiden was increased 30-fold while that in carriers of both hyperhomocysteinemia and prothrombin G20210A was increased 50-fold.[13] However, other studies disagreed with these results[14] and a recent meta-analysis[15] found no evidence of interactions between factor V Leiden and hyperhomocysteinemia or *MTHFR* C677T genotype in VTE.

### Improvement of clinical phenotype

Potentially harmful polymorphisms may confer a selective advantage in some critical conditions. For instance, Corral *et al.*[16] found that the presence of factor V Leiden caused a 5-fold reduction in the risk of primary intracranial hemorrhage (1% *vs.* 4.9%, $p$=0.019), and that the G202109 prothrombin mutation was half as frequent in patients than in controls (1.5% *vs.* 3%, $p$=0.31). On the other hand, carriers of the –323 inser-

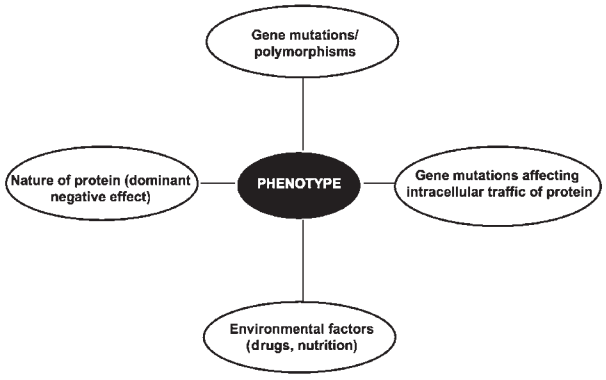


**Figure 1.** Interrelation between congenital and acquired factors in modulating the clinical phenotype of blood coagulation disorders.

tion in the factor VII gene promoter, associated with lower than normal factor VII levels, increased the risk of intracranial hemorrhage 1.54-fold. These results are in accordance with the protective role of factor V Leiden in other bleeding conditions. For instance, Lindqvist *et al.*[17] compared intra-partum blood loss between women with or without factor V Leiden. The latter had significantly lower intra-partum blood loss (318 mL *vs*. 380 mL, $p$=0.018) and a smaller difference between pre- and post-partum hemoglobin levels (0.30 g/dL *vs*. 0.80 g/dL, $p$=0.02) than women not carrying factor V Leiden. Subsequently, the same authors demonstrated that carriership of factor V Leiden was associated with higher hemoglobin and serum ferritin concentration in early pregnancy and reduced menstrual blood loss,[18] suggesting that the gain-of-function mutation might have conferred female carriers an evolutionary advantage by protecting them against blood loss.[1] However, a recent large study did not find a beneficial influence of factor V Leiden on the amount of peri-partum blood loss, assessed visually.[19]

### Conclusions

During the last decades, substantial progress has been made in understanding the multifactorial nature of hemostatic diseases. It is clear that the clinical phenotypic variability of blood coagulation disorders is often the result of gene-gene or gene-enviroment interactions that are, however, only partially understood (Figure 1). Further insights into the molecular mechanisms underlying these associations will help us to develop personally tailored therapeutic strategies aimed at preventing or treating these hemostatic disorders.

References a-x are listed in the *Online Supplementary Index*.

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