Genetic determinants of venous thrombosis

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CHAPTER 1

General introduction and outline

GENERAL INTRODUCTION

Venous thrombosis (VT), the occlusion of the venous system by a blood clot, is a multicausal disorder affecting 1-2 per 1000 individuals annually.[1,2] The most common manifestations are deep vein thrombosis (DVT) of the lower extremities and pulmonary embolism (PE). Mortality and morbidity after a thrombotic event are considerable: PE has a case-fatality rate of about 10% within the first month,[1-4] whereas 20 to 60% of the DVT patients develop the post-thrombotic syndrome.[5-7] In addition, VT recurs in 20 to 30% of the patients within five years of the first event.[5,8] The risk of VT and its complications is not equal for all individuals. For example, the incidence of first events increases exponentially with age and men have approximately a twofold higher risk of recurrence than women.[9-11] The established risk factors for VT are often present concurrently and include recent immobilization, surgery, cancer, pregnancy or postpartum period, and hormone use (see Table for a short overview).[12,13] Most, if not all, risk factors relate to hypercoagulability, vascular endothelial injury, or stasis, also known as Virchow's triad, and trigger a shift in the hemostatic balance towards clotting.

Table. Main risk factors for venous thrombosis

Factor	Relation with venous thrombosis*
Increasing age	Weak to strong
Male sex	Weak
Genetic factors	Weak to strong
Active cancer	Strong
Surgery, trauma, immobilization	Strong
Long-haul (air) travel	Moderately strong
Oral contraceptive use	Moderately strong
Hormone replacement therapy	Moderately strong
Pregnancy and postpartum period	Moderately strong
Overweight or obesity	Moderately strong

*Strong denotes a relative risk >5; moderately strong: relative risk 2-5; weak: relative risk <2
For an extensive review on risk factors for venous thrombosis see Lijfering *et al*.[40]

Many individuals who develop VT do not have any of the established risk factors,[2] which suggests that as yet unrecognized factors must play a role in VT pathophysiology. This is also in line with the observation that patients whose first thrombotic event

is not provoked by any of the established risk factors have a 2- to 3-fold increased recurrence risk.[5,8,14-16] Furthermore, (prophylactic) treatment of VT by anticoagulant use is not without risks, as all currently available anticoagulants are associated with bleeding complications.[17] In order to have better prevention and treatment strategies, we need to advance our knowledge on risk factors for VT and their underlying biological mechanisms.

In addition to clinical or acquired risk factors, genetic variation contributes to the risk of VT. Individuals with a positive family history of VT have an increased risk of developing VT compared with individuals with a negative family history,[18] with the risk being proportional to the degree of relatedness to the affected family member.[19] Overall, VT has a strong genetic basis with heritability estimates between 50 and 60% based on family and twin studies.[20-22] To identify genes and specific genetic variants contributing to VT pathophysiology, different strategies have been employed including linkage analysis, candidate gene studies, genome-wide association studies, and (next-generation) DNA sequencing. Variants in seventeen genes have so far been identified as well-established genetic risk factors for VT.[23]

Among the first identified genetic risk factors for VT are the deficiencies in the natural anticoagulant proteins, i.e., antithrombin, protein C, and protein S (encoded by *SERPINC1, PROC*, and *PROS1,* respectively).[24-26] These deficiencies are mainly caused by rare or even family-specific variants and have a large effect on VT risk. Other major genetic risk variants for VT include factor V (FV) Leiden (in *F5*, rs6025) and prothrombin (PT) G20210A (in *F2,* rs1799963), which reach an average population frequency of 5% and 2% in Northwest Europe, respectively.[23,27,28] FV Leiden was identified in individuals with activated protein C (APC) resistance, as the missense variant demolishes one of the APC cleavage sites in activated FV.[27,29] The absence of this cleavage site also hampers the cofactor function of FV in degrading activated factor VIII by APC and protein S.[29] As a result, FV Leiden carriers have a 3-fold increased risk of VT,[23,27] which can be further increased in combination with other risk factors such as oral contraception use.[30] The 2-fold increased VT risk observed in carriers of PT G20210A is due to a substitution in the 3' untranslated region of *F2,* which affects the post-transcriptional regulation of PT mRNA and thereby increases PT plasma levels.[23,28,31] The remaining established genetic risk factors are common variants associated with modest effects on VT risk. Similar to FV Leiden and PT G20210A, most risk variants are located in or near genes coding

for proteins involved in hemostasis.[23] However, for some of the identified genetic loci, such as the locus in *TSPAN15*,[32] the causal variant and biological mechanism remain unknown. In addition, the established genetic risk factors explain around 5% of the phenotypic variance,[33] suggesting that there exist genetic risk factors for VT that have not yet been identified.

For recurrence, previous studies have mainly focussed on genetic variants associated with a first thrombotic event. For most variants no association with recurrence or much smaller effect sizes have been observed.[34-37] For example, carriers of FV Leiden have a 1.4-fold increased risk of recurrent VT compared with non-carriers, whereas PT G20210A is associated with a risk increase of recurrence of around 20 to 70 %.[34,35] In part, these findings can be explained by the difference in absolute risks of first and recurrent VT, resulting in incomparability of effects on a relative risk scale.[38] In addition, research into risk factors for recurrence risk may be hindered by index event bias, although this could lead to both under- and overestimation of the risk estimate.[39] This all assumes that the risk factors and underlying biological mechanisms for a first and recurrent VT are the same, whereas different genetic mechanisms may be involved in recurrence. For example, genetic variants that control the response to damaged vessels and valves after a thrombotic event could play a role in recurrence pathophysiology, but few studies have investigated recurrence-specific variants.

The main aim of the research conducted for this thesis was to identify novel genetic risk factors for a first and recurrent VT. This will not only advance our understanding of the genetic architecture of (recurrent) VT, but also aid in unravelling the biological mechanisms, improve risk stratification, and help to identify potential drug targets. In addition, we aim to show potential applications of genetic risk variants in risk stratification and causal inference.

OUTLINE

In **chapter 2**, we aim to identify novel genetic risk factors for a first VT by studying common and rare genetic variants in mainly coding regions of over 700 genes involved in hemostasis and related pathways using targeted next-sequencing. A more agnostic approach is used in **chapter 3**, where we conduct a genome-wide association study to

uncover common genetic variants associated with recurrent VT. To explore whether the difference in (recurrent) VT risk between men and women can be explained by variations on the Y chromosome, we study in **chapter 4** the association between common European Y haplogroups and the association with the risk of a first and recurrent VT. In **chapter 5**, our aim is to validate the synergistic effect of variation in *CADM1* and protein C deficiency which was previously observed in a family with thrombophilia. For this, we study the joint effects on VT risk of over 300 common variants in *CADM1* and abnormalities in the protein C pathway. The discriminative value of a risk score based on genetic risk factors for a first VT is assessed and compared with a clinical risk model in **chapter 6**. In addition, in **chapter 7**, we discuss the basic concepts of Mendelian randomisation analyses and their use in causal inference.

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