# Understanding the biological mechanisms underlying acquired risk factors for venous thrombosis : studies in mice

Cleuren, A.C.A.

# Chapter 2

## Transgenic mouse models of venous thrombosis: fulfilling the expectations?

Audrey CA Cleuren, Bart JM van Vlijmen, Pieter H Reitsma

*Semin Thromb Hemost. 2007 Sep;33(6):610-616*

## Summary

Over the past 15 years, transgenic mice have been generated that carry defective and/or mutant alleles of the natural anticoagulant genes, thereby displaying a spontaneous thrombotic phenotype. With the generation of these mouse lines, better opportunities became available for investigating both existing and novel risk factors for venous thrombosis. In addition, these models could serve as a tool for evaluating novel antithrombotic strategies. In this review, we will summarize these mouse models and evaluate whether they have fulfilled the expectations.

## Introduction

Mice are at present the most frequently used experimental animals. Their popularity is mainly due to their short breeding time, and easy low-cost maintenance. More importantly, as we have gained extensive knowledge of the mouse genome, mice have also proven to be extremely useful for carrying out genetic modification like transgenesis and gene targeting. However, because regular inbred mice do not develop venous thrombosis spontaneously, their use in animal research on venous thrombosis has been quite limited. A thrombotic phenotype could only be obtained by crude experimental approaches like ferric chloride induced injury, mechanical injury, photochemical injury or ligation induced stasis (reviewed in [1,2]). Although such models provide some insight into the thrombotic tendency under different genetic and environmental circumstances, there are important limitations. The major drawback is that these models lead to thrombus formation in normal blood vessels by inducing endothelial damage (in a largely platelet dependent process), whereas venous thrombi in humans are formed under conditions of stasis and/or hypercoagulability. In addition, subtle effects on the thrombotic tendency, which are a hall-mark of human studies, cannot be detected in these inducible mouse models. Finally, these models do not allow evaluation of the effects of prolonged exposure to certain inherited and acquired risk factors.

A potential breakthrough to circumvent these limitations came with the development of transgenesis and gene targeting in mice. These techniques allow the introduction of genes, the mutation of genes, or the knock-out of complete genes in the mouse genome. This technological advance opened an avenue to study risk factors for venous thrombosis in a more clinically relevant manner. In the past 15 years, most genes known to be involved in primary hemostasis, secondary hemostasis and fibrinolysis have indeed been targeted in mice, resulting in complete deficiencies, mutated gene products or altered protein levels, and these models have been extensively reviewed elsewhere.[3-11]

This review will focus on genetic mouse models for venous thrombosis and therefore we evaluate the results of deletions and modifications of natural anticoagulant factors, as they are key elements in altering the venous thrombotic risk. Gene modifications in murine tissue factor pathway inhibitor, antithrombin and the proteins involved in the protein C pathway, including protein C, thrombomodulin, the endothelial protein C receptor and factor V (factor V Leiden) yielded mouse lines with spontaneous thrombotic phenotypes. These phenotypes

range from mildly enhanced tissue fibrin deposition, to lethal thrombosis-induced consumptive coagulopathy.
With the generation of these mouse lines, better opportunities became available for investigating both existing and novel risk factors for venous thrombosis. In addition, these models could serve as a tool for evaluating novel antithrombotic strategies. Here we will summarize the mouse models that lack or carry mutant anticoagulant factors and display a spontaneous thrombotic phenotype. Furthermore, we evaluate whether these mouse models based on genetic modification have fulfilled the expectations, i.e. whether these models contributed to our understanding of risk factors that modulate the venous thrombotic risk, and if they have provided novel strategies for evaluating antithrombotic therapies.

## Tissue factor pathway inhibitor

Tissue factor pathway inhibitor (TFPI) directly inhibits activated factor X (FXa), and subsequently inhibits the tissue factor/factor VIIa (TF/FVIIa) complex which is responsible for the initiation of coagulation. Mice deficient in TFPI were generated by disruption of the Kunitz domain 1 ($TFPI^{K1-/-}$), which is required for TF/FVIIa inhibition.[12-14] Homozygosity for this mutation results in embryonic lethality and examination of $TFPI^{K1-/-}$ embryos revealed a severe thrombotic phenotype including signs of yolk sac hemorrhage, immunoreactive fibrin(ogen) in the liver and intravascular thrombi, symptoms that are indicative for a consumptive coagulopathy. Mice heterozygous for the TFPI mutation have plasma TFPI activity levels about 50% of wild-type mice, are fertile and do not display a thrombotic phenotype.[12-14]
Chan et al. showed that the survival rate of $TFPI^{K1-/-}$ mice depends on the level of FVII.[15] When FVII levels are decreased genetically - by crossing with FVII knock-out mice - homozygous TFPI deficient mice survive the embryonic period. However, after birth the combined knock-out still succumbs due to a perinatal consumptive coagulopathy. In line with this study, it appeared that a low level of tissue factor (TF) (obtained by crossing the TFPI mutant mice with mice expressing a low levels of human TF) also rescues the intrauterine lethality of TFPI deficient embryos.[15,16] Surprisingly, in contrast to the perinatal death observed in TFPI deficient mice having a decreased FVII level, $TFPI^{-/-}$/low TF mice are viable and show no signs of thrombosis. Also, $TFPI^{-/-}$/low TF mice do not show signs of hemorrhage in the lung nor cardiac fibrosis, which is seen in pure low TF mice.

These studies demonstrated that the severe thrombotic phenotype seen in tissue factor pathway inhibitor deficient mice can partly be rescued by restoring the hemostatic balance, i.e. by lowering tissue factor or FVII levels.

## Antithrombin

Antithrombin (AT) is a serine protease inhibitor that inactivates several coagulation proteases that are generated during blood coagulation, particularly thrombin and FXa. AT deficient mice ($AT^{-/-}$) die in utero and the embryos show extensive subcutaneous and intracranial hemorrhages. Fibrin deposition was present in the liver and myocardium but no fibrin was present at the site of hemorrhage, which might be due to consumptive coagulopathy and/or liver dysfunction, indicating a severe thrombotic phenotype.[17]

Although $AT^{+/-}$ mice have significantly reduced plasma antithrombin antigen and activity levels, their external appearance is normal and they do not show signs of spontaneous thrombosis.[17] However, an additional thrombogenic stimulus results in a mild thrombotic phenotype with increased tissue fibrin deposition as compared to challenged wild-type mice.[18]

Because heparin is an important enhancer of antithrombin activity, mice have been generated that carry a mutation in the heparin binding site of AT, which corresponds to the R47 mutation frequently seen in AT deficient patients.[19] Mice homozygous for this mutation ($AT^{m/m}$) are born, but exhibit extensive fibrin deposition and develop spontaneous life-threatening thrombosis, predominantly in the heart and liver.

To evaluate whether diminished tissue factor activity could rescue the hypercoagulability of $AT^{-/-}$ mice, AT deficiency was introduced on a low TF background.[20] However, the resulting $AT^{-/-}$/low TF mice die in utero and exhibit disseminated thrombosis in the liver and heart.

The rather mild thrombotic phenotype of $AT^{+/-}$ mice and the more severe phenotype of $AT^{m/m}$ mice, make these mice a potentially suitable model for studying risk factors of thrombosis and antithrombotic strategies. Until now, only $AT^{m/m}$ mice have been used for this purpose. Dewerchin et al. have shown that the human monoclonal anti-FVIII antibody LCL-mAb-LE2E9 efficiently prevents thrombosis (presented as priapism) in male $AT^{m/m}$ mice, indicating that FVIII is a potential target for anticoagulation therapy.[21]

## Protein C pathway

The protein C pathway consists of two circulating proteins, protein C (PC) and protein S, and two endothelial transmembrane proteins, thrombomodulin (TM) and the endothelial protein C receptor (EPCR). Furthermore, the factor V Leiden (FVL) mutation also affects the protein C pathway as FVL leads to an increased APC resistance. All of these genes, except protein S, have been inactivated and modified in mice, and will be discussed below.

*Protein C*

Activated protein C, in association with its cofactor protein S, can cleave FVIIIa and FVa, thereby inhibiting coagulation. Homozygous PC deficient embryos show early signs of thrombosis, beginning at the embryonic age of 12.5 days (E12.5).[22] Although some $PC^{-/-}$ pups are born, they do not survive beyond 24 hours after delivery. Microscopically, scattered microvascular thrombosis in the brain and focal liver necrosis was seen, as well as hemorrhages in the brain. This combination of thrombosis and bleeding in the brain is indicative of a consumptive coagulopathy.

Whereas complete PC deficiency leads to embryonic lethality, $PC^{+/-}$ mice appear healthy and reproduce normally.[22] As is seen in heterozygous AT deficient mice, heterozygous PC deficient mice also exhibit more pronounced intravascular fibrin deposition, but only after a thrombogenic stimulus.[23]

Mice with severe PC deficiency (PC levels less than 4%; these mice were generated by introducing a PC gene construct back into the PC deficient mice) survive beyond birth and develop spontaneous thrombosis characterized by large thrombi and fibrin depositions in the lungs, liver and heart.[24] The survival of these mice, and the onset and severity of the thrombotic phenotype, strongly depends on the actual plasma PC levels.

To determine whether the coagulopathy in $PC^{-/-}$ embryos can be compensated by additional deletion of the FVII gene, double deficient mice were generated.[25] $PC^{-/-}$ embryos with heterozygous FVII deficiency are born but die of consumptive coagulopathy. Double homozygous deficient embryos show from day E12.5 intra- and extravascular coagulopathy, resulting in hemorrhages and immediate death after birth.

However, PC deficient mice can be rescued from embryonic lethality by a combination with FXI deficiency.[26] The resulting double homozygous mice can live up to 3 months but eventually die of thrombotic diseases as is demonstrated by massive systemic fibrin depositions.

Taken together, these data show that interruption of the protein C pathway by deleting protein C itself generates several prothrombotic phenotypes. These phenotypes range from a slightly increased thrombotic risk as is seen in $PC^{+/-}$ mice, to severe spontaneous thrombotic events present in mice with very low PC levels. Several gene-gene interactions have been studied, resulting in the rescue of completely PC deficient mice when it is combined with FXI deficiency, although these mice develop spontaneous thrombosis later in life. This has led to the opportunity to study protein C deficiency in adult mice, and suggests a role for the intrinsic pathway to manipulate the thrombotic outcome.

*Endothelial protein C receptor*

EPCR can bind protein C, thereby facilitating the rate of PC activation by the thrombin-thrombomodulin complex. Total EPCR deficiency leads to intrauterine death.[27] It has been suggested that thrombosis at the maternal-embryonic interface might contribute to embryonic death, as the developing placenta is positively stained for fibrin.

$EPCR^{+/-}$ mice develop normally, appear healthy and do not show apparent thrombotic complications upon a thrombogenic stimulus.[27,28] In contrast to the $EPCR^{-/-}$ embryos, mice with very low EPCR levels are viable, and do not show increased fibrin deposition after a challenge as compared to wild-type mice.[29]

In an attempt to determine the cause of the embryonic lethality, Li et al. used conditional knock-out strategies to selectively express EPCR in the embryo or in the placenta.[30] Embryonic EPCR expression in the absence of placental expression could not circumvent the embryonic lethality. In contrast, ablation of EPCR in the embryo but not in the placenta resulted in viable EPCR deficient mice with an age-dependent spontaneous thrombotic phenotype. In addition, the combination of EPCR deficiency with low TF activity (less than 1%) is also able to rescue EPCR deficient mice by preventing local thrombin generation at the maternal-embryonic interface.[30]

Summarizing, EPCR expression in the placenta (in particular trophoblast cells) is essential for the maintenance of pregnancy, as only embryonic EPCR can be selectively knocked-out without resulting in embryonic lethality. A low level of EPCR expression is able to maintain a normal phenotype and only a total deficiency of EPCR results in a severe thrombotic phenotype.

**Table 1: transgenic mouse models of venous thrombosis.**

| Gene | Genotype¶ | Thrombotic phenotype# | Reference |
|---|---|---|---|
| Tissue factor pathway inhibitor | $TFPI^{K1-/-}$ | Embryonic lethality, consumptive coagulopathy | 12,13 |
| | $TFPI^{K1+/-}$ | Normal phenotype | 12,13 |
| | $TFPI^{K1-/-}/FVII^{+/-}$ or $TFPI^{K1-/-}/FVII^{-/-}$ | Embryonic lethality, consumptive coagulopathy | 15 |
| | $TFPI^{K1-/-}$/low TF | Normal phenotype | 16 |
| Antithrombin | $AT^{-/-}$ | Embryonic lethality, consumptive coagulopathy | 17 |
| | $AT^{+/-}$ | Normal phenotype, unless challenge | 18 |
| | $AT^{m/m}$ | Fatal thrombosis at later age | 19 |
| | $AT^{-/-}$/low TF | Embryonic lethality, consumptive coagulopathy | 20 |
| Protein C | $PC^{-/-}$ | Embryonic lethality, consumptive coagulopathy | 22 |
| | $PC^{+/-}$ | Normal phenotype, unless challenge | 23 |
| | Low PC (<4%) | Fatal thrombosis at later age | 24 |
| | $PC^{-/-}/FVII^{-/-}$ or $PC^{-/-}/FVII^{+/-}$ | Perinatal death, consumptive coagulopathy | 25 |
| | $PC^{-/-}/FXI^{-/-}$ | Fatal thrombosis at later age | 26 |
| Endothelial protein C receptor | $EPCR^{-/-}$ | Embryonic lethality, placental thrombosis | 27 |
| | $EPCR^{+/-}$ | Normal phenotype | 27 |
| | Low EPCR | Normal phenotype | 29 |
| | $EPCR^{-/-}$/low TF | Normal phenotype, unless challenge | 30 |
| Thrombomodulin | $TM^{-/-}$ | Embryonic lethality, developmental retardation | 31,32 |
| | $TM^{+/-}$ | Normal phenotype | 31,32 |
| | $*TM^{pro/pro}$ | Fibrin deposition | 32-34 |
| | TMLox | Partial embryonic lethality, Fatal thrombosis at later age | 38 |
| Factor V Leiden | $* FV^{Q/Q}$ | Fibrin deposition | 42 |
| | $FV^{Q/Q}/FVIII^{-/-}$ or $FV^{Q/Q}/FIX^{-/-}$ | Restore ability clot formation | 43 |
| | $FV^{Q/Q}/GLA^{-/-}$ | Fibrin deposition, non-fatal occlusive thrombi | 44 |
| | $FV^{Q/Q}/TFPI^{+/-}$ | Perinatal lethality | 45 |
| | $FV^{Q/Q}/PZ^{-/-}$ | Perinatal lethality | 46-48 |

¶ Additional abbreviations: $TFPI^{K1}$: tissue factor pathway inhibitor with a disrupted Kunitz 1 domain; low TF: reduced tissue factor levels; $AT^{m/m}$: antithrombin with R48C mutation; $TM^{pro/pro}$: thrombomodulin with E387P mutation; TMLox: endothelium-specific thrombomodulin loss; $FV^{Q/Q}$: factor V with R504Q mutation (factor V Leiden); GLA: α-galactidose A; PZ: protein Z.
# Normal phenotype implicates no thrombosis present, evaluated or challenged. * Phenotypes depend on genetic strain.

*Thrombomodulin*

Endothelial membrane bound thrombomodulin (TM) forms a complex with thrombin and inhibits the amplification loop of the coagulation cascade by activating protein C. Mice with a complete TM deficiency die before embryonic day 9.5 and it is suggested that this is due to an inhibition of growth and development, secondary to a defect in the parietal yolk sac.[31,32] $TM^{+/-}$ mice show a 50% reduction in TM levels compared to wild-type mice, but they appear normal and are free of thrombotic events.[31,32]

The knock-in of a TM mutation at position 387 (glutamic acid to proline), which is thought to cause a loss of protein C co-factor activity, bypasses embryonic lethality.[32] The $TM^{pro/pro}$ mutation leads to a severely reduced capacity to generate activated PC and to inhibit thrombin. This in turn results in a mild thrombotic phenotype with fibrin depositions in the spleen, heart and lung.[32-35] Overt thrombosis occurs only in the presence of an additional pathological challenge.[34,36] Interestingly, these results depend on the genetic background of the mouse. $TM^{pro/pro}$ mice with a mixed 129Sv/C57BL/6J background show intravascular fibrin depositions whereas these depositions are not present in $TM^{pro/pro}$ mice with a C57BL/6J background.[34]

Using tetraploid aggregation, Isermann et al. showed that restoring TM expression in extraembryonic tissue can rescue $TM^{-/-}$ mice from early embryonic lethality. However, absence of TM from the endothelium still causes late embryonic lethality due to consumptive coagulopathy.[37] Furthermore, using a conditional (Cre/LoxP) approach the same research group showed that the endothelium-specific loss of TM results in partial embryonic lethality.[38,39] Mice that survive to birth, and beyond, are born healthy and lack a visible thrombotic phenotype until the age of two to three weeks. After this period, all mutant mice develop severe thrombosis, terminating in a lethal consumptive coagulopathy.

In conclusion, these studies have been extremely valuable in unraveling the functions of thrombomodulin. As the thrombotic phenotype of $TM^{pro/pro}$ mice is variable and dependent on interaction with secondary genetic modifiers, this model is attractive as a tool for future research on risk factors that modulate the venous thrombotic risk and to study novel strategies for antithrombotic therapies.

*Factor V Leiden*

Normally, factor V (FV) is degraded by activated protein C (APC), but due to an arginine to glutamine substation at position 506 in the human FV gene (also known

as the factor V Leiden (FVL) mutation[40]), FV is more resistant to the actions of APC. This mutation of the FV gene is the most common genetic risk factor for venous thrombosis in humans.

In 1998 Yang et al. showed that the murine R504Q mutation in the FV gene, which corresponds to the human FVL mutation, results in partial APC resistance.[41] Cui et al. generated mice carrying this homologous mutation in the FV gene by a gene-targeting knock-in approach.[42] Adult hetero- and homozygous mice ($FV^{+/Q}$ and $FV^{Q/Q}$) are fertile and exhibit normal survival, but $FV^{Q/Q}$ mice reveal evidence for chronic low-grade thrombin generation, resulting in fibrin deposition in multiple tissues. As with the $TM^{pro/pro}$ mice, the genetic background of the mouse is important for the thrombotic phenotype, with the mixed 129Sv/C57BL/6J background being more prone to developing fibrin depositions than C57BL/6J mice.

$FV^{Q/Q}$ mice have been crossed with hemophilia A ($FVIII^{-/-}$) and hemophilia B ($FIX^{-/-}$) mice.[43] The hemophilia mice show spontaneous hemorrhages and are not able to form thrombi after vascular injuries. However, an additional FVL mutation ($FV^{+/Q}$ or $FV^{Q/Q}$) can restore the ability to form a thrombus after injury.

Fabry disease, a deficiency in α-galactidose A (GLA), is associated with premature vascular events that, in human, may be thrombotic in nature.[44] When $GLA^{-/-}$ mice are mated with $FV^{Q/Q}$ mice, the offspring shows greatly increased fibrin deposition and occlusive thrombus formation as compared with mice with only one defect. This indicates a synergistic interaction between GLA deficiency and factor V Leiden.

Eitzman et al. demonstrated that a modest reduction in TFPI levels, which is by itself not sufficient to cause a thrombotic phenotype, combined with $FV^{Q/Q}$ is nearly completely fatal in the early perinatal period.[45] $FV^{Q/Q}$/ $TFPI^{+/-}$ mice show increased fibrin deposition, suggesting disseminated thrombosis. The same holds for protein Z (PZ) deficiency: $PZ^{-/-}$ mice appear healthy and have no signs of vascular thrombosis or hepatic fibrin depositions, whereas in combination with $FV^{Q/Q}$ mutation intrauterine and perinatal thrombosis results, eventually leading to mortality.[46-48]

Recently, Sood et al. described a mouse model in which TM and EPCR variants are experimentally validated as modulators of pregnancy success in $FV^{Q/Q}$ mothers.[49] It appears that the interaction between these thrombotic risk factors has different consequences on disease manifestation, depending on the vascular bed in which the interaction occurs.

In the FVL mouse model, the severity and phenotypic expression of spontaneous thrombosis is proven to be variable and dependent on interaction with secondary genetic or environmental modifiers, including the genetic background of the mouse, FVIII, FIX, α-galactidose, TFPI and protein Z. Thus, the FVL model represents important aspects of thrombophilia and thrombosis in humans, and has proven to be valuable to validate existing, and develop novel, concepts of disease mechanisms in human.

## Discussion

Mouse models that lack or carry mutant anticoagulant factors yielded several insights regarding the role of these factors and provided several mouse models that display a spontaneous thrombotic phenotype. In mice, complete deficiencies of natural anticoagulants results in many cases in embryonic lethality, mostly related to hemodynamic dysfunction. This demonstrates that anticoagulant factors are crucial for embryonic survival, and explains why complete deficiencies are rare in humans.[3] In contrast to complete deficiencies, heterozygous deficiencies coincide with normal survival. For antithrombin and protein C, deletion of one allele results in an increased thrombotic tendency, however, only when an additional thrombotic stimulus is applied. These latter two models, as well as the $TM^{pro/pro}$ and $FV^{Q/Q}$ mice, display a mild thrombotic phenotype characterized by tissue fibrin deposition as a sign of chronic low-grade thrombosis.

To circumvent embryonic lethality, different models have been developed, resulting in viable mice with a strong thrombotic tendency or a spontaneous thrombotic phenotype. These models include the homozygous mutation in the heparin binding site of AT, severe protein C deficiency, combined complete PC/FXI deficiency, $TM^{pro/pro}$ and deletion of TM expression on endothelial cells. Interestingly, some of the thrombotic phenotypes in these models are dependent on the genetic background of the mice.

The generation of genetic thrombosis models was expected to provide better opportunities to study risk factors for venous thrombosis, and novel antithrombotic drugs. The question is whether the models discussed in this review indeed have fulfilled these expectations. Severe spontaneous thrombosis models can be useful for testing new antithrombotic strategies. However, to our knowledge, Dewerchin et al. were the only to employ such a model for evaluating the use of a monoclonal anti-FVIII antibody in preventing thrombosis.[21]

Of the mouse thrombosis models that followed from deletion and/or mutation of anticoagulant factors, the $FV^{Q/Q}$ model appears to be particularly successful in studying risk factors for venous thrombosis. The $FV^{Q/Q}$ model demonstrates that the intensity of the thrombotic phenotype can be modified by other apparently neutral prothrombotic factors. Examples are TFPI and protein Z; since they emerged as candidate modifying genes for thrombotic risk in human FV Leiden carriers.

It is possible that the use of mice displaying a spontaneous thrombotic phenotype will become more popular in studying gene-gene and gene-environment interactions. For example, pro- and anti-inflammatory genes are potential candidate genes in modulating thrombotic risk, and prothrombotic mice like $AT^{+/-}$, $PC^{+/-}$, $TM^{pro/pro}$ and $FV^{Q/Q}$ mice could be of great value in a first validation of these candidate genes. In addition, epidemiological findings in large case-control studies have yielded several environmental risk factors, like estrogens, progestins, obesity, pregnancy and malignancies. Perhaps it is possible to recapitulate the effect of these risk factors in prothrombotic mice, which would give a unique opportunity to dissect the underlying mechanisms.

A major breakthrough in the application of mice displaying spontaneous thrombotic phenotypes could also come from whole genome mutagenesis screens that are set up to identify genes that counteract the thrombotic phenotype. In these studies mice with a lethal prothrombotic phenotype are randomly mutagenized, in the expectation that some mutations will rescue the mice. To our knowledge such screens are currently ongoing but have not been published in full.[50] In the future, these screening programs can provide additional information on protective gene-gene and gene-environment interactions. This is of particular interest, as the mouse models also have taught us that thrombosis is a multi-causal disease that not only depends on prothrombotic risk factors but also on protective factors like the deficiency in a procoagulant factor.

Although the use of genetically altered mice in thrombosis research was thought to be a major breakthrough, providing excellent opportunities to study the interaction between different risk factors and to study novel antithrombotic strategies, we consider their application at present limited. However, we are convinced of the potential of these models and think that the limited use of these models is because they are time-consuming and labour-intensive, rather than that these are inappropriate for studying venous thrombosis.

## References


1 Day SM, Reeve JL, Myers DD, et al. Murine thrombosis models. Thromb Haemost 2004;92:486-94.

2 Dorffler-Melly J, Schwarte LA, Ince C, et al. Mouse models of focal arterial and venous thrombosis. Basic Res Cardiol 2000;95:503-9.

3 Hogan KA, Weiler H, Lord ST. Mouse models in coagulation. Thromb Haemost 2002;87:563-74.

4 Mackman N. Mouse models in haemostasis and thrombosis. Thromb Haemost 2004;92:440-3.

5 Emeis JJ, Jirouskova M, Muchitsch EM, et al. A guide to murine coagulation factor structure, function, assays, and genetic alterations. J Thromb Haemost 2007;5:670-9.

6 Carmeliet P, Moons L, Collen D. Mouse models of angiogenesis, arterial stenosis, atherosclerosis and hemostasis. Cardiovasc Res 1998;39:8-33.

7 Sood R, Weiler H. Embryogenesis and gene targeting of coagulation factors in mice. Best Pract Res Clin Haematol 2003;16:169-81.

8 Mackman N. Tissue-specific hemostasis in mice. Arterioscler Thromb Vasc Biol 2005;25:2273-81.

9 Tsakiris DA, Scudder L, Hodivala-Dilke K, et al. Hemostasis in the mouse (Mus musculus): a review. Thromb Haemost 1999;81:177-88.

10 Preissner KT. Hemostatic protease receptors and endothelial cell function: insights from gene targeting in mice. Semin Thromb Hemost 2000;26:451-62.

11 Car BD, Eng VM. Special considerations in the evaluation of the hematology and hemostasis of mutant mice. Vet Pathol 2001;38:20-30.

12 Huang ZF, Broze G, Jr. Consequences of tissue factor pathway inhibitor gene-disruption in mice. Thromb Haemost 1997;78:699-704.

13 Huang ZF, Higuchi D, Lasky N, et al. Tissue factor pathway inhibitor gene disruption produces intrauterine lethality in mice. Blood 1997;90:944-51.

14 Dusse LM, Carvalho MG, Cooper AJ, et al. Tissue factor and tissue factor pathway inhibitor: a potential role in pregnancy and obstetric vascular complications? Clin Chim Acta 2006;372:43-6.

15 Chan JC, Carmeliet P, Moons L, et al. Factor VII deficiency rescues the intrauterine lethality in mice associated with a tissue factor pathway inhibitor deficit. J Clin Invest 1999;103:475-82.

16 Pedersen B, Holscher T, Sato Y, et al. A balance between tissue factor and tissue factor pathway inhibitor is required for embryonic development and hemostasis in adult mice. Blood 2005;105:2777-82.

17 Ishiguro K, Kojima T, Kadomatsu K, et al. Complete antithrombin deficiency in mice results in embryonic lethality. J Clin Invest 2000;106:873-8.

18 Yanada M, Kojima T, Ishiguro K, et al. Impact of antithrombin deficiency in thrombogenesis: lipopolysaccharide and stress-induced thrombus formation in heterozygous antithrombin-deficient mice. Blood 2002;99:2455-8.

19 Dewerchin M, Herault JP, Wallays G, et al. Life-threatening thrombosis in mice with targeted Arg48-to-Cys mutation of the heparin-binding domain of antithrombin. Circ Res 2003;93:1120-6.

20 Hayashi M, Matsushita T, Mackman N, et al. Fatal thrombosis of antithrombin-deficient mice is rescued differently in the heart and liver by intercrossing with low tissue factor mice. J Thromb Haemost 2006;4:177-85.
21 Dewerchin M, Van der Elst L, Singh I, et al. Inhibition of factor VIII with a partially inhibitory human recombinant monoclonal antibody prevents thrombotic events in a transgenic model of type II HBS antithrombin deficiency in mice. J Thromb Haemost 2004;2:77-84.
22 Jalbert LR, Rosen ED, Moons L, et al. Inactivation of the gene for anticoagulant protein C causes lethal perinatal consumptive coagulopathy in mice. J Clin Invest 1998 ;102:1481-8.
23 Levi M, Dorffler-Melly J, Reitsma P, et al. Aggravation of endotoxin-induced disseminated intravascular coagulation and cytokine activation in heterozygous protein-C-deficient mice. Blood 2003;101:4823-7.
24 Lay AJ, Liang Z, Rosen ED, et al. Mice with a severe deficiency in protein C display prothrombotic and proinflammatory phenotypes and compromised maternal reproductive capabilities. J Clin Invest 2005;115:1552-61.
25 Chan JC, Cornelissen I, Collen D, et al. Combined factor VII/protein C deficiency results in intrauterine coagulopathy in mice. J Clin Invest 2000;105:897-903.
26 Chan JC, Ganopolsky JG, Cornelissen I, et al. The characterization of mice with a targeted combined deficiency of protein c and factor XI. Am J Pathol 2001;158:469-79.
27 Gu JM, Crawley JT, Ferrell G, et al. Disruption of the endothelial cell protein C receptor gene in mice causes placental thrombosis and early embryonic lethality. J Biol Chem 2002;277:43335-43.
28 Zheng X, Li W, Gu JM, et al. Effects of membrane and soluble EPCR on the hemostatic balance and endotoxemia in mice. Blood 2007;109:1003-9.
29 Castellino FJ, Liang Z, Volkir SP, et al. Mice with a severe deficiency of the endothelial protein C receptor gene develop, survive, and reproduce normally, and do not present with enhanced arterial thrombosis after challenge. Thromb Haemost 2002;88:462-72.
30 Li W, Zheng X, Gu JM, et al. Extraembryonic expression of EPCR is essential for embryonic viability. Blood 2005;106:2716-22.
31 Healy AM, Rayburn HB, Rosenberg RD, et al. Absence of the blood-clotting regulator thrombomodulin causes embryonic lethality in mice before development of a functional cardiovascular system. Proc Natl Acad Sci U S A 1995;92:850-4.
32 Rosenberg RD. Thrombomodulin gene disruption and mutation in mice. Thromb Haemost 1997;78:705-9.
33 Weiler-Guettler H, Christie PD, Beeler DL, et al. A targeted point mutation in thrombomodulin generates viable mice with a prethrombotic state. J Clin Invest 1998;101:1983-91.
34 Weiler H, Lindner V, Kerlin B, et al. Characterization of a mouse model for thrombomodulin deficiency. Arterioscler Thromb Vasc Biol 2001;21:1531-7.
35 Weiler H, Isermann BH. Thrombomodulin. J Thromb Haemost 2003;1:1515-24.
36 Dorffler-Melly J, de Kruif M, Schwarte LA, et al. Functional thrombomodulin deficiency causes enhanced thrombus growth in a murine model of carotid artery thrombosis. Basic Res Cardiol 2003;98:347-52.
37 Isermann B, Hendrickson SB, Hutley K, et al. Tissue-restricted expression of thrombomodulin in the placenta rescues thrombomodulin-deficient mice from early lethality and reveals a secondary developmental block. Development 2001;128:827-38.

38 Isermann B, Hendrickson SB, Zogg M, et al. Endothelium-specific loss of murine thrombomodulin disrupts the protein C anticoagulant pathway and causes juvenile-onset thrombosis. J Clin Invest 2001;108:537-46.
39 Weiler H. Mouse models of thrombosis: thrombomodulin. Thromb Haemost 2004;92:467-77.
40 Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64-7.
41 Yang TL, Cui J, Rehumtulla A, et al. The structure and function of murine factor V and its inactivation by protein C. Blood 1998;91:4593-9.
42 Cui J, Eitzman DT, Westrick RJ, et al. Spontaneous thrombosis in mice carrying the factor V Leiden mutation. Blood 2000;96:4222-6.
43 Schlachterman A, Schuettrumpf J, Liu JH, et al. Factor V Leiden improves in vivo hemostasis in murine hemophilia models. J Thromb Haemost 2005;3:2730-7.
44 Shen Y, Bodary PF, Vargas FB, et al. Alpha-galactosidase A deficiency leads to increased tissue fibrin deposition and thrombosis in mice homozygous for the factor V Leiden mutation. Stroke 2006;37:1106-8.
45 Eitzman DT, Westrick RJ, Bi X, et al. Lethal perinatal thrombosis in mice resulting from the interaction of tissue factor pathway inhibitor deficiency and factor V Leiden. Circulation 2002;105:2139-42.
46 Yin ZF, Huang ZF, Cui J, et al. Prothrombotic phenotype of protein Z deficiency. Proc Natl Acad Sci U S A 2000;97:6734-8.
47 Corral J, Gonzalez-Conejero R, Hernandez-Espinosa D, et al. Protein Z/Z-dependent protease inhibitor (PZ/ZPI) anticoagulant system and thrombosis. Br J Haematol 2007;137:99-108.
48 Broze GJ, Jr. Protein Z-dependent regulation of coagulation. Thromb Haemost 2001;86:8-13.
49 Sood R, Zogg M, Westrick RJ, et al. Fetal gene defects precipitate platelet-mediated pregnancy failure in factor V Leiden mothers. J Exp Med 2007 Apr 16.
50 Westrick RJ, Manning SL, Dobies SL, et al. A Sensitized Genome-Wide ENU Mutagenesis Screen in the Mouse to Identify Genetic Modifiers of Thrombosis. ASH Annual Meeting Abstracts 2004;104:2.