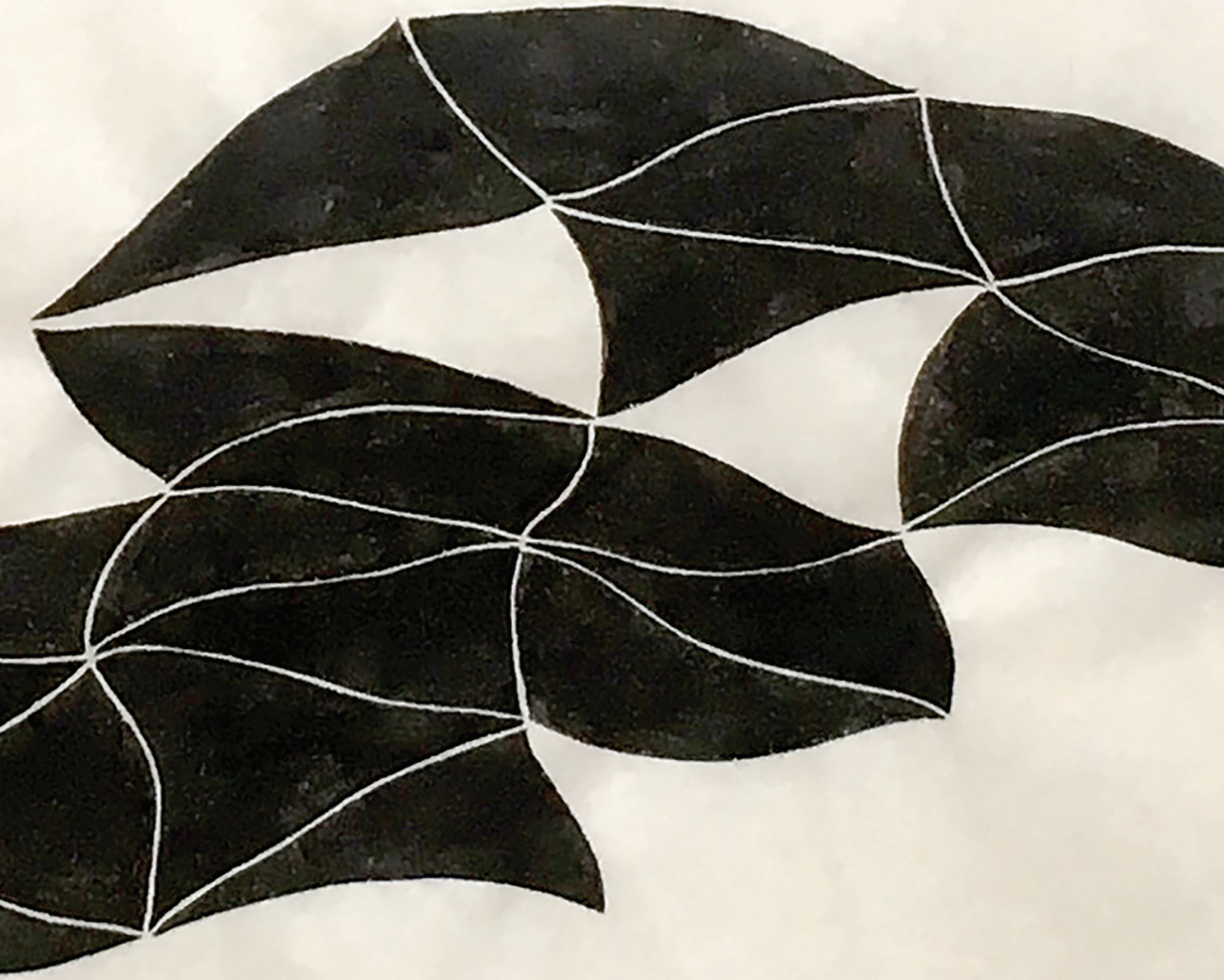
# THE ROLE OF ANTITHROMBIN IN VENOUS AND ARTERIAL THROMBOSIS

Frederik Nanne Croles



ISBN 978-94-6361-190-9
Layout: Egied Simons
Cover: Hiroe Okubo
Printing: Optima
The work described in this thesis was performed at the Department of Hematology at the Erasmus Medical Center Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands and at the department of hematology at the University Medical Center, University Groningen, Groningen, the Netherlands.

Printing of this thesis was financially supported by Bayer, Federatie van Nederlandse Trombosediensten (FNT) and Erasmus University Rotterdam.

# THE ROLE OF ANTITHROMBIN IN VENOUS AND ARTERIAL THROMBOSIS

DE ROL VAN ANTITROMBINE IN VENEUZE EN ARTERIËLE TROMBOSE

**Proefschrift**

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

prof.dr. R.C.M.E. Engels

**en volgens besluit van het College voor Promoties.**
**De openbare verdediging zal plaatsvinden op**

dinsdag 27 november 2018 om 11:30 uur

door

**FREDERIK NANNE CROLES**

geboren te ‘s-Hertogenbosch

Erasmus University Rotterdam

## DOCTORAL COMMITTEE

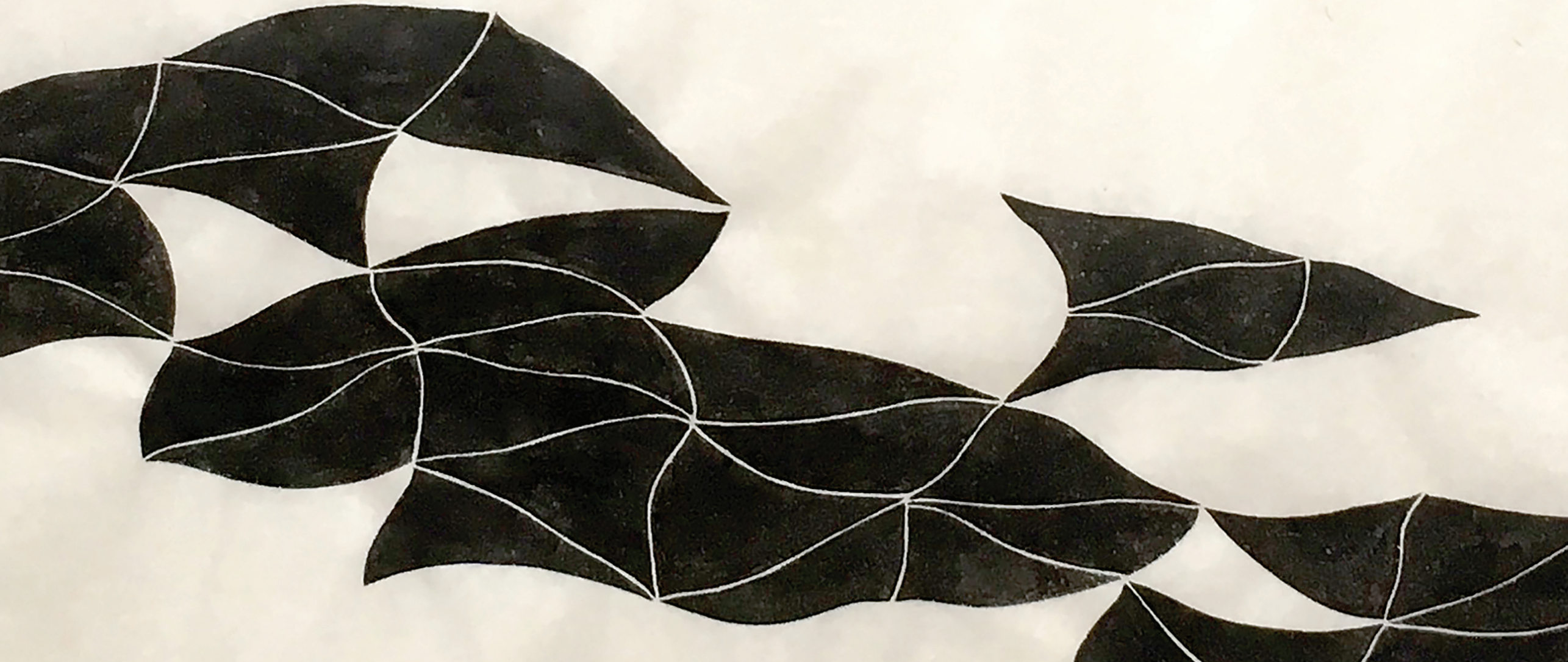
**Promotors:** Prof. dr. F.W.G. Leebeek
Prof. dr. K. Meijer

**Other members:** Prof. dr. J.A. Lisman
Prof. dr. M.V. Huisman
Prof. dr. ir. H. Boersma

## CONTENTS

# 1

# GENERAL INTRODUCTION AND OUTLINE OF THE THESIS



**The hemostatic system**

The hemostatic system is a complex and balanced system that has a function to stop bleeding in response to injury. In short, hemostasis can be simplified tot three distinct phases. These phases may occur simultaneously. Primary hemostasis results in a platelet plug, through interactions of collagen, Von Willebrand factor and platelets. Secondary hemostasis is the process of strengthening the clot by fibrin formation. This process of secondary hemostasis is initiated by tissue factor, which results in activation of coagulation factors, leading to fibrin formation, and is counterbalanced by natural anticoagulants. After overcoming the hemostatic challenge and after wound healing, the process of fibrinolysis resolves the blood clot to restore normal blood flow. Figure 1 shows the main processes of primary and secondary hemostasis as based on a recent review of its physiology[1].

**The anticoagulant systems**

The process of hemostasis is regulated by three important pathways with an anticoagulant effect, mainly on secondary hemostasis: by tissue factor pathway inhibitor (TFPI), by the protein C system and by antithrombin, as shown in figure 1. The focus of this thesis is on the role of antithrombin, which is the most important naturally occurring anticoagulant and is the major circulating plasma inhibitor of thrombin and factors IXa and Xa. A lack of inhibition of hemostasis may result in accelerated thrombus formation, which may lead to thrombosis

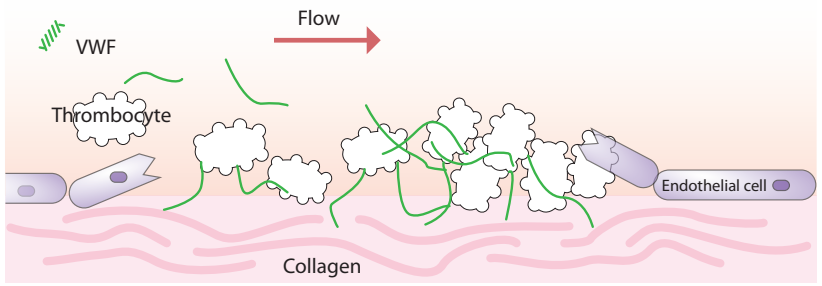
**Thrombosis**

First described by Rudolf Virchow (1821-1902), thrombosis encompasses pathologic formation of thrombi, causing obstruction of blood vessels and subsequent symptoms. If this occurs in the arterial system, it may cause diseases such as acute myocardial infarction or ischemic stroke. If this occurs in the venous system it may cause deep venous thrombosis and/or pulmonary embolism (venous thromboembolism, VTE). The main triad of causes of thrombosis described by Virchow were stasis, hypercoagulability, and vessel wall pathology[3]. Inherited or acquired deficiencies of natural anticoagulants including antithrombin deficiency results in hypercoagulability, thereby contributing to the risk of thrombosis.

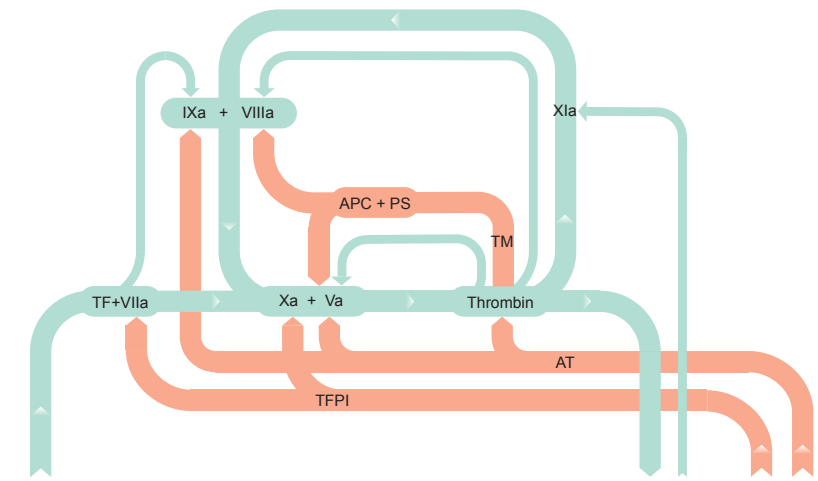
The global burden of thrombosis is huge. The 2010 Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), showed that globally about one in four deaths is caused by ischemic heart disease and stroke collectively[4]. Venous thrombosis was found to be a major global disease burden as well, with incidence rates ranging from 0.75 to 2.69 per 1000 individuals in the population[5]. In the Netherlands, incidence is estimated at 1 per 1000 individuals[6]. Furthermore, following deep venous thrombosis, post-thrombotic complaints, also known as post-thrombotic syndrome (PTS) may develop in up to 50% of patients with deep venous thrombosis. This results in severe morbidity and an increased risk of permanent work-related disability[7].

**Figure 1. Overview of primary and secondary hemostasis, and of the anticoagulant systems.**

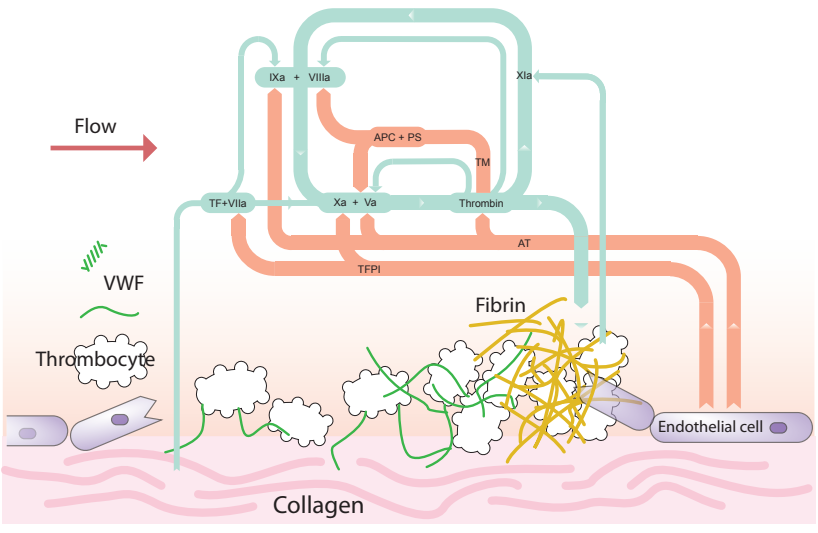
A




B




C




**Panel A: Primary hemostasis.** This panel shows a damaged vessel wall, with absent/damaged endothelial cells, exposing collagen to the circulation. Von Willebrand factor (VWF) binds to collagen, unfolds, and binds platelets. Upon platelet activation, a platelet plug is formed. **Panel B: Secondary hemostasis and natural anticoagulants.** The platelet plug must be reinforced with fibrin, which is the result of a simultaneous process called secondary hemostasis. The secondary hemostasis is depicted in green. *The initiation phase* of secondary hemostasis starts with tissue factor (TF) present on or derived from damaged tissue. Tissue factor acts as a cofactor of factor VII, resulting in formation of factor VIIa (FVIIa). The TF+FVIIa complex activates factor IX and X to IXa and Xa respectively. Factor Xa associates with factor Va to form the prothrombinase complex (Xa+Va). Through this interaction prothrombin (factor II) is converted into thrombin (factor IIa). In the *amplification phase* thrombin production and coagulation is amplified, through activation of platelets, activation of platelet-derived factor V to factor Va, factor VIII to VIIIa and factor IX to IXa. Factor VIII associates with factor IXa on the surface of platelets to support the generation of factor Xa. This illustrates the importance of platelets not only in primary but also in secondary hemostasis. The *propagation phase* occurs on surfaces containing procoagulant phospholipids, such as activated platelets. Factor XIa converts factor IX to IXa, which in turn associates with factor VIIIa to form sufficient amounts of thrombin to allow for a burst in formation of fibrin fibers. In addition, thrombin activates factor XIII, which propagates formation of crosslinks between fibrin fibers. This results in an elastic, polymerized fibrin clot. **The anticoagulant systems** are depicted in red. Antithrombin (AT) mainly inhibits factors IXa and Xa, and thrombin. Although antithrombin is present in the circulation in large quantities, it may bind to glycoasminoglycans (GAGs) on the vascular endothelium[2]. These are thought to be the main activators of antithrombin in vivo. Tissue factor pathway inhibitor (TFPI) inhibits coagulation by binding to factor Xa alone, or to the TF-FVIIa-Xa complex. The TFPI-Xa interaction can be enhanced by the cofactor protein S (not depicted). The protein C/protein S pathway is initiated by binding of thrombin to thrombomodulin (TM). TM-bound thrombin activates protein C (APC) that is bound to nearby endothelial protein C receptors. APC in complex with protein S (PS) inhibits factors VIIIa and Va. In addition, PS may bind to TFPIa to inhibit factor Xa [1]. **Panel C: fusion of primary and secondary hemostasis, and the anticoagulant systems.** The fibrinolytic pathway, which results in the resolution of blood clots, is not part of this figure

## Risk factors for thrombosis

Both arterial and venous thrombosis are recognized as multifactorial diseases. Well-known long-term risk factors for venous thrombosis are male sex, increasing age, family history of VTE and inherited thrombophilia. Inherited thrombophilias are a group of alterations in the hemostatic system that promote a prothrombotic phenotype. In addition, certain provoking factors can temporarily increase the risk of VTE, such as surgery, bed rest, oral contraceptive use, hormonal replacement therapy, pregnancy, caesarian section, or puerperium and immobilization. Other factors that can provoke thrombosis may occur later in life, and may persist for a longer time, such as cancer or inflammatory bowel disease. Assessing these risk factors is important for prevention, and for estimation of the risk of recurrence after a VTE[8].

Current concepts of arterial thromboembolism (ATE) point at several mechanisms at play, including formation of atherosclerotic lesions, plaque rupture and subsequent thrombus formation. Atherosclerosis is a process driven by chronic inflammation, in which factors like age, sex, smoking, blood pressure diabetes and lipids play an important role. The likelihood of plaque rupture depends on the tissue composition of the atherosclerotic lesion[9,10]. Both chronic inflammation and thrombus formation following plaque rupture are mediated by the hemostatic system. Interestingly, natural anticoagulants have been associated with inflammation. Deficiency of TFPI and reduced protein C activation have been associated with increased atherosclerosis in mice[11]. Deficiencies of protein C and S have been linked to increased incidence of ATE in humans[12]. Antithrombin has been implicated with a leukocyte activation inhibiting role in the pathologic condition of sepsis[13].

**Antithrombin**

Antithrombin's main function is inhibition of hemostasis through the inactivation of thrombin and factor Xa. Antithrombin physiologically circulates mostly in an inactive form. Its functionality is increased approximately 1000-fold in the presence of heparin and other heparin-like substances. However, under physiological conditions heparin is not present in the circulation, and it is not exactly clear how antithrombin is activated *in vivo*[14]. Heparan sulphate on the vascular endothelium is believed to be the physiological activator of antithrombin[2,14], although there are no data in humans to substantiate this. Unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) can both activate antithrombin, but do so in different ways: UFH and LMWH directly activate antithrombin in its inhibitory interaction with factors IXa and Xa through allosteric activation, whilst UFH also inhibits thrombin through an extra bridging mechanism[2].

Antithrombin consists of two isoforms, 90% of circulating antithrombin being α-antithrombin and 10% β-antithrombin. Due to differences in glycosylation, the N-glycan at Asn135 is not present in β-antithrombin. β-antithrombin has a higher affinity for heparin[15,16]. Following the theory that antithrombin is activated under physiological conditions by heparan sulphate, it has been hypothesized that β-antithrombin is the most important isoform of antithrombin in vivo[17]. It has been shown that the oligosaccharide at Asn135 present in α-antithrombin decreases the heparin affinity of antithrombin by interfering with the heparin-induced shape change that is required for activation of antithrombin[18]. However the importance of these subtypes *in vivo* are unknown.

**Antithrombin deficiency**

Inherited antithrombin deficiency was first described in 1965 by Egeberg, when he described a family with a high incidence of thromboembolic disease. In affected family members antithrombin activity was about half of normal average activity. The family tree showed that it was an autosomal dominant inherited disease[19]. Hereditary antithrombin deficiency is usually caused by heterozygous mutations. Homozygous antithrombin deficiency is generally lethal in utero, although rare homozygous and compound heterozygous patients have been described[17,20,21]. Current estimates of the prevalence of antithrombin deficiency in healthy populations range from 1:500-1:645, although heritability was not established in all of these studies[22-24]. Antithrombin deficiency is subdivided in quantitative defects (type I) and qualitative defects (type II)[25].

Antithrombin deficiency has been reported to be associated with a 16.3-fold increased risk of first VTE[26]. Moreover, also individuals with relatively low antithrombin levels within the normal range are at an increased risk of venous thrombosis: Antithrombin levels between 81-90 and of 70-80 IU/dL are associated with a 1.65-fold and 2.11-fold increased risk of thrombosis, respectively[27]. Subtypes of antithrombin deficiency and specific mutations in the gene that encodes antithrombin, SERPINC1, are reported to modify VTE risk. These data however, are biased by proband selection[28-30]. The added value of measuring the α- and β-isoforms of antithrombin in antithrombin deficiency with respect to VTE risk is currently unknown. Therefore, it is currently not possible to reliably establish if some individuals may be at lower risk amongst those with antithrombin deficiency.

For arterial thromboembolism, a pathogenetic role of low antithrombin levels is largely unknown. It has been studied in ischemic stroke patients. No antithrombin-deficient subject were found in one study[31], whereas in a case-control study, both in stroke patients and the controls prevalence of antithrombin deficiency was exceptionally high (5.2% and 4.1%, respectively)[32]. In the only study on familial thrombophilia and ischemic stroke, no increased risk of stroke was found in 92 antithrombin-deficient subjects, who were relatively young (age range 29-49 years)[33]. This lack of informative data precludes any conclusions on possible associations between antithrombin deficiency and arterial thromboembolism.

**Other inherited thrombophilias**

After the first report on antithrombin deficiency by Egeberg, several other abnormalities in proteins involved in hemostasis have been implicated as inherited thrombophilias. These include dysfibrinogenemia[34], hypoplasminogenemia[35], protein C deficiency[36], protein S deficiency[37], factor V Leiden[38-41], prothrombin G20210A mutation[42], and many more. The most commonly tested thrombophilic abnormalities include antithrombin, protein C and protein S deficiency, factor V Leiden and prothrombin G20210A mutations. With respect to VTE, antithrombin, protein C and protein S are currently recognized as rare but high-risk thrombophilic abnormalities, whereas heterozygous factor V Leiden or prothrombin G20210A mutations are more prevalent but are relatively low-risk abnormalities[43].

With respect to arterial thromboembolism, a large pooled analysis of four family studies has shown an association between inherited thrombophilia (antithrombin, protein C or S deficiency, factor V Leiden mutation or prothrombin G20210A mutation) and arterial thromboembolism, HR 1.7, 95%CI: 1.18-2.58). This association tended to be stronger in females as compared to men (HR 2.60, 95%CI: 1.42-4.73) and in individuals aged <55 years as compared to older individuals (HR 2.74, 95%CI:1.63-4.60)[44].

**Pregnancy, venous thrombosis and thrombophilia.**

Venous thrombosis is a well-recognized and major cause of pregnancy associated mortality[45] and morbidity[46]. Pregnancy increases the risk of venous thrombosis five- to six-fold compared with age-matched controls[47]. The risk of VTE is further increased (3.7-8.5-fold) by a positive family history of VTE. In a systematic review, thrombophilia had been reported to further increase the risk of VTE up to 34-fold[48]. However, absolute risk estimates, for instance used in the guidelines of the American College of Chest Physicians of 2012, are based on a limited number of cohort studies and on an estimated baseline incidence of venous thrombosis multiplied by the odds ratios found asystematic review [48,49]. Because of

the limitations of the available data guidelines differ considerably in their recommendations on management issues such as prescription of thrombosis prophylaxis to pregnant women with thrombophilia[50].

**Anticoagulant therapy**

The risk of recurrence of unprovoked VTE after stopping anticoagulant therapy after 3-6 months is around 25-30% in 5 years. The use of anticoagulant drugs aims to reduce the risk of thrombus growth and to prevent recurrent thrombosis. It has long been debated whether inherited thrombophilias increase the risk of recurrent VTE, and study results pertaining to recurrence risk often group antithrombin, protein C and S deficiency together. Relative risk for recurrent VTE in individuals with severe inherited thrombophilias has been estimated to be 1.9–2.6[51]. However, absolute risk estimates for recurrent VTE for individuals with antithrombin deficiency vary and are influenced by the duration of anticoagulant therapy. The recurrence risk in individuals with factor V Leiden or prothrombin G20210A mutation is considered not to be higher than in non-carriers with a previous VTE [52].

The most commonly used classes of drugs to treat venous thrombosis are heparins, vitamin K antagonists (VKA) and direct acting oral anticoagulants (DOAC ). When anticoagulant treatment is initiated for treatment of VTE, patients treated with VKA or the DOAC dabigatran or edoxaban are first given a short course of LMWH, usually 5-7 days. VTE patients treated with apixaban or rivaroxaban only are given an increased dose of the drug initially. In certain subgroups of VTE patients, such as patients with cancer-associated VTE or pregnant VTE patients LMWH is given for 3-6 months, or even longer. LMWH therapy can be monitored by measuring the anti-factor-Xa activity (aXa). Interestingly, in patients with cirrhosis, in whom antithrombin levels are reduced, aXa and antithrombin activity levels seem to be related: lower antithrombin levels are correlated with lower aXa levels. This may result in an underestimation of the anticoagulant effect, as cirrhosis patients have multiple changes in their hemostatic system[53,54]. Monitoring of aXa is not a standard practice in patients receiving LMWH, and it is not known if in antithrombin deficient subjects with an indication for anticoagulant treatment measuring aXa levels is reliable and useful.

**Acquired antithrombin deficiency**

Acquired antithrombin deficiency is a well-known phenomenon in a number of diseases, and may result from decreased synthesis (cirrhosis), loss of antithrombin (in nephrotic syndrome or protein loosing enteropathy) or increased antithrombin consumption (disseminated intravascular coagulation). Acquired antithrombin deficiency may also be drug induced[14]. In patients with cirrhosis, reduced synthesis of coagulation factors, including antithrombin is common[55]. Acquired antithrombin deficiency is also known to occur during severe inflammatory responses, either due to decreased synthesis, increased consumption or both[56]. Acquired antithrombin deficiency associated with disseminated intravascular coagulation has been found in cancer patients, such as acute myeloid leukemia patients. The prevalence of thrombosis in this study was as high as 8.7%, and low antithrombin levels were associated with a 3.45-fold risk of thrombotic events[57]. Acute lymfophoblastic leukemia (ALL) and its treatment has also been associated with a high incidence of VTE, estimates varying from 1-36%, depending on the populations investigated[58]. In adults VTE risk is higher than in children, and VTE-rates of about 10% are typical[59,60]. VTE is an important complication in ALL patients, because development of VTE in ALL patients has been associated with a 40% increase in the risk of death within one year[61]. An important component of ALL treatment, asparaginase, decreases liver synthesis of procoagulant and anticoagulant proteins[59,60,62-64].

## AIM AND OUTLINE OF THIS THESIS

The aim of this thesis is to investigate the role of inherited antithrombin deficiency and antithrombin levels in the pathogenesis of arterial and venous thrombosis.
The first part of this thesis focusses on inherited antithrombin deficiency. In **chapter 2** we will report a systematic review and Bayesian meta-analysis of the current literature. The relative and absolute risks of a first VTE in antithrombin deficient individuals, and the impact of subtypes will be investigated. In addition, the relative risks of recurrent VTE and the absolute risks of recurrent VTE depending on duration of anticoagulant therapy are investigated. In **chapter 3** we investigate whether antithrombin deficiency subtypes and β-antithrombin levels have impact on VTE risk in antithrombin-deficient individuals. In **chapter 4** we investigate if the efficacy of heparins is altered by antithrombin levels and antithrombin deficiency. In plasma samples from subjects from the family study described in chapter 3, spiked with UFH and LMWH, we investigate the relation between aXa levels and antithrombin and β-antithrombin levels and SERPINC1 mutations. In **chapter 5** we investigate the spectrum of SERPINC1 mutations in the family study reported in chapter 3, to explain the antithrombin deficiencies.

In **chapter 6** we report a systematic review and Bayesian meta-analysis to investigate the absolute risks of pregnancy-associated VTE in women with or without thrombophilia. Based on our study finding, we explain how the perception of the risks of pregnancy-associated VTE should be altered, and how this should lead to changes in treatment recommendations.

The last part of this thesis investigates the role of antithrombin in the pathogenesis of thrombosis in a relatively new area. In **chapter 7** we investigate antithrombin levels and the association with a first arterial thrombotic event at a young age. Additionally, we investigate the association with recurrent arterial thrombotic events in subject with coronary heart disease at a young age. Finally, In **chapter 8,** the implications of our findings are discussed, putting them in a clinical perspective and reflecting on the implications of these findings for future clinical management of antithrombin deficiency. Also, suggestions for further research are given.



## REFERENCES

1. Versteeg HH, Heemskerk JW, Levi M, Reitsma PH. New fundamentals in hemostasis. *Physiol Rev*. 2013;93(1):327-358.
2. Olson ST, Richard B, Izaguirre G, Schedin-Weiss S, Gettins PG. Molecular mechanisms of antithrombin-heparin regulation of blood clotting proteinases. A paradigm for understanding proteinase regulation by serpin family protein proteinase inhibitors. *Biochimie*. 2010;92(11):1587-1596.
3. Rosendaal FR, Raskob GE. On World Thrombosis Day. *Lancet*. 2014;384(9955):1653-1654.
4. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-2128.
5. ISTH, Steering, Committee, et al. Thrombosis: a major contributor to the global disease burden. *J Thromb Haemost*. 2014;12(10):1580-1590.
6. Lambooij M, Drewes H. Risico's en gebrekkige afstemming in de trombosezorg. *RIVM*. 2010.
7. Braekkan SK, Grosse SD, Okoroh EM, et al. Venous thromboembolism and subsequent permanent work-related disability. *J Thromb Haemost*. 2016;14(10):1978-1987.
8. Kearon C, Ageno W, Cannegieter SC, et al. Categorization of patients as having provoked or unprovoked venous thromboembolism: guidance from the SSC of ISTH. *J Thromb Haemost*. 2016;14(7):1480-1483.
9. Emerging Risk Factors C, Wormser D, Kaptoge S, et al. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet*. 2011;377(9771):1085-1095.
10. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999;340(2):115-126.
11. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. *N Engl J Med*. 2011;364(18):1746-1760.
12. Mahmoodi BK, Brouwer JL, Veeger NJ, van der Meer J. Hereditary deficiency of protein C or protein S confers increased risk of arterial thromboembolic events at a young age: results from a large family cohort study. *Circulation*. 2008;118(16):1659-1667.
13. Okajima K. Regulation of inflammatory responses by natural anticoagulants. *Immunol Rev*. 2001;184:258-274.
14. Patnaik MM, Moll S. Inherited antithrombin deficiency: a review. *Haemophilia*. 2008;14(6):1229-1239.
15. Picard V, Ersdal-Badju E, Bock SC. Partial glycosylation of antithrombin III asparagine-135 is caused by the serine in the third position of its N-glycosylation consensus sequence and is responsible for production of the beta-antithrombin III isoform with enhanced heparin affinity. *Biochemistry*. 1995;34(26):8433-8440.
16. McCoy AJ, Pei XY, Skinner R, Abrahams JP, Carrell RW. Structure of beta-antithrombin and the effect of glycosylation on antithrombin's heparin affinity and activity. *J Mol Biol*. 2003;326(3):823-833.
17. Martinez-Martinez I, Navarro-Fernandez J, Ostergaard A, et al. Amelioration of the severity of heparin-binding antithrombin mutations by posttranslational mosaicism. *Blood*. 2012;120(4):900-904.
18. Turk B, Brieditis I, Bock SC, Olson ST, Bjork I. The oligosaccharide side chain on Asn-135 of alpha-antithrombin, absent in beta-antithrombin, decreases the heparin affinity of the inhibitor by affecting the heparin-induced conformational change. *Biochemistry*. 1997;36(22):6682-6691.
19. Egeberg O. Thrombophilia Caused by Inheritable Deficiency of Blood Antithrombin. *Scand J Clin Lab Invest*. 1965;17:92.
20. Aguila S, Martinez-Martinez I, Collado M, et al. Compound heterozygosity involving Antithrombin Cambridge II (p.Ala416Ser) in antithrombin deficiency. *Thromb Haemost*. 2013;109(3):556-558.

21. Lane DA, Bayston T, Olds RJ, et al. Antithrombin mutation database: 2nd (1997) update. For the Plasma Coagulation Inhibitors Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost*. 1997;77(1):197-211.
22. Sakata T, Okamoto A, Mannami T, Matsuo H, Miyata T. Protein C and antithrombin deficiency are important risk factors for deep vein thrombosis in Japanese. *J Thromb Haemost*. 2004;2(3):528-530.
23. Tait RC, Walker ID, Perry DJ, et al. Prevalence of antithrombin deficiency in the healthy population. *Br J Haematol*. 1994;87(1):106-112.
24. Wells PS, Blajchman MA, Henderson P, et al. Prevalence of antithrombin deficiency in healthy blood donors: a cross-sectional study. *Am J Hematol*. 1994;45(4):321-324.
25. Lane DA, Kunz G, Olds RJ, Thein SL. Molecular genetics of antithrombin deficiency. *Blood Rev*. 1996;10(2):59-74.
26. Di Minno MND, Ambrosino P, Ageno W, Rosendaal F, Di Minno G, Dentali F. Natural anticoagulants deficiency and the risk of venous thromboembolism: A meta-analysis of observational studies. *Thromb Res*. 2015;135(5):923-932.
27. Di Minno MN, Dentali F, Veglia F, Russolillo A, Tremoli E, Ageno W. Antithrombin levels and the risk of a first episode of venous thromboembolism: a case-control study. *Thromb Haemost*. 2013;109(1):167-169.
28. Mitsuguro M, Sakata T, Okamoto A, et al. Usefulness of antithrombin deficiency phenotypes for risk assessment of venous thromboembolism: type I deficiency as a strong risk factor for venous thromboembolism. *Int J Hematol*. 2010;92(3):468-473.
29. Luxembourg B, Pavlova A, Geisen C, et al. Impact of the type of SERPINC1 mutation and subtype of antithrombin deficiency on the thrombotic phenotype in hereditary antithrombin deficiency. *Thromb Haemost*. 2014;111(2):249-257.
30. Alhenc-Gelas M, Plu-Bureau G, Hugon-Rodin J, Picard V, Horellou MH, Thrombophilia GsgoG. Thrombotic risk according to SERPINC1 genotype in a large cohort of subjects with antithrombin inherited deficiency. *Thromb Haemost*. 2017;117(6):1040-1051.
31. Carod-Artal FJ, Nunes SV, Portugal D, Silva TV, Vargas AP. Ischemic stroke subtypes and thrombophilia in young and elderly Brazilian stroke patients admitted to a rehabilitation hospital. *Stroke*. 2005;36(9):2012-2014.
32. Hankey GJ, Eikelboom JW, van Bockxmeer FM, Lofthouse E, Staples N, Baker RI. Inherited thrombophilia in ischemic stroke and its pathogenic subtypes. *Stroke*. 2001;32(8):1793-1799.
33. Vossen CY, Rosendaal FR, Group ES. Risk of arterial thrombosis in carriers of familial thrombophilia. *J Thromb Haemost*. 2006;4(4):916-918.
34. Egeberg O. Inherited fibrinogen abnormality causing thrombophilia. *Thromb Diath Haemorrh*. 1967;17(1-2):176-187.
35. Aoki N, Moroi M, Sakata Y, Yoshida N, Matsuda M. Abnormal plasminogen. A hereditary molecular abnormality found in a patient with recurrent thrombosis. *J Clin Invest*. 1978;61(5):1186-1195.
36. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest*. 1981;68(5):1370-1373.
37. Comp PC, Nixon RR, Cooper MR, Esmon CT. Familial protein S deficiency is associated with recurrent thrombosis. *J Clin Invest*. 1984;74(6):2082-2088.
38. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature*. 1994;369(6475):64-67.
39. Greengard JS, Sun X, Xu X, Fernandez JA, Griffin JH, Evatt B. Activated protein C resistance caused by Arg506Gln mutation in factor Va. *Lancet*. 1994;343(8909):1361-1362.





40. Voorberg J, Roelse J, Koopman R, et al. Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. *Lancet*. 1994;343(8912):1535-1536.
41. Zoller B, Dahlback B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. *Lancet*. 1994;343(8912):1536-1538.
42. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996;88(10):3698-3703.
43. Middeldorp S. Inherited thrombophilia: a double-edged sword. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):1-9.
44. Mahmoodi BK, Veeger NJ, Middeldorp S, et al. Interaction of Hereditary Thrombophilia and Traditional Cardiovascular Risk Factors on the Risk of Arterial Thromboembolism: Pooled Analysis of Four Family Cohort Studies. *Circ Cardiovasc Genet*. 2016;9(1):79-85.
45. Bezemer ID, van der Meer FJ, Eikenboom JC, Rosendaal FR, Doggen CJ. The value of family history as a risk indicator for venous thrombosis. *Arch Intern Med*. 2009;169(6):610-615.
46. Wik HS, Jacobsen AF, Sandvik L, Sandset PM. Prevalence and predictors for post-thrombotic syndrome 3 to 16 years after pregnancy-related venous thrombosis: a population-based, cross-sectional, case-control study. *J Thromb Haemost*. 2012;10(5):840-847.
47. Melis F, Vandenbrouke JP, Buller HR, Colly LP, Bloemenkamp KW. Estimates of risk of venous thrombosis during pregnancy and puerperium are not influenced by diagnostic suspicion and referral basis. *Am J Obstet Gynecol*. 2004;191(3):825-829.
48. Robertson L, Wu O, Langhorne P, et al. Thrombophilia in pregnancy: A systematic review. *Br J Haematol*. 2006;132(2):171-196.
49. Bates SM, Greer IA, Middeldorp S, et al. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e691S-736S.
50. Bates SM, Middeldorp S, Rodger M, James AH, Greer I. Guidance for the treatment and prevention of obstetric-associated venous thromboembolism. *J Thromb Thrombolysis*. 2016;41(1):92-128.
51. Middeldorp S, van Hylckama Vlieg A. Does thrombophilia testing help in the clinical management of patients? *Br J Haematol*. 2008;143(3):321-335.
52. Kyrle PA, Rosendaal FR, Eichinger S. Risk assessment for recurrent venous thrombosis. *Lancet*. 2010;376(9757):2032-2039.
53. Senzolo M, Rodriguez-Castro KI, Rossetto V, et al. Increased anticoagulant response to low-molecular-weight heparin in plasma from patients with advanced cirrhosis. *J Thromb Haemost*. 2012;10(9):1823-1829.
54. Potze W, Arshad F, Adelmeijer J, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. *Br J Haematol*. 2013;163(5):666-673.
55. Lisman T, Leebeek FW. Hemostatic alterations in liver disease: a review on pathophysiology, clinical consequences, and treatment. *Dig Surg*. 2007;24(4):250-258.
56. Levi M, van der Poll T. The role of natural anticoagulants in the pathogenesis and management of systemic activation of coagulation and inflammation in critically ill patients. *Semin Thromb Hemost*. 2008;34(5):459-468.
57. Libourel EJ, Klerk CPW, van Norden Y, et al. Disseminated intravascular coagulation at diagnosis is a strong predictor for thrombosis in acute myeloid leukemia. *Blood*. 2016;128(14):1854-1861.
58. Goyal G, Bhatt VR. L-asparaginase and venous thromboembolism in acute lymphocytic leukemia. *Future Oncol*. 2015;11(17):2459-2470.

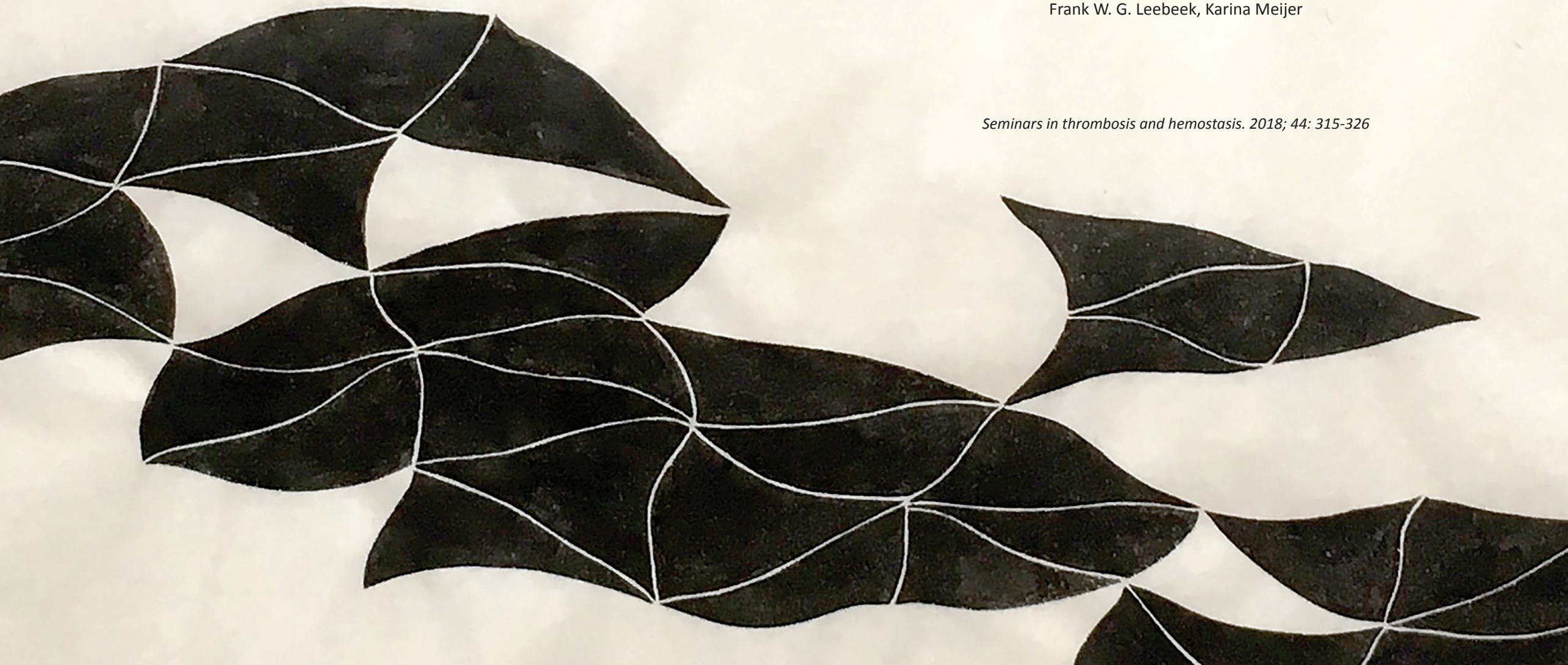
59. Hunault-Berger M, Chevallier P, Delain M, et al. Changes in antithrombin and fibrinogen levels during induction chemotherapy with L-asparaginase in adult patients with acute lymphoblastic leukemia or lymphoblastic lymphoma. Use of supportive coagulation therapy and clinical outcome: the CAPELAL study. *Haematologica*. 2008;93(10):1488-1494.
60. Lauw MN, Van der Holt B, Middeldorp S, Meijers JC, Cornelissen JJ, Biemond BJ. Venous thromboembolism in adults treated for acute lymphoblastic leukaemia: Effect of fresh frozen plasma supplementation. *Thromb Haemost*. 2013;109(4):633-642.
61. Ku GH, White RH, Chew HK, Harvey DJ, Zhou H, Wun T. Venous thromboembolism in patients with acute leukemia: incidence, risk factors, and effect on survival. *Blood*. 2009;113(17):3911-3917.
62. Appel IM, Hop WC, Pieters R. Changes in hypercoagulability by asparaginase: a randomized study between two asparaginases. *Blood Coagul Fibrinolysis*. 2006;17(2):139-146.
63. Appel IM, Hop WC, van Kessel-Bakvis C, Stigter R, Pieters R. L-Asparaginase and the effect of age on coagulation and fibrinolysis in childhood acute lymphoblastic leukemia. *Thromb Haemost*. 2008;100(2):330-337.
64. Ranta S, Heyman MM, Jahnukainen K, et al. Antithrombin deficiency after prolonged asparaginase treatment in children with acute lymphoblastic leukemia. *Blood Coagul Fibrinolysis*. 2013;24(7):749-756.

2

# RISK OF VENOUS THROMBOSIS IN ANTI-THROMBIN DEFICIENCY: A SYSTEMATIC REVIEW AND BAYESIAN META-ANALYSIS

F. Nanne Croles,Jaime Borjas-Howard, Kazem Nasserinejad,
Frank W. G. Leebeek, Karina Meijer

*Seminars in thrombosis and hemostasis. 2018; 44: 315-326*



## SUMMARY

Antithrombin deficiency is a strong risk factor for venous thromboembolism (VTE), but the absolute risk of first and recurrent VTE is unclear. The objective of this paper is to establish the absolute risks of first and recurrent VTE and mortality in individuals with antithrombin deficiency. The databases Embase, Medline Ovid, Web of Science, the Cochrane library and Google scholar were systematically searched for case-control and cohort studies. Bayesian random-effects meta-analysis was used to calculate odds ratios (ORs), absolute risks, and probabilities of ORs being above thresholds. Thirty-five publications were included in the systematic review and meta-analysis. Based on 19 studies OR-estimates for first VTE showed a strongly increased risk for antithrombin deficient individuals, OR 14.0; 95% credible interval (CrI), 5.5-29.0. Based on 10 studies, meta-analysis showed that the annual VTE risk was significantly higher in antithrombin-deficient than in non-antithrombin-deficient individuals: 1.2% (95% CrI, 0.8-1.7) vs. 0.07% (95% CrI, 0.01-0.14). In prospective studies, the annual VTE risk in antithrombin deficient individuals was as high as 2.3%; 95% CrI, 0.2-6.5%. Data on antithrombin deficiency subtypes are too limited for reliable risk-differentiation. The OR for recurrent VTE based on 10 studies was 2.1; 95% CrI, 0.2-4.0. The annual recurrence risk without long-term anticoagulant therapy based on 4 studies was 8.8% (95% CrI, 4.6-14.1) for antithrombin-deficient and 4.3% (95% CrI, 1.5-7.9) for non-antithrombin-deficient VTE patients. The probability of the recurrence risk being higher in antithrombin-deficient patients was 95%.

We conclude that antithrombin deficient individuals have a high annual VTE risk, and a high annual recurrence risk. Antithrombin deficient patients with VTE require long-term anticoagulant therapy.






## INTRODUCTION

The first family with hereditary antithrombin deficiency was described in 1965 by Egeberg [1]. Since then many studies have shown that antithrombin deficiency is characterized by a high incidence of venous thromboembolism (VTE), although risk estimates vary. A subdivision of inherited antithrombin deficiency is made in type I deficiency (quantitative defect) or type II deficiency (qualitative defects). Type II deficiencies are subdivided in type II RS (reactive site), type II HBS (heparin binding site) and type II PE (pleiotropic effects) [2]. These subtypes may mitigate thrombosis risk [3,4].

The absolute annual risk of VTE in antithrombin deficient individuals is not yet clear. However, recently we have shown that in antithrombin deficient women, the risk of a first VTE is so high during pregnancy and puerperium that thrombosis prophylaxis is warranted [5]. Absolute risk estimates of VTE are essential in making well-informed decisions in managing other high-risk situations.

Current guidelines on prevention and treatment of first and recurrent VTE recommend to guide treatment based on both risk of thrombosis recurrence and risk of bleeding on anticoagulation. The American College of Chest Physicians (ACCP) and European Society of Cardiology (ESC) guidelines mention an increased risk of recurrence in patients with hereditary thrombophilia, but without specifically mentioning antithrombin deficiency [6,7]. It is unknown whether in individuals with antithrombin deficiency the absolute risk of recurrent VTE is so high that it outweighs the risk of bleeding of long-term-treatment with anticoagulants. So far meta-analyses of the absolute risks of a first VTE or recurrent VTE have not been performed [8].

Because of the paucity of specific data for antithrombin deficiency we performed a systematic review of all published studies to gather all available evidence on the absolute and relative risks of first and recurrent VTE in patients with antithrombin deficiency to help guide clinical decisions in these patients in an evidence based way. We collected data on the role of antithrombin subtype and the role of mutations or mutation types on risks of first and recurrent VTE in patients with antithrombin deficiency based on available studies to enable a personalized approach in these patients as much as possible.

## METHODS

### Searches

The databases Embase, Medline Ovid, Web of Science, Cochrane and Google Scholar were searched systematically using a complex search strategy that was constructed with the aid of a biomedical information specialist. For details: see the data supplement. The search was last performed on May 12th 2017.

### Study selection

Two reviewers (F.N.C., J.B-H.) independently screened the titles and abstracts of the database search results. The full article was retrieved when the information in the title or abstract appeared to meet the inclusion criteria of this systematic review. Studies selected for full text review were independently assessed for inclusion by both reviewers, and the reference list was checked for relevant publications. Disagreements between the two reviewers were solved by consensus. If no consensus could be reached, K.M. acted as referee.

### Inclusion and exclusion criteria

We systematically searched for studies describing the risk of first VTE, use of anticoagulants and recurrent VTE in antithrombin deficient patients.

Eligible studies were cohort-studies or case-control studies that contained separate information on antithrombin deficiency (as was defined in studies), and relative or absolute risk of first VTE or recurrent VTE. Studies on VTE were only included if VTE was confirmed by objective means, or when the patient had received full course of a full dose unfractionated heparin and a vitamin K antagonist without objective testing (as was described in studies). Studies on VTE were not included if the data were limited to specific high risk situations, such as surgery, pregnancy, hormonal replacement therapy, oral contraceptives or immobilization. Studies were published in peer-reviewed journals, in English during 1970-2016. In case of duplicate publications the most informative was included.

### Data extraction and quality assessment

For eligible studies, data were extracted using a data extraction form that was created for this review and adapted after pilot extractions. Relevant data included study characteristics, inclusion criteria, study type, antithrombin activity assay used. Specific items to identify sources of bias were assessed: the items selection bias, diagnostic criteria, comparability of cases and controls and adequacy of reported follow-up of cohort studies were assessed using the method of the Newcastle-Ottawa Scale (NOS) quality assessment score [9]. Studies were classified as cohort or case-control studies; cohort studies were classified as prospective (VTE occurred after inclusion) or retrospective (VTE occurred before inclusion). As a positive family history for VTE influences the VTE risk, studies were classified as a family or a non-family study. VTE events were deep venous thrombosis and/or pulmonary embolism or non-separately described superficial vein thrombosis events. Separately reported superficial vein thrombosis events were not considered as VTE events. Information on diagnosis of VTE was reported as objective only or objective and/or treatment. We reported the number of individuals in the antithrombin deficient group and in the non-thrombophilic controls, as well as the number of (first of recurrent) VTE events in those groups. Of articles that contained both review data and original data, only the original data were used in the analysis. For absolute risk estimates information on age at study entry and at onset of VTE was collected. Post-hoc, as data on subtypes was scant on antithrombin subtypes, we e-mailed authors asking for separate data on probands and family members.

### Statistical analysis

To estimate odds ratios (ORs) of first or recurrent VTE in antithrombin deficiency versus controls, a Bayesian random-effects meta-analysis was used, assuming heterogeneity among studies. In contrast to frequentist statistics, Bayesian statistics assume that estimated ORs or absolute risks are not fixed values, but have a distribution. Bayesian statistics combines previous knowledge and available data to estimate (posterior) distributions of ORs and absolute risks. This chance distribution can be summarized by the median as a point estimate and 95% area under the posterior distribution, i.e. credible interval (CrI; Bayesian terminology for confidence interval) [10]. The outcomes of this Bayesian meta-analysis model provide information on the probability of events to occur, whereas frequentist meta-analysis would provide a fixed point estimate, with a 95% confidence interval to estimate where the point estimate would be 95% of the time should the experiments be repeated infinitely. Therefore, Bayesian meta-analysis is more relevant from a clinical perspective. In addition, the probability of ORs and absolute risks being above a threshold of interest were estimated. For OR calculations we used a relatively non-informative normal distribution as a prior. For a more detailed explanation of Bayesian meta-analysis we refer to the technical appendix of previous work [5].

To estimate absolute risks of VTE in antithrombin deficient individuals and controls, a Bayesian random effects meta-analysis model was used with non-informative priors. Absolute annual risks were reported as percentage. To test the statistical difference between two groups, exceedance probability (counterpart of p-value in frequentist statistics) was calculated. Exceedance probability is defined as the posterior probability that the estimated parameter in a group is greater than the estimated parameter in another group. Like the p-value in frequentist statistics, extreme values for the exceedance probability (i.e., <5% or >95%) indicate a significant difference. Even if 95% CrIs overlap, due to skewed posterior distributions, the exceedance probability may still be extreme, indicating a significant difference.

All statistical computations and graphics were performed using the R program [11]. All Bayesian computations were performed using the Markov chain Monte Carlo (MCMC) sampler via Jags interface in R.[12] MCMC sampling was run for each analysis for 1,000,000 iterations after discarding the first 75,000 iterations (burn-in).

Post-hoc, we investigated how the absolute risk of VTE in cohort studies was influenced by age of participants at onset of VTE by performing a Bayesian regression meta-analysis where age at onset of VTE was a predictor in the model. We used this model to predict the annual probability of VTE by age of participants at onset of VTE. Since the age at onset was not available for all studies, age at onset was modelled based on age of participants at study entry and study type (retro- or prospective) via a multiple linear regression model.

### Risk of bias or heterogeneity across studies

To analyze bias across studies, factors that could influence the results of the meta-analyses, such as selection issues, diagnostic issues (objective diagnosis/objective diagnosis or treatment), comparability of cases and controls and adequacy of reported follow-up of cohort studies were separately scored as present/absent. Other factors were: age at study entry and age at onset of first VTE, study type, retrospective/prospective study data, family or non-family study, established heritability and method of antithrombin diagnosis. A sensitivity analysis was planned for all OR and absolute risk estimates by performing separate analyses for all these items if applicable.



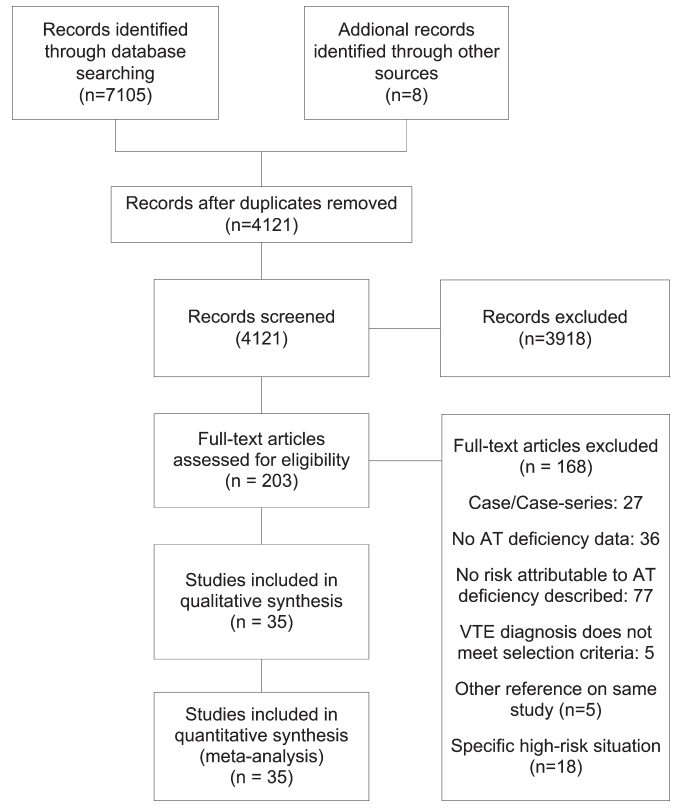


## RESULTS

### Study selection

The search revealed 7105 articles. After removal of duplicates, 4113 articles were screened on title and abstract for further reading. Through checking of references we identified another 8 articles for full review. In total, 203 articles were selected for full article review (figure 1). After exclusion of 168 articles, we included a total of 35 studies for the systematic review and meta-analysis.

**Figure 1: Study selection process**




### Study characteristics

The study characteristics are shown in table 1. Twenty-five studies reported on first VTE [3,4,13-35]. Of those studies, 9 were case-control studies and 16 were cohort studies. Eleven cohort studies on first VTE reported on absolute risk, 22 studies reported on relative risk. Only three of these studies reported on antithrombin subtype [3,4,13]. Ten studies reported on recurrent VTE [28,31,33,36-42].

**Table 1. Characteristics of the 35 included studies**

| 1st author | year | prospective/ retrospective | Study type | Family study | Selection bias | Diagnostic bias | Comparability issues | Follow-up issues | Type of AT test used | n of AT deficient individuals | n of controls | VTE diagnosis | Risk type reported |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| First VTE studies | | | | | | | | | | | | | |
| Brouwer [14] | 2006 | Retro | cohort | Yes | No | Yes | No | No | IIa | 67 | 56 | O or T | both |
| Bucciarelli [15] | 2012 | Retro | case-control | No | No | No | No | | Xa | 11 | 2340 | O | relative |
| Bucciarelli [16] | 1999 | Retro | cohort | Yes | No | No | Yes | No | NA | 95 | NA | O | absolute |
| Chougule [17] | 2016 | Retro | case-control | No | No | No | Yes | | IIa | 2 | 244 | O | relative |
| Cogo [18] | 1996 | Retro | case-control | No | No | No | Yes | | IIa | 2 | 92 | O | relative |
| Cohen [19] | 2012 | Retro | cohort | Yes | No | Yes | No | No | IIa | 28 | 699 | O or T | both |
| De Stefano [20] | 1994 | Pro | cohort | No | Yes | No | Yes | No | NA | 94 | NA | O | absolute |
| Di Minno [21] | 2013 | Retro | case-control | No | No | No | No | | IIa | 53 | 2803 | O | relative |
| Folsom [22] | 2002 | Pro | cohort | No | Yes | No | No | No | IIa or Xa | 704 | 13654 | O | relative |
| Koster [23] | 1995 | Retro | case-control | No | No | No | No | | IIa | 6 | 912 | O | relative |
| Mahmoodi [24] | 2010 | Pro | cohort | Yes | No | No | No | No | IIa | 46 | 233 | O | relative |
| Martinelli [25] | 1998 | Retro | cohort | Yes | No | Yes | No | No | NA | 85 | 327 | O or T | both |
| Mateo [26] | 1998 | Retro | cohort | Yes | No | No | No | No | NA | 9 | 270 | O | relative |
| Okumus [27] | 2008 | Retro | case-control | No | No | No | No | | IIa | 3 | 379 | O | relative |
| Rossi [28] | 2011 | Retro | cohort | Yes | No | No | Yes | No | NA | 17 | 463 | O | relative |
| Sakata [29] | 2004 | Retro | case-control | No | No | No | Yes | | IIa | 13 | 4599 | O | relative |
| Sanson [30] | 1999 | Pro | cohort | Yes | No | No | Yes | Yes | IIa | 45 | NA | O | absolute |
| Shen [31] | 2000 | Retro | case-control | No | No | No | Yes | | NA | 14 | 150 | O | relative |
| Suchon [32] | 2016 | Retro | case-control | No | Yes | yes | No | | IIa | 6 | 1413 | O or T | relative |
| Tormene [33] | 2005 | Pro | cohort | Yes | No | No | Yes | yes | IIa | 41 | 147 | O | relative |
| Vossen [35] | 2004 | Retro | cohort | No | No | No | No | No | NA | 145 | 1212 | O | both |
| Vossen [34] | 2005 | Pro | cohort | No | No | No | No | No | NA | 96 | 1118 | O | both |
| First VTE in AT subtype studies | | | | | | | | | | | | | |
| Alhenc-Gelas [13] | 2017 | Retro | cohort | Yes | Yes | No | Yes | No | various | 540 | NA | O | relative |
| Luxembourg [4] | 2014 | Retro | cohort | Yes | Yes | Yes | Yes | No | IIa or Xa | 133 | NA | O or T | relative |
| Mitsuguro [3] | 2010 | Retro | cohort | No | Yes | No | Yes | No | IIa | 31 | NA | O | relative |
| Recurrence studies | | | | | | | | | | | | | |
| Baglin [36] | 2003 | Pro | cohort | No | No | No | No | No | NA | 8 | 562 | O | relative |
| Brouwer [37] | 2009 | Retro | cohort | Yes | Yes | Yes | Yes | No | IIa | 25 | 8 | O or T | both |
| De Stefano[38] | 2006 | Retro | cohort | No | No | No | Yes | Yes | NA | 14 | 538 | O | both |
| Di Minno [39] | 2014 | Pro | cohort | No | No | No | No | No | IIa | 80 | 743 | O | both |
| Kearon [40] | 2008 | Pro | cohort | No | Yes | No | No | Yes | NA | 23 | 280 | O | relative |
| Prandoni [43] | 2007 | Pro | cohort | No | Yes | No | Yes | No | NA | 7 | 724 | O | relative |
| Santamaria [45] | 2005 | Pro | cohort | No | No | No | No | Yes | NA | 3 | 138 | O | relative |
| Taliani [44] | 2009 | Pro | cohort | No | Yes | Yes | Yes | Yes | NA | 3 | 206 | O | relative |
| Vossen [41] | 2005 | Pro | cohort | Yes | No | No | Yes | No | NA | 11 | 79 | O | both |
| Weingarz [42] | 2015 | Pro | cohort | No | No | No | No | No | Xa | 31 | 585 | O | relative |

AT: antithrombin. n = number. VTE: venous thromboembolism. Retro: retrospective. Pro: prospective. O: Objective. O or T: Objective or treatment. NA: data not available in this study. IIa: factor IIa-based test for AT deficiency. Xa: Factor Xa-based test for AT deficiency.

### Study quality assessment and risk of bias within studies

The sources of bias per study are reported in table 1. Selection issues were identified in 3/22 studies on first VTE and in 4/10 recurrence studies. Diagnostic issues (such as VTE diagnoses based on treatment) occurred in 4/22 first VTE studies and in 3/10 recurrence studies. Comparability of antithrombin deficient subjects and controls was an issue in 9/22 first VTE studies, and in 5/10 recurrence studies. In the three studies on subtypes of antithrombin deficiency, proband selection occurred, and no non-deficient control group was available [3,4,13]. Of all studies, 15 studies used factor IIa-based tests as antithrombin activity assay, 2 studies used factor Xa-based tests, 3 used various tests, and 15 studies did not report the tests used. The number of tests performed per patient was reported in 16/35 studies. VTE diagnoses were objective in 28 studies. Seven studies were included in which treatment of VTE without objective tests was considered as a VTE.

Of the 10 studies on recurrence, two studies were family cohort studies, and 8 were non-family cohort studies. Four studies had an additional selection bias: one study allowed multiple previous episodes of VTE [40]. Two studies left thrombophilia testing to the discretion

of the treating physician, which also influences control selection [43,44]. One study pooled data from probands and family members and lacked a control group[37]. Two of the studies reported documented treatment for a VTE diagnosis, apart from objectively established VTE [37,38]. Only 3 studies on recurrent VTE reported the tests used for antithrombin activity, and only 3 studies reported repeated antithrombin testing when low values were found. In one study, carriers of factor V Leiden were used as controls [41].

**Table 2. Relative risk of first venous thromboembolism for antithrombin deficiency, and between subtypes.**

| Analysis | Odds ratio | 95% credible interval | Probability of OR>1 |
|---|---|---|---|
| All studies (n=19) | 14.0 | 5.5-29.0 | 100 |
| Case-control (n=9) | 9.7 | 1.2-28.1 | 99.9 |
| Cohort studies (n=10) | 19.7 | 1.9-69.1 | 99.9 |
| Retrospective cohort studies (n=6) | 26.4 | 1.5-100.1 | 99.9 |
| Prospective cohort studies (n=4) | 11.6 | 0.0-148.8 | 97.0 |
| High quality studies, NOS≥8 (n=12) | 17.0 | 8.3-30.3 | 100 |
| Family studies (n=7) | 32.4 | 1.8-159.1 | 99.9 |
| Non-family studies (n=12) | 8.3 | 1.8-19.6 | 99.9 |
| Only factor IIa based AT tests (n=11) | 17.1 | 4.8-40.2 | 100 |
| Only objective VTE diagnosis (n=15) | 12.6 | 3.9-29.3 | 100 |
| No selection issues (n=17) | 17.4 | 7.0-33.8 | 100 |
| No diagnostic issues (n=15) | 12.7 | 3.8-28.5 | 100 |
| No comparability issues (n=12) | 12.7 | 2.4-29.5 | 99.9 |
| No bias issues (n=8) | 15.0 | 5.7-25.7 | 100 |
| Comparisons among AT subtypes | | | |
| All studies, all data (n=3) | 9.3 | 0.0-30.9 | 96.5 |
| Only family member data (n=2) | 2.6 | 0.0-19.5 | 87.9 |
| Type I vs IIHBS, all data (n=2) | 4.3 | 0.0-21.3 | 94.9 |
| Type I vs IIRS, all data (n=2) | 1.2 | 0.0-10.0 | 58.8 |
| Type I vs IIPE, all data (n=2) | 1.6 | 0.0-9.5 | 75.5 |

Probability of OR>1: the probability that the odds ratio is greater than 1. n= number. NOS: Newcastle-Ottawa Scale score. VTE: venous thromboembolism. AT: antithrombin





## Results of individual studies and synthesis of the results

*First VTE*

Nineteen studies were used for the OR-estimates of first VTE, containing data on 1381 antithrombin-deficient individuals and 31,111 controls [14,15,17-19,21-29,31-35]. Antithrombin-deficient individuals have an increased risk of a first VTE-event as compared to controls, OR 14.0; 95% CrI, 5.5-29.0. An increased risk was found for antithrombin deficient individuals in all meta-analyses of subgroups of studies. When removing studies (n=11) that were deemed to have bias issues (in terms of selection, diagnosis or comparability), the OR was 15.0, with a narrower 95% CrI of 5.7-25.7. The probability of the OR being >1 is 100% overall, and >97% in all sub-analyses, see Table 2.

Three studies were used for risk calculation of subtypes of antithrombin deficiency. These studies contain data on 343 type I antithrombin-deficient individuals and 326 type II antithrombin-deficient individuals [4,13,24]. The OR of type I vs. type II overall was 9.3 (95% CrI, 0.0-30.9) and the probability of the OR being >1 was 96.5%. From two studies we extracted separate proband and family member data, and analyzed the family members separately, to minimize bias. In this sub-analysis of antithrombin-deficient family members 132 had type I and 115 had type II antithrombin deficiency. The OR of type I vs. type II was lower in family members only as compared to all subjects: 2.6, 95% CrI, 0.0-19.4. The probability of OR>1 was 87.9%.[4,13] When type I antithrombin deficiency was compared to the subtypes of type II antithrombin deficiency, the VTE risk in type IIHBS antithrombin deficiency seems lower, OR 4.3 with 95% CrI 0.0-21.3, see Table 2.

In the analysis of absolute risk of first VTE, ten studies were used, containing data of 718 antithrombin-deficient individuals [14,16,19,20,24,25,28,30,34,35]. The results are summarized in Table 3. From one study, retrospective data and prospective data were analyzed separately [20]. In total, 190 events occurred in 18,190 observation years. The annual absolute risk of VTE in antithrombin-deficient individuals in all studies was 1.2%; 95% CrI, 0.8-1.7%. This risk estimate was higher in prospective studies than in retrospective studies, 2.3% (95% CrI, 0.2-6.5) versus 1.0% (95% CrI, 0.6-1.5), exceedance probability= 0.95. The annual absolute risk of VTE was high in both family- and non-family studies, 1.3% (95% CrI, 0.8-2.1) vs. 1.0% (95% CrI, 0.0-2.7). Several sub-analyses were performed to see if selection, diagnostic, comparability and follow-up issues influenced results. In the 3 studies that were not deemed to have such issues the absolute risk was 1.2%/year; 95% CrI, 0.0-4.3, see table 3.

Seven studies reported on non-antithrombin-deficient individuals [14,19,24,25,28,34,35]. In 3686 non-antithrombin-deficient individuals 67 VTE events occurred in 95,924 observation years. Therefore, in non-antithrombin-deficient individuals the annual absolute risk of VTE was 0.07%; 95% CrI, 0.01-0.14. In non-antithrombin-deficient individuals the risk estimate was higher in prospective studies than in retrospective studies, 0.17% (95% CrI, 0.00-1.99) vs. 0.04% (95% CrI, 0.00-0.11), exceedance probability= 0.87. The annual absolute risks of VTE were similar in family- and non-family studies, 0.08% (95% CrI, 0.00-0.21) vs. 0.06% (95% CrI, 0.00-1.80).

**Table 3. Absolute risk of first venous thromboembolism in antithrombin deficiency**

| Analysis | Studies (n) | median AR in AT deficiency (%) | 95% CrI of AR in AT deficiency | Studies (n) | median AR in control (%) | 95% CrI of AR in control | Probability of VTE risk being higher in antithrombin deficiency |
|---|---|---|---|---|---|---|---|
| Overall AR | 10 | 1.2 | 0.8-1.7 | 7 | 0.07 | 0.01-0.14 | 100 |
| High quality studies, NOS≥8 | 7 | 1.1 | 0.6-1.7 | 7 | 0.07 | 0.01-0.14 | 100 |
| Retrospective cohort studies | 7 | 1.0 | 0.6-1.5 | 5 | 0.04 | 0.00-0.11 | 99 |
| Prospective cohort studies | 3 | 2.3 | 0.2-6.5 | 2 | 0.17 | 0.00-2.14 | 94 |
| Family studies | 7 | 1.3 | 0.8-2.1 | 5 | 0.07 | 0.00-0.21 | 99 |
| Non-family studies | 3 | 1.0 | 0.0-2.7 | 2 | 0.05 | 0.00-1.06 | 93 |
| Only factor IIa based AT test | 4 | 1.8 | 0.4-3.9 | 3 | 0.12 | 0.00-0.64 | 97 |
| Studies with control population | 7 | 1.1 | 0.6-1.7 | 7 | 0.07 | 0.01-0.14 | 100 |
| No selection issues | 9 | 1.2 | 0.7-1.9 | 7 | 0.07 | 0.01-0.14 | 100 |
| No diagnostic issues | 6 | 1.3 | 0.5-2.4 | 4 | 0.08 | 0.00-0.25 | 99 |
| No comparability issues | 6 | 1.1 | 0.5-2.0 | 6 | 0.07 | 0.00-0.16 | 99 |
| No follow-up issues | 9 | 1.1 | 0.7-1.6 | 7 | 0.07 | 0.01-0.14 | 100 |
| No bias issues | 3 | 1.2 | 0.0-4.3 | 3 | 0.08 | 0.00-0.51 | 96 |

AR: absolute risk. AT: antithrombin. n=number. CrI: credible interval. VTE: venous thromboembolism. NOS: Newcastle-Ottawa Scale score. No bias issues is a combination of no selection, diagnostic or comparability issues.

*Risk of recurrent VTE*

A total of 10 studies were used. In six studies anticoagulant treatment was short-term [36,37,42-45], in one study long-term [40], and in three studies both long-term and short-term [38,39,41]. These studies contained data on 231 antithrombin-deficient individuals, of which 97 (42%) had a recurrence [36-45]. Nine-hundred-twenty-nine out of 3863 controls (24%) had a recurrence. The OR was 2.1; 95% CrI, 0.2-4.0 and the probability of the OR being >1 was 88%. In various sub-analyses 95% CrIs of ORs were wide, and the probability of OR being >1 varied between 64-98%, see Table 4. No data on recurrent VTE regarding antithrombin subtypes was found.

**Table 4. Risk of recurrent venous thromboembolism in antithrombin deficiency**

| Analysis | Odds ratio | 95% credible interval | Probability of OR>1 |
|---|---|---|---|
| All studies (n=10) | 2.1 | 0.2-4.0 | 88.4 |
| Retrospective cohort studies (n=2) | 10.4 | 0.0-204.8 | 93.8 |
| Prospective cohort studies (n=8) | 1.5 | 0.1-2.9 | 73.8 |
| High quality studies, NOS≥6.5 (n=5) | 2.4 | 0.3-4.4 | 94.7 |
| Family studies (n=2) | 6.5 | 0.0-184.4 | 88.5 |
| Non-family studies (n=8) | 1.6 | 0.1-3.3 | 74.6 |
| Only objective VTE diagnosis (n=8) | 1.5 | 0.1-2.9 | 74.2 |

Probability of OR>1: the probability that the odds ratio is greater than 1. NOS: Newcastle-Ottawa Scale score. n=number.

Absolute risk estimates of recurrent VTE were made separately for antithrombin-deficient patients with and without long-term anticoagulant therapy. Four studies reported on 93 antithrombin-deficient VTE patients without long-term anticoagulation [37-39,41], of which only one was deemed at low risk for bias [39]. During 740 observation years in 4 studies 65 recurrent VTE events occurred. Median follow-up was reported in three studies and ranged from 4.6-8.7 years. The annual absolute risk of recurrent VTE in antithrombin-deficient patients was 8.8%; 95% CrI, 4.6-14.1. Three studies reported on non-antithrombin-deficient VTE patients (heterozygous FVL carriers in one of three studies) without long-term anticoagulant therapy. In 1096 non-antithrombin-deficient VTE patients, 352 events occurred in 7743 observation years. The annual absolute risk of recurrence was 4.3%; 95% CrI, 1.5-7.9. When comparing the absolute risk of recurrence without long-term anticoagulation between antithrombin-deficient and non-antithrombin-deficient VTE patients, the probability of recurrence risk being higher in antithrombin-deficient patients than in non-antithrombin-deficient patients was 95% (exceedance probability 5%). The study data did not allow for a separate analysis of recurrence risk after a provoked or an unprovoked VTE.

The results of a sensitivity analysis, which included removing the results from studies with sources of bias are shown in table 5. This did not lead to in major changes in the results.

Two studies reported on 63 antithrombin-deficient VTE patients with long-term anticoagulant therapy [39,41]. During 469 observation years 13 recurrent events occurred. The annual absolute risk of recurrent VTE in antithrombin-deficient patients was 2.6%; 95% CrI, 0.0-10.5. Both studies reported recurrence risk in non-antithrombin-deficient VTE patients (heterozygous FVL carriers in one of two studies). Of 277 non-antithrombin-deficient VTE patients on long-term anticoagulation 24 had a recurrent event during 2351 observation years. The annual absolute risk of recurrence was 0.7%; 95% CrI, 0.0-4.3. The exceedance probability of the recurrence risk being higher in antithrombin-deficient patients as compared to non-antithrombin-deficient patients was 16%.

**Table 5. Absolute risk of recurrent venous thromboembolism in antithrombin deficiency.**

| Analysis | Studies (n) | median AR in AT deficiency (%) | 95% CrI of AR in AT deficiency | Studies (n) | median AR control (%) | 95% CrI HPD AR control | Probability of risk being higher in AT deficiency(%) |
|---|---|---|---|---|---|---|---|
| **Results of studies on absolute risk of recurrence without long-term anticoagulation** | | | | | | | |
| All studies (n=4) | 4 | 8.8 | 4.6-14.1 | 3 | 4.3 | 1.5-7.9 | 95 |
| No selection issues (n=3) | 3 | 8.4 | 1.6-16.7 | 3 | 4.3 | 1.5-7.9 | 91 |
| No diagnostic issues (n=3) | 3 | 8.4 | 1.6-16.7 | 3 | 4.3 | 1.5-7.9 | 91 |
| No comparability issues (n=1)* | 1 | 8.0 | 5.4-11.5 | 1 | 4.6 | 3.9-5.3 | NA |
| No follow-up issues (n=3) | 3 | 9.1 | 2.8-18.2 | 2 | 4.1 | 0.0-13.2 | 88 |
| No issues (n=1)* | 1 | 8.0 | 5.4-11.5 | 1 | 4.6 | 3.9-5.3 | NA |
| **Results of studies on absolute risk of recurrence with long-term anticoagulation** | | | | | | | |
| All studies (n=2) | 2 | 2.6 | 0.00-10.5 | 2 | 0.71 | 0.00-4.43 | 84 |

AR: absolute risk. AT: antithrombin. n=number. CrI: credible interval. No issues is a combination of no selection, diagnostic, comparability or follow-up issues.
*= Fisher exact test performed, the risk is higher in antithrombin deficient individuals p<0.05

### Risk of bias across studies/ Sensitivity analyses

The results of assessment of bias risk and the sensitivity analyses are shown in tables 2-5. Information on tests used to determine antithrombin levels revealed that many studies did not report what tests were used, and that most reported tests were factor IIa based tests (table 1). For first VTE OR and absolute risk estimates, the studies that used factor IIa based test, showed results that were not clearly different than the results of all studies (Table 2 and 3). As the largest difference in absolute risk of a first VTE was found between retrospective (1.0%/yr; 95% CrI, 0.6-1.5) and prospective (2.3%/year; 95% CrI, 0.2-6.5) studies, we investigated how these study types, age of participants and age at VTE were related. Figure 2 shows how the annual probability of VTE risk increases in antithrombin deficient individuals by increasing age from approximately 1%/year at the age of 20 to 3% per year at the age of 60.





## DISCUSSION

### Main findings

In this systematic review and Bayesian meta-analysis we observed that in antithrombin-deficient patients the annual incidence of a first VTE is high, varying from approximately 1.0% (95%CrI, 0.6-1.5%) per year in retrospective studies to 2.3% (95%CrI, 0.2-6.5%) per year in prospective studies. The relative risk for antithrombin-deficient individuals compared to population- and family controls to develop VTE is clearly increased. The observation that the prospective studies on first VTE in antithrombin-deficient patients show a higher annual incidence than the retrospective studies may be explained by better capture of events or by an increasing risk of a first VTE with increasing age as shown in figure 2. Increasing VTE risk with increasing age has been shown before in a population study [46].

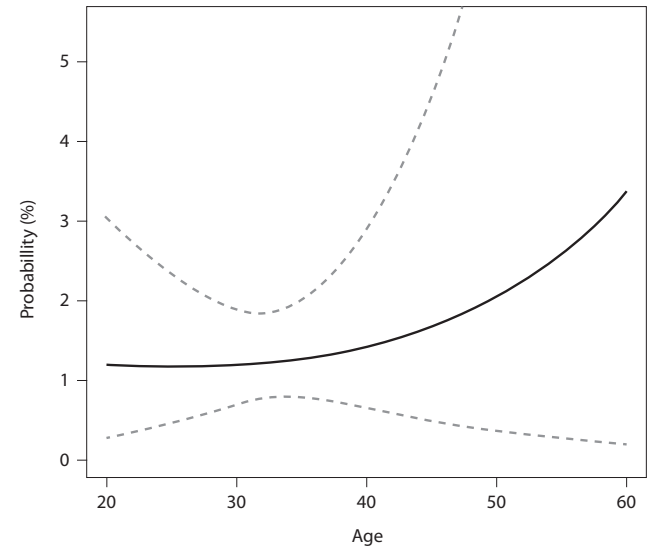



**Figure 2: Increase of the annual probability of a first VTE with age in antithrombin deficient individuals.**
The annual probability of a first VTE according to age. The dotted lines depict the 95% credible interval of these estimates.

For antithrombin-deficient VTE patients, the risk of recurrent VTE was increased compared to non-antithrombin deficient VTE patients (OR 2.1; probability of OR being >1, 88.4%) in 10 studies encompassing 231 antithrombin-deficient patients, and 3863 non-antithrombin-deficient controls. In antithrombin-deficient VTE patients the absolute risk estimates of recurrence are considerably higher than in non-antithrombin-deficient individuals. Without anticoagulant therapy the annual recurrence risk was higher in antithrombin-deficient VTE patients than in non-antithrombin-deficient VTE patients, 8.8% vs 4.3%, with a probability of recurrence risk being higher in antithrombin deficient patients of 95%. Although bias in

studies may have influenced outcomes (one study lacked a control group and in one study controls had a milder inherited thrombophilia, heterozygous factor V Leiden), sensitivity analysis showed no major differences between these studies. In antithrombin-deficient VTE patients on anticoagulant therapy the annual risk of recurrence was 2.6%, versus 0.7% in non-antithrombin-deficient VTE patients. Based on limited evidence (2 studies, 13 events in 470 observation years in antithrombin deficient individuals) it seems that even with anticoagulant therapy (vitamin K antagonists in these studies) antithrombin deficient individuals have a higher recurrence risk.

Only three studies showed data on subtype[3,4,13]. Intriguingly, they show a higher risk of a first VTE in type I patients as compared to type II patients (OR 9.3). Because many of these observations are in probands, conferring a high risk of selection bias, we separately analyzed only the family-members from two of those studies, showing a more modestly increased of risk in type I patients (OR 2.6). As for the separate subtypes, type IIHBS may confer a lower risk as compared to type I antithrombin deficiency, while the other type II subtypes are similar to type I. As these risk estimates are influenced by proband selection it is not feasible to estimate reliably by how much the VTE risk is lower in type IIHBS antithrombin deficient individuals.

**Strengths**

Our systematic review and meta-analysis is by far the most complete reported to date. As compared to a recent review by Di Minno et al [8], this review encompasses more studies on first (19 vs. 13 studies for ORs) and on recurrent VTE (10 vs. 4 studies) despite more restrictive inclusion criteria such as the requirement for objective VTE diagnosis or treatment. Three studies in the previous review were not used in this systematic review. Two studies reporting on first VTE were excluded because they actually contained many patients with recurrences [47,48], and one study failed our criteria for a VTE diagnosis [49]. The OR-estimate of first VTE (14.0) compares quite well to the OR of 16.3 found in the previous review [8]. The recurrence OR estimates of 2.1 is more conservative than the 3.6 found by the previous review,[8] and the Bayesian meta-analysis shows far wider CrIs.

The use of Bayesian random-effects meta-analysis allowed us to perform absolute risk analyses. The inferences provided by Bayesian meta-analysis are conditional on the data and provides CrIs that are straightforward in terms of interpretation: the 95% CrIs for a risk is that region of the risk is believed to be with a probability of 95% [50].This is the first meta-analysis of absolute risk in antithrombin deficiency.

**Limitations**

Most of the studies included in this review and meta-analysis come from dedicated clinics performing VTE research. This may introduce referral bias, which may lead to overestimation of VTE risk. Furthermore, in many studies the investigated patients were family members of probands. This point is important as the risk of VTE in family members of a symptomatic proband is estimated to be 12-fold higher than in family members of an asymptomatic proband [51]. However, the absolute risk of first VTE in family studies was only slightly higher than the risk in non-family studies in our meta-analyses, 1.3% vs. 1.0%.

In several of the analyses the credible intervals are wide. In the OR-estimates of first and recurrent VTE, this reflects heterogeneity through study type and bias issues, see table 2 and 4. Removing biased studies in the OR-estimates of first VTE resulted in narrower credible intervals. In the subtype analysis and in the absolute risk of recurrence analyses wide credible intervals are the result of limited data.

**Relevance for daily practice**

The risk of a first VTE is clearly increased in antithrombin deficient individuals. After removal of probands from the analyses the OR of type I versus type II overall is estimated at 2.6 (95% CrI, 0.0-19.5). Considering the 14-fold increased risk in antithrombin deficient individuals as compared to controls, in type II antithrombin deficient individuals VTE risk is still expected to be considerably increased, although risk may vary among subtypes. Therefore, all antithrombin deficient individuals should be considered to be at a high risk for VTE. The high thrombosis risk should be considered when managing antithrombin deficient individuals in high-risk situations such as pregnancy [5].

Current ACCP guidelines recommend a treatment duration of 3 months in provoked VTE. In unprovoked VTE, after 3 months of treatment, it is recommended to weigh the risk of recurrent VTE against the bleeding risk of prolonged anticoagulation, not mentioning thrombophilia such as antithrombin deficiency [52]. However, the ESC and the National Institute for Health and Clinical Excellence (NICE) guidelines do take this into account [6,53]. In as systematic review, the annual recurrence risks two years after the initial events were calculated. For provoked VTE the annualized recurrence rates were estimated to be 0.7% after surgery and 4.2% after a non-surgical transient risk factor. For unprovoked VTE the annualized recurrence rate two years after the initial event was estimated to be 7.4% [54].These risk estimates are all lower than the annual recurrence risk without long-term anticoagulation of 8.8% in antithrombin deficient VTE patients.

Antithrombin deficiency should therefore be regarded as a thrombophilia that confers a high risk of recurrence, which should certainly be taken into account when deciding upon duration of anticoagulant therapy. Based on the height of the absolute risk of recurrence without long-term anticoagulant therapy of 8.8%, the probability of the risk being higher than in non-deficient populations of 95%, and given that in antithrombin deficient individuals it is not known by how much recurrence risk changes if the first VTE was provoked or not, we advise to treat any VTE patient with antithrombin deficiency with anticoagulant drugs indefinitely unless bleeding risk is very high. Even so, the annual recurrence risk seems still to be quite high on long-term anticoagulant therapy (2.7%). During anticoagulant therapy,

2 of the recurrence studies calculated major bleeding risk in the combined groups of patients with antithrombin, protein C or protein S deficiency. Annual major bleeding risk varied from 0.5% to 0.8%, which is lower than the 2-3 percent in unselected patients with VTE during treatment with vitamin K antagonists [34,37]. Thrombophilic defects may protect against major bleeding, or the younger age of thrombophilic patients at their first thrombotic event may result in a lower bleeding risk [37]. In one recurrence study in antithrombin deficient patients annual overall bleeding risk during anticoagulant therapy in all subjects was reported to be 1.27%, the risk being higher in subjects on long-term anticoagulant therapy [39]. Considering these data, recurrence risk in antithrombin deficiency seems higher than bleeding risk during anticoagulant therapy. As all the data on anticoagulant therapy in antithrombin deficiency are on vitamin K antagonists, the role of direct oral anticoagulants in this defect is currently unknown.

To our knowledge this is the most comprehensive review on VTE risk antithrombin deficiency, not only providing estimates of relative risks, but also on absolute risks of antithrombin deficiency. This will help clinicians to guide prophylactic and therapeutic management of antithrombin-deficient individuals. The data clearly show high absolute risks of a first VTE, and of recurrent VTE. Our meta-analyses indicate that antithrombin-deficient patients with VTE should be treated with long-term anticoagulation.

Authorship details

F. Nanne Croles designed the study protocol, performed the search, performed the data abstraction and statistics, and wrote the manuscript. Jaime Borjas-Howard performed the search, performed the data abstraction and critically revised the manuscript. Kazem Nasserinejad designed and performed the statistics. Frank W. G. Leebeek and Karina Meijer designed the study protocol and critically revised the manuscript.

**Acknowledgements**

The authors thank Wichor Bramer from the Medical Library of Erasmus MC for his help in designing the search strategies of this review.

**Financial Disclosures**

F.N.C., J.B.-H. and K.N. have no financial disclosures. K.M. partly used an unrestricted research grant from Bayer to finance this work. F.W.L. is a consultant for Shire, UniQure and NovoNordisk and has received an unrestricted research grant from CSL Behring and Shire; all outside the submitted work.





## REFERENCES

1. Egeberg O. Thrombophilia Caused by Inheritable Deficiency of Blood Antithrombin. *Scand J Clin Lab Invest*. 1965;17:92.
2. Lane DA, Kunz G, Olds RJ, Thein SL. Molecular genetics of antithrombin deficiency. *Blood Rev*. 1996;10(2):59-74.
3. Mitsuguro M, Sakata T, Okamoto A, et al. Usefulness of antithrombin deficiency phenotypes for risk assessment of venous thromboembolism: type I deficiency as a strong risk factor for venous thromboembolism. *Int J Hematol*. 2010;92(3):468-473.
4. Luxembourg B, Pavlova A, Geisen C, et al. Impact of the type of SERPINC1 mutation and subtype of antithrombin deficiency on the thrombotic phenotype in hereditary antithrombin deficiency. *Thromb Haemost*. 2014;111(2):249-257.
5. Croles FN, Nasserinejad K, Duvekot JJ, Kruip MJ, Meijer K, Leebeek FW. Pregnancy, thrombophilia, and the risk of a first venous thrombosis: systematic review and bayesian meta-analysis. *BMJ*. 2017;359:j4452.
6. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J*. 2014;35(43):3033-3069, 3069a-3069k.
7. Guyatt GH, Akl EA, Crowther M, et al. Executive summary: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):7S-47S.
8. Di Minno MND, Ambrosino P, Ageno W, Rosendaal F, Di Minno G, Dentali F. Natural anticoagulants deficiency and the risk of venous thromboembolism: A meta-analysis of observational studies. *Thromb Res*. 2015;135(5):923-932.
9. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp Accessed October 5th 2015. .
10. Smith TC, Spiegelhalter DJ, Thomas A. Bayesian approaches to random-effects meta-analysis: a comparative study. *Stat Med*. 1995;14(24):2685-2699.
11. R_Core_Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Accessed July 1st 2016.
12. Plummer M. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. *Proceedings of the 3rd international workshop on distributed statistical computing*. 2003;124.
13. Alhenc-Gelas M, Plu-Bureau G, Hugon-Rodin J, Picard V, Horellou MH, Thrombophilia GsgoG. Thrombotic risk according to SERPINC1 genotype in a large cohort of subjects with antithrombin inherited deficiency. 2017.
14. Brouwer JL, Veeger NJ, Kluin-Nelemans HC, van der Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. *Ann Intern Med*. 2006;145(11):807-815.
15. Bucciarelli P, Passamonti SM, Biguzzi E, et al. Low borderline plasma levels of antithrombin, protein C and protein S are risk factors for venous thromboembolism. *J Thromb Haemost*. 2012;10(9):1783-1791.
16. Bucciarelli P, Rosendaal FR, Tripodi A, et al. Risk of venous thromboembolism and clinical manifestations in carriers of antithrombin, protein C, protein S deficiency, or activated protein C resistance: a multicenter collaborative family study. *Arterioscler Thromb Vasc Biol*. 1999;19(4):1026-1033.
17. Chougule A, Rajpal S, Ahluwalia J, et al. Coagulation factor VIII, IX and XI levels in north Indian patients with venous thromboembolism: First study from India. *Blood Coagul Fibrinolysis*. 2016;27(1):58-63.
18. Cogo A, Lensing AWA, Prandoni P, et al. Relevance of inherited risk factors in young patients with deep-vein thrombosis. *Clin Appl Thromb-Hemost*. 1996;2(1):55-59.

19. Cohen W, Castelli C, Alessi MC, et al. ABO blood group and von Willebrand factor levels partially explained the incomplete penetrance of congenital thrombophilia. *Arterioscler Thromb Vasc Biol*. 2012;32(8):2021-2028.
20. De Stefano V, Leone G, Mastrangelo S, et al. Clinical manifestations and management of inherited thrombophilia: retrospective analysis and follow-up after diagnosis of 238 patients with congenital deficiency of antithrombin III, protein C, protein S. *Thromb Haemost*. 1994;72(3):352-358.
21. Di Minno MN, Dentali F, Veglia F, Russolillo A, Tremoli E, Ageno W. Antithrombin levels and the risk of a first episode of venous thromboembolism: a case-control study. *Thromb Haemost*. 2013;109(1):167-169.
22. Folsom AR, Aleksic N, Wang L, Cushman M, Wu KK, White RH. Protein C, antithrombin, and venous thromboembolism incidence: a prospective population-based study. *Arterioscler Thromb Vasc Biol*. 2002;22(6):1018-1022.
23. Koster T, Rosendaal FR, Briet E, et al. Protein C deficiency in a controlled series of unselected outpatients: an infrequent but clear risk factor for venous thrombosis (Leiden Thrombophilia Study). *Blood*. 1995;85(10):2756-2761.
24. Mahmoodi BK, Brouwer JL, Ten Kate MK, et al. A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. *J Thromb Haemost*. 2010;8(6):1193-1200.
25. Martinelli I, Mannucci PM, De Stefano V, et al. Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. *Blood*. 1998;92(7):2353-2358.
26. Mateo J, Oliver A, Borrell M, Sala N, Fontcuberta J. Increased risk of venous thrombosis in carriers of natural anticoagulant deficiencies. Results of the family studies of the Spanish Multicenter Study on Thrombophilia (EMET study). *Blood Coagul Fibrinolysis*. 1998;9(1):71-78.
27. Okumus G, Kiyan E, Arseven O, et al. Hereditary thrombophilic risk factors and venous thromboembolism in Istanbul, Turkey: the role in different clinical manifestations of venous thromboembolism. *Clin Appl Thromb Hemost*. 2008;14(2):168-173.
28. Rossi E, Ciminello A, Za T, Betti S, Leone G, De Stefano V. In families with inherited thrombophilia the risk of venous thromboembolism is dependent on the clinical phenotype of the proband. *Thromb Haemost*. 2011;106(4):646-654.
29. Sakata T, Okamoto A, Mannami T, Matsuo H, Miyata T. Protein C and antithrombin deficiency are important risk factors for deep vein thrombosis in Japanese. *J Thromb Haemost*. 2004;2(3):528-530.
30. Sanson BJ, Simioni P, Tormene D, et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. *Blood*. 1999;94(11):3702-3706.
31. Shen MC, Lin JS, Tsay W. Protein C and protein S deficiencies are the most important risk factors associated with thrombosis in Chinese venous thrombophilic patients in Taiwan. *Thromb Res*. 2000;99(5):447-452.
32. Suchon P, Al Frouh F, Henneuse A, et al. Risk factors for venous thromboembolism in women under combined oral contraceptive: The PILL genetic RIsk monitoring (PILGRIM) study. *Thromb Haemost*. 2016;115(1):135-142.
33. Tormene D, Fortuna S, Tognin G, et al. The incidence of venous thromboembolism in carriers of antithrombin, protein C or protein S deficiency associated with the HR2 haplotype of factor V: a family cohort study. *J Thromb Haemost*. 2005;3(7):1414-1420.
34. Vossen CY, Conard J, Fontcuberta J, et al. Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). *J Thromb Haemost*. 2005;3(3):459-464.
35. Vossen CY, Conard J, Fontcuberta J, et al. Familial thrombophilia and lifetime risk of venous thrombosis. *J Thromb Haemost*. 2004;2(9):1526-1532.







36. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. *Lancet*. 2003;362(9383):523-526.
37. Brouwer JL, Lijfering WM, Ten Kate MK, Kluin-Nelemans HC, Veeger NJ, van der Meer J. High long-term absolute risk of recurrent venous thromboembolism in patients with hereditary deficiencies of protein S, protein C or antithrombin. *Thromb Haemost*. 2009;101(1):93-99.
38. De Stefano V, Simioni P, Rossi E, et al. The risk of recurrent venous thromboembolism in patients with inherited deficiency of natural anticoagulants antithrombin, protein C and protein S. *Haematologica*. 2006;91(5):695-698.
39. Di Minno MN, Dentali F, Lupoli R, Ageno W. Mild antithrombin deficiency and risk of recurrent venous thromboembolism: a prospective cohort study. *Circulation*. 2014;129(4):497-503.
40. Kearon C, Julian JA, Kovacs MJ, et al. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: results from a randomized trial. *Blood*. 2008;112(12):4432-4436.
41. Vossen CY, Walker ID, Svensson P, et al. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. *Arterioscler Thromb Vasc Biol*. 2005;25(9):1992-1997.
42. Weingarz L, Schindewolf M, Schwonberg J, et al. Thrombophilia and risk of VTE recurrence according to the age at the time of first VTE manifestation. *Vasa Euro J Vasc Med*. 2015;44(4):313-323.
43. Prandoni P, Noventa F, Ghirarduzzi A, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. *Haematologica*. 2007;92(2):199-205.
44. Taliani MR, Becattini C, Agnelli G, et al. Duration of anticoagulant treatment and recurrence of venous thromboembolism in patients with and without thrombophilic abnormalities. *Thromb Haemost*. 2009;101(3):596-598.
45. Santamaria MG, Agnelli G, Taliani MR, et al. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. *Thromb Res*. 2005;116(4):301-306.
46. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost*. 2007;5(4):692-699.
47. Mishra MN, Bedi VS. Prevalence of common thrombophilia markers and risk factors in Indian patients with primary venous thrombosis. *Sao Paulo Med J*. 2010;128(5):263-267.
48. Heijboer H, Brandjes DP, Buller HR, Sturk A, ten Cate JW. Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis. *N Engl J Med*. 1990;323(22):1512-1516.
49. Bombeli T, Basic A, Fehr J. Prevalence of hereditary thrombophilia in patients with thrombosis in different venous systems. *Am J Hematol*. 2002;70(2):126-132.
50. Higgins J, Green S, (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. *The Cochrane Collaboration*. 2011.
51. Rossi E, Za T, Ciminello A, Leone G, De Stefano V. The risk of symptomatic pulmonary embolism due to proximal deep venous thrombosis differs in patients with different types of inherited thrombophilia. *Thromb Haemost*. 2008;99(6):1030-1034.
52. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. *Chest*. 2016;149(2):315-352.
53. Howard LS, Hughes RJ. NICE guideline: management of venous thromboembolic diseases and role of thrombophilia testing. *Thorax*. 2013;68(4):391-393.
54. Iorio A, Kearon C, Filippucci E, et al. Risk of recurrence after a first episode of symptomatic venous thromboembolism provoked by a transient risk factor: a systematic review. *Arch Intern Med*. 2010;170(19):1710-1716.

## SUPPLEMENTAL DATA

**Search yields, before and after removing duplicates**

| | | |
|---|---|---|
| Embase.com | 2720 | 2688 |
| Medline Ovid | 1900 | 368 |
| Web of science | 2093 | 873 |
| Cochrane | 124 | 43 |
| Google scholar | 268 | 141 |
| **Total** | **7105** | **4113** |

## SEARCH STRATEGY

### Embase.com

('antithrombin deficiency'/exp OR 'antithrombin III deficiency'/exp OR ((antithrombin/de OR 'antithrombin III'/de OR 'thrombophilia'/de) AND ('risk factor'/de OR 'disease predisposition'/de OR 'genetic predisposition'/exp OR 'recurrence risk'/de OR 'genetic risk'/de OR inheritance/de OR heredity/de OR 'risk assessment'/de )) OR (((thrombophil* OR antithrombin* OR anti-thrombin*) NEAR/6 (heredit* OR inherit* OR genetic* OR mutat* OR familial* OR deficien* OR deficit* OR decrease*)) OR ((antithrombin* OR thrombophili*) AND ('risk factor*' OR predispos* OR susceptib*)) OR SERPINC1):ab,ti) AND ('venous thromboembolism'/exp OR 'vein thrombosis'/de OR (thromboembolism/de AND 'peripheral vascular system'/exp) OR (((venous OR vein) NEAR/3 (thromboembol* OR thrombo-embol* OR thrombosis OR thrombotic)) OR ((lung OR pulmonar*) NEAR/3 embolism*)):ab,ti) NOT ((child/exp OR adolescent/exp ) NOT adult/exp) AND ('observational study'/exp OR 'cohort analysis'/exp OR 'longitudinal study'/exp OR 'retrospective study'/exp OR 'prospective study'/exp OR 'case control study'/de OR 'major clinical study'/de OR 'follow up'/de OR 'clinical study'/de OR 'clinical article'/de OR 'clinical trial'/exp OR 'randomization'/exp OR 'intervention study'/de OR 'open study'/de OR 'community trial'/de OR (((observation* OR famil* OR comparativ* OR communit*) NEAR/6 (stud* OR data OR research)) OR cohort* OR longitudinal* OR retrospectiv* OR prospectiv* OR population* OR ((case OR cases OR match*) NEAR/3 control*) OR correlation* OR 'follow up' OR followup* OR clinical* OR trial OR random*):ab,ti) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim) AND [english]/lim

### Medline Ovid

("Antithrombin III Deficiency"/ OR Antithrombins/df OR ((antithrombins/ OR "Antithrombin III"/ OR "Thrombophilia"/) AND ("Risk Factors"/ OR exp "Disease Susceptibility"/ OR heredity/ OR "risk assessment"/ )) OR (((thrombophil* OR antithrombin* OR anti-thrombin*) ADJ6 (heredit* OR inherit* OR genetic* OR mutat* OR familial* OR deficien* OR deficit* OR decrease*)) OR ((antithrombin* OR thrombophili*) AND ("risk factor*" OR predispos* OR susceptib*)) OR SERPINC1).ab,ti.) AND ("venous thromboembolism"/ OR "Venous Thrombosis"/ OR (((venous OR vein) ADJ3 (thromboembol* OR thrombo-embol* OR thrombosis OR thrombotic)) OR ((lung OR pulmonar*) ADJ3 embolism*)).ab,ti.) NOT ((child/ OR adolescent/ ) NOT adult/) AND ("observational study"/ OR exp "Cohort Studies"/ OR "Case-Control Studies"/ OR exp "clinical study"/ OR "Random Allocation"/ OR (((observation* OR famil* OR comparativ* OR communit*) ADJ6 (stud* OR data OR research)) OR cohort* OR longitudinal* OR retrospectiv* OR prospectiv* OR population* OR ((case OR cases OR match*) ADJ3 control*) OR correlation* OR "follow up" OR followup* OR clinical* OR trial OR random*).ab,ti.) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt. AND english.la.

### Cochrane

((((thrombophil* OR antithrombin* OR anti-thrombin*) NEAR/6 (heredit* OR inherit* OR genetic* OR mutat* OR familial* OR deficien* OR deficit* OR decrease*)) OR ((antithrombin* OR thrombophili*) AND ('risk factor*' OR predispos* OR susceptib*)) OR SERPINC1):ab,ti) AND ((((venous OR vein) NEAR/3 (thromboembol* OR thrombo-embol* OR thrombosis OR thrombotic)) OR ((lung OR pulmonar*) NEAR/3 embolism*)):ab,ti)

### Web of science

TS=(((((thrombophil* OR antithrombin* OR anti-thrombin*) NEAR/5 (heredit* OR inherit* OR genetic* OR mutat* OR familial* OR deficien* OR deficit* OR decrease*)) OR ((antithrombin* OR thrombophili*) AND ("risk factor*" OR predispos* OR susceptib*)) OR SERPINC1)) AND ((((venous OR vein) NEAR/2 (thromboembol* OR thrombo-embol* OR thrombosis OR thrombotic)) OR ((lung OR pulmonar*) NEAR/2 embolism*))) AND ((((observation* OR famil* OR comparativ* OR communit*) NEAR/5 (stud* OR data OR research)) OR cohort* OR longitudinal* OR retrospectiv* OR prospectiv* OR population* OR ((case OR cases OR match*) NEAR/2 control*) OR correlation* OR "follow up" OR followup* OR clinical* OR trial OR random*)) NOT ((child* OR infan* OR adolescen*) NOT (adult*))) AND DT=(article) AND LA=(english)

**Google scholar**
First 200:

“Antithrombin deficiency|deficit” “risk factor”|predisposition|susceptibility “venous|vein thromboembolism|thrombosis”|”lung|pulmonary embolism”

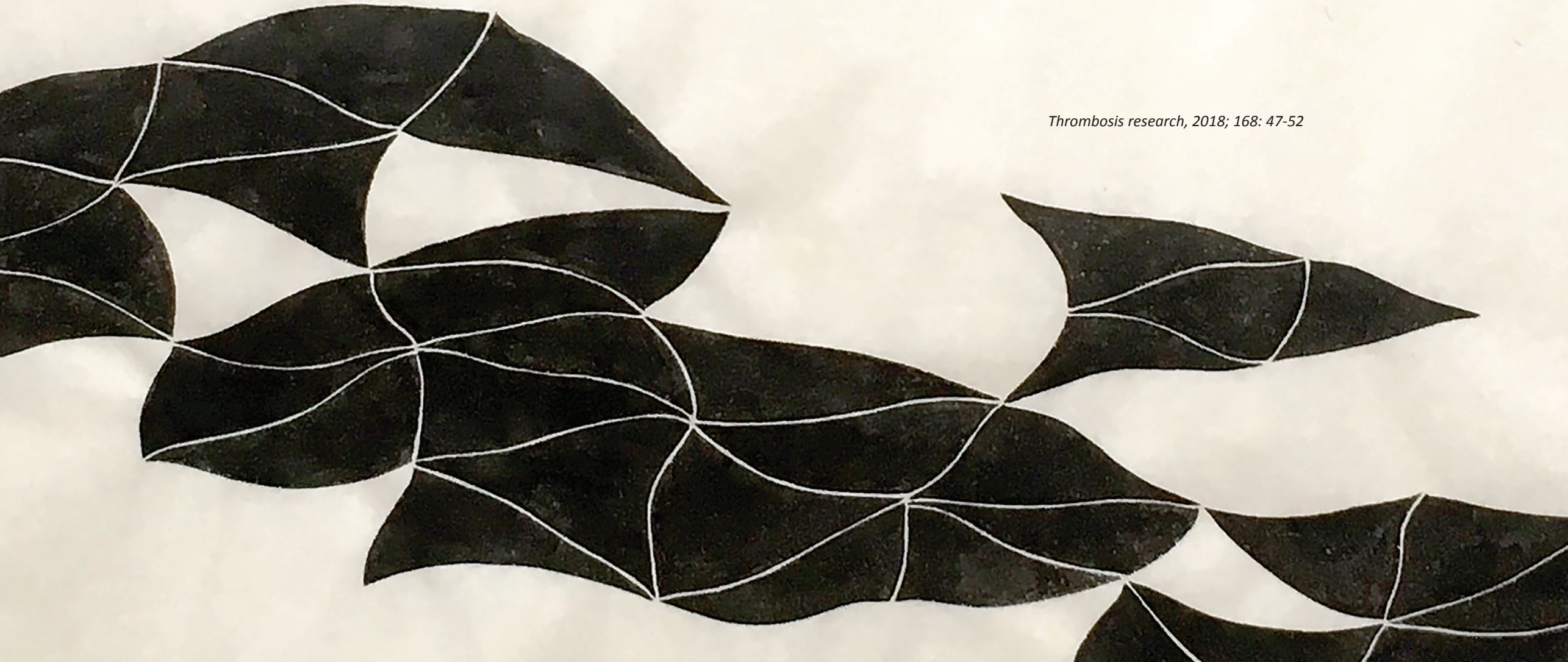
allintitle:”Antithrombin deficiency|deficit” “venous|vein thromboembolism|thrombosis”|”lung|pulmonary embolism”

3

# B-ANTITHROMBIN, SUBTYPE OF ANTI-THROMBIN DEFICIENCY AND THE RISK OF VENOUS THROMBOEMBOLISM IN HEREDITARY ANTITHROMBIN DEFICIENCY: A FAMILY COHORT STUDY.

F. N. Croles, R. Mulder, A.B. Mulder, M.V. Lukens, Karina Meijer

*Thrombosis research, 2018; 168: 47-52*



## ABSTRACT

### Introduction

Hereditary antithrombin deficiency is associated with a high incidence of venous thromboembolism (VTE) , but VTE risk differs between families. Beta-antithrombin is reported to be the most active isoform of antithrombin *in vivo*. Whether β-antithrombin activity and subtypes in antithrombin deficiency have impact on VTE risk has not been investigated outside the proband setting.

### Methods

We performed a retrospective family cohort study to investigate whether subtypes of antithrombin deficiency or β-antithrombin levels are associated with the risk of first or recurrent VTE.

### Results

Eighty-one subjects from 21 families were included, of which 52 were antithrombin deficient. Beta-antithrombin levels were decreased in most type I and type IIPE subjects, but normal levels were found in all subtypes of antithrombin deficiency.

The annual incidence of VTE in antithrombin-deficient family members was 1.24%, 95%CI:0.72-1.99%, in low β-antithrombin 1.36% (95%CI:0.76-2.25%) and in normal β-antithrombin 0.79% (95%CI:0.10-2.77). The annual incidence of recurrence in family members was 3.1% (95%CI 0.9-7.1%). Duration of anticoagulation had an impact on recurrence risk: In family members annual recurrence with fixed duration was 10% (95%CI:2.1-29.2%), with indefinite duration 1.5% (95%CI:0.2-5.4%), p<0.05. Beta-antithrombin levels were not associated with the risk for first or recurrent VTE in antithrombin deficient subjects.

### Conclusions

In this high-risk antithrombin-deficient population, both subjects with low and normal plasma β-antithrombin activity had high risks of first and recurrent VTE. This puts the importance of β-antithrombin into question. Long-term anticoagulation is warranted in antithrombin-deficient VTE patients.






## INTRODUCTION

Antithrombin deficiency (OMIM 613118) is an inherited autosomal dominant defect, characterized by a high incidence of venous thromboembolism (VTE). Antithrombin inhibits blood coagulation mainly through inactivation of thrombin and factor Xa [1] . Hereditary antithrombin deficiency is usually caused by heterozygous mutations. Homozygous antithrombin deficiency is generally lethal in utero, although rare homozygous and compound heterozygous patients have been described [2-4]. A subdivision is made in type I deficiency (quantitative defect) and type II deficiency (qualitative defects). Type II antithrombin deficiencies are subdivided in type IIRS (reactive site), type IIHBS (heparin binding site) and type IIPE (pleiotropic effects) [5].

Antithrombin deficiency is rare. The prevalence in healthy blood donors was found to be between 1:500 and 1:645 [6-8]. In patients with VTE the prevalence is 1-5% [9-12]. In a recent systematic review a 14-fold increased risk for VTE was found in antithrombin-deficient individuals as compared to controls (OR: 14.0, 95% credible interval (CrI): 5.5-29.0) [13]. Type II antithrombin deficiency is more common in healthy donors [6-8], while type I seems more common in patients [14-16]. In two studies type IIHBS antithrombin deficiency confers a lower VTE risk [14,15]. Di Minno et al showed in a case-control study that not only antithrombin deficiency, but also lower levels of antithrombin activity within the normal range are associated with a higher risk of first [17] and recurrent VTE [9,18]. These data suggest that hereditary antithrombin deficiency is a condition that consists of a risk-spectrum for VTE defined by the levels of antithrombin activity, the type of deficiency, and (considering the healthy blood donor data versus family study data) family history of VTE.

Antithrombin is activated by heparin-like substances such as glycosaminoglycans on the vessel wall, or heparins [19]. Antithrombin consists of two isoforms, 90% of circulating antithrombin being α-antithrombin and 10% is β-antithrombin. The N-glycan at Asn135 is not present in β-antithrombin. Beta-antithrombin has a higher affinity for heparin [20,21], and binds better to the endothelium [22] and may therefore serve as the major inhibitor of coagulation *in vivo*. Specific antithrombin mutations have been described that mainly affect α- but not β-antithrombin, thereby retaining enough antithrombin activity to allow homozygous mutants to survive [4]. Considering this, β-antithrombin may also be an important modulator of VTE risk in antithrombin-deficient individuals, but this has not previously been investigated.

We performed a retrospective analysis of antithrombin-deficient families and investigated the relation between the clinical phenotype, subtype of antithrombin deficiency and β-antithrombin.

## METHODS

### Patients

We performed a cross-sectional cohort study in an academic thrombosis center. Eligible subjects were patients with previously diagnosed hereditary antithrombin deficiency, their first-degree relatives, and second- degree relatives if the first degree-relative was antithrombin deficient. Through pedigree analysis we identified probands and relatives. Probands were those participants who had been the first to be diagnosed with antithrombin deficiency in their family. Patients (≥18 years) were enrolled after written informed consent, obtained during an outpatient clinic visit, between January 2013 and January 2015. Detailed information was collected on previous VTE episodes, exposure to other risk factors, obstetric history and use of anticoagulant drugs. A modified standardized questionnaire[23] was used, and medical records were reviewed. The participants largely stem from referrals to our tertiary center for thrombophilia screening because of venous thrombotic events. A number of subjects (7 probands, 35 relatives) were also included in a previous prospective cohort study, of which follow-up had ended between September 2005 and December 2007 [24,25], and were asked to enter this new study through a new informed consent procedure.

Our policy is to discourage antithrombin-deficient individuals from using oral contraceptives or hormonal replacement therapy, and we encourage them to use thrombosis prophylaxis in high risk situations, such as surgery, immobilizations, pregnancy and puerperium. Many antithrombin-deficient patients with VTE were encouraged to use oral anticoagulants indefinitely.

This study was approved by the institutional Medical Ethics Committee of the University Medical Center Groningen (ABR nr. NL41324).

### Definitions

Only symptomatic VTE was evaluated. The first episode of symptomatic VTE was considered established if proximal deep-vein thrombosis (DVT) was confirmed by compression ultrasound or venography, and pulmonary embolism (PE) by ventilation perfusion lung scanning or CT-scanning of the lung or pulmonary angiography, or when the patient had received full dose heparin and a VKA for at least three months without objective testing when these techniques were not yet available. In DVT patients, PE was only investigated when symptoms were suggestive of PE. VTE at unusual sites was considered established if confirmed by objective means. Isolated calf-vein thrombosis and superficial thrombophlebitis were not classified as a thrombotic event. Recurrence of symptomatic VTE was considered proven if it was demonstrated by objective techniques at another site than the first event, or at the same site if a previously repeated test showed no residual thrombus at the original site. If recurrence at the same site was suspected, but objective tests were inconclusive, it was diagnosed when the patient revealed pronounced signs and symptoms of recurrence without preceding post-thrombotic syndrome, or when pulmonary embolism was objectively demonstrated. VTE was considered provoked, if it had occurred at or within three months after exposure to one or more exogenous risk factors, including major surgery, trauma, immobilization for more than 7 days, oral contraceptives, hormone replacement therapy pregnancy and malignant disease. If these factors were absent, VTE was considered unprovoked.

### Blood collection

Blood samples were collected once at the outpatient clinic. Venous blood samples were anticoagulated with a 1:10 volume of 0.109 M trisodium citrate. Platelet-poor plasma was prepared by centrifugation at 2500 x g for 15 minutes. Next, samples were aliquoted and immediately frozen at -80 °C and analyzed after rapid thawing at 37 °C. Genomic DNA was obtained from EDTA samples using the Qiacube system.

### Laboratory studies

Patients were tested for deficiencies of all natural anticoagulants (antithrombin, protein C, and protein S), factor V Leiden mutation and prothrombin G20210A mutation. Antithrombin activity measurements were performed with an assay based on factor Xa inhibition (INNOVANCE® Antithrombin assay, Siemens, Marburg, Germany), normal levels >80%. Antithrombin antigen levels were measured with an enzyme-linked immunosorbent assay (ELISA) with reagents obtained from Affinity Biologicals (Ancaster, Ontario, Canada), normal levels >80 %. For the heparin-antithrombin binding (HAB) ratio the assay of Moore et al was adopted [26]. This assay quantifies heparin-binding capacity of antithrombin by deriving a ratio of antithrombin activities generated from short (30s) and prolonged incubation times (290s) with heparin. A reduced HAB ratio (<0.8) distinguishes type II HBS antithrombin deficiency from the other subtypes. The subdivision of antithrombin deficiency was made according to the classification by Lane[5].

To quantify β-antithrombin we used a previously published protocol that was adapted by V. Karlaftis[27]. Based on previously published data from this method, and verified in our controls, normal levels were defined as >80 %[27,28]. Briefly, the UFH binding antithrombin activity of patient plasma was measured at 405nm on the Varioskan LUX with Skanit Software 4.1 using reagents from Stachrom ATIII kit (Diagnostica Stago, USA). The protocol works on the premise of plasma samples being incubated with a known excess of thrombin and the residual thrombin being measured by its amidolytic action on the supplied chromogenic substrate. Furthermore, the protocol is designed to result in a final high salt concentration (1.1 M NaCl) allowing for specific activation of UFH binding AT only [28].

Analysis of causative mutations in the SERPINC1gene coding for antithrombin was performed as described elsewhere [29]. Mutalyzer 2.0.22 was used to check the descriptions of each SERPINC1 sequence variant according to the standard Human Genome Sequence Variation Society (HGVS) nomenclature [http://www.hgvs.org/mutnomen) of the UK Association for Clinical Genetic Science and the American College for Medical Genetics [30,31]. Reference sequences used were NP_000479.1, NM_000488.3 (cDNA) and NG_012462.1 (genomic).

In many subjects protein C and protein S plasma level, factor V Leiden mutation and prothrombin G20210A mutation testing had been performed previously [24,25]. We only tested subjects in whom these tests had not previously been done. Protein C and protein S antigen levels were measured by ELISA (ASSERACHROM®PROTEIN C and ASSERACHROM® TOTAL PROTEIN S, Diagnostica Roche, France). Protein C activity was tested by a chromogenic substrate assay (Berichrom® Protein C, Siemens, Marburg, Germany). Free protein S testing was performed with an assay based on latex beads containing C4BP with reagents (HemosIL™) obtained from Instrumentation Laboratory, Milano, Italy. Plasma level of protein C and protein S were decreased at levels below < 65 IU/dL. Genetic analysis of factor V Leiden mutation and prothrombin G20210A mutation was performed by real-time PCR.

### Statistical analysis

Only relatives (not probands) were analyzed to estimate the risk of a first VTE, as proband selection may inflate risk estimates through selection by outcome. Annual incidences of a first VTE were calculated by dividing the number of events by the number of observation years. For first VTE analyses, observation started at birth and ended on the day of enrollment or on the date of the first VTE, whichever happened first. For recurrent VTE observation started at the date of the first VTE and ended on the date of the consecutive recurrent VTE or the day of enrollment, whichever happened first. Confidence intervals for annual incidences were calculated using the Fisher's exact test.

Annual recurrence rates were calculated by dividing the number of relatives with a second episode of VTE by the total number of observation years. Observation time was defined as the period from the first episode of VTE until the onset of the second episode or the end of follow-up. Recurrence free survival was estimated by the use of the Kaplan-Meier method. To investigate if antithrombin and β-antithrombin were associated with VTE free survival, a Cox regression model adjusted for age at enrollment and sex was used.

Continuous variables were expressed as median values and ranges and categorical data as counts or percentages.

All analysis were performed using IBM SPSS Statistics version 22.



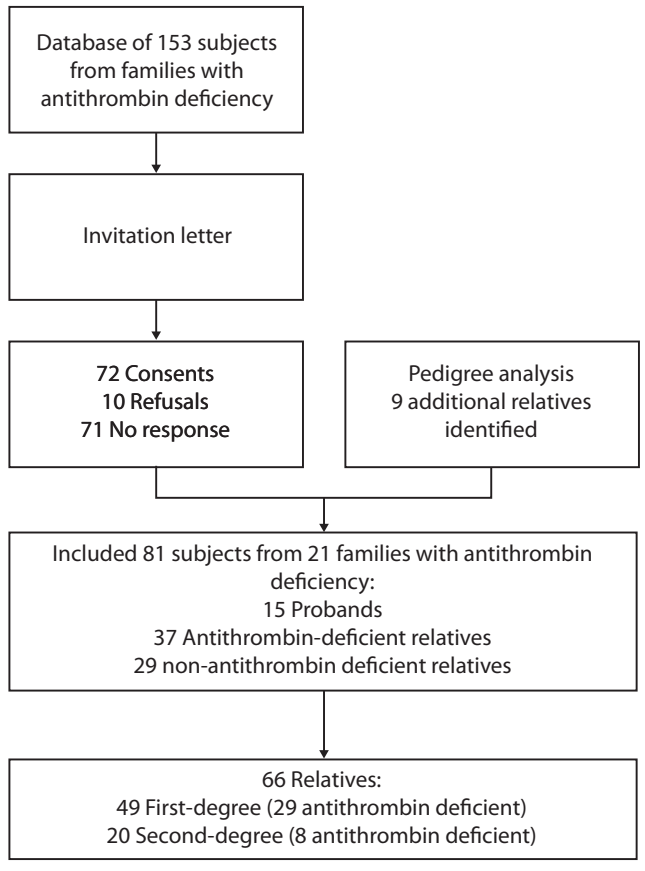


## RESULTS

### Patients

The selection of patients is shown in figure 1. We approached 153 subjects by letter with a return envelope. These were members of families with known antithrombin deficiency. Of those, 72 were included, 10 refused and 71 did not respond. A further 9 subjects were identified by pedigree analysis. We finally included 81 subjects belonging to 21 families in the study. Fifteen subjects were the probands of the family. From six families the original probands could not be included; one had died prior to the study, four did not respond and 1 refused participation. Sixty-six subjects were relatives of probands, of which 37 were antithrombin-deficient and 29 were not.

**Figure 1: Subject selection.**



The characteristics of all subjects are summarized in Table 1, including information on anticoagulant therapy.

**Table 1: Characteristics of the study population**

| | Type I antithrombin deficiency | | Type II antithrombin deficiency | | Non-deficient |
|---|---|---|---|---|---|
| | Probands (N=6) | Family members (N=29) | Probands (N=9) | Family members (N=8) | Family members (N=29) |
| Age, median (y) | 57 | 42 | 47 | 38.5 | 54 |
| Men/all subjects (n/N) | 2 | 14 | 4 | 3 | 12 |
| Antithrombin activity, mean (% ±SD) | 58.1 ±8.9 | 55.9 ±6.5 | 61.5 ±8.9 | 55.9 ±6.2 | 106.0 ±13.0 |
| Antithrombin antigen, mean (% ±SD) | 52.5 ±12.3 | 57.2 ±10.3 | 97.1 ±23.2 | 86.6 ±17.3 | 117.1 ±16.5 |
| Beta-antithrombin activity, mean (% ±SD) | 62.5 ±8.9 | 64.3 ±14.1 | 91.6 ±21.2 | 79.9 ±26.8 | 108.1 ±16.1 |
| Mutation found (n/N) | 4 | 26 | 9 | 8 | 0 |
| Current anticoagulant therapy | 5 | 13 | 8 | 4 | 4 |
| - ASA/clopidogrel | 0 | 1 | 0 | 1 | 3 |
| - LMWH | 1 | 0 | 0 | 0 | 0 |
| - VKA | 4 | 12 | 8 | 3 | 1 |
| First VTE (n/N) | 6 | 14 | 8‡ | 3 | 0 |
| Median age at VTE (y) | 40.5 | 29 | 19 | 55 | NA |
| Provoked/Spontaneous | 5 | 6 | 7 | 0 | |
| Fixed anticoagulation | 1 | 5 | 5 | 0 | |
| Indefinite anticoagulation | 5 | 9 | 3 | 3 | |
| Recurrent VTE (n/N) | 1 | 5 | 6 | 0 | NA |
| Fixed anticoagulation | 0 | 3 | 4 | 0 | |
| Indefinite anticoagulation | 1 | 2 | 2 | 0 | |

Six probands were men, of whom 2 had a spontaneous VTE, three had a postoperative VTE and one proband(‡) had a cerebral infarction at the age of 17. All 9 female probands had a provoked VTE: 3 postoperative VTEs, 1 pregnancy-related VTE, 4 oral contraceptives related VTEs and 1 VTE related to a trauma during oral contraceptives use. Provoked VTE in type 1 family members: 1 male, immobilization, 5 female, of whom 2 pregnancy-associated, 2 contraceptives, 1 immobilization





Type I antithrombin deficiency was found in 6 probands and 29 family members (the six probands that could not be included all had type I AT deficiency). Type II was found in 9 probands and 8 family members. Of the subtypes, type IIHBS was found in 3 probands and 3 family members. Type IIRS was found in 2 probands, while none of their 5 included family members were antithrombin-deficient. Type IIPE was diagnosed in 4 probands and 5 family members.

In 18 out of 21 families a mutation was found in the SERPINC1gene that fully segregated with the phenotype of antithrombin deficiency, as described elsewhere[29]. In 3 families no mutation was found (Table 2).

Protein C and protein S were tested in all patients, and no deficiency of these proteins was found. Heterozygous factor V Leiden mutations were found in 2 family members of different families, one with normal antithrombin levels and one that was antithrombin deficient. Heterozygous prothrombin G20210 mutations was found in 1 proband and 2 antithrombin-deficient family members of the same family (Table 2).

**Table 2, Mutations and relation to subtypes and β-antithrombin**

| Family | Nucleotide change | Mutation class | | Antithrombin activity, range(%) | Antithrombin antigen, mean (%) | Antithrombin antigen, range(%) | HAB ratio | Antithrombin deficiency subtype | β-antithrombin activity, mean (%) | β-antithrombin activity, range (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| C, Q‡ | c.218C>T | Missense | 65 | (56-72) | 108 | (96-128) | (0.69-077) | IIHBS | 103 | 93-112 |
| F, I‡ | c.377C>G | Missense | 61 | (53-71) | 73 | (60-82) | (0.88-0.99) | IIPE | 64 | 42-88 |
| K | c.337_338delCT | Deletion | 72 | 72 | 46 | 46 | 0.94 | I | 94 | 94 |
| A | c.536T>G | Missense | 49 | 49 | 70 | 70 | 0.97 | IIPE | 116 | 116 |
| G, T | c.569A>G | Missense | 57 | (50-63) | 80 | (75-93) | (0.85-0.94) | IIPE | 72 | 49-102 |
| L | c.749C>T | Missense | 63 | (57-68) | 106 | (99-112) | (0.73-0.82) | IIPE | 99 | 97-101 |
| B, E, S | c.857A>C | Missense | 53 | (46-62) | 53 | (43-67) | (0.9-0.94) | I | 62 | 48-72 |
| H‡ | c.763-1G>A | Splice variant | 50 | (47-53) | 46 | (41-51) | (0.88-0.96) | I | 53 | 50-56 |
| J | c.979dup | Insertion | 60 | (58-61) | 54 | (51-58) | (0.93-0.98) | I | 80 | 77-84 |
| M | c.1056del | Deletion | 57 | (51-67) | 54 | (44-62) | (0.91-0.93) | I | 70 | 62-85 |
| N | c.1033_1035del | Deletion | 51 | (44-58) | 69 | (65-73) | (0.95-0.97) | I | 52 | 48-55 |
| O | c.1246G>T | Missense | 51 | 51 | 103 | 103 | 1.01 | II | 106 | 106 |
| R | c.1274G>A | Missense | 70 | 70 | 136 | 136 | 0.90 | IIRS | 98 | 98 |
| D, P, U | No mutation† | No mutation | 56 | (48-69) | 53 | (42-63) | | I | 57 | 42-81 |

‡In family Q, a non-deficient family member had heterozygous factor V Leiden mutation. In family H an antithrombin deficient family member had heterozygous factor V Leiden mutation. In family I three antithrombin-deficient family members had heterozygous prothrombin G20210A mutation.
†In three families no SERPINC1 mutations could be detected despite direct sequencing of all 7 exons and regulatory regions, and multiplex ligation-dependent probe amplification (MLPA).

### β-antithrombin and antithrombin deficiency subtypes.

Normal β-antithrombin activity levels were found in subjects of all antithrombin deficiency subtypes (Table 2). In the type I antithrombin-deficient subjects the mean antithrombin activity was 56%, the mean antigen level was 56% and the mean β-antithrombin activity was 64%. In type IIHBS antithrombin deficiency the mean antithrombin activity level was 64%, the mean antigen level was 107% and the mean β-antithrombin activity was 102%. In type IIRS antithrombin deficiency the mean antithrombin activity level was 61%, the mean antigen level was 119% and the mean β-antithrombin activity was 102%. In type IIPE antithrombin deficiency the mean antithrombin activity level was 55%, the mean antigen level was 76% and the mean β-antithrombin activity was 72%.



### Probands

Fourteen out of 15 probands had had a VTE. One proband had a cerebral infarction at the age of 17. Twelve first VTE events were provoked, and 2 were unprovoked. The median age of onset of the first VTE in type II antithrombin-deficient probands was significantly lower (19y) than in type I antithrombin-deficient probands (41y, p=0.006).

### First VTE

In 37 antithrombin-deficient family members, 17 first VTE events were recorded. In 29 non-deficient family members no VTE occurred. In a total of 1367 observation years in 37 antithrombin-deficient family members 17 first VTE events occurred. Annual incidence in family members was 1.24% (95%CI 0.72-1.99%).

Using a Cox regression model adjusted for sex and age, antithrombin and β-antithrombin activity, antithrombin activity levels were associated with VTE risk in family members (p=0.026), whereas β-antithrombin levels were not associated with VTE risk (p=0.35). Within antithrombin deficient family members β-antithrombin levels were not associated with VTE risk (p=0.60).

Low β-antithrombin (≤80%) family members (n=31) had 15 events in 1099 observation years, annual incidence 1.36% (95%CI 0.76-2.25%), normal β-antithrombin (>80%) family members (n=6) had 2 events in 252 observation years, annual incidence 0.79 (95%CI 0.10-2.77). The difference in annual incidence was not statistically different (p=0.70). VTE-free survival analysis in antithrombin deficient family members with and without preserved beta-antithrombin was not significantly different (p=0.11).

Survival analysis according to antithrombin subtype in family members demonstrated no difference in VTE-free survival between type I and type II antithrombin deficiency in family members (p=0.68).

In 29 family members with type I antithrombin deficiency 14 first VTE occurred in 1044 observation years, annual incidence 1.34% (95%CI 0.73-2.25%). In the 8 type II antithrombin-deficient family members 3 events in 323 observation years occurred, annual incidence 0.93% (95%CI 0.19-2.71%). The difference in annual incidence was not statistically different (p=0.60).

### Recurrent VTE

Of the 31 subjects (probands and family members) with a first VTE, 18 had a provoked VTE and 13 had a spontaneous first VTE. Recurrent VTE occurred in 4 out of 7 subjects with a provoked first event and a fixed treatment duration (mean initial duration: 1.1yr, range 0.2y-2.4y) and in 3 out of 11 with a provoked event and indefinite treatment duration (mean duration: 19.2y, range 0.4-45.0). Recurrent VTE occurred in 3 out of 4 subjects with a spontaneous first event and a fixed treatment duration (mean initial duration 0.7y, range 0.5-1.0y), and in 2 out of 9 with an indefinite treatment duration (mean duration 7.0y, range

0.3-38y). The annual rate of recurrence in family members was 3.1% (95%CI 0.9-7.1%). With a fixed duration of anticoagulation (mean initial duration: 0.92y, range 0.2-2.4y) the annual incidence of recurrence in family members was 10% (95%CI 2.1-29.2%). The VTEs occurred after cessation of anticoagulant therapy. With an indefinite duration of anticoagulation (mean duration 13.7y, range 0.3-45y) the annual incidence of recurrence in family members was 1.5% (95%CI 0.2-5.4%). The difference in recurrence free survival was statistically significant ($p<0.05$).

We performed the β-antithrombin analyses in all subjects, according to coagulation duration. As proband selection may inflate the incidence rate of VTE but not the relative risk [32], we only report relative risks. In a Cox regression analysis adjusted for sex and coagulation duration, no significant difference in recurrent VTE-free survival was observed between subjects with normal β-antithrombin levels and subjects with low β-antithrombin levels (HR 0.95, 95%CI 0.25-3.65).





## DISCUSSION

To our knowledge, this is the first cohort study to investigate the impact of the β-antithrombin isoform in relation to antithrombin subtypes, mutations and the risk of VTE. In this typical antithrombin-deficient population our investigation, β-antithrombin function in plasma was preserved in a proportion of subjects of all subtypes of antithrombin deficiency. Preserved β-antithrombin activity levels had no major impact on VTE risk in our study. The absolute annual risks of a first VTE in subjects with low or preserved β-antithrombin activity were both high (1.36% vs.0.79%). Furthermore, no significant difference in recurrent VTE-free survival was observed between subjects with normal β-antithrombin levels and subjects with low β-antithrombin levels (HR 0.95, 95%CI 0.25-3.65).

These findings are novel. In this family cohort study the absolute annual risk of VTE in antithrombin-deficient family members was as high as 1.2% (95%CI 0.7-2.0%). In the family members, the proportion of provoked VTE was lower than in probands, supporting our policy of identifying affected family members and actively trying to lower VTE risk in high-risk situations. Furthermore, the high annual risk of recurrent VTE (3.1%, 95%CI:0.9-7.1%) was mostly influenced by the duration of anticoagulation. With a fixed duration of anticoagulation the annual incidence of recurrence in family members was as high as 10.0% (95%CI 2.1-29.2%), and with an indefinite duration of anticoagulation the annual recurrence risk was 1.5% (95%CI 0.2-5.4%). The difference in recurrence free survival was statistically significant ($p<0.05$) and is of high clinical relevance.

Our study is a representative family study on antithrombin deficiency: the absolute VTE risk is high and in agreement with a recent systematic review and meta-analysis on absolute risk of VTE in antithrombin deficient subjects: In retrospective studies the annual risk of VTE was estimated at 1.0% (95%CrI 0.6-1.5) [13]. The annual VTE risk in prospective studies was even higher, over 2.3% (95%CrI 0.2-6.5). VTE risk has also been shown to increase with age, and was as high as 1.94% in a study counting from the age of 15 onwards (as opposed to from birth)[24]. Therefore, our choice of counting events from birth onwards may, while avoiding overestimation of VTE risk, have led to an underestimation of VTE risk in the adult age. As antithrombin deficiency has not been associated with increased mortality[33,34], we don't think that death before enrollment leads to bias explaining the different findings between retrospective and prospective studies. We have found additional heterozygous factor V Leiden and prothrombin G20210A mutations in our cohort, but these groups were too small to analyze for impact on VTE risk.

In a systematic review, the annual recurrence rate without long-term use of anticoagulants in antithrombin-deficient individuals was estimated to be 8.8% (95%CrI 4.6-14.2) and with long-term anticoagulation 2.6% (95%CrI 0.0-10.5) [13]. Our results are similar: In subjects that had received anticoagulation of indefinite duration annual recurrence risk was eight percent lower than in subjects with a fixed treatment duration. Our study adds to the evidence that

may justify a policy of long-term anticoagulation in antithrombin-deficient VTE patients, regardless of the first VTE being provoked or not.

The analysis of β-antithrombin levels challenges the insights gained by other studies[4,21,28]. Previously it had been found that β-antithrombin as compared to α-antithrombin had an increased affinity for heparin. Therefore the hypothesis was that β-antithrombin was the main inhibitor of coagulation in vivo[21,28]. Based on this, mutations that affect α- but not β-antithrombin were thought to cause a milder type of antithrombin deficiency[4]. In our work, we show that in some families total antithrombin activity levels were decreased, whereas β-antithrombin activity levels were preserved. This occurred in families with all subtypes of antithrombin deficiency.

Preserved β-antithrombin activity levels had no major impact on VTE risk in our study. Although the small sample size may have limited the power to detect a difference in VTE-free survival between subjects with low or preserved β-antithrombin activity, in both groups the annual absolute risks of a first VTE in subjects with low or preserved β-antithrombin activity were high (1.36% vs.0.79%), and no difference in recurrence-free survival was observed. The high VTE risk in antithrombin-deficient subjects both with and without preserved β-antithrombin levels calls into question the hypothesis that maintained β-antithrombin function can explain an amelioration of clinical phenotype. The maintained interaction of β-antithrombin with heparin may not at all be important *in vivo*, as the interaction with heparin may be a bad predictor of the interaction between antithrombin and its supposed natural targets, the glycosaminoglycans in the vessel walls.

Our study was too small to show differences between the four subtypes. Three studies have investigated the impact of subtypes on clinical phenotypes[14-16]. In a recent systematic review and meta-analysis Type IIHBS antithrombin deficiency had an up to six-fold lower risk of VTE as compared to type I antithrombin deficiency. However, results of both studies may have been affected by proband selection: In a recent meta-analysis the VTE risk in type I patients as compared to type II patients including probands was increased 9.3-fold (95%CrI 0.0-30.9) [13]. However, this risk was only increased 2.6-fold (95%CrI 0.0-19.5) when considering only family member data and the probability of the risk being higher in type I subjects was only 88%. Bearing in mind the 14-fold increase of antithrombin deficiency as a whole, antithrombin deficient subjects should generally be considered to have a high risk for VTE.

In conclusion, antithrombin deficiency is a serious prothrombotic condition with high rates of first and recurrent VTE. Antithrombin deficient subjects with either reduced or with preserved β-antithrombin activity levels in plasma had high absolute risks of first and recurrent VTE. The suspected importance of β-antithrombin can therefore be questioned. Testing for antithrombin deficiency may be useful in family members, as preventative measures may decrease the rate of provoked VTE. Our family study shows such a high risk of recurrence that long-term anticoagulation is warranted in antithrombin-deficient individuals with VTE.



**Acknowledgements**

We would like to acknowledge Dr Gary Moore and Naomi de Jager (Viapath Haemostasis & Thrombosis Laboratories at Guy's & St. Thomas' Hospitals, London, UK) for providing the assay settings for the heparin-antithrombin binding assay.

Furthermore, we like to acknowledge Ms Vasiliki Karlaftis (Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia) for providing the protocol to quantify β-antithrombin.

Sources of funding: An unrestricted research grant from Bayer to K. Meijer was partly used to fund this study.

**Disclosures**

The authors report no conflicts of interest regarding this work.

## REFERENCES


1. Patnaik MM, Moll S. Inherited antithrombin deficiency: a review. *Haemophilia*. 2008;14(6):1229-1239.
2. Aguila S, Martinez-Martinez I, Collado M, et al. Compound heterozygosity involving Antithrombin Cambridge II (p.Ala416Ser) in antithrombin deficiency. *Thromb Haemost*. 2013;109(3):556-558.
3. Lane DA, Bayston T, Olds RJ, et al. Antithrombin mutation database: 2nd (1997) update. For the Plasma Coagulation Inhibitors Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost*. 1997;77(1):197-211.
4. Martinez-Martinez I, Navarro-Fernandez J, Ostergaard A, et al. Amelioration of the severity of heparin-binding antithrombin mutations by posttranslational mosaicism. *Blood*. 2012;120(4):900-904.
5. Lane DA, Kunz G, Olds RJ, Thein SL. Molecular genetics of antithrombin deficiency. *Blood Rev*. 1996;10(2):59-74.
6. Sakata T, Okamoto A, Mannami T, Matsuo H, Miyata T. Protein C and antithrombin deficiency are important risk factors for deep vein thrombosis in Japanese. *J Thromb Haemost*. 2004;2(3):528-530.
7. Tait RC, Walker ID, Perry DJ, et al. Prevalence of antithrombin deficiency in the healthy population. *Br J Haematol*. 1994;87(1):106-112.
8. Wells PS, Blajchman MA, Henderson P, et al. Prevalence of antithrombin deficiency in healthy blood donors: a cross-sectional study. *Am J Hematol*. 1994;45(4):321-324.
9. Di Minno MN, Dentali F, Lupoli R, Ageno W. Mild antithrombin deficiency and risk of recurrent venous thromboembolism: a prospective cohort study. *Circulation*. 2014;129(4):497-503.
10. Rossi E, Za T, Ciminello A, Leone G, De Stefano V. The risk of symptomatic pulmonary embolism due to proximal deep venous thrombosis differs in patients with different types of inherited thrombophilia. *Thromb Haemost*. 2008;99(6):1030-1034.
11. De Stefano V, Simioni P, Rossi E, et al. The risk of recurrent venous thromboembolism in patients with inherited deficiency of natural anticoagulants antithrombin, protein C and protein S. *Haematologica*. 2006;91(5):695-698.
12. Heijboer H, Brandjes DP, Buller HR, Sturk A, ten Cate JW. Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis. *N Engl J Med*. 1990;323(22):1512-1516.
13. Croles FN, Borjas-Howard J, Nasserinejad K, Leebeek FWG, Meijer K. Risk of Venous Thrombosis in Antithrombin Deficiency: A Systematic Review and Bayesian Meta-analysis. *Semin Thromb Hemost*. 2018.
14. Alhenc-Gelas M, Plu-Bureau G, Hugon-Rodin J, Picard V, Horellou MH, Thrombophilia GsgoG. Thrombotic risk according to SERPINC1 genotype in a large cohort of subjects with antithrombin inherited deficiency. *Thromb Haemost*. 2017;117(6):1040-1051.
15. Luxembourg B, Pavlova A, Geisen C, et al. Impact of the type of SERPINC1 mutation and subtype of antithrombin deficiency on the thrombotic phenotype in hereditary antithrombin deficiency. *Thromb Haemost*. 2014;111(2):249-257.
16. Mitsuguro M, Sakata T, Okamoto A, et al. Usefulness of antithrombin deficiency phenotypes for risk assessment of venous thromboembolism: type I deficiency as a strong risk factor for venous thromboembolism. *Int J Hematol*. 2010;92(3):468-473.
17. Di Minno MN, Dentali F, Veglia F, Russolillo A, Tremoli E, Ageno W. Antithrombin levels and the risk of a first episode of venous thromboembolism: a case-control study. *Thromb Haemost*. 2013;109(1):167-169.
18. Sokol J, Timp JF, le Cessie S, et al. Mild antithrombin deficiency and risk of recurrent venous thromboembolism: results from the MEGA follow-up study. *J Thromb Haemost*. 2018.
19. Olson ST, Richard B, Izaguirre G, Schedin-Weiss S, Gettins PG. Molecular mechanisms of antithrombin-heparin regulation of blood clotting proteinases. A paradigm for understanding proteinase regulation by serpin family protein proteinase inhibitors. *Biochimie*. 2010;92(11):1587-1596.
20. Picard V, Ersdal-Badju E, Bock SC. Partial glycosylation of antithrombin III asparagine-135 is caused by the serine in the third position of its N-glycosylation consensus sequence and is responsible for production of the beta-antithrombin III isoform with enhanced heparin affinity. *Biochemistry*. 1995;34(26):8433-8440.
21. McCoy AJ, Pei XY, Skinner R, Abrahams JP, Carrell RW. Structure of beta-antithrombin and the effect of glycosylation on antithrombin's heparin affinity and activity. *J Mol Biol*. 2003;326(3):823-833.
22. Witmer MR, Hatton MW. Antithrombin III-beta associates more readily than antithrombin III-alpha with uninjured and de-endothelialized aortic wall in vitro and in vivo. *Arterioscler Thromb*. 1991;11(3):530-539.
23. Frezzato M, Tosetto A, Rodeghiero F. Validated questionnaire for the identification of previous personal or familial venous thromboembolism. *Am J Epidemiol*. 1996;143(12):1257-1265.
24. Brouwer JL, Veeger NJ, Kluin-Nelemans HC, van der Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. *Ann Intern Med*. 2006;145(11):807-815.
25. Mahmoodi BK, Brouwer JL, Ten Kate MK, et al. A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. *J Thromb Haemost*. 2010;8(6):1193-1200.
26. Moore GW, de Jager N, Cutler JA. Development of a novel, rapid assay for detection of heparin-binding defect antithrombin deficiencies: the heparin-antithrombin binding (HAB) ratio. *Thromb Res*. 2015;135(1):161-166.
27. de la Morena-Barrio ME, Garcia A, Martinez-Martinez I, et al. A new method to quantify beta-antithrombin glycoform in plasma reveals increased levels during the acute stroke event. *Thromb Res*. 2015;136(3):634-641.
28. Karlaftis V, Sritharan G, Attard C, Corral J, Monagle P, Ignjatovic V. Beta (beta)-antithrombin activity in children and adults: implications for heparin therapy in infants and children. *J Thromb Haemost*. 2014;12(7):1141-1144.
29. Mulder R, Croles FN, Mulder AB, Huntington JA, Meijer K, Lukens MV. SERPINC1 gene mutations in antithrombin deficiency. *Br J Haematol*. 2017.
30. Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PE. Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. *Hum Mutat*. 2008;29(1):6-13.
31. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
32. Lijfering WM, Middeldorp S, Veeger NJ, et al. Risk of recurrent venous thrombosis in homozygous carriers and double heterozygous carriers of factor V Leiden and prothrombin G20210A. *Circulation*. 2010;121(15):1706-1712.
33. Pabinger I, Vossen CY, Lang J, et al. Mortality and inherited thrombophilia: results from the European Prospective Cohort on Thrombophilia. *J Thromb Haemost*. 2012;10(2):217-222.
34. van Boven HH, Vandenbroucke JP, Westendorp RG, Rosendaal FR. Mortality and causes of death in inherited antithrombin deficiency. *Thromb Haemost*. 1997;77(3):452-455.

4

# MONITORING OF HEPARINS IN ANTITHROMBIN-DEFICIENT PATIENTS

F. Nanne Croles, Michaël V. Lukens, René Mulder, Moniek P.M. De Maat,
André B. Mulder, Karina Meijer

*Submitted*

## ABSTRACT

### Background

Heparins exert their anticoagulant effect through activation of antithrombin. Whether deficiency of antithrombin leads to clinically relevantly reduced anti-Xa activity of heparins is unknown. We investigated the effect of inherited antithrombin deficiency on the monitoring of heparins and determined the relation between antithrombin deficiency, subtypes of antithrombin deficiency and anti-Xa activity measurements of plasma samples spiked with unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH).

### Patients/methods

Plasma samples from 34 antithrombin deficient subjects and 17 family controls were spiked with UFH and LMWH (nadroparin) to a final concentration aimed to correspond with an anti-Xa activity of 0.8 IU/mL. Antithrombin, β-antithrombin and anti-Xa activities were measured.

### Results

Mean anti-Xa activity with LWMH was 0.55 IU/mL (0.30-0.74) (recovery 69%, 38-93%) in antithrombin-deficient subjects and 0.82 (0.71-0.89) IU/mL in controls (recovery 103%, 89-111%). Expected anti-Xa measurements after LMWH spiking were found in 17/17 non-deficient subjects and in 8/34 antithrombin-deficient subjects. Anti-Xa measurements in the expected range (0.6-1.0 IU/mL) after UFH spiking were found in 17/17 non-deficient subjects and in 1/22 antithrombin-deficient subjects. Antithrombin activity correlated with anti-Xa activity of UFH (R= 0.77) and LMWH (R=0.66). Mixing studies of pooled normal plasma and antithrombin deficient plasma showed a clear relation between antithrombin levels and anti-Xa measurements: anti-Xa recovery was linearly reduced with antithrombin levels decreasing below 100%.

### Conclusions

We conclude that reduced antithrombin levels cause clinically significant reductions of anti-Xa levels. Standard doses of LWMH or UFH are likely to lead to under treatment in antithrombin-deficient individuals.




## INTRODUCTION

Heparins are widely used anticoagulant drugs that exert their effect by activation of antithrombin. Antithrombin is a slow-working anticoagulant molecule circulating in a repressed state in the blood. Its anticoagulant function is greatly enhanced after activation by heparin. Different types of heparin molecules, such as unfractionated heparin (UFH) or low-molecular weight heparin(LWMH), exert their anticoagulant effects in different ways, either predominantly via factors Xa, IXa and thrombin (UFH) or via factors Xa and IXa (LMWH) [1].

Antithrombin deficiency (OMIM 613118) is an inherited autosomal dominant defect, clinically characterized by a fourteen-fold increased risk of a first venous thromboembolism (VTE) [2]. In high-risk periods such as pregnancy and puerperium, thrombotic risk is even higher, with current risk-estimates as high as 7.3% for the antepartum period and 11.1% for the postpartum period. Antithrombin deficiency is therefore a recognized indication for prophylactic treatment with LMWH[3]. However, despite prophylactic treatment with LMWH, VTE events have been described in pregnant antithrombin-deficient women [4-6]. We hypothesized that antithrombin-deficient individuals may have less therapeutic effect of heparins due to the antithrombin deficiency itself.

A subdivision of antithrombin deficiency is made in type I deficiency (quantitative defect) and type II deficiency (qualitative defects) [7]. Antithrombin consists of two isoforms, 90% of circulating antithrombin being α-antithrombin and 10% β-antithrombin. The N-glycan at Asn135 is not present in β-antithrombin. β-antithrombin has a higher affinity for heparin, and binds better to the endothelium [8] and may therefore serve as the major inhibitor of coagulation *in vivo*, and as the major target for heparins [9,10]. In the setting of inherited antithrombin deficiency the interplay between antithrombin deficiency types, β-antithrombin levels and types of heparins and activity measurements of these drugs is unknown.

The aim of this study is to investigate the relation between antithrombin deficiency, subtypes of antithrombin deficiency, β-antithrombin levels and anti-Xa activity measurements of plasma samples spiked with UFH or LMWH.

## METHODS

### Participants

In a cross-sectional and retrospective family cohort study we included patients with inherited antithrombin deficiency and their first-degree relatives. Inclusion of participants is described elsewhere in more detail [11].Eighty participants in the study did not use LMWH/UFH at inclusion. From these 80, we had blood samples available from 51 subjects for the present study. Of those 51 subjects, all samples were spiked with LMWH, and for 39 subjects enough plasma remained for spiking with UFH. This study was approved by the institutional medical ethics committee of the University Medical Center Groningen (ABR nr. NL41324).

Blood collection, specialized antithrombin measurements and SERPINC1 gene mutation analyses have been described in detail elsewhere [11,12]. Standard antithrombin activity measurements were performed with an assay based on anti-FXa inhibition (INNOVANCEAntithrombin assay; Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) (normal levels >80%). Measurements of β-antithrombin activity were performed using a previously published protocol that was adapted by V. Karlaftis [13]. Anti-Xa assay measurements were performed using the HemosIL Liquid anti-Xa assay (this test does not contain exogenous antithrombin) and APTT measurements were performed using the HemosIL Liquid APTT, both according to the protocols from the manufacturer on a ACLTOP 500, both from Werfen, Bedford, MA, USA.

### Addition of anticoagulants to plasma

UFH (LEO Pharma BV, Amsterdam, The Netherlands) and LWMH (nadroparin, Aspen Pharma, Dublin, Ireland) were added to plasma samples of the participants. The final concentrations in plasma were aimed to correspond with anti-Xa levels of 0.8 IU/mL, in order to perform the study in the therapeutic anti-Xa levels of 0.6-1.0 IU/mL that are seen with twice daily dosing of LMWH [14]. In the APTT mixing studies the UFH levels were aimed to prolong the APTT 2-2,5 time in the normal pool plasma, corresponding to a final heparin concentration of 0.3 IU/mL.

### Mixing studies

In addition to the studies in patient samples, we performed mixing studies of pooled normal plasma and antithrombin-deficient plasma to analyse the relation between anti-Xa activity measurements, APTT measurements and antithrombin levels. Antithrombin-deficient plasma was obtained from Diagnostica Stago (Asnières sur Seine, France). Antithrombin-deficient plasma was mixed with pooled normal plasma to obtain a range of antithrombin concentrations from 0-100%. Pooled normal plasma was obtained from 60 healthy donors and immediately aliquoted and frozen at -80$^0$C after processing.

### Statistical analysis:

Data are expressed as mean ± standard deviation (SD), medians (with ranges), or numbers (with percentages) as appropriate. Means of two groups were compared by Student's T-test. Spearman's rho correlation coefficient was used to assess correlation between continuous variables. P-values of 0.05 or less were considered statistically significant. IBM SPSS version 21 (New York, USA) was used for all analyses. Figures were created using GraphPad PRISM version 5.01 (San Diego, USA).

## RESULTS

Of 51 subjects available for this study, 18 participants had type I antithrombin deficiency, 5 had type II heparin binding site (type IIHBS) antithrombin deficiency, 2 had type II reactive site (type IIRS) antithrombin deficiency, 9 had type II pleiotropic effect (type IIPE) antithrombin deficiency, and 17 had no antithrombin deficiency. The levels of antithrombin activity, β-antithrombin activity and antithrombin antigen were not normally distributed. Antithrombin and anti-Xa measurements according to subtypes are shown in table 1.

**Table 1: Measurements in antithrombin-deficient and non-antithrombin-deficient subjects.**

| Group | Antithrombin activity (%) (Mean, ±SD) | β-antithrombin activity (%) (Mean ±SD) | Antithrombin antigen (%) (Mean ±SD) | Anti-Xa activity with UFH (IU/mL) (Mean ±SD) | Anti-Xa activity with LMWH (IU/mL) (Mean ±SD) |
|---|---|---|---|---|---|
| No antithrombin deficiency (n=17) | 108.4 ±9.7 | 107.2 ±16.3 | 117.0 ±15.3 | 0.79 ±0.09 (n=17) | 0.82 ±0.04 |
| All antithrombin deficiency (n=34) | 58.6 ±7.6 | 74.6 ±21.4 | 73.3 ±24.0 | 0.50 ±0.07 (n=22) | 0.55 ±0.08 |
| Type I (n=18) | 58.4 ±7.2 | 65.8 ±14.5 | 57.0 ±11.0 | 0.52 ±0.07 (n=12) | 0.55 ±0.05 |
| Type II (n=16) | 58.7 ±8.3 | 84.6 ±23.9 | 90.6 ±22.3 | 0.49 ±0.08 (n=10) | 0.55 ±0.10 |

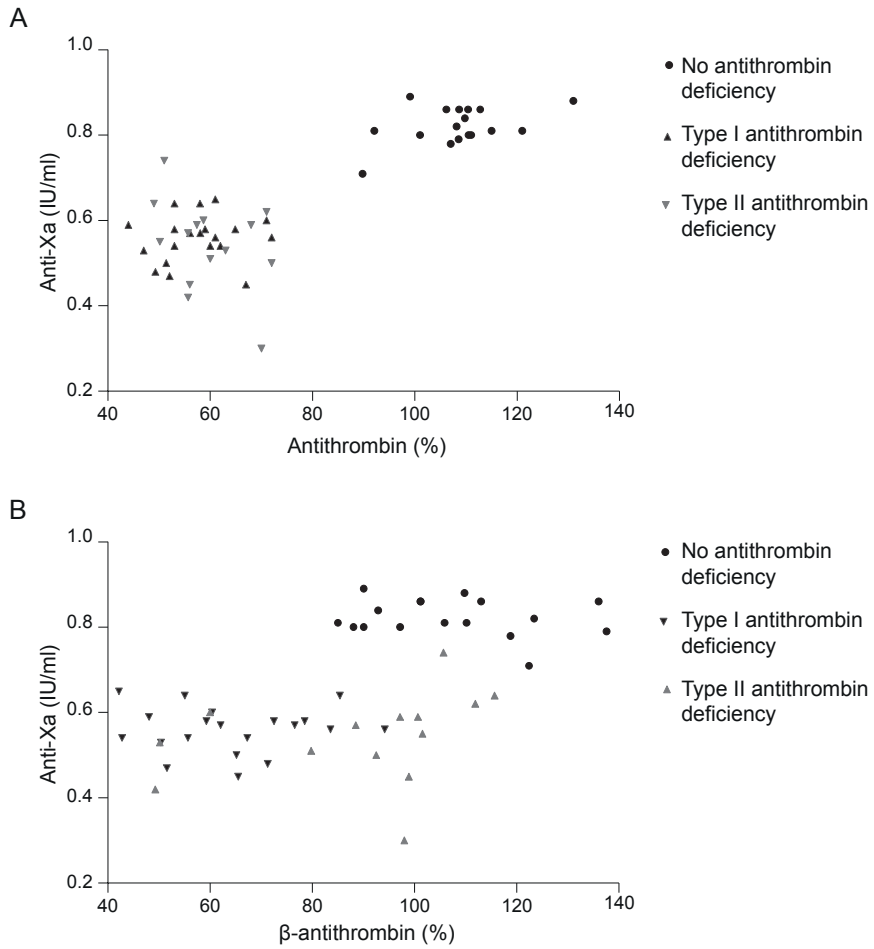
Data are presented as mean, ± standard deviation. UFH: unfractionated heparin. LMWH: low-molecular-weight heparin. For the anti-Xa measurements after addition of UFH less samples were available, the numbers are shown in the UFH column.

In antithrombin-deficient individuals, spiking with LMWH resulted in lower mean anti-Xa activity than in non-deficient individuals (0.55 IU/mL, range 0.30-0.74 vs. 0.82 IU/mL, 0.71-0.89, p<0.001). Mean anti-Xa recovery with LWMH was 103% (89-111%) in controls and 69% (38-93%) in antithrombin deficient individuals. In antithrombin-deficient individuals, after spiking with UFH, the mean anti-Xa was also lower than in non-deficient individuals (0.50 IU/mL, 0.32-0.63 vs. 0.79 IU/mL, 0.64-0.93, p<0.001). Between type I and type II antithrombin-deficient individuals the mean anti-Xa was not significantly different for both heparins. Expected anti-Xa levels (0.6-1.0 U/mL) after UFH spiking were found in all non-deficient subjects and in 1/22 (5%) of antithrombin-deficient subjects. Expected anti-Xa levels after LMWH spiking were found in all non-deficient subjects and in 8/34 (24%) of the antithrombin-deficient subjects.

Scatterplots of antithrombin- and β-antithrombin activity and anti-Xa measurements of all investigated samples are shown in figure 1. Using Spearman's rho correlation analysis, antithrombin activity was correlated with anti-Xa activity of UFH (R= 0.77, p<0.001) and of LMWH (R=0.66, p<0.001). β-antithrombin activity also correlated with anti-Xa activity of UFH (R=0.59, p<0.001) and of LMWH (R=0.56, p<0.001), but less strongly.



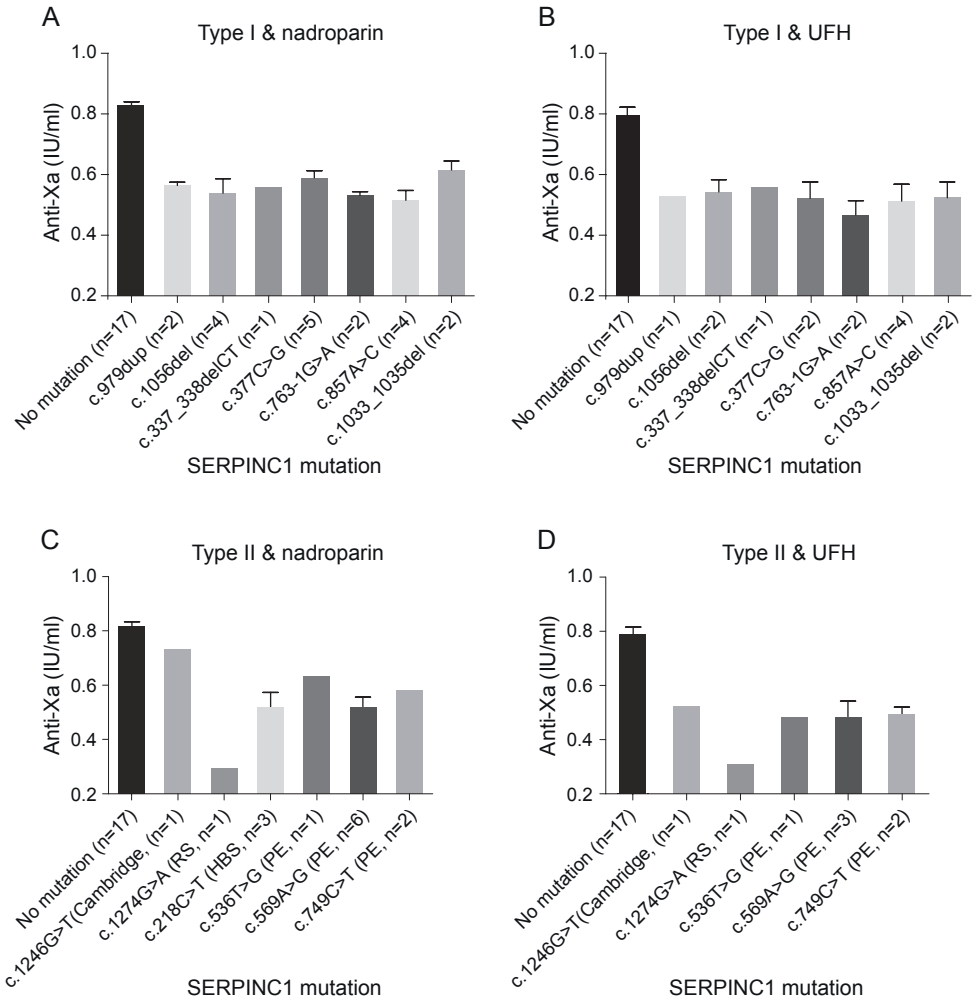
**Figure 1: Scatterplots of antithrombin activity and anti-Xa activity measurements.**




Panel A: antithrombin and anti-Xa activity measurements after spiking with low-molecular-weight heparin (LMWH). Panel B: β-antithrombin and anti-Xa activity measurements after spiking with LMWH.

In order to investigate if anti-Xa measurements were linked to specific SERPINC1 mutations underlying the antithrombin deficiency, we related anti-Xa measurements to the underlying mutations. With all mutations, mean anti-Xa levels were lower than in the non-antithrombin-deficient family members. Only 2 mutations differed from the other mutations: the anti-Xa level in the c.1246G>T mutation (n=1) (antithrombin Cambridge II) was somewhat higher with LMWH, and in the c.1274G>A mutation (n=1) (antithrombin Glasgow) the anti-Xa level was lower with both UFH and LMWH, as compared to the other mutations. These results are shown in figure 2.

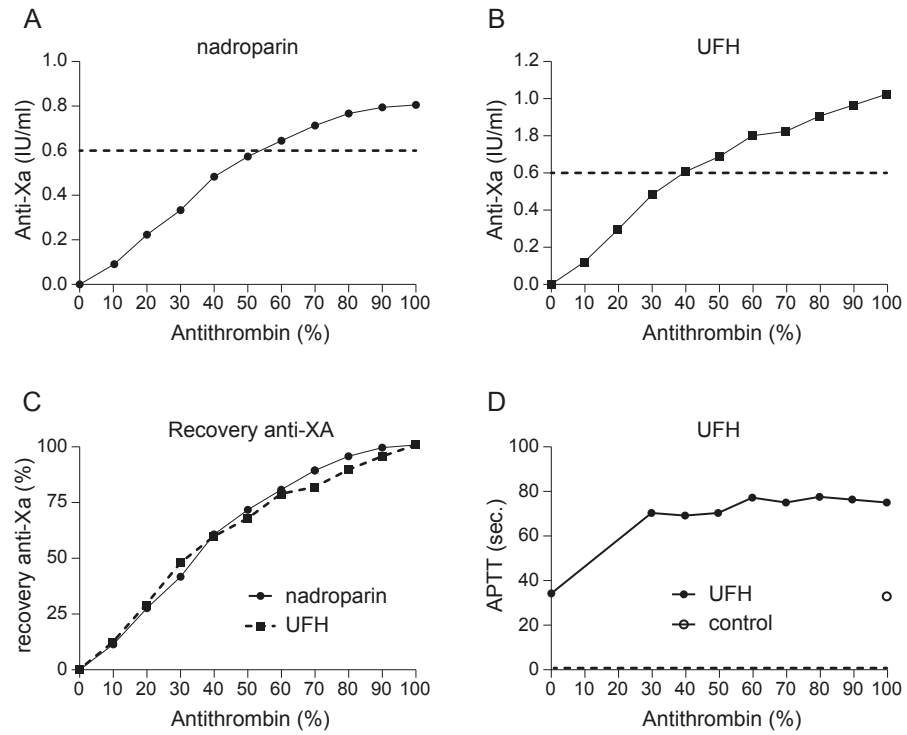
**Figure 2. Anti-Xa activity measurements according to antithrombin subtype, SERPINC1 mutations, and heparin type.**




Panel A and B: mutations causing type I antithrombin deficiency. Panel C and D: mutations causing type II antithrombin deficiency. Panel A and C: nadroparin. Panel B and D: unfractionated heparin. UFH: unfractionated heparin. In the type II samples the subtypes are given between brackets. Abbreviations: RS: reactive site. HBS: Heparin binding site. PE: pleiotropic effects. For amino acid sequences we refer to previous work[11].



**Figure 3. Anti-Xa and APTT measurements in mixing studies of antithrombin-deficient and pooled normal plasma.**




Panel A: anti-Xa activity according to antithrombin levels after adding nadroparin. Panel B: anti-Xa activity according to antithrombin levels after adding unfractionated heparin (UFH). Panel C: Recovery of anti-Xa according to antithrombin level, normalized for maximum anti-Xa activity. UFH: unfractionated heparin. Panel D: APTT measurements according to antithrombin levels after adding UFH.

In order to further investigate the relation between antithrombin levels and anti-Xa measurements, we performed a similar spiking experiment in mixtures with increasing concentrations of antithrombin (0-100%) of commercially available antithrombin-deficient plasma with pooled normal plasma. Anti-Xa levels after spiking these samples with either UFH or LMWH are clearly related to antithrombin concentrations (figure 3). These results prompted us to perform the same experiment with APTT measurements. Only in the completely antithrombin-deficient sample APTT normalised to non-heparin values, but with all other concentrations APTT measurements were equally prolonged as the normal-pool plasma spiked with heparin. We therefore did not measure the APTT in UFH spiked samples from antithrombin-deficient subjects.

## DISCUSSION

Our experiments show that anti-Xa measurements in the presence of LMWH or UFH are clearly correlated with antithrombin activity levels. Mean anti-Xa levels were about 35% lower than expected in antithrombin-deficient subjects as compared to non-deficient subjects. Of antithrombin-deficient subjects, 76-95% had levels that would be classified as subtherapeutic in clinical setting, whereas all controls had expected anti-Xa levels.

The correlation of antithrombin activity levels with anti-Xa measurements is stronger than the correlation of β-antithrombin activity levels with anti-Xa measurements. These data therefore do not support the theory of β-antithrombin being the strongest inhibitor of coagulation in vivo, although the natural activator of antithrombin is not currently known [9,10]. The weaker correlation of β-antithrombin activity with anti-Xa measurement may be explained by either β-antithrombin not being a stronger inhibitor than overall antithrombin, or due to limitations of the β-antithrombin measurement itself. Either way, antithrombin activity measurements are easier to perform, and have a stronger correlation with anti-Xa measurements than β-antithrombin measurements. However, it cannot be excluded that using different antithrombin activity assays would lead to different correlations, as no available antithrombin activity test is able to detect all types of antithrombin deficiency[15-17].

In the mixing studies, a sharp contrast was seen between the anti-Xa measurements and APTT measurements after spiking with UFH with respect to its sensitivity to reduced antithrombin concentrations. Whereas in samples with reduced antithrombin concentrations anti-Xa measurements were reduced as well, APTT measurements were only influenced by the complete absence of antithrombin. In a study by Mitchell et al. various anti-Xa and anti-IIa based chromogenic heparin-activity tests were performed in antithrombin-depleted plasma with added antithrombin and UFH in various concentrations. All chromogenic heparin activity tests were similarly influenced by antithrombin levels. Interestingly, in samples without added heparin but with 2-fold increased antithrombin concentration "heparin activity" was measured [18]. This implies that the lower anti-Xa activities we found in UFH-spiked samples with low antithrombin activity are more likely to be related to the antithrombin levels than to the type of anti-Xa test we have used. From a clinical point of view however, it is yet unknown which test best reflects the anticoagulant efficacy of UFH in vivo.

Different types of LMWH may have different profiles regarding the relation between antithrombin- and β-antithrombin concentrations, different SERPINC1 mutations and anti-Xa measurements. Our study was limited to only one type of LMWH, as nadroparin is a commonly used LMWH in The Netherlands.

The lower anti-Xa activity found in the c.1274G>A mutation (antithrombin Glasgow) may be explained by a different conformation of the antithrombin Glasgow molecule than the normal heparin-activated conformation of normal antithrombin [19].

The relation between antithrombin levels and anti-Xa measurements has been found before in subjects with liver cirrhosis, with antithrombin levels as low as 23% [20]. This led the authors to conclude that drug levels of antithrombin-dependent drugs were underestimated. No statement could be made on clinical efficacy in liver patients, who generally have multiple defects of the haemostatic system. Despite lower anti-Xa measurements due to the acquired antithrombin deficiency of liver disease the procoagulant effect may still be significant, as other procoagulant factors are decreased as well. Acquired antithrombin deficiency resulting in decreased anti-Xa activity has been found in other patients groups with combinations of alterations in the haemostatic system as well, such as ICU patients and patients with sepsis or nephrotic syndrome [21]. The relation between anti-Xa activity measurements and antithrombin concentrations has also been shown in children during cardiopulmonary bypass [22]. Lower antithrombin levels were associated with measures of increased thrombin generation, such as thrombin-antithrombin complexes, prothrombin activation fragment 1.2 and D-dimer levels. In contrast, in our study antithrombin deficiency is the single major abnormality in the haemostatic system. In most of our antithrombin-deficient subjects anti-Xa measurements after spiking with UFH and LMWH were below the expected range of 0.6-1.0 U/mL, whereas in all anti-Xa measurements non-deficient subjects were in the expected range. Therefore, it is possible that antithrombin-deficient subjects are undertreated with regular doses of UFH/LMWH. This may explain the occurrence of thrombotic events in antithrombin-deficient individuals despite treatment with LMWH [4-6]. However, it is unknown if the interaction of antithrombin and anti-Xa activity is a cause of the high risk of recurrent VTE in antithrombin deficiency [2], and in patients with mildly reduced antithrombin levels (<87%) [23,24]. Although the use of LMWH generally requires no routine monitoring [14], the knowledge of inherited antithrombin deficiency causing a reduced anticoagulant effect of heparins, as measured by anti-Xa assays, in combination with the clear prothrombotic phenotype supports measuring anti-Xa activity in antithrombin deficient subjects who are treated with heparins. Doses of LMWH should be adjusted to achieve the target range in order to achieve a sufficient antithrombotic effect.

Since most anti-Xa assays used in clinical laboratories (including the test used in our study) do not contain exogenous antithrombin, anti-Xa activity assays are often fully dependent on the patient's own antithrombin. This situation reflects the in vivo situation. Adding exogenous antithrombin to anti-Xa activity assays may lead to overestimation of the anticoagulant effect of heparins in patients with antithrombin deficiency.

In conclusion, we have found that anti-Xa activity measurements of LMWH and UFH are clearly correlated with antithrombin levels, and that even mildly reduced antithrombin levels may result in a reduced anti-Xa recovery. In most antithrombin-deficient plasma samples anti-Xa measurements were below the expected range of 0.6-1.0 U/mL. Antithrombin-deficient individuals, already at an increased risk for recurrent venous thrombosis, may therefore be undertreated using standard doses of UFH/LMWH.

## REFERENCES

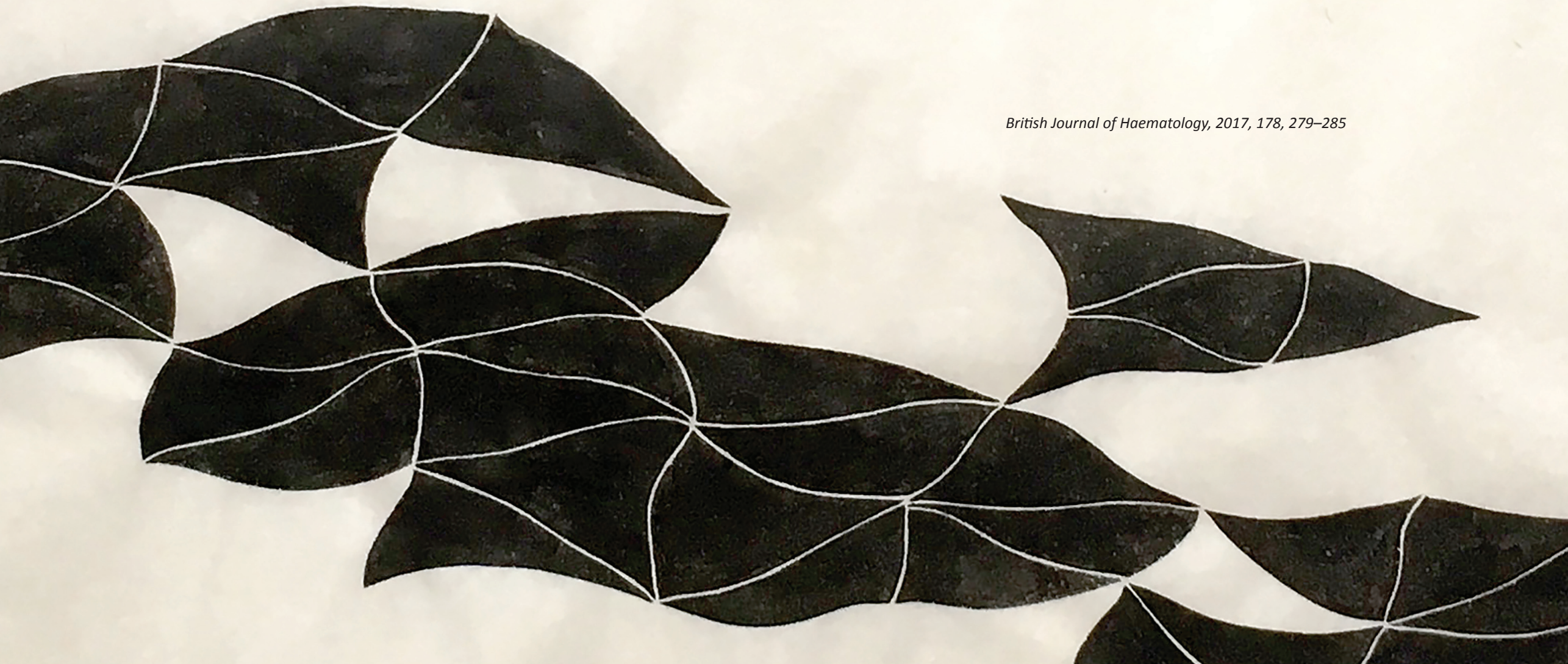

1. Olson ST, Richard B, Izaguirre G, Schedin-Weiss S, Gettins PG. Molecular mechanisms of antithrombin-heparin regulation of blood clotting proteinases. A paradigm for understanding proteinase regulation by serpin family protein proteinase inhibitors. *Biochimie*. 2010;92(11):1587-1596.
2. Croles FN, Borjas-Howard J, Nasserinejad K, Leebeek FWG, Meijer K. Risk of Venous Thrombosis in Antithrombin Deficiency: A Systematic Review and Bayesian Meta-analysis. *Semin Thromb Hemost*. 2018;44(4):315-326.
3. Croles FN, Nasserinejad K, Duvekot JJ, Kruip MJ, Meijer K, Leebeek FW. Pregnancy, thrombophilia, and the risk of a first venous thrombosis: systematic review and bayesian meta-analysis. *BMJ*. 2017;359:j4452.
4. Rogenhofer N, Bohlmann MK, Beuter-Winkler P, et al. Prevention, management and extent of adverse pregnancy outcomes in women with hereditary antithrombin deficiency. *Ann Hematol*. 2014;93(3):385-392.
5. Sanson BJ, Simioni P, Tormene D, Moia M. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. *Blood*. 1999.
6. Simioni P, Tormene D, Prandoni P, Girolami A. Pregnancy-related recurrent events in thrombophilic women with previous venous thromboembolism. *Thromb Haemost*. 2001;86(3):929.
7. Lane DA, Kunz G, Olds RJ, Thein SL. Molecular genetics of antithrombin deficiency. *Blood Rev*. 1996;10(2):59-74.
8. Witmer MR, Hatton MW. Antithrombin III-beta associates more readily than antithrombin III-alpha with uninjured and de-endothelialized aortic wall in vitro and in vivo. *Arterioscler Thromb*. 1991;11(3):530-539.
9. Picard V, Ersdal-Badju E, Bock SC. Partial glycosylation of antithrombin III asparagine-135 is caused by the serine in the third position of its N-glycosylation consensus sequence and is responsible for production of the beta-antithrombin III isoform with enhanced heparin affinity. *Biochemistry*. 1995;34(26):8433-8440.
10. McCoy AJ, Pei XY, Skinner R, Abrahams JP, Carrell RW. Structure of beta-antithrombin and the effect of glycosylation on antithrombin's heparin affinity and activity. *J Mol Biol*. 2003;326(3):823-833.
11. Mulder R, Croles FN, Mulder AB, Huntington JA, Meijer K, Lukens MV. SERPINC1 gene mutations in antithrombin deficiency. *Br J Haematol*. 2017;178(2):279-285.
12. Croles FN, Mulder R, Mulder AB, Lukens MV, Meijer K. beta-Antithrombin, subtype of antithrombin deficiency and the risk of venous thromboembolism in hereditary antithrombin deficiency: A family cohort study. *Thromb Res*. 2018;168:47-52.
13. de la Morena-Barrio ME, Garcia A, Martinez-Martinez I, et al. A new method to quantify beta-antithrombin glycoform in plasma reveals increased levels during the acute stroke event. *Thromb Res*. 2015;136(3):634-641.
14. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J*. 2014;35(43):3033-3069, 3069a-3069k.
15. Corral J, Vicente V. Puzzling questions on antithrombin: Diagnostic limitations and real incidence in venous and arterial thrombosis. *Thromb Res*. 2015;135(6):1047-1048.
16. Javela K, Engelbarth S, Hiltunen L, Mustonen P, Puurunen M. Great discrepancy in antithrombin activity measured using five commercially available functional assays. *Thromb Res*. 2013;132(1):132-137.
17. Kristensen SR, Rasmussen B, Pedersen S, Bathum L. Detecting antithrombin deficiency may be a difficult task--more than one test is necessary. *J Thromb Haemost*. 2007;5(3):617-618.
18. Mitchell LG, Vegh P. Conventional chromogenic heparin assays are influenced by patient's endogenous plasma Antithrombin levels. *Klin Padiatr*. 2010;222(3):164-167.
19. Owen MC, George PM, Lane DA, Boswell DR. P1 variant antithrombins Glasgow (393 Arg to His) and Pescara (393 Arg to Pro) have increased heparin affinity and are resistant to catalytic cleavage by elastase. Implications for the heparin activation mechanism. *FEBS Lett*. 1991;280(2):216-220.
20. Potze W, Arshad F, Adelmeijer J, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. *Br J Haematol*. 2013;163(5):666-673.
21. Vera-Aguilera J, Yousef H, Beltran-Melgarejo D, et al. Clinical Scenarios for Discordant Anti-Xa. *Adv Hematol*. 2016;2016:4054806.
22. Manlhiot C, Gruenwald CE, Holtby HM, et al. Challenges with heparin-based anticoagulation during cardiopulmonary bypass in children: Impact of low antithrombin activity. *J Thorac Cardiovasc Surg*. 2016;151(2):444-450.
23. Di Minno MN, Dentali F, Lupoli R, Ageno W. Mild antithrombin deficiency and risk of recurrent venous thromboembolism: a prospective cohort study. *Circulation*. 2014;129(4):497-503.
24. Sokol J, Timp JF, le Cessie S, et al. Mild antithrombin deficiency and risk of recurrent venous thromboembolism: results from the MEGA follow-up study. *J Thromb Haemost*. 2018.

5

# SERPINC1 GENE MUTATIONS IN ANTITHROMBIN DEFICIENCY

R. Mulder, F.N. Croles, A.B. Mulder, J.A. Huntington, Karina Meijer and M.V. Lukens

*British Journal of Haematology, 2017, 178, 279–285*



## ABSTRACT

Existing evidence suggests that in most cases antithrombin deficiency can be explained by mutations in its gene *SERPINC1*.

We investigated the molecular background of antithrombin deficiency in a single centre family cohort study.

We included a total of 21 families comprising 15 original probands and sixty-six relatives, 6 of whom were surrogate probands for the genetic analysis. Antithrombin activity and antigen levels were measured. The heparin-antithrombin binding (HAB) ratio assay was used to distinguish between the different subtypes of antithrombin deficiency. *SERPINC1* mutations were detected by direct sequencing of all 7 exons and regulatory regions, and multiplex ligation-dependent probe amplification.

Eighty-six percent of the families had a detrimental *SERPINC1* gene mutation that segregated in the family. We detected 13 different *SERPINC1* gene mutations of which 5 novel. Among all these mutations, 44% was associated with type I deficiency, whereas the remainder was associated with type II heparin binding site (11%), type II pleiotropic effect (33%), type II reactive site (6%) or had the antithrombin Cambridge II mutation (6%).

The current study reports several novel *SERPINC1* mutations, thereby adding to our knowledge of the molecular background of antithrombin deficiency. Finally, our results point out the importance of future research outside the conventional *SERPINC1* gene approach.




## INTRODUCTION

Antithrombin is a member of the serine proteinase inhibitor (serpin) superfamily, which has a highly conserved structure, with three β-sheets (A–C) and eight to nine α-helices (A–I) [1]. Antithrombin is the major physiological inhibitor of coagulation but also exhibits anti-inflammatory properties, thereby accentuating the crosstalk between both pathways [2].

The inhibitory function of antithrombin is mediated principally through targeting thrombin, factor Xa and factor IXa. However, the inhibitory function of antithrombin is remarkably slow due to its repressed reactivity state. Heparin or heparan sulfate-type glycosaminoglycan cofactors transform antithrombin into a fast inhibitor by relieving this repression. This explains the therapeutic use of heparin as an anticoagulant.

The clinical significance of antithrombin function is clearly demonstrated by the fact that its deficiency (Online Mendelian Inheritance in Man (OMIM) reference 107300) is associated with an increased risk of venous thrombosis. Antithrombin deficiency can be classified into type I (quantitative defect) and type II (qualitative defect). Type I is characterized by a parallel reduction of both the plasma antigen levels and functional activity. Type II is characterized by low activity levels and normal antithrombin plasma antigen levels. Depending on the location of the mutations in the gene of antithrombin, *SERPINC1*, type II deficiency can be further subdivided in type II RS (reactive site), type II HBS (heparin binding site) and type II PE (pleiotropic effects) [3]. Noteworthy, the p.Ala416Ser (antithrombin Cambridge II) is converted into a substrate in the presence of heparin [4]. Although it is in the RCL, we cannot call it an RS mutant. Instead this mutation forms a unique class of among the type II mutations.

Antithrombin deficiency is rare, the prevalence in healthy blood donors was found to be between 1:500 and 1:645 [3,5,6], whereas in patients with venous thrombosis the prevalence was 1-5% [7-10].

*SERPINC1* is located on chromosome 1q23-25 [11], contains seven exons and six introns, and spans 13.578 bp of genomic DNA.

To date, more than 250 *SERPINC1* gene mutations have been reported to be associated with antithrombin deficiency [http://www.hgmd.cf.ac.uk/ac/; https://www1.imperial.ac.uk/departmentofmedicine/divisions/experimentalmedicine/haematology/coag/antithrombin/]. The mutation profile of the *SERPINC1* gene is heterogeneous, being typically comprised of point mutations, splice site variants and small insertion/deletion mutations, whereas gross rearrangements account for only a small proportion.

In this study, we investigated the molecular background of antithrombin deficiency in a single centre family cohort study.

## MATERIAL AND METHODS

### Patients

The subjects in this single centre family cohort study were patients with previously diagnosed hereditary antithrombin deficiency or their first degree relatives. Our study enrolled adult patients (≥18 years) after written informed consent, between January 2013 and January 2015. Some of the probands were included in a previous cohort study[12,13]. They largely stem from referrals to our tertiary centre for thrombophilia screening because of venous or arterial thrombotic events. First-degree relatives of probands were identified by pedigree analysis.

### Blood collection

Venous blood samples were anticoagulated with a 1:10 volume of 0.109 mol/l trisodium citrate. Platelet-poor plasma was prepared by centrifugation at 2500 x g for 15 minutes. The samples were then aliquoted and immediately frozen at -80°C until use, and analysed after rapid thawing at 37°C. Genomic DNA was obtained from EDTA samples using the Qiacube® system (QIAGEN, Hilden, Germany).

### Antithrombin assays

Antithrombin activity measurements were performed with an assay based on anti-FXa inhibition (INNOVANCE® Antithrombin assay, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) (normal levels >80%). Antithrombin antigen levels were measured with an enzyme-linked immunosorbent assay (ELISA) with reagents obtained from Affinity Biologicals (Ancaster, ON, Canada) (normal levels > 80 %). For the heparin-antithrombin binding (HAB) ratio we adopted the assay of Moore et al [14]. This assay quantifies heparin-binding capacity of antithrombin by deriving a ratio of antithrombin activities generated from short (30s) and prolonged incubation times (290s) with heparin. A reduced HAB ratio (<0.8) distinguishes type II HBS antithrombin deficiency from the other subtypes.

### Additional thrombophilic assays

Total protein S antigen levels were measured with an ELISA using the Asserachrom Protein S kit (Diagnostica Stago, Asnières sur Seine, France). Free protein S antigen levels were measured with a latex-based test (HemosIL; Instrumentation Laboratory Spa, Milan, Italy). Protein C antigen levels were measured with an ELISA using the Asserachrom Protein C kit from Diagnostica Stago. Protein C activity levels were measured with the Berichrom protein C test from Siemens. The presence of F5 R506Q (Factor V Leiden) and F2 G20210A (prothrombin mutation) was tested using the Cepheid Xpert® FII & FV on the Cepheid GeneXpert System (Cepheid, Sunnyvale, CA, USA).

### Genomic DNA amplification and sequence analysis of SERPINC1

Direct sequencing analysis of all 7 exons and flanking introns of *SERPINC1* gene was performed to detect sequence variations in the (surrogate) probands. Additionally, MLPA using the SALSA MLPA probemix P227-B2 SERPINC1 (MRC-Holland, Amsterdam, The Netherlands, lot B2-0314), and direct sequencing of the promotor region (1500 bp upstream of exon 1) [15] was performed if no sequence variation was detected by direct sequencing of all 7 exons and flanking introns. If a sequence variation was found in the proband, all relatives were tested for that specific variant. Primer sequences and PCR conditions can be obtained from the authors upon request.

### In silico prediction tools

*In silico* tools for pathogenicity prediction of exonic *SERPINC1* variants were chosen based on having the highest performance characteristics in a study by Luxembourg et al [16]. These tools included MutPred [17], PhD-SNP [18], and PolyPhen-2 HumDiv [19]. In addition, we used MutationTaster [20] because it enabled us to perform predictions of pathogenicity at base pair level. MutationTaster also uses a locally installed third party prediction program, called NNSplice [21] that analyses possible changes in splice sites. This program together with Human Splicing Finder (HSF) V3 [22] were used to analyse possible changes in splice sites.

### Protein sequence alignment of antithrombin from vertebrates

To determine the degree of conservation we used the results of the protein sequence alignment of antithrombin from 45 vertebrate genomes [23].

### Structural visualization

For structural visualization we used the native structure of α-antithrombin (1E05) [24].

### Nomenclature

Mutalyzer 2.0.22 (https://www.mutalyzer.nl/) was used to check the descriptions of each SERPINC1 sequence variant according to the standard Human Genome Sequence Variation Society (HGVS) nomenclature [http://www.hgvs.org/mutnomen) of the UK Association for Clinical Genetic Science and the American College for Medical Genetics [25,26]. Reference sequences used were NP_000479.1, NM_000488.3 (cDNA) and NG_012462.1 (genomic). For convenience, the mature amino acid numbering (minus the 32 amino acid signal peptide) is also shown in parenthesis.

## RESULTS

A total of 21 families were included, of which the original proband was available for 15 families. For the other 6 families, a family member with previously diagnosed antithrombin deficiency and a history of VTE was used as surrogate proband for genetic testing. Sixty-six relatives were available for testing. No deficiency of protein C or S was found. Families H and Q had surrogate probands that carry the F5 R506Q. Furthermore, 3 members of Family I, including the proband carry the F2 G20210A.

### *SERPINC1* gene analysis and *in silico* prediction

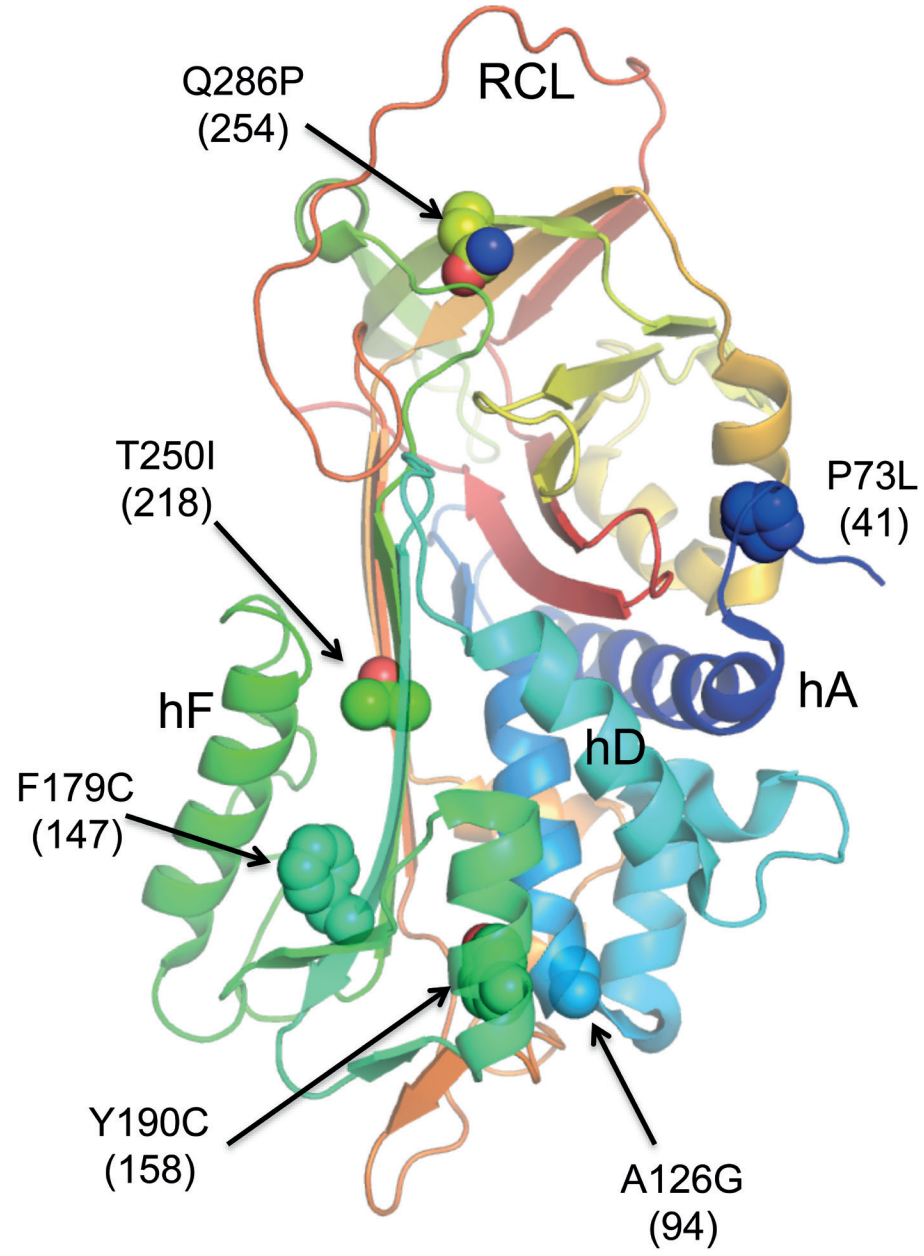
Eighteen out of 21 families had a detrimental SERPINC1 gene mutation that was only found in antithrombin-deficient individuals (Table I). Three families (D, P and U) remained mutation-negative after application of additional mutation screening (MLPA and promotor region). In total, we identified 13 different SERPINC1 gene mutations, with 8 missense mutations, 3 deletions, 1 insertion, and 1 splice site variant (Table I). This mutation profile is in agreement with previous reports [http://www.hgmd.cf.ac.uk/ac/; https://www1.imperial.ac.uk/departmentofmedicine/divisions/experimentalmedicine/haematology/coag/antithrombin/].

Eight of these mutations have been reported previously, whereas 5 were novel (Fig 1). One of the novel mutations occurred in 3 probands/families. Upon further questioning, we learned that two families were related.

All SERPINC1 gene mutations were found in a heterozygous state. All in silico predictions confirmed the pathogenicity of all mutations, except for p.Tyr190Cys (158), p.Val327Glyfs*16 (285), and p.Glu345del (303). In case of p.Tyr190Cys (158), all in silico prediction tools, except for MutPred, predicted this mutation to be pathogenic. UsingMutPred, p.Tyr190Cys (158) had a score of 0.31, not high enough (0.50) for it to be designated as a pathogenic mutation. For the novel p.Val327Glyfs*16 (285) we retrieved a prediction with only Mutationtaster, which was 'disease causing'. Interestingly, mutationtaster classified p.Glu345del (303) as being a polymorphism, which is in contrast with Chowdhury et al. [27], who clearly stated that it is a known disease causing mutation at this position. Based on the protein sequence alignment of antithrombin from 45 vertebrate genomes, all missense mutations were shown to affect a site that was highly conserved (70%), whereas three out of 8 missense mutations affected a site that was fully conserved (100%) [23]. These results suggest a strong negative effect on the overall structure of antithrombin.



**Figure 1. Structure of native antithrombin showing position of missense mutations.**




Native alpha antithrombin (1E05) is shown as a ribbon diagram, coloured in rainbow, from N-terminus (blue) to C-terminus red. It is shown in the standard orientation (reactive centre loop on top, RCL), but turned to the side to better visualize the sites of the mutations. Helices (h) A, D and F are indicated. For convenience, we also included in the text the mature amino acid numbering (minus the 32 amino acid signal peptide) between brackets.

**Table 1. Characteristics of 21 antithrombin deficient families**

| Family | Sex | Age at VTE | AT:act (%) | AT:ag (%) | HAB ratio | Type | Location | cNomen | pNomen | References | In silico prediction[a] | Tested family members | AT def/ mutation | Normal/ mutation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | M | 17 | 49 | 70 | 0.97 | IIPE | Exon 3 | c.536T>G | p.Phe179Cys | None | Pathogenic | 1 | 1/1 | 0/0 |
| B | V | 24 | 56 | 67 | 0.93 | I | Exon 5 | c.857A>C | p.Gln286Pro | None | Pathogenic | 8 | 8/8 | 0/0 |
| C | M | No VTE | 72 | 96 | 0.77 | IIHBS[f] | Exon 2 | c.218C>T | p.Pro73Leu | [41] | Pathogenic | 4 | 2/2 | 2/0 |
| D | M | 70 | 61 | 42 | 0.91 | I | No mutation detected | | | | NA | 2 | NA | NA |
| E | V | 27 | 49 | 46 | 0.90 | I | Exon 5 | c.857A>C | p.Gln286Pro | None | Pathogenic | 4 | 3/3 | 1/0 |
| F | M | No VTE | 55 | 82 | 0.95 | IIPE | Exon 2 | c.377C>G | p.Ala126Gly | [28] | Pathogenic | 4 | 3/3 | 1/0 |
| G | V | 16 | 59 | 93 | 0.94 | IIPE | Exon 3 | c.569A>G | p.Tyr190Cys | [40] | Pathogenic[b] | 7 | 4/4 | 3/0 |
| H | M | 17 | 47 | 41 | 0.88 | I | Intron 4 | c.763-1G>A | | None | Pathogenic | 4 | 2/2 | 2/0 |
| I | V | 40 | 53 | 77 | 0.93 | IIPE | Exon 2 | c.377C>G | p.Ala126Gly | [28] | Pathogenic | 4 | 4/4 | 0/0 |
| J | V | 66 | 61 | 58 | 0.93 | I | Exon 5 | c.979dup | p.Val327Glyfs*16 | None | Pathogenic[c] | 5 | 2/2 | 3/0 |
| K | V | No VTE | 72 | 46 | 0.94 | I | Exon 2 | c.337_338delCT | p.Leu113Efs*15 | [27] | Pathogenic[c] | 1 | 1/1 | 0/0 |
| L | M | 29 | 68 | 99 | 0.83 | IIPE | Exon 4 | c.749C>T | p.Thr250Ile | None | Pathogenic | 2 | 2/2 | 0/0 |
| M | M | 41 | 67 | 53 | 0.93 | I | Exon 5 | c.1056del | p.Met352Ilefs*12 | [42] | Pathogenic[c] | 7 | 4/4 | 3/0 |
| N | V | 17 | 58 | 73 | 0.95 | I | Exon 5 | c.1033_1035del | p.Glu345del | [27] | Pathogenic[d] | 2 | 2/2 | 0/0 |
| O | V | 21 | 51 | 103 | 1.01 | II[e] | Exon 7 | c.1246G>T | p.Ala416Ser | [39] | Pathogenic | 2 | 1/1 | 1/0 |
| P | V | 26 | 69 | 48 | 0.96 | I | No mutation detected | | | | NA | 2 | NA | NA |
| Q | V | 25 | 71 | 128 | 0.72 | IIHBS[f] | Exon 2 | c.218C>T | p.Pro73Leu | [41] | Pathogenic | 4 | 2/2 | 2/0 |
| R | M | 4 | 70 | 136 | 0.90 | IIRS | Exon 7 | c.1274G>A | p.Arg425His | [38] | Pathogenic | 3 | 1/1 | 2/0 |
| S | V | 62 | 49 | 49 | 0.93 | I | Exon 5 | c.857A>C | p.Gln286Pro | None | Pathogenic | 6 | 3/3 | 3/0 |
| T | V | 27 | 56 | 76 | 0.89 | IIPE | Exon 3 | c.569A>G | p.Tyr190Cys | [40] | Pathogenic[b] | 5 | 2/2 | 3/0 |
| U | M | No VTE | 53 | 58 | 0.88 | I | No mutation detected | | | | NA | 2 | NA | NA |

[a] Results of in silico prediction for pathogenicity was combined. For more details on type of tools used see methods section.
[b] All in silico prediction tools, except for MutPred, predicted this mutation to be pathogenic.
[c] We retrieved a prediction with only Mutationtaster, which was 'disease causing'.
d We retrieved a prediction with only Mutationtaster, which was 'polymorphism'.
[e] This mutation is called antithrombin Cambridge II and is known to form an unique class among the type II mutations [4].
[f] Questionable association (see text). NA denotes not applicable.



### Genotype-phenotype associations

Overall, 52% of probands had a type I, and 48% had a type II antithrombin deficiency. All three mutation-negative probands had a type I antithrombin deficiency with a mean antithrombin antigen of 51% (range 42-63%), and a mean antithrombin activity of 60% (range 50-69%). The mean HAB ratio in these probands was 0.93, which indicates that the interaction between antithrombin and heparin is normal (>0.8). Of the families where we could detect an causative mutation, 44% had a type I antithrombin deficiency and 56% had a type II antithrombin deficiency. In families with a type I, the mean antithrombin antigen was 53% (range 41-73%), and the mean antithrombin activity was 54% (range 44-72% ).

The families with a type II antithrombin deficiency had a mean antithrombin antigen of 88% (range 60-136%), and the mean antithrombin activity of 60% (range 49-72% ). The type II could be further subdivided on basis of the type of mutation and HAB ratio, where a HAB ratio below 0.8 indicates a type II HBS while a HAB ratio above 0.8 indicates either type I or the type II (RS of PE). We found the following subtypes, type II PE, (33%), type II RS (6%), type II HBS (11%), and those carrying the antithrombin Cambridge II mutation (6%). In the HBS patients the mean HAB ratio was 0.73 (range 0.67-0.77).

## DISCUSSION

Antithrombin deficiency (OMIM 107300) is associated with an increased risk of venous thrombosis. Therefore, unravelling the molecular background of this thrombophilic disorder is important to give more insight in risk of developing venous thrombosis. Our study detected a causal mutation in 86% of the families with antithrombin deficiency. These results are in agreement with a recent study by Luxembourg et al [28] and Caspers et al [29], who found a candidate gene mutation in 80% and 84% of all patients, respectively.

The results of the tests remained uninformative in three type I families (D, P and U). Linkage studies would have increased our chance of finding the molecular defect in these three families, but were not part of this study. Nonetheless, these three families did include first- and second-degree relatives with antithrombin type I deficiency. This makes it likely that this phenotype is caused by the genetic defect found in these families. Despite the absence of linkage data, other potential causes for antithrombin deficiency in these patients may be found outside the direct molecular context of SERPINC1 gene regulation, such as microRNAs [30] or the antithrombin-modulating gene LARGE1 [31]. Recently, de la Morena-Barrio et al. identified hypoglycosylation, not only of antithrombin but also of other N-glycoproteins in 8 out of 30 antithrombin-deficient cases without alterations in SERPINC1 [32]. Moreover, evidence is mounting with regard to the association between DNA methylation and thrombophilic risk factors [33-35].

The novel mutations included 3 missense mutations, 1 splice site variant, and 1 insertion. The novel missense mutation p.Phe179Cys (147) was identified in 1 family (A). This mutation is on s2A under helix F and will probably result in rapid conversion from native to latent. This would explain the relative normal antigen level and low activity. Another novel missense mutation p.Thr250Ile (218) was also found in only 1 family (L). We termed this mutation as being novel, because there was no published clinical data on this variant. The primary submitter was NHLBI Exome Sequencing Project (ESP). The minor allele frequency was A=0.00021978. This mutation is on s3A and is underneath hF and the hF-loop. It should increase the rate of latent conversion. Both mutations are expected to allow correct folding to native, but to increase the rate of conversion to latent. This seems similar to the wibble/wobble mutations described by Beauchamp et al. [36], and fully explains the discrepancy between antigen level and activity. We therefore termed these mutations as type II PE.

The novel missense mutation, p.Gln286Pro (254) was associated with type I antithrombin deficiency. Its position is consistent with induced polymerisation via the C-terminal mechanism, resulting in a secretion defect, with similar low antigen and activity levels. Noteworthy, another mutation at p.Gln286 (p.Gln286His) has been reported in dbSNP (rs139463995). This mutation has an allele frequency of 8.247e-06 [http://exac.broadinstitute.org/]. Furthermore, *in silico* prediction tools predicted this mutation to be pathogenic. Taken together, these results suggest that a mutation at this site is rare and unfavourable.

We identified 1 novel splice site variant at the acceptor site of exon 4. This variant was found in 1 family (H). Using NNSplice [21] and Human Splicing Finder (HSF) V3 [18], this variant was predicted to have a negative effect on splicing. This variant was associated with type I antithrombin deficiency. Such an association has been described before [37].

The final novel mutation p.Val327Glyfs*16 was detected in exon 5 in 1 family (J). The apparent conformational sensitivity of serpins suggests that this insertion would never tolerate secretion. This is in agreement with the type I deficiency status.

In all families the mutations were only found in antithrombin-deficient individuals. However, for families A and K only 1 individual was available for testing (Table I). For families O and R, all tested family members, beside the proband, were non-deficient but did not carry a SERPINC1 mutation (Table I). Not enough family members were tested in these four families to be sure that the molecular defect is disease causing. Three out of four SERPINC1 mutations have been described previously[27,38,39].

In this study, almost half of the SERPINC1 mutations were associated with type I antithrombin deficiency, whereas the other half was associated with a type II antithrombin deficiency. In general, these associations do not deviate from the literature. However, in the study of Luxembourg et al. p.Ala126Gly (94) was associated with a type I phenotype[28], whereas our results led us to conclude that it is clearly a type II. There might be some folding defect leading to slightly normal antigen levels (82%), but the activity is clearly decreased



(50%). The position of the mutation, highlighted in Fig 1, would also probably result in conversion to latent. Furthermore, there are some discrepancies between phenotype and in silico predictions of some type II mutations. For instance, on the basis of antithrombin levels and HAB ratio, p.Pro73Leu can be classified as being a type II HBS[38]. However, structural assessment of this mutation may question the association with type II, let alone with being type II HBS. The location is the flexible N-terminus, and although this is in position to affect the binding of heparin, there is no structural reason why it should do so. Further research is required to determine how the Pro to Leu substitution will affect heparin binding, hence, support the association with type II HBS phenotype. Additionally, p.Tyr190Cys (158) is on helix E and underlies sheet A. It would be predicted to result in a rapid conversion to latent. In the literature the reported association with antithrombin deficiency was said to be controversial for p.Tyr190Cys mutation as it did not cosegregate with the phenotype in the pedigrees from a previous study [40]. However, our study showed a complete segregation and strong association with a type II. Based on the structural prediction we designated this mutation as type II PE.

Finally, in our study, the individual carrying the Cambridge II mutation had low antithrombin activity, which is in line with what would be expected using full-length heparin in the activity assay [4].

In conclusion, our study showed that, in most cases, antithrombin deficiency can be explained by an underlying SERPINC1 mutation, but that approximately 14% of clearly hereditary deficiencies cannot be explained. These results also highlight the importance of future research outside the conventional

SERPINC1 gene approach. Finally, this study added several novel mutations to the already growing list of SERPINC1 mutations, thereby adding to our knowledge of the molecular background of antithrombin deficiency.

**Acknowledgements**

We would like to acknowledge Dr Gary Moore and Ms Naomi de Jager (Viapath Haemostasis & Thrombosis Laboratories at Guy's & St. Thomas' Hospitals, London, UK) for providing the assay settings for the heparin-antithrombin binding assay. Finally, we want to thank Krista A. Kooi BSc (Department of Genetics, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands) for her technical support with the MLPA. This study was partly funded by an unrestricted grant from Bayer.

**Author contribution**

RM, FNC, ABM, KM and MVL performed the research. RM,FNC, ABM, KM and MVL designed the research study. RM,FNC, ABM, JAH, KM and MVL analysed the data. RM,FNC, ABM, JAH, KM and MVL wrote the paper.

## REFERENCES


1. Levy JH, Sniecinski RM, Welsby IJ, Levi M. Antithrombin: anti-inflammatory properties and clinical applications. *Thromb Haemost*. 2016;115(4):712-728.
2. Law RH, Zhang Q, McGowan S, et al. An overview of the serpin superfamily. *Genome Biol*. 2006;7(5):216.
3. Lane DA, Bayston T, Olds RJ, et al. Antithrombin mutation database: 2nd (1997) update. For the Plasma Coagulation Inhibitors Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost*. 1997;77(1):197-211.
4. Mushunje A, Zhou A, Carrell RW, Huntington JA. Heparin-induced substrate behavior of antithrombin Cambridge II. *Blood*. 2003;102(12):4028-4034.
5. Wells PS, Blajchman MA, Henderson P, et al. Prevalence of antithrombin deficiency in healthy blood donors: a cross-sectional study. *Am J Hematol*. 1994;45(4):321-324.
6. Sakata T, Okamoto A, Mannami T, Matsuo H, Miyata T. Protein C and antithrombin deficiency are important risk factors for deep vein thrombosis in Japanese. *J Thromb Haemost*. 2004;2(3):528-530.
7. De Stefano V, Simioni P, Rossi E, et al. The risk of recurrent venous thromboembolism in patients with inherited deficiency of natural anticoagulants antithrombin, protein C and protein S. *Haematologica*. 2006;91(5):695-698.
8. Di Minno MN, Dentali F, Lupoli R, Ageno W. Mild antithrombin deficiency and risk of recurrent venous thromboembolism: a prospective cohort study. *Circulation*. 2014;129(4):497-503.
9. Heijboer H, Brandjes DP, Buller HR, Sturk A, ten Cate JW. Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis. *N Engl J Med*. 1990;323(22):1512-1516.
10. Rossi E, Za T, Ciminello A, Leone G, De Stefano V. The risk of symptomatic pulmonary embolism due to proximal deep venous thrombosis differs in patients with different types of inherited thrombophilia. *Thromb Haemost*. 2008;99(6):1030-1034.
11. Bock SC, Harris JF, Balazs I, Trent JM. Assignment of the human antithrombin III structural gene to chromosome 1q23-25. *Cytogenet Cell Genet*. 1985;39(1):67-69.
12. Brouwer JL, Veeger NJ, Kluin-Nelemans HC, van der Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. *Ann Intern Med*. 2006;145(11):807-815.
13. Mahmoodi BK, Brouwer JL, Ten Kate MK, et al. A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. *J Thromb Haemost*. 2010;8(6):1193-1200.
14. Moore GW, de Jager N, Cutler JA. Development of a novel, rapid assay for detection of heparin-binding defect antithrombin deficiencies: the heparin-antithrombin binding (HAB) ratio. *Thromb Res*. 2015;135(1):161-166.
15. de la Morena-Barrio ME, Anton AI, Martinez-Martinez I, et al. Regulatory regions of SERPINC1 gene: identification of the first mutation associated with antithrombin deficiency. *Thromb Haemost*. 2012;107(3):430-437.
16. Luxembourg B, D'Souza M, Korber S, Seifried E. Prediction of the pathogenicity of antithrombin sequence variations by in silico methods. *Thromb Res*. 2015;135(2):404-409.
17. Li B, Krishnan VG, Mort ME, et al. Automated inference of molecular mechanisms of disease from amino acid substitutions. *Bioinformatics*. 2009;25(21):2744-2750.
18. Capriotti E, Calabrese R, Casadio R. Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. *Bioinformatics*. 2006;22(22):2729-2734.
19. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249.
20. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods*. 2014;11(4):361-362.
21. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. *J Comput Biol*. 1997;4(3):311-323.
22. Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Res*. 2009;37(9):e67.
23. Kumar A, Bhandari A, Sarde SJ, Goswami C. Sequence, phylogenetic and variant analyses of antithrombin III. *Biochem Biophys Res Commun*. 2013;440(4):714-724.
24. McCoy AJ, Pei XY, Skinner R, Abrahams JP, Carrell RW. Structure of beta-antithrombin and the effect of glycosylation on antithrombin's heparin affinity and activity. *J Mol Biol*. 2003;326(3):823-833.
25. Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PE. Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. *Hum Mutat*. 2008;29(1):6-13.
26. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
27. Chowdhury V, Olds RJ, Lane DA, et al. Identification of nine novel mutations in type I antithrombin deficiency by heteroduplex screening. *Br J Haematol*. 1993;84(4):656-661.
28. Luxembourg B, Delev D, Geisen C, et al. Molecular basis of antithrombin deficiency. *Thromb Haemost*. 2011;105(4):635-646.
29. Caspers M, Pavlova A, Driesen J, et al. Deficiencies of antithrombin, protein C and protein S - practical experience in genetic analysis of a large patient cohort. *Thromb Haemost*. 2012;108(2):247-257.
30. Teruel-Montoya R, Rosendaal FR, Martinez C. MicroRNAs in hemostasis. *J Thromb Haemost*. 2015;13(2):170-181.
31. de la Morena-Barrio ME, Buil A, Anton AI, et al. Identification of antithrombin-modulating genes. Role of LARGE, a gene encoding a bifunctional glycosyltransferase, in the secretion of proteins? *PLoS One*. 2013;8(5):e64998.
32. de la Morena-Barrio ME, Martinez-Martinez I, de Cos C, et al. Hypoglycosylation is a common finding in antithrombin deficiency in the absence of a SERPINC1 gene defect. *J Thromb Haemost*. 2016;14(8):1549-1560.
33. El-Maarri O, Becker T, Junen J, et al. Gender specific differences in levels of DNA methylation at selected loci from human total blood: a tendency toward higher methylation levels in males. *Hum Genet*. 2007;122(5):505-514.
34. Dick KJ, Nelson CP, Tsaprouni L, et al. DNA methylation and body-mass index: a genome-wide analysis. *Lancet*. 2014;383(9933):1990-1998.
35. Aissi D, Dennis J, Ladouceur M, et al. Genome-wide investigation of DNA methylation marks associated with FV Leiden mutation. *PLoS One*. 2014;9(9):e108087.
36. Beauchamp NJ, Pike RN, Daly M, et al. Antithrombins Wibble and Wobble (T85M/K): archetypal conformational diseases with in vivo latent-transition, thrombosis, and heparin activation. *Blood*. 1998;92(8):2696-2706.
37. Jochmans K, Lissens W, Yin T, et al. Molecular basis for type 1 antithrombin deficiency: identification of two novel point mutations and evidence for a de novo splice site mutation. *Blood*. 1994;84(11):3742-3748.
38. Erdjument H, Lane DA, Panico M, Di Marzo V, Morris HR. Single amino acid substitutions in the reactive site of antithrombin leading to thrombosis. Congenital substitution of arginine 393 to cysteine in antithrombin Northwick Park and to histidine in antithrombin Glasgow. *J Biol Chem*. 1988;263(12):5589-5593.

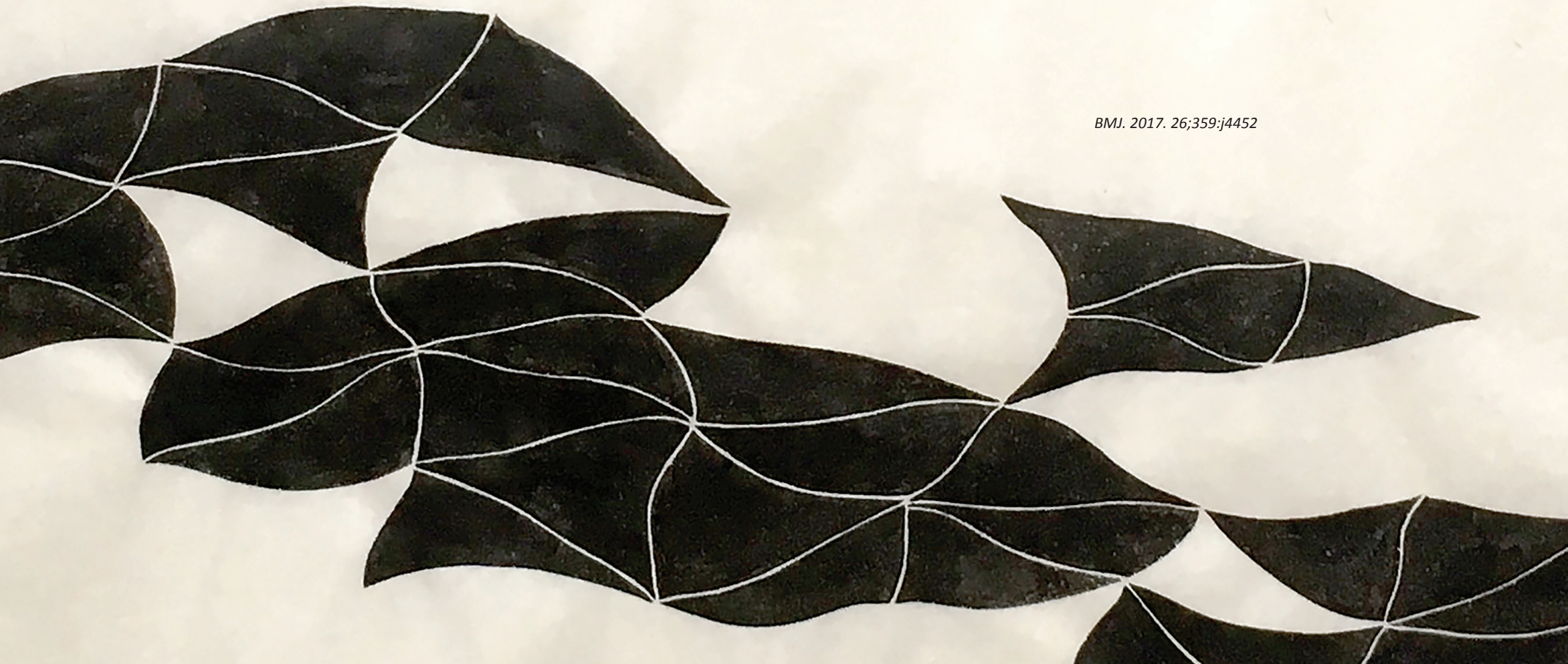
39. Perry DJ, Daly M, Harper PL, et al. Antithrombin Cambridge II, 384 Ala to Ser. Further evidence of the role of the reactive centre loop in the inhibitory function of the serpins. *FEBS Lett*. 1991;285(2):248-250.
40. Millar DS, Wacey AI, Ribando J, et al. Three novel missense mutations in the antithrombin III (AT3) gene causing recurrent venous thrombosis. *Hum Genet*. 1994;94(5):509-512.
41. Tran TH, Bounameaux H, Bondeli C, Honkanen H, Marbet GA, Duckert F. Purification and partial characterization of a hereditary abnormal antithrombin III fraction of a patient with recurrent thrombophlebitis. *Thromb Haemost*. 1980;44(2):87-91.
42. van Boven HH, Olds RJ, Thein SL, et al. Hereditary antithrombin deficiency: heterogeneity of the molecular basis and mortality in Dutch families. *Blood*. 1994;84(12):4209-4213.

# 6

## PREGNANCY, THROMBOPHILIA AND THE RISK OF A FIRST VENOUS THROMBOSIS: A SYSTEMATIC REVIEW AND BAYESIAN META-ANALYSIS

F. Nanne Croles, Kazem Nasserinejad, Johannes J. Duvekot,
Marieke J.H.A. Kruip, Karina Meijer, Frank W.G. Leebeek

*BMJ. 2017. 26;359:j4452*



## ABSTRACT

### Background
Pregnancy and thrombophilia increase the risk of venous thromboembolism (VTE). Current guidelines on management of pregnant women with thrombophilia without prior VTE are mainly based on family-cohort studies and a systematic review of nine studies from 2006.

### Objective
To provide evidence to support updated guidelines for the management of pregnant women with hereditary thrombophilia in order to reduce the risk of a first venous thromboembolism (VTE) in pregnancy.

### Design
Systematic review and bayesian meta-analysis.

### Data sources
Embase, Medline, Web of Science, Cochrane Library, and Google Scholar from inception through 14 November 2016.

### Review methods
Observational studies that reported on pregnancies without the use of anticoagulants and the outcome of first VTE for women with thrombophilia were eligible for inclusion. VTE was considered established if it was confirmed by objective means, or when the patient had received a full course of a full dose anticoagulant treatment without objective testing.

### Results
36 studies were included in the meta-analysis. All thrombophilias increased the risk for pregnancy associated VTE (probabilities ≥91%). Regarding absolute risks of pregnancy associated VTE, high risk thrombophilias were antithrombin deficiency (antepartum: 7.3%, 95% credible interval 1.8% to 15.6%; post partum: 11.1%, 3.7% to 21.0%), protein C deficiency (antepartum: 3.2%, 0.6% to 8.2%; post partum: 5.4%, 0.9% to 13.8%), protein S deficiency (antepartum: 0.9%, 0.0% to 3.7%; post partum: 4.2%; 0.7% to 9.4%), and homozygous factor V Leiden (antepartum: 2.8%, 0.0% to 8.6%; post partum: 2.8%, 0.0% to 8.8%). Absolute combined antepartum and postpartum risks for women with heterozygous factor V Leiden, heterozygous prothrombin G20210A mutations, or compound heterozygous factor V Leiden and prothrombin G20210A mutations were all below 3%.

### Conclusions
Women with antithrombin, protein C, or protein S deficiency or with homozygous factor V Leiden should be considered for antepartum or postpartum thrombosis prophylaxis, or both. Women with heterozygous factor V Leiden, heterozygous prothrombin G20210A mutation, or compound heterozygous factor V Leiden and prothrombin G20210A mutation should generally not be prescribed thrombosis prophylaxis on the basis of thrombophilia and family history alone. These data should be considered in future guidelines on pregnancy associated VTE risk.

## WHAT THIS PAPER ADDS

### What is already known on this topic

- Women with thrombophilia have an increased risk of pregnancy-associated venous thrombosis (VTE)
- Absolute risk estimates vary considerably between studies and guidelines
- Guidance on whom should be given thrombosis prophylaxis varies considerably between guidelines

### What this study adds

- Thrombosis prophylaxis is generally not warranted for heterozygous factor V Leiden mutation, heterozygous prothrombin G20210A mutation, or compound heterozygous factor V Leiden and prothrombin G20210A mutation
- Antepartum and postpartum thrombosis prophylaxis is warranted in women with antithrombin deficiency or protein C deficiency and a positive family history of VTE
- Postpartum prophylaxis only is warranted for women with protein S deficiency and a positive family history of VTE



## INTRODUCTION

Venous thromboembolism (VTE) is a major causes of pregnancy related mortality[1,2] and morbidity[3]. VTE increases the risk of permanent work-related disability[4]. Pregnancy increases the risk of VTE in women fivefold to sixfold compared to age matched controls[5]. A positive family history for VTE further increases the risk of pregnancy-associated VTE 3.7-fold to 8.5 fold[6,7]. Hereditary thrombophilia increases the risk of pregnancy associated VTE up to 34-fold as reported by Robertson et al[8] in a systematic review including nine studies in 2006. Women are at an even higher risk for pregnancy-associated VTE in the six-week postpartum period than during pregnancy.

Absolute risk estimates of pregnancy-associated VTE in women with inherited thrombophilia mentioned in the American College of Chest Physicians' (ACCP) 2012 guidelines[9] are based on a limited number of cohort studies, and on an estimated baseline VTE incidence multiplied by the odds ratios found in the systematic review by Robertson et al[8]. To date, no systematic review or meta-analysis of absolute risk of pregnancy associated VTE for women with thrombophilia has been published. Data on risks of pregnancy associated VTE of compound heterozygous factor V Leiden and prothrombin G20210A mutation (FVL&FII) were not included in Robertson's systematic review[8] nor in the ACCP 2012 guidelines[9]. As several studies on thrombophilia and the risk of pregnancy-associated VTE have been published since 2006, an updated systematic review is needed.

Owing to limitations of the data available, guidelines on how to manage pregnant women with thrombophilia without previous VTE differ considerably in their recommendations on the use of thrombosis prophylaxis in the antepartum and postpartum period[10]. We performed an updated systematic review and meta-analysis of studies on the relative and absolute risks of pregnancy associated VTE in women with established thrombophilia with no previous VTE to improve prevention of pregnancy associated VTE in women.

## METHODS

The results of this systematic review and meta-analysis are reported according to PRISMA guidelines[11]. Details of the study protocol are available in web appendix 1.

### Data sources and Searches

We systematically searched Embase, Medline, Web of Science, Cochrane Library, and Google Scholar using a search strategy constructed with the aid of a biomedical information specialist (see web appendix 1 for details). References were checked for additional articles missed by the primary search. The search was first performed on 8 February 2016, and updated on 14 November 2016. Two authors (F.N.C. and J.J.D) independently selected articles for further reading. All articles were included or excluded based on predefined selection criteria. Decisions were based on consensus. Authors were contacted by e-mail if clarification was required.

### Study selection

Case-control or cohort studies that contained extractable information on the number of pregnancies and the outcome first VTE were eligible. The studies contained information on participants with specific inherited thrombophilias: antithrombin deficiency, protein C deficiency, protein S deficiency, factor V Leiden mutation (heterozygous or homozygous), prothrombin G20210A mutation (heterozygous or homozygous) and/or compound heterozygous factor V Leiden and prothrombin G20210A mutations. Studies were included if the definition of VTE was compatible with the following: VTE was considered established if it was confirmed by objective means, or when the patient had received a full course of a full dose anticoagulant therapy without objective testing. Studies contained data on pregnancies without use of anticoagulants. To minimise bias we excluded cohort studies that did not separate proband data from family member data. Studies were peer-reviewed, written in English, and published from 1970-2016. In the event of duplicate publications, we chose the most informative report.

### Data extraction and quality assessment

Data were extracted in duplicate using standardized forms. Disagreements were resolved by consensus. VTE events were deep venous thrombosis, pulmonary embolism, deep venous thrombosis and pulmonary embolism, or non-separately described superficial venous thrombosis events. Separately reported superficial venous thrombosis events were not considered as VTE events. In case-control studies, we recorded the number of women with the selected thrombophilia per number of cases and for each number of respective controls. For each group in cohort studies, we recorded the number of pregnancy associated first VTE events for each number of pregnancies. These events were classified as antepartum or postpartum if this information was available. For the calculation of odds ratios, in case-control studies we compared women with separate thrombophilias with their respective study controls and in cohort studies we compared women with separate thrombophilias with women without established thrombophilia (non-carriers). Information on women in cohort studies who carried less-established thrombophilic abnormalities, such as MTHFR mutations[8] and associated hyperhomocysteinemia, was analysed in the non-carriers group. A family history for VTE influences the VTE risk[6,12], so we classified studies as family studies or a non-family studies. Quality assessment was performed using the Newcastle-Ottawa Scale (NOS)[13], scoring: see web appendix 1.



### Statistical analysis

Bayesian random-effects meta-analysis was used to estimate odds ratios (ORs) and absolute risks of VTE for each thrombophilia. See web appendix 2 for more information on the Bayesian analysis used. Risk distributions are summarised by the median as a point estimate and 95% credible intervals (ie, area under the posterior distribution). Relatively non-informative priors were used for all variables (see web appendix 2). In addition, we estimated the probability of odds ratios and absolute risks being above thresholds of interest [14]. For calculation of the absolute risk of postpartum VTE, we disregarde pregnancies that had resulted in a VTE antepartum. To handle partly missing information on time of VTE events, Bayesian multiple imputation was used (ie, the assumption that data were missing completely at random). Computations and graphics were performed with "R"[15]. All Bayesian computations were performed using the Marcov chain Monte Carlo (MCMC) sampler via Jags interface in R[16]. MCMC sampling was run for each analysis for 1,500,000 iterations after discarding the first 50,000 iterations (burn-in).

### Data synthesis and analysis

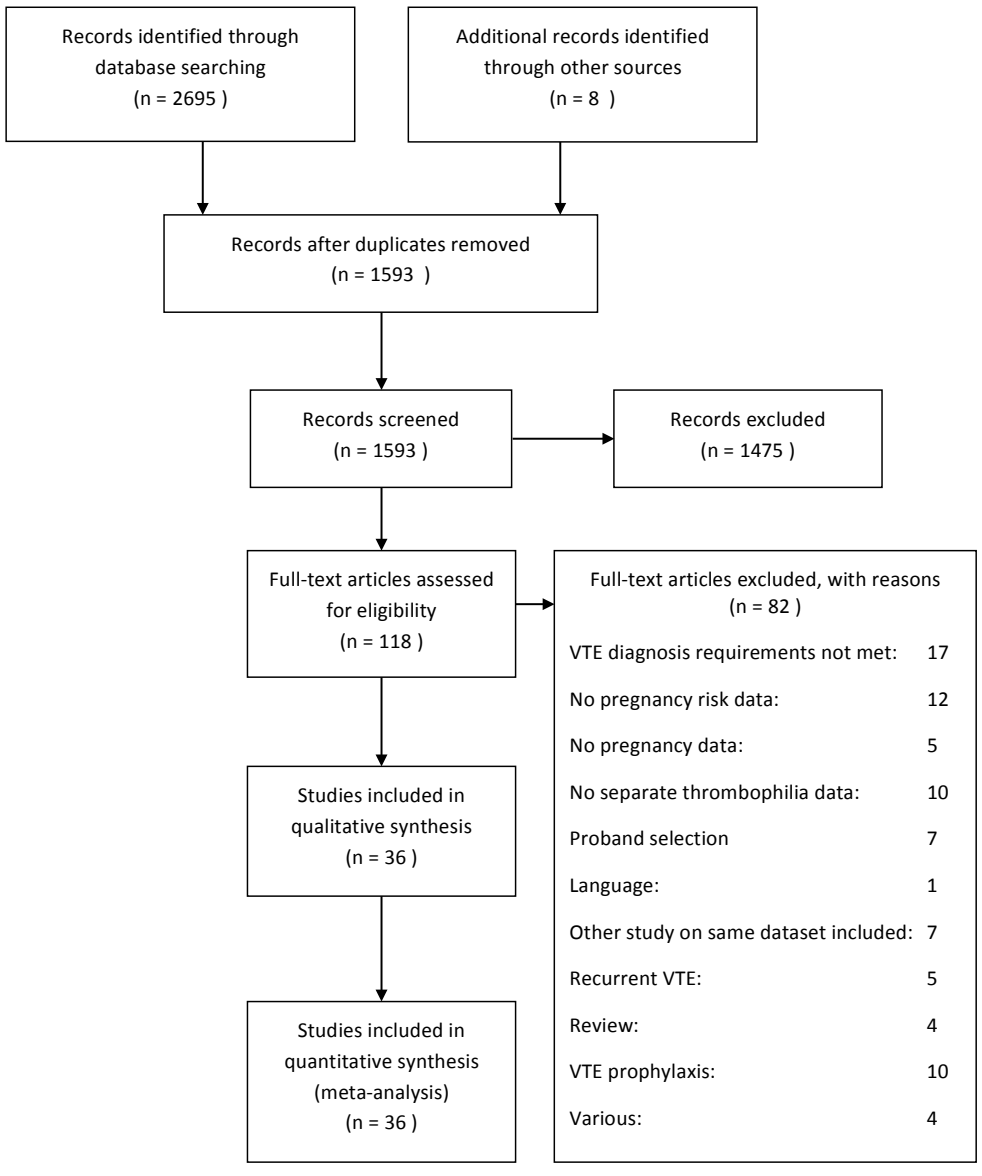
As a different absolute risk of pregnancy-associated VTE was expected in non-carriers in family studies as compared to non-family studies, we used the labels "family study" and "non-family study" as a covariate in the analyses. In addition, the analyses of the absolute risks of pregnancy-associated VTE in family studies and non-family studies are shown separately for women with thrombophilia and for non-carriers. We performed a sensitivity analysis by comparing the results of meta-analysis of all studies with the results of meta-analysis of studies remaining after elimination of studies not requiring objective VTE diagnoses (odds ratio only) and after elimination of low quality studies (defined as studies with a score below the median on the NOS). For the odds ratios, we also carried out separate case-control and cohort-study analyses.

### Patient involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for design or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no

plans to disseminate the results of the research to study participants or the relevant patient community. It was not evaluated whether the studies included in the review had any patient involvement

**Figure 1. Study selection process**






## RESULTS

### Summary of selection process

The search yielded 2695 articles. We identified another eight articles for full review by checking references. After removal of duplicates 1593 articles were screened on title and abstract for further reading. Figure 1 shows that a total of 118 articles were selected for full review. Eighty two articles were excluded for various reasons. Finally, 36 articles were selected for the systematic review and meta-analysis.

Most studies covered a selection of the thrombophilias of interest. The following numbers of studies were included on the separate thrombophilias: antithrombin deficiency: 4 cohort studies [17-20] and 7 case-control studies[21-27], protein C deficiency: 3 cohort studies[18-20] and 8 case-control studies[21,23-29], protein S deficiency: 3 cohort studies[18-20] and 8 case-control studies[21-27,29], heterozygous factor V Leiden: 17 cohort studies[7,30-45] and 11 case-control studies[21-26,28,29,46-48], homozygous factor V Leiden: 6 cohort studies[7,30,31,43,45,49] and 6 case-control studies[21,23,24,26,47,48], heterozygous prothrombin G20210A mutation: 5 cohort studies[31,32,39,50,51] and 10 case-control studies[21-26,28,46-48], homozygous prothrombin G20210A mutation: 1 cohort study[31] and 3 case-control studies[22,26,47], compound heterozygous factor V Leiden and prothrombin G20210A mutation: 3 cohort studies[39,45,49] and 5 case-control studies[21,22,24,26,47].

### Study characteristics

Web appendix 1 summarises the study characteristics. The total number of pregnancies reported was 41297, of which 5994 were in women with thrombophilia and 35303 pregnancies were in controls or non-carriers. Of 36 studies, 12 were case-control studies, 15 were family cohort studies and nine were non-family cohort studies. Fourteen cohort studies were retrospective and 10 were prospective. Of 24 cohort studies, 15 contained data on the time of venous thromboembolism (VTE) events (antepartum or postpartum).

### Risk of bias within studies

Web appendix 2 provides details of study quality assessment as reflected by NOS-scoring. The median of NOS scores was 8 in both case-control and cohort studies. Two cohort studies had no non-carrier group[18,41]. Most studies required an objective VTE diagnosis. The six studies that allowed antithrombotic treatment without an objective VTE diagnosis were not allowed a star in the quality assessment score for “selection of cases” in case-control studies or for “assessment of outcome” in cohort studies[7,17,30,31,36,50].

### Relative risk of pregnancy-associated VTE:

Table 1 summarises the meta-analyses of the relative risk of pregnancy-associated VTE expressed as odds ratios. High odds ratios were found for antithrombin deficiency (9.5;

95% credible interval: 1.6-31.9), protein C deficiency (9.3; 95%CrI:2.1-43.1), Protein S deficiency (7.0; 95%CrI:1.3-21.9), heterozygous factor V Leiden mutation (6.4; 95%CrI:4.0-9.7), homozygous factor V Leiden (35.8; 95%CrI:0.4-137.8), heterozygous prothrombin G20210A mutation (5.1; 95%CrI:2.6-9.8), and compound heterozygous factor V Leiden and prothrombin G20210A mutation (21.2; 95%CrI:1.6-89.0). Odds ratios were high but not statistically significant for homozygous prothrombin G20210A mutation (21.1; 95%CrI:0.0-727.4). Web appendix 1 shows the forest-plots for the odds ratio calculations.

**Absolute risk of pregnancy-associated VTE:**

Table 2 summarises the results of the meta-analyses of absolute risks of pregnancy-associated VTE for women with the separate thrombophilias. The forest plots are given in web appendix 1.

**Table 1. Relative risk of pregnancy-associated VTE**

| Thrombophilic defect | No. of women with thrombophilia, with VTE/Total* | No. of controls, with VTE/Total* | No. of studies | OR, all studies (95% CrI) | OR, only case-control studies (95% CrI) | OR, only cohort studies (95% CrI) | OR, studies requiring objectively confirmed VTE (95% CrI) | No. of high quality studies | OR, high quality studies (95% CrI) | Probability (%) of OR > 1 for high quality studies |
|---|---|---|---|---|---|---|---|---|---|---|
| Antithrombin deficiency | 48/153 | 710/2178 | 10 | 9.5 (1.6-31.9) | 5.0 (0.6-24.7) | 25.9 (0.0-176.3) | 7.9 (1.2-25.5) | 7 | 8.9 (0.3-34.7) | 99 |
| Protein C deficiency | 49/180 | 691/2024 | 10 | 9.3 (2.1-43.1) | 12.3 (0.0-139.8) | 5.9 (0.0-49.6) | 9.3 † (2.1-43.1) | 6 | 7.7 (0.0-48.1) | 99 |
| Protein S deficiency | 53/192 | 700/2212 | 10 | 7.0 (1.3-21.9) | 6.7 (0.2-34.7) | 7.2 (0.0-35.4) | 7.0 † (1.3-21.9) | 7 | 6.9 (0.2-24.6) | 91 |
| Factor V Leiden, heterozygous | 305/3345 | 923/34626 | 27 | 6.4 (4.0-9.7) | 7.2 (4.3-12.6) | 3.9 (0.2-11.9) | 5.9 (3.8-9.0) | 21 | 6.4 (3.9-10.6) | 100 |
| Factor V Leiden, homozygous | 27/80 | 919/26906 | 12 | 35.8 (0.4-137.8) | 128.9 (3.0-3093.9) | 12.0 (0.0-69.9) | 31.8 (0.2-145.3) | 9 | 46.7 (4.1-193.1) | 100 |
| Prothrombin G20210A mutation heterozygous | 94/1433 | 1002/21736 | 15 | 5.1 (2.6-9.8) | 4.9 (2.0-11.4) | 4.9 (0.0-23.7) | 4.5 (2.2-8.5) | 12 | 4.3 (2.0-8.8) | 100 |
| Prothrombin G20210A mutation homozygous | 4/5 | 559/19692 | 4 | 21.1 (0.0-727.4) | 18.2 (0.0-1073.7) | NA | 14.8 (0.0-1052.9) | 3 | 13.4 (0.0-584.2) | 99 |
| Compound heterozygous factor V Leiden & prothrombin G20210A mutation | 45/242 | 803/2652 | 8 | 21.2 (1.6-89.0) | 45.4 (0.6-478.6) | 8.6 (0.5-62.3) | 21.2 † (1.6-89.0) | 7 | 26.9 (1.1-147.1) | 100 |

Odds ratios for pregnancy associated VTE for each thrombophilia compared with controls or non-carriers, including sensitivity analyses. The sensitivity analyses show the meta-analyses per study type, meta-analyses of only studies requiring an objective VTE diagnosis and of only high-quality studies (NOS-score≥8). The final column shows that the probability for the odds ratio being >1 for high quality studies are ≥99% for all thrombophilias. n/No: number of women with VTE out of the total number of women in the study population. OR: Odds ratio. 95%CrI: 95% credible interval. NOS score: Newcastle-Ottawa scale assessment score. NA: Not available: only 1 study, meta-analysis was not performed. *Case-control and cohorts with non-carriers group. † All studies for this thrombophilia required objectively confirmed VTE.

**Table 2: absolute risks of pregnancy-associated VTE**

| Thrombophilic defect | Studies on selected thrombophilia, n | n/No. Women with thrombophilia in cohorts | Absolute risk of VTE antepartum and postpartum combined, % pregnancies (95% CrI) | n/No. Of events with information on time of occurence | Absolute risk of VTE antepartum, all studies, % pregnancies (95% CrI) | Probability of antepartum AR>1%, for all studies, % | Probability of antepartum AR>3%, for all studies, % | Absolute risk of VTE postpartum based on all data, % pregnancies (95% CrI) | Probability of postpartum AR>1%, for all studies, % | Probability of postpartum AR>3%, for all studies, % | No of studies with NOS≥8 | n/No. Women with thrombophilia in cohorts with NOS≥8 | Absolute risk of VTE antepartum and postpartum combined, % pregnancies (95% CrI) for studies with NOS≥8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antithrombin deficiency | | | | | | | | | | | | | |
| Family cohort studies | 4 | 23/125 | 16.6 (0.0-45.1) | 23/23 | 7.3 (1.8-15.6) | 100 | 96 | 11.1 (3.7-21.0) | 100 | 99 | 3 | 13/105 | 10.5 (0.0-30.3) |
| Protein C deficiency | | | | | | | | | | | | | |
| Family cohort studies | 3 | 10/137 | 7.8 (0.0-33.8) | 10/10 | 3.2 (0.6-8.2) | 96 | 54 | 5.4 (0.9-13.8) | 99 | 83 | 2 | 4/123 | 2.6 (0.0-13.5) |
| Protein S deficiency | | | | | | | | | | | | | |
| Family cohort studies | 3 | 7/135 | 4.8 (0.0-20.0) | 7/7 | 0.9 (0.0-3.7) | 47 | 9 | 4.2 (0.7-9.4) | 98 | 73 | 2 | 6/130 | 3.6 (0.0-37.0) |
| Factor V Leiden, heterozygous | 17 | 45/3031 | 1.1 (0.3-1.9)† | 37/45 | 0.4 (0.1-0.9) | 2 | 0 | 2.0 (0.9-3.7) | 97 | 11 | 13 | 43/2604 | 1.3 (0.5-2.2) |
| Family cohort studies | 8 | 35/1359 | 2.4 (0.9-4.4) | 34/35 | 0.4 (0.0-0.9) | 3 | 0 | 2.5 (1.2-4.4) | 100 | 25 | 6 | 33/1291 | 2.4 (0.6-5.4) |
| Non-family cohort studies | 9 | 10/1672 | 0.4 (0.0-0.9) | 3/10 | 0.7 (0.0-2.6) | 31 | 4 | 0.4 (0.0-1.8) | 15 | 2 | 7 | 10/1313 | 0.6 (0.0-1.2) |
| Factor V Leiden, homozygous | 6 | 5/58 | 6.2 (0.0-18.0) | 5/5 | 2.8 (0.0-8.6) | 86 | 47 | 2.8 (0.0-8.8) | 85 | 46 | 5 | 5/56 | 7.9 (0.4-23.0) |
| Family cohort studies | 3 | 4/35 | 8.3 (0.0-29.6) | 4/4 | NA | NA | NA | NA | NA | NA | 2 | 4/33 | 9.9 (0.0-39.7) |
| Non-family cohort studies | 3 | 1/23 | 5.6 (0.0-34.3) | 1/1 | NA | NA | NA | NA | NA | NA | 3 | 1/23 | 5.6 (0.0-34.3) |
| Prothrombin G20210A mutation heterozygous | 5 | 14/1322 | 0.9 (0.2-2.0) | 9/14 | 0.0 (0.0-0.2) | 0 | 0 | 0.9 (0.2-2.0) | 41 | 1 | 5 | 14/1322 | 0.9 (0.2-2.0) |
| Family cohort studies | 4 | 11/998 | 1.0 (0.0-2.5) | 9/11 | NA | NA | NA | NA | NA | NA | 4 | 11/998 | 1.0 (0.0-2.5) |
| Non-family cohort studies | 1 | 3/324 | 0.8 (0.1-2.0) | 0/3 | NA | NA | NA | NA | NA | NA | 1 | 3/324 | 0.8 (0.1-2.0) |
| Combined heterozygous factor V Leiden & prothrombin G20210A mutation | | | | | | | | | | | | | |
| Family cohort studies | 3 | 5/199 | 2.5 (0.0-9.5) | 3/5 | NA | NA | NA | NA | NA | NA | 3 | 5/199 | 2.5 (0.0-9.5) |
| Non-carriers, overall | | | | | | | | | | | | | |
| Family cohort studies | 13 | 14/2330 | 0.5 (0.2-1.0) | 13/14 | NA | NA | NA | NA | NA | NA | 12 | 12/2293 | 0.4 (0.1-0.8) |
| Non-family cohort studies | 9 | 20/31245 | 0.1 (0.0-0.1) | 9/20 | NA | NA | NA | NA | NA | NA | 7 | 18/30791 | 0.1 (0.0-0.1) |

Absolute risks for pregnancy-associated VTE per thrombophilia, per study type. The analyses shown are the meta-analyses of absolute risks of all studies, including absolute risk of antepartum VTE and postpartum VTE, and the meta-analyses of high-quality studies (NOS-score≥8) only. The probability of absolute risks of pregnancy-associated VTE being above the treatment-thresholds of 1% and 3% are shown as well.
n/No: number of women with VTE out of studied number of women with selected thrombophilia.
95%CrI: 95% credible interval. NNT: number of women needed to treat to prevent one VTE. NOS score: Newcastle-Ottawa scale assessment score. NA: Not available: (As overall risk is below any treatment threshold: no relevance).
†Significant effect of family studies as compared to non-family studies.

### Antithrombin, protein C and protein S deficiency

Only family studies were available For women with antithrombin, protein C, and protein S deficiency. The highest absolute risk of pregnancy associated VTE was found in antithrombin deficient women (Overall: 16.6%; 95%CrI:0.0-45.1%, antepartum: 7.3%; 95%CrI:1.8-15.6, postpartum: 11.1%; 95%CrI:3.7-21.0%). Absolute risks were also high for protein C deficiency (Overall: 7.8%; 95%CrI:0.0-33.8%, antepartum: 3.2%, 95%CrI:0.6-8.2%, postpartum 5.4%; 95%CrI:0.9-13.8%), and protein S deficiency (overall: 4.8%; 95%CrI:0.0-20.0%, antepartum: 0.9% 95%CrI:0.0-3.7%, postpartum: 4.2%; 95%CrI:0.7-9.4).

### Heterozygous and homozygous factor V Leiden mutations

For women with heterozygous factor V Leiden mutation, the absolute risk of pregnancy-associated VTE was 1.1% overall (95%CrI:0.3-1.9%, antepartum 0.4%; 95%CrI:0.1-0.9%, postpartum: 2.0%; 95%CrI:0.9-3.7%). The absolute risk for pregnancy-associated VTE derived from family studies (2.4%) was higher than the risk derived from non-family studies (0.4%). For women with homozygous factor V Leiden mutation, the absolute risk of pregnancy-associated VTE was 6.2% overall (95%CrI:0.0-18.0%), 2.8% antepartum (95%CrI:0.0-8.6%), 2.8% postpartum (95%CrI:0.0-8.8%). The absolute risk of pregnancy-associated VTE derived from family studies (8.3%, 95%CrI:0.0-29.6%) was higher than the risk derived from non-family studies (5.6%; 95%CrI:0.0-34.3%).

### Heterozygous and homozygous prothrombin G20210A mutation

For women with heterozygous prothrombin G20210A mutation, the absolute risk of pregnancy-associated VTE was 0.9% overall (95%CrI:0.2-2.0%), 0.0% antepartum (95%CrI: 0.0-0.2%), and 0.9% postpartum (95%CrI:0.2-2.0%). The absolute risk of pregnancy associated VTE derived from family studies (1.0%) was higher than the risk derived from nonfamily studies (0.8%).

The one woman with homozygous prothrombin G20210A mutation in the cohort study that qualified for inclusion did not have a VTE event.

### Combined heterozygous factor V Leiden and prothrombin G20210A mutation

For women with heterozygous factor V Leiden and prothrombin G20210A combined, the absolute risk of pregnancy associated VTE in family studies was 2.5% overall (95%CrI:0.0-9.5%). Data were too limited for an analysis of antepartum and postpartum risk. No non-family studies without thrombosis prophylaxis were found.

### Antepartum versus postpartum VTE

Of 143 VTE events in cohort studies, information on time of occurrence was available in 115 events. Thirty (26%) occurred antepartum, 85 (74%) events occurred postpartum. In high-risk thrombophilias (antithrombin deficiency, protein C deficiency, protein S deficiency, and homozygous factor V Leiden mutation) 17/44 events (44%) occurred antepartum, in low risk thrombophilias (heterozygous factor V Leiden, heterozygous prothrombin G20210A mutation, or compound heterozygous factor V Leiden and prothrombin G20210A mutation) or non-carriers 13/71 events (18%) occurred antepartum. For each thrombophilia the absolute risk of pregnancy-associated VTE antepartum or post partum was calculated (table 2). As posterior distributions of the absolute risk estimates were skewed, the probabilities of absolute risks of pregnancy-associated VTE being above the thresholds of 1% and 3% were calculated to aid in clinical decision making (table 2). We were not able to perform this analysis for compound heterozygous factor V Leiden and prothrombin G20210A mutation or for the family and non-family subgroups for homozygous factor V Leiden mutation or heterozygous prothrombin G20210A mutation owing to a lack of data.



### Risk of bias across studies: Additional and sensitivity analyses

In family studies on non-carriers the absolute risk of pregnancy associated VTE was higher (0.5%; 95%CrI:0.2-1.0%) than in the non-family studies (0.1%; 95%CrI:0.0-0.2%). Table 1 shows the results of the sensitivity-analyses of relative risks. To investigate if study type (case-control v cohort) had an impact on odds ratio estimates, we performed separate analyses for these study types. Higher estimates were found in case-control studies for protein C deficiency and heterozygous factor V Leiden, homozygous factor V Leiden and compound heterozygous factor V Leiden and prothrombin G20210A mutation. For antithrombin deficiency the odds ratio in cohort studies was higher. Table 1 shows that removing studies which did not require objective VTE diagnoses had only limited impact on odds ratio estimates. To investigate if study quality had impact on relative risk estimates of pregnancy-associated VTE, the meta-analyses were repeated only including “high-quality” studies (NOS-score ≥ 8, the median score). Table 1 shows that similar odds ratio distributions and point estimates were obtained. The small number of pregnancies and VTE events per study and variable results per study cause skewed posterior distributions and hence statistical insignificance in the odds ratios for some thrombophilias. When calculating the probability of the odds ratios being greater than 1 in the high quality studies, for all thrombophilias we found a probability of 91% for protein S deficiency and 99% or higher for the others.

Table 2 summarises the results of most sensitivity analyses of absolute risk estimates. To investigate if study quality had an impact on absolute risk estimates of pregnancy associated VTE, the meta-analyses were repeated including only high quality studies. Using this criterion, cohorts that lacked a non-carriers group (n=2) were also excluded. Web appendix 1 shows the results of all individual meta-analyses of high quality studies for all investigated thrombophilias. Overall, removal of low quality studies had an impact on the point estimates of absolute risks of pregnancy associated VTE, which are generally lower in studies with a high NOS score (≥8). However, the 95% credible intervals for all studies and for high quality studies remain similar for most thrombophilias.

## DISCUSSION

Women with hereditary thrombophilia have an increased risk of pregnancy-associated venous thromboembolism (VTE). High absolute risk estimates for pregnancy-associated VTE were found for women with the rare thrombophilias antithrombin, protein C or protein S deficiency, and homozygous factor V Leiden mutation, but not for compound heterozygous factor V Leiden and prothrombin G20210A mutation. For women with the more prevalent heterozygous factor V Leiden or heterozygous FII mutations the absolute risk was mildly increased, and limited to women with a positive family history. Most VTE occurred in the postpartum period. The absolute risk of pregnancy-associated VTE was higher for non-carriers in family studies as compared to non-carriers in non-family studies. Sensitivity analysis shows that point estimates and the 95% credible intervals of absolute risks were influenced by study quality in family cohort studies only. Compared with American College of Chest Physicians' (ACCP) guidelines[9], our meta-analysis regarding only high quality studies shows markedly higher absolute risk estimates for women with antithrombin deficiency and protein C deficiency, and more conservative absolute risk estimates for protein S deficiency. In addition, high risks of pregnancy-associated VTE were found for women with combined heterozygous factor V Leiden and prothrombin G20210A mutation. Antithrombin deficiency, protein C deficiency, protein S deficiency, homozygous factor V Leiden mutation, and homozygous prothrombin G20210A mutation should all be considered high risk thrombophilias for pregnant women based on our assessment of all studies and only high quality studies. In contrast, absolute risk estimates for heterozygous factor V Leiden and for heterozygous prothrombin G20210A mutation were lower compared with ACCP guidelines [9].

### Strengths and weaknesses of this study

Bayesian statistics enabled us to perform both a meta-analysis of the relative risks and the first meta-analysis of the absolute risks of pregnancy-associated VTE in women with thrombophilia. It allowed for calculating the antepartum and postpartum risk using multiple imputation when only partial information was available, and for calculating the probability for risks being greater than treatment thresholds. It is useful to take specific thresholds of interest into account, especially with skewed risk distributions as is the case in all analyses. 95% Credible intervals are not constructed the same way as in classic statistics (see technical appendix). Therefore, the probability of the OR being >1 can still be above 99%, even though the 95% credible intervals include 1 (table 1). In contrast with classical statistics, Bayesian statistics provide inferences that are conditional on the data and are exact. Therefore, Bayesian analysis interpretation is more straightforward and more direct in terms of belief (e.g., a 95% credible interval for a risk is that region in which we believe the risk to be with a probability of 95%). Classic statistics' 95% confidence intervals describe the range where the exact risk estimate will be should the same experiment be repeated infinitely. Bayesian random-effects meta-analysis is better able to compute study-specific credible intervals depending on data from other studies[52]. Bayesian analysis is the most suited methodology for this dataset of rare events: classical statistical methods cannot deal with infinite variance of individual studies, when event rates in a group of 0 or 100 percent occur[14]. Most classical meta-analytical software automatically replace 0 by a fixed value (typically 0.5). This usually has the undesirable effect of biasing study estimates[53]. Regarding the studies included our review, this issue would have occurred in all odds ratio estimates if classical meta-analysis software would have been used, but this poses no problem for Bayesian analysis.

Odds ratio estimates correlate poorly with absolute risk estimates in our meta-analysis. This can be explained by differences of VTE risk of non-carriers: The absolute risk of pregnancy-associated VTE for non-carriers from family studies is sixfold higher than the risk from non-family studies. Only the absolute risk of pregnancy-associated VTE found in the non-family study non-carriers resembles the risk in the general population of 0.08% found by Simpson et al[54], whereas most odds ratio data stem from family studies and case-control studies. Controls from those studies do not resemble the general population, and therefore odds ratios are not easily translated to absolute risks in the general population.

The sensitivity analysis shows the impact of study quality on point estimates of VTE risk. This is most clear for the absolute risk of pregnancy-associated VTE in women with deficiencies of antithrombin, protein C or protein S. Nonetheless, the meta-analyses including only high quality studies still show increased absolute risk-estimates, with similar risk distributions (table 2). Overall, for women with high-risk thrombophilias risk estimates of pregnancy-associated VTE have wide credible intervals. This is caused by the low number of women with rare thrombophilias investigated in studies and variable results between studies. All available data were used because the number of thrombotic events was low for the antepartum- and postpartum analyses. For protein C and protein S deficiency, this causes a discrepancy between the antepartum, postpartum, and high-quality overall risk estimates, which could change recommendations for protein C deficiency.

Non-family cohort studies only rarely contain information on women with high-risk thrombophilias. The data therefore provide little information on women with high-risk thrombophilias that do not have a positive family history for VTE. The most recent study included in our review by Gerhardt et al[21] is a case-control study that provide absolute VTE risk estimates in pregnant women with high-risk thrombophilias regardless of family history. However, unusual cut-off values (40-90% of normal) for defining mild and severe deficiencies of antithrombin, protein C and protein S were used. Furthermore, the calculation of absolute risk estimates from case-control data required making assumptions of the absolute risk in control women. The absolute VTE risk estimates for non-carriers in their analyses (0.105-0.20%) did not take family- or non-family setting into account, which we have shown to differ significantly (0.5% v 0.1%).

A limitation of our meta-analysis is that it has not taken into account the changes in the methods of diagnosis of thrombophilia that have occurred, especially for protein S deficiency[55], as we were limited to the methodology used in the individual studies. Preferably studies require objective diagnosis of VTE. Using studies that allowed treatment without objective diagnoses as a diagnostic criterion for VTE is not optimal. However, data on rare thrombophilias are sparse, and removing those studies in the sensitivity analysis of odds ratio estimates did not show major changes to the main results.

**Strengths and weaknesses in relation to other studies**

This systematic review has identified the largest number of studies on thrombophilia and pregnancy-associated VTE so far, including 41297 pregnancies[8,56-58]. When comparing the present review on thrombophilia and pregnancy-associated VTE to the review by Robertson et al[8] published in 2006, some differences should be appreciated. The review by Robertson et al included nine case-control studies, of which the latest was reported in 2003. Fifteen out of 36 studies included in our study predate 2003. This reflects a more inclusive search strategy. Our meta-analysis, based on more data, revealed higher odds ratios for pregnancy-associated VTE for many thrombophilias, as compared with controls. These differences between both reviews are important, as absolute risk estimates in current guidelines are mainly based on the review by Robertson et al[9,10]. In the ACCP 2012 Guidelines[9] 10 additional studies have been reviewed[20,33,40,41,45,49,50,59-61]. We excluded three of these additional studies because they failed to meet the inclusion criteria of "separate data per thrombophilia"[61], "objective VTE diagnosis"[60] and "no use of VTE prophylaxis"[59]. Nevertheless, our review included 36 studies, compared with 19 studies used in the ACCP 2012 guidelines. For women with deficiencies of antithrombin, protein C, or protein S, the results of our meta-analysis show notably higher relative and absolute risks of pregnancy-associated VTE as compared to the ACCP guidelines. Lower absolute risks were found for women with heterozygous factor V Leiden or heterozygous prothrombin G20210A mutation.

**Recommendations**

In order to determine an acceptable number of women needed to treat to prevent a VTE, the benefits of reducing the risk of pregnancy-associated VTE through treatment have to be weighed against the burdens of self-injecting LMWH during a certain period. However, efficacy and optimal doses of LMWH prophylaxis are still a matter of debate[62]. Using data on hip arthroplasty, ACCP guidelines estimate the relative effects of prophylaxis using low molecular weight heparins (LMWH) in women [9]. A 2014 Cochrane review found insufficient evidence on which to base recommendations for dose and duration of thrombosis prophylaxis during pregnancy and the postpartum period[63]. To data, evidence on LMWH prophylaxis in pregnant women is insufficient to establish a number needed to treat. Fortunately, prospective investigator-initiated studies in women with thrombophilia are



being performed, and the results are eagerly awaited[64,65]. In guidelines thresholds for LMWH prophylaxis of the absolute risk of pregnancy-associated VTE ante- or postpartum vary from 1% to 3%[10,66,67]. It must be considered that recommendations would differ considerably between thresholds of 1% or 3% (see web appendix 1). In the most recent guideline, experts set the threshold of the absolute risk of VTE for each antepartum or postpartum period at 3% by consensus [10]. Therefore, for our recommendations this threshold of 3% per antepartum or postpartum period was also used. We used all study data for our recommendations because credible intervals of all studies overlapped with those of high-quality studies, and because more study data allowed for more precise risk estimates.

Based on the thresholds of 3% and our meta-analyses recommendations of the current ACCP 2012 guidelines[9] but also other guidelines as summarized by Bates et al.[10] can be challenged with regard to thrombophilia. In the ACCP 2012 guidelines, LMWH prophylaxis in both the antepartum and postpartum period is only suggested for women with homozygous factor V Leiden or homozygous prothrombin G20210A mutations. We suggest antepartum prophylaxis and prophylaxis up to six weeks postpartum for women with no previous VTE with antithrombin or protein C deficiency if they have a positive family history. For women with homozygous factor V Leiden mutations, thrombosis prophylaxis may be considered after consideration of family history and additional risk factors for VTE, because the probabilities of ante- and postpartum VTE risk being >3% are 47% and 46%, and risk estimates from family studies are higher than the risk estimates from non-family studies. For women with protein S deficiency and a positive family history for VTE, we suggest prophylaxis using low molecular weight heparins only up to six weeks post partum. For homozygous prothrombin G20210A mutation we cannot give recommendations because of the lack of cohort data and the poor correlation of odds ratios with absolute risks. ACCP guidelines suggest thrombosis prophylaxis both antepartum and post partum [9].

In contrast with ACCP guidelines, for women with heterozygous factor V Leiden or heterozygous prothrombin G20210A mutations we suggest clinical vigilance only, even with a positive family history of VTE. The same applies for women with compound heterozygous factor V Leiden and prothrombin G20210A mutations. Additional personal risk factors for VTE such as obesity and immobilisation, should be taken into account when deciding on thrombosis prophylaxis in individual patients.

## CONCLUSIONS

In this systematic review and meta-analysis we show that all women with inherited thrombophilia have an increased risk of pregnancy associated VTE. We have calculated high absolute risk estimates for pregnancy associated VTE for women with deficiency of antithrombin, protein C, or protein S and a positive family history for VTE, and for all women with homozygous factor V Leiden mutation. Although further research is needed for more precise risk estimates, in these women routine thrombosis prophylaxis should be considered. The risk estimates and chosen thresholds for treatment do not support routine use of thrombosis prophylaxis in women with heterozygous factor V Leiden or heterozygous prothrombin G20210A mutations or compound heterozygous factor V Leiden and prothrombin G20210A mutation. The data in this review should be considered in future management guidelines on pregnancy associated VTE risk in women with hereditary thrombophilia.



**Acknowledgements**
The authors thank Wichor Bramer from the Medical Library of the Erasmus MC for his help in designing the search strategies of this review.

**Competing interests**
All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisation that might have an interest in the submitted work in the previous three years;

M. Kruip has received research funding from Ferring Pharmaceuticals, Pfizer, Daiichi Sankyo, Boehringer Ingelheim, Bayer Nederland and from Pfizer Nederland. K. Meijer received research support from Bayer, Baxter, Sanquin and Pfizer; speaker fees from Bayer, Sanquin, Boehringer Ingelheim and BMS; consulting fees from Uniqure. F.W.G. Leebeek is consultant for Uniqure, Shire and Novonordisk (fees go to the institution), received unrestricted research grants of CSL Behring and Baxalta (Shire), and travel support from Roche.

**Funding**
No external funding was used for this manuscript.

**Contributors**
FNC conceived the study and is the guarantor. FNC and KN designed the study methods. FNC and JJD identified the relevant studies. FNC and KN did the analysis. FNC prepared the initial manuscript. All authors were involved in the revision of this manuscript. All authors read and approved the final manuscript and are accountable for all aspects of the work, including the accuracy and integrity.

**Ethics approval**
Not required.

**Data sharing**
The data from this study and/or statistical code are available upon request from the corresponding author or from the articles referred to in this manuscript.

**Transparency**
The manuscript's guarantor (FNC) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

## REFERENCES

1. Hasegawa J, Sekizawa A, Tanaka H, et al. Current status of pregnancy-related maternal mortality in Japan: a report from the Maternal Death Exploratory Committee in Japan. *BMJ Open*. 2016;6(3):e010304.
2. Cantwell R, Clutton-Brock T, Cooper G, et al. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom. *BJOG*. 2011;118 Suppl 1:1-203.
3. Wik HS, Jacobsen AF, Sandvik L, Sandset PM. Prevalence and predictors for post-thrombotic syndrome 3 to 16 years after pregnancy-related venous thrombosis: a population-based, cross-sectional, case-control study. *J Thromb Haemost*. 2012;10(5):840-847.
4. Braekkan SK, Grosse SD, Okoroh EM, et al. Venous thromboembolism and subsequent permanent work-related disability. *J Thromb Haemost*. 2016;14(10):1978-1987.
5. Melis F, Vandenbrouke JP, Buller HR, Colly LP, Bloemenkamp KW. Estimates of risk of venous thrombosis during pregnancy and puerperium are not influenced by diagnostic suspicion and referral basis. *Am J Obstet Gynecol*. 2004;191(3):825-829.
6. Tosetto A, Frezzato M, Rodeghiero F. Prevalence and risk factors of non-fatal venous thromboembolism in the active population of the VITA Project. *J Thromb Haemost*. 2003;1(8):1724-1729.
7. Lindqvist PG, Svensson PJ, Maršál K, Grennert L, Luterkort M, Dahlbäck B. Activated protein C resistance (FV:Q506) and pregnancy. *Thromb Haemost*. 1999;81(4):532-537.
8. Robertson L, Wu O, Langhorne P, et al. Thrombophilia in pregnancy: A systematic review. *Br J Haematol*. 2006;132(2):171-196.
9. Bates SM, Greer IA, Middeldorp S, et al. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e691S-736S.
10. Bates SM, Middeldorp S, Rodger M, James AH, Greer I. Guidance for the treatment and prevention of obstetric-associated venous thromboembolism. *J Thromb Thrombolysis*. 2016;41(1):92-128.
11. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151(4):264-269, W264.
12. Bezemer ID, van der Meer FJ, Eikenboom JC, Rosendaal FR, Doggen CJ. The value of family history as a risk indicator for venous thrombosis. *Arch Intern Med*. 2009;169(6):610-615.
13. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp Accessed October 5th 2015. .
14. Smith TC, Spiegelhalter DJ, Thomas A. Bayesian approaches to random-effects meta-analysis: a comparative study. *Stat Med*. 1995;14(24):2685-2699.
15. R_Core_Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Accessed July 1st 2016.
16. Plummer M. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. *Proceedings of the 3rd international workshop on distributed statistical computing*. 2003;124.
17. Boven HHV, Vandenbroucke JP, Briet E. Gene-gene and gene-environment interactions determine risk of thrombosis in families with inherited antithrombin deficiency. Vol. 94: Am Soc Hematology; 1999.
18. Bucciarelli P, Rosendaal FR, Tripodi A. Risk of Venous Thromboembolism and Clinical Manifestations in Carriers of Antithrombin, Protein C, Protein S Deficiency, or Activated Protein C Resistance. A Multicenter Collaborative Family Study. *ARTERIOSCLER THROMB VASC BIOL*. 1999;19:1026-1033.

19. Folkeringa N, Brouwer JLP, Korteweg FJ, Veeger NJGM, Erwich JJHM, Van Der Meer J. High risk of pregnancy-related venous thromboembolism in women with multiple thrombophilic defects. *Br J Haematol*. 2007;138(1):110-116.
20. Friederich PW, Sanson BJ, Simioni P, et al. Frequency of pregnancy-related venous thromboembolism in anticoagulant factor-deficient women: Implications for prophylaxis. *ANN INTERN MED*. 1996;125(12):955-960.
21. Gerhardt A, Scharf RE, Greer IA, Zotz RB. Hereditary risk factors of thrombophilia and probability of venous thromboembolism during pregnancy and the puerperium. *Blood*. 2016.
22. Grandone E, Margaglione M, Colaizzo D, et al. Genetic susceptibility to pregnancy-related venous thromboembolism: Roles of factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations. *Am J Obstet Gynecol*. 1998;179(5):1324-1328.
23. Klai S, Fekih-Mrissa N, Sassi RB, Mrissa R, Rachdi R, Gritli N. Effects of prothrombotic markers and non-O blood group in maternal venous thromboembolism during pregnancy and postpartum. *Blood Coagul Fibrinolysis*. 2012;23(7):649-652.
24. Martinelli I, De Stefano V, Taioli E, Paciaroni K, Rossi E, Mannucci PM. Inherited thrombophilia and first venous thromboembolism during pregnancy and puerperium. *Thromb Haemost*. 2002;87(5):791-795.
25. Meglič L, Stegnar M, Milanez T, et al. Factor V Leiden, prothrombin 20210G → A, methylenetetrahydrofolate reductase 677C → T and plasminogen activator inhibitor 4G/5G polymorphism in women with pregnancy-related venous thromboembolism. *Eur J Obstet Gynecol Reprod Biol*. 2003;111(2):157-163.
26. Mitic G, Kovac M, Jurisic D, et al. Clinical characteristics and type of thrombophilia in women with pregnancy-related venous thromboembolic disease. *Gynecol Obstet Invest*. 2011;72(2):103-108.
27. Tam WH, Ng MHL, Yiu AKW, Lau KM, Cheng GYM, Li CY. Thrombophilia among chinese women with venous thromboembolism during pregnancy. *Gynecol Obstet Invest*. 2012;73(3):183-188.
28. Ogunyemi D, Cuellar F, Ku W, Arkel Y. Association between inherited thrombophilias, antiphospholipid antibodies, and lipoprotein A levels and venous thromboembolism in pregnancy. *Am J Perinatol*. 2003;20(1):17-23.
29. Vora S, Ghosh K, Shetty S, Salvi V, Satoskar P. Deep venous thrombosis in the antenatal period in a large cohort of pregnancies from western India. *Thromb J*. 2007;5:5-9.
30. Clark P, Walker ID, Govan L, Wu O, Greer IA. The GOAL study: A prospective examination of the impact of factor V Leiden and ABO(H) blood groups on haemorrhagic and thrombotic pregnancy outcomes. *Br J Haematol*. 2008;140(2):236-240.
31. Cochery-Nouvellon É, Mercier É, Lissalde-Lavigne G, et al. Homozygosity for the C46T polymorphism of the F12 gene is a risk factor for venous thrombosis during the first pregnancy. *J Thromb Haemost*. 2007;5(4):700-707.
32. Cordoba I, Pegenaute C, González-López TJ, et al. Risk of placenta-mediated pregnancy complications or pregnancy-related VTE in VTE-asymptomatic families of probands with VTE and heterozygosity for factor V Leiden or G20210 prothrombin mutation. *Eur J Haematol*. 2012;89(3):250-255.
33. Couturaud F, Leroyer C, Mottier D, Groupe dEtude de la Thrombose de Bretagne O. Risk factors and clinical presentation of venous thromboembolism according to the age of relatives of patients with factor V Leiden. *Thromb Haemost*. 2008;99(4):793-794.
34. Dizon-Townson D, Miller C, Sibai B, et al. The relationship of the factor V Leiden mutation and pregnancy outcomes for mother and fetus. *Obstet Gynecol*. 2005;106(3):517-524.
35. Eichinger S, Weltermann A, Philipp K, et al. Prospective evaluation of hemostatic system activation and thrombin potential in healthy pregnant women with and without factor V Leiden. *Thromb Haemost*. 1999;82(4):1232-1236.





36. Hammerová L, Chabada J, Drobný J, Bátorová A. Factor V Leiden mutation and its impact on pregnancy complications. *Acta Medica (Hradec Kralove)*. 2011;54(3):117-121.
37. Heit JA, Sobell JL, Li H, Sommer SS. The incidence of venous thromboembolism among Factor V Leiden carriers: A community-based cohort study. *J Thromb Haemost*. 2005;3(2):305-311.
38. Kjellberg U, Van Rooijen M, Bremme K, Hellgren M. Factor v Leiden mutation and pregnancy-related complications. *Am J Obstet Gynecol*. 2010;203(5):469.e461-469.e468.
39. Martinelli I, Battaglioli T, De Stefano V, et al. The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A. *J Thromb Haemost*. 2008;6(3):494-498.
40. Middeldorp S, Henkens CMA, Koopman MMW, et al. The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. *Ann Intern Med*. 1998;128(1):15-20.
41. Middeldorp S, Meinardi JR, Koopman MMW, et al. A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. *Ann Intern Med*. 2001;135(5):322-327.
42. Murphy RP, Donoghue C, Nallen RJ, et al. Prospective evaluation of the risk conferred by factor V Leiden and thermolabile methylenetetrahydrofolate reductase polymorphisms in pregnancy. *Arterioscler Thromb Vasc Biol*. 2000;20(1):266-270.
43. Samama MM, Simon D, Horellou MH, Trossaërt M, Elalamy I, Conard J. Diagnosis and clinical characteristics of inherited activated protein C resistance. *HAEMOSTASIS*. 1996;26(SUPPL. 4):315-330.
44. Simioni P, Tormene D, Prandoni P, Zerbinati P. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Vol. 99: Am Soc Hematology; 2002.
45. Tormene D, Simioni P, Prandoni P, et al. Factor V Leiden mutation and the risk of venous thromboembolism in pregnant women. *Haematologica*. 2001;86(12):1305-1309.
46. Dilley A, Austin H, El-Jamil M, et al. Genetic factors associated with thrombosis in pregnancy in a United States population. *Am J Obstet Gynecol*. 2000;183(5):1271-1277.
47. Jacobsen AF, Dahm A, Bergrem A, Jacobsen EM, Sandset PM. Risk of venous thrombosis in pregnancy among carriers of the factor V Leiden and the prothrombin gene G20210A polymorphisms. *J Thromb Haemost*. 2010;8(11):2443-2449.
48. Yilmazer M, Kurtay G, Sonmezer M, Akar N. Factor V Leiden and prothrombin 20210 G-A mutations in controls and in patients with thromboembolic events during pregnancy or the puerperium. *Arch Gynecol Obstet*. 2003;268(4):304-308.
49. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannuccio Mannucci P. Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia. *Thromb Haemost*. 2001;86(3):800-803.
50. Bank I, Libourel EJ, Middeldorp S, et al. Prothrombin 20210A mutation: A mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study. *Arch Intern Med*. 2004;164(17):1932-1937.
51. Tormene D, De Stefano V, Grandone E, et al. The G20210A prothrombin variant and the risk of venous thromboembolism or fetal loss in pregnant women: A family study. *J Thromb Haemost*. 2007;5(11):2193-2196.
52. Higgins J, Green S, (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. *The Cochrane Collaboration*. 2011.
53. Sweeting MJ, Sutton AJ, Lambert PC. What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. *Stat Med*. 2004;23(9):1351-1375.

54. Simpson EL, Lawrenson RA, Nightingale AL, Farmer RD. Venous thromboembolism in pregnancy and the puerperium: incidence and additional risk factors from a London perinatal database. *BJOG*. 2001;108(1):56-60.
55. Mulder R, Ten Kate MK, Kluin-Nelemans HC, Mulder AB. Low cut-off values increase diagnostic performance of protein S assays. *Thromb Haemost*. 2010;104(3):618-625.
56. Ziakas PD, Poulou LS, Pavlou M, Zintzaras E. Thrombophilia and venous thromboembolism in pregnancy: A meta-analysis of genetic risk. *Eur J Obstet Gynecol Reprod Biol*. 2015;191:106-111.
57. Rheaume M, Weber F, Durand M, Mahone M. Pregnancy-Related Venous Thromboembolism Risk in Asymptomatic Women With Antithrombin Deficiency: A Systematic Review. *Obstet Gynecol*. 2016;127(4):649-656.
58. Biron-Andreani C, Schved JF, Daures JP. Factor V Leiden mutation and pregnancy-related venous thromboembolism: What is the exact risk? results from a meta-analysis. *Thromb Haemost*. 2006;96(1):14-18.
59. Coppens M, van de Poel MH, Bank I, et al. A prospective cohort study on the absolute incidence of venous thromboembolism and arterial cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. *Blood*. 2006;108(8):2604-2607.
60. Middeldorp S, Libourel EJ, Hamulyák K, Van Meer JD, Büller HR. The risk of pregnancy-related venous thromboembolism in women who are homozygous for factor V Leiden. *Br J Haematol*. 2001;113(2):553-555.
61. Simioni P, Sanson BJ, Prandoni P, et al. Incidence of venous thromboembolism in families with inherited thrombophilia. *Thromb Haemost*. 1999;81(2):198-202.
62. Roeters van Lennep JE, Meijer E, Klumper FJ, Middeldorp JM, Bloemenkamp KW, Middeldorp S. Prophylaxis with low-dose low-molecular-weight heparin during pregnancy and postpartum: is it effective? *J Thromb Haemost*. 2011;9(3):473-480.
63. Bain E, Wilson A, Tooher R, Gates S, Davis LJ, Middleton P. Prophylaxis for venous thromboembolic disease in pregnancy and the early postnatal period. *Cochrane Database of Systematic Reviews*. 2014(2).
64. Bleker SM, Buchmuller A, Chauleur C, et al. Low-molecular-weight heparin to prevent recurrent venous thromboembolism in pregnancy: Rationale and design of the Highlow study, a randomised trial of two doses. *Thromb Res*. 2016;144:62-68.
65. de Jong PG, Quenby S, Bloemenkamp KW, et al. ALIFE2 study: low-molecular-weight heparin for women with recurrent miscarriage and inherited thrombophilia--study protocol for a randomized controlled trial. *Trials*. 2015;16:208.
66. Chan WS, Rey E, Kent NE, et al. Venous thromboembolism and antithrombotic therapy in pregnancy. *J Obstet Gynaecol Can*. 2014;36(6):527-553.
67. Huisman MV, Bakx R, Coppens M, et al. Richtlijn antitrombotisch beleid. Veneuze trombo-embolie tijdens de zwangerschap en postpartum periode. *https://richtlijnendatabasenl/richtlijn/antitrombotisch_beleid/preventie_vte_in_de_verloskunde/trombo-embolie_bij_zwangerschap_en_postpartumhtml*. 2016; Accessed November 1st 2016.




## DATA SUPPLEMENT

**Contents**

## TECHNICAL APPENDIX

Bayesian hierarchical modelling is a specific methodology used to combine results from multiple studies (e.g., meta-analysis) to obtain estimates[1]. Hierarchical modelling allows borrowing of information across studies, which means for larger studies we have good estimates, but for the smaller studies we may be able to borrow information from other schools to obtain more accurate estimates. The strength of borrowing depends on the homogeneity of these studies.

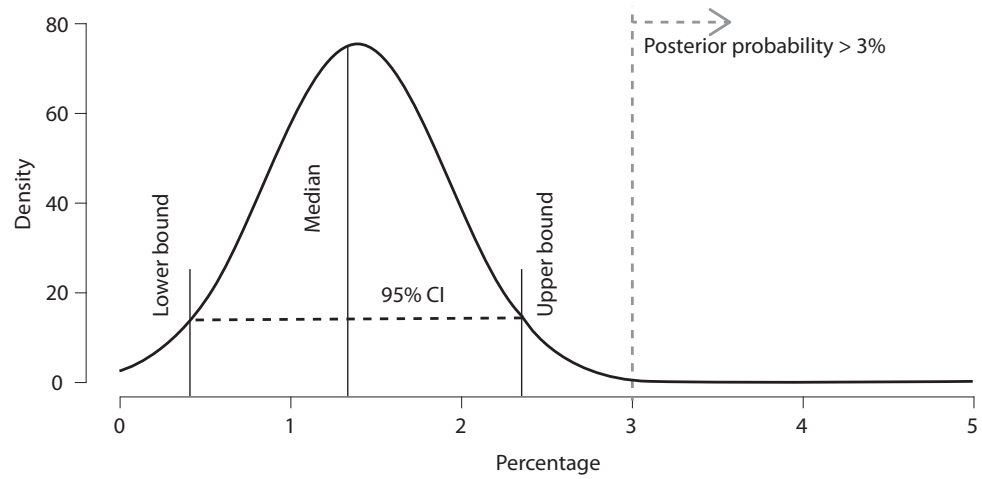
In contrast to classical statistics inference, Bayesian statistics inference assumes that parameters in the model are not fixed values, but stochastic and have a distribution. Bayesian inference enables us to combine previous available knowledge as a prior information with current data to estimate posterior distributions of parameters. The priors information of the parameters of interest can be derived via historical studies and/or with expert knowledge, but without observing the current data. In case there is no prior information about the parameters in the model, a non-informative prior is often implemented, where the idea is the posterior distributions is computed based on the current data solely (objective Bayesian).

Parameter distributions are summarized by the median as a point estimate and the 95% area under the posterior distribution of the Parameter, i.e. credible intervals (Bayesian terminology for confidence interval). Figure 1A and 1B show graphically what a risk distribution may look like, and what calculations were performed.

A wide credible interval (or confidence interval in classical statistics inference) indicates the lack of information (i.e., small sample size), which means that the data is consistent with a wide range of possible hypotheses. As an advantage, Bayesian statistics inference allows us to compute the probability that the posterior distribution is greater/smaller than a certain value (e.g., OR being >1, AR being >3%). In a very skewed posterior distribution cases, this probability (OR being >1) might be in more than 99% while the credible interval includes 1.

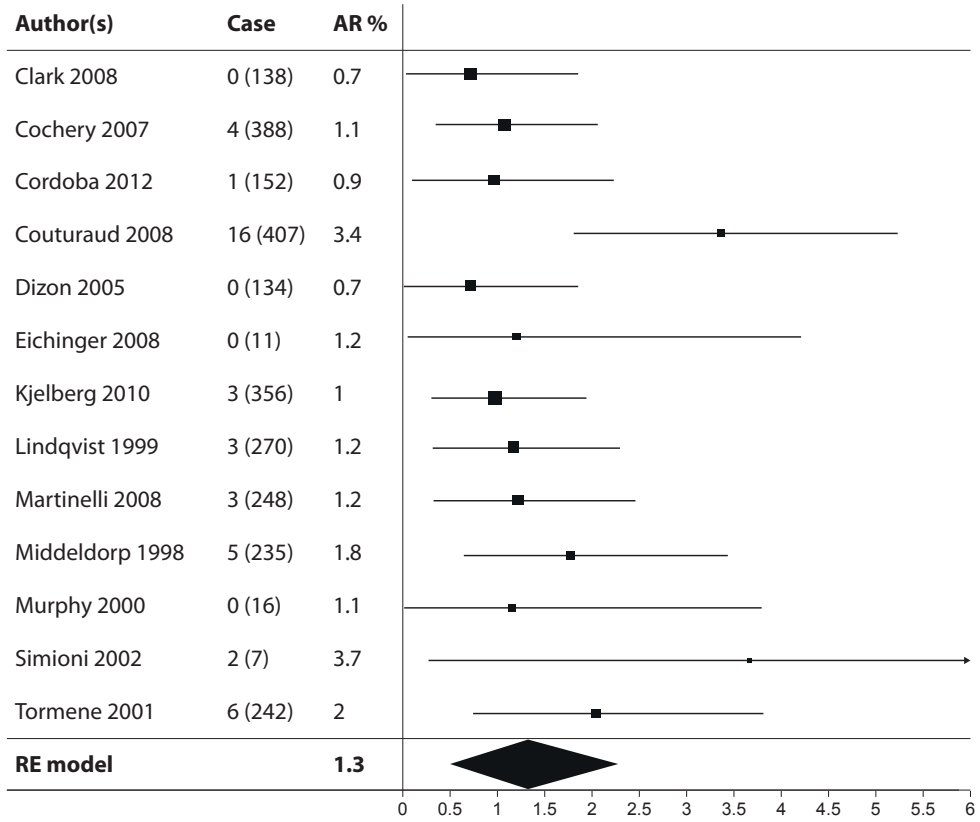


**Figure 1A. displays the posterior distribution of the absolute risk of pregnancy- associated VTE in women with heterozygous FVL mutation in family studies with NOS≥8.**




In this figure the following is demonstrated: the median of the posterior as point estimate, the 95% credible interval (the 95% area under posterior distribution, which is the interval between lower bound and upper bound), and the probability of that posterior being greater than 3% (selected cut-off).
The selected cut-off for the probability of the absolute risk of pregnancy-associated VTE being larger than 3% is small (1%), as most of the area under the curve (99%) is to the left of this cut-off value.

**Figure 1B. Forest plot for absolute risk calculations of pregnancy-associated VTE for women with heterozygous FVL mutation in family studies with NOS≥8.**




This is an example of a forest-plot of the absolute risk of pregnancy-associated VTE in women with heterozygous FII in family studies. AR: absolute risk. RE: Random effects.

## REFERENCE

1. Jackman S. *Bayesian analysis for the social sciences.* : Wiley & Sons, 2009



## SEARCH STRATEGY:

In this section we describe the search-strategy used for our systematic review for the separate databases. The number designates the number of hits per database.

| | | |
|---|---|---|
| Embase.com | 932 | 921 |
| Medline Ovid | 679 | 152 |
| Web of science | 869 | 402 |
| Cochrane | 15 | 4 |
| Google scholar | 200 | 106 |
| **Total** | **2695** | **1585** |

**Embase.com**

('pregnancy'/exp OR 'pregnant woman'/de OR 'pregnancy outcome'/de OR 'pregnancy disorder'/de OR 'pregnancy complication'/de OR 'puerperal disorder'/de OR 'puerperium'/de OR 'high risk pregnancy'/de OR (pregnan* OR puerper* OR postpartum* OR 'post partum*' OR antenatal ):ab,ti) AND ('venous thromboembolism'/exp OR 'vein thrombosis'/exp OR thrombosis/de/mj OR thromboembolism/de/mj OR (((venous OR vein ) NEAR/3 (thrombo*)) OR ((lung OR pulmonar*) NEAR/3 embolism*)):ab,ti) AND ('risk'/exp OR 'incidence'/de OR 'high risk pregnancy'/de OR (risk OR incidence*):ab,ti) AND ('thrombophilia'/de OR 'antithrombin'/de OR 'blood clotting factor 5 Leiden'/de OR 'protein S'/de OR 'protein C'/de OR 'protein C deficiency'/de OR 'protein S deficiency'/de OR 'antithrombin deficiency'/de OR 'antithrombin III deficiency'/de OR 'prothrombin'/de OR (thrombophil* OR Hypercoagulabilit* OR antithrombin* OR '5 Leiden' OR 'V Leiden' OR 'protein S' OR 'protein C' OR (natural* NEAR/3 anticoagulant*) OR prothromb* OR g20210a OR 20201A):ab,ti) AND ('observational study'/exp OR 'cohort analysis'/exp OR 'longitudinal study'/exp OR 'retrospective study'/exp OR 'prospective study'/exp OR 'epidemiological data'/de OR 'case control study'/de OR 'cross-sectional study'/de OR 'correlational study'/de OR 'population research'/de OR 'family study'/de OR 'major clinical study'/de OR 'multicenter study'/de OR 'comparative study'/de OR 'follow up'/de OR 'clinical study'/de OR 'clinical article'/de OR 'clinical trial'/exp OR 'randomization'/exp OR 'intervention study'/de OR 'population based case control study'/de OR (((observation* OR epidemiolog* OR famil* OR comparativ*) NEAR/6 (stud* OR data OR research)) OR cohort* OR longitudinal* OR retrospectiv* OR prospectiv* OR population* OR (national* NEAR/3 (stud* OR survey)) OR ((case OR cases OR match*) NEAR/3 control*) OR (cross NEXT/1 section*) OR correlation* OR multicenter* OR (multi* NEXT/1 center*) OR 'follow up' OR followup* OR clinical* OR trial OR random*):ab,ti) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim) AND [english]/lim

**Medline Ovid**

(exp “pregnancy”/ OR exp “pregnant women”/ OR “pregnancy outcome”/ OR “Pregnancy Complications”/ OR “Puerperal Disorders”/ OR “Postpartum Period”/ OR “Pregnancy, High-Risk”/ OR (pregnan* OR puerper* OR postpartum* OR “post partum*” OR antenatal ).ab,ti.) AND (“Venous Thromboembolism”/ OR “Venous Thrombosis”/ OR * thrombosis/ OR * thromboembolism/ OR (((venous OR vein ) ADJ3 (thrombo*)) OR ((lung OR pulmonar*) ADJ3 embolism*)).ab,ti.) AND (“risk”/ OR “incidence”/ OR “Pregnancy, High-Risk”/ OR (risk OR incidence*).ab,ti.) AND (exp “thrombophilia”/ OR “antithrombins”/ OR “protein S”/ OR “protein C”/ OR “protein C deficiency”/ OR “protein S deficiency”/ OR “prothrombin”/ OR (thrombophil* OR Hypercoagulabilit* OR antithrombin* OR “5 Leiden” OR “V Leiden” OR “protein S” OR “protein C” OR (natural* ADJ3 anticoagulant*) OR prothromb* OR g20210a OR 20201A).ab,ti.) AND (“observational study”/ OR exp “Cohort Studies”/ OR “Epidemiologic Studies”/ OR “Epidemiological Monitoring”/ OR “Case-Control Studies”/ OR “Cross-Sectional Studies”/ OR “multicenter study”/ OR “comparative study”/ OR exp “clinical study”/ OR “Random Allocation”/ OR (((observation* OR epidemiolog* OR famil* OR comparativ*) ADJ6 (stud* OR data OR research)) OR cohort* OR longitudinal* OR retrospectiv* OR prospectiv* OR population* OR (national* ADJ3 (stud* OR survey)) OR ((case OR cases OR match*) ADJ3 control*) OR (cross ADJ section*) OR correlation* OR multicenter* OR (multi* ADJ center*) OR “follow up” OR followup* OR clinical* OR trial OR random*).ab,ti.) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt. AND english.la.

**Cochrane**

((pregnan* OR puerper* OR postpartum* OR ‘post partum*’ OR antenatal ):ab,ti) AND ((((venous OR vein ) NEAR/3 (thrombo*)) OR ((lung OR pulmonar*) NEAR/3 embolism*)):ab,ti) AND ((risk OR incidence*):ab,ti) AND ((thrombophil* OR Hypercoagulabilit* OR antithrombin* OR ‘5 Leiden’ OR ‘V Leiden’ OR ‘protein S’ OR ‘protein C’ OR (natural* NEAR/3 anticoagulant*) OR prothromb* OR g20210a OR 20201A):ab,ti)

**Web of science**

TS=(((pregnan* OR puerper* OR postpartum* OR “post partum*” OR antenatal )) AND ((((venous OR vein ) NEAR/2 (thrombo*)) OR ((lung OR pulmonar*) NEAR/2 embolism*))) AND ((risk OR incidence*)) AND ((thrombophil* OR Hypercoagulabilit* OR antithrombin* OR “5 Leiden” OR “V Leiden” OR “protein S” OR “protein C” OR (natural* NEAR/2 anticoagulant*) OR prothromb* OR g20210a OR 20201A)) AND ((((observation* OR epidemiolog* OR famil* OR comparativ*) NEAR/5 (stud* OR data OR research)) OR cohort* OR longitudinal* OR retrospectiv* OR prospectiv* OR population* OR (national* NEAR/2 (stud* OR survey)) OR ((case OR cases OR match*) NEAR/2 control*) OR (cross NEAR/1 section*) OR correlation* OR multicenter* OR (multi* NEAR/1 center*) OR “follow up” OR followup* OR clinical* OR trial OR random*)))



**Google scholar**

pregnant|pregnancy|puerperal|postpartum “venous|vein thromboembolism|thrombosis”|”lung|pulmonary embolism” risk|incidence thrombophilia|Hypercoagulability|antithrombins|”5|V Leiden”|”protein S|C”|”natural anticoagulants”|prothrombin|g20210a|20201A

**List of excluded studies:**

| Author | year | Main reason for exlcusion (more than 1 reason may apply) |
|---|---|---|
| Adachi [2] | 2001 | Review |
| Allaart [3] | 1993 | Lacks requirements for VTE diagnosis |
| Antovic [4] | 2003 | No pregnancy risk data |
| Bergrem [5] | 2010 | No separate thrombophilia data |
| Bergrem [6] | 2012 | Other study on same dataset included |
| Blanco-Molina [7] | 2007 | No separate thrombophilia data |
| Bombeli [8] | 2001 | Lacks requirements for VTE diagnosis |
| Brouwer [9] | 2006 | No Pregnancy data |
| Chopra [10] | 2002 | No separate thrombophilia data |
| Conard [11] | 1990 | Lacks requirements for VTE diagnosis |
| Coppens [12] | 2006 | VTE prophylaxis |
| Coriu [13] | 2014 | Lacks requirements for VTE diagnosis |
| Couturaud [14] | 2006 | Other study on same dataset included |
| Danilenko [15] | 2001 | No separate thrombophilia data |
| Dargaud [16] | 2009 | VTE prophylaxis |
| De Stefano [17] | 1994 | No Pregnancy data |
| De Stefano [18] | 2003 | no pregnancy risk data |
| De Stefano [19] | 2006 | Recurrent VTE |
| DeSancho [20] | 2010 | no pregnancy risk data |
| Dulicek [21] | 2000 | no pregnancy risk data |
| Emmerich [22] | 1997 | No pregnancy risk data |
| Faioni [23] | 1997 | no pregnancy risk data |
| Finazzi [24] | 1994 | No Pregnancy data |
| Gadelha [25] | 2010 | no pregnancy risk data |
| Gat [26] | 2014 | VTE prophylaxis |
| Gerhardt [27] | 2000 | Other study on same dataset included |
| Gerhardt [28] | 2003 | Other study on same dataset included |
| Grandone [29] | 2008 | VTE prophylaxis |

| Author | year | Main reason for exlcusion (more than 1 reason may apply) |
|---|---|---|
| Hallak [30] | 1997 | no pregnancy risk data |
| Hellgren [31] | 1995 | Lacks requirements for VTE diagnosis |
| Hiltunen [32] | 2007 | Lacks requirements for VTE diagnosis |
| Hirsh [33] | 1996 | no pregnancy risk data |
| Hvas [34] | 2009 | Lacks requirements for VTE diagnosis |
| Ilonczai [35] | 2015 | VTE prophylaxis |
| Jacobsen [36] | 2008 | No separate thrombophilia data |
| James [37] | 2006 | Lacks requirements for VTE diagnosis |
| Khalafallah [38] | 2014 | Recurrent VTE |
| Knight [39] | 2008 | No separate thrombophilia data |
| Koster [40] | 1995 | No Pregnancy data |
| Kovac [41] | 2010 | Other study on same dataset included |
| Laroche [42] | 2001 | Various |
| Lensen [43] | 2000 | Lacks requirements for VTE diagnosis |
| Lenz [44] | 2016 | Lacks requirements for VTE diagnosis |
| Lim [45] | 2016 | Various |
| Lindqvist [46] | 1999 | No separate thrombophilia data |
| Lindqvist[47] | 2002 | Review |
| Linnemann[48] | 2014 | no pregnancy risk data |
| Lykke [49] | 2012 | Lacks requirements for VTE diagnosis |
| Mahmoodi [50] | 2010 | Other study on same dataset included |
| McColl [51] | 2000 | Recurrent VTE |
| McColl [52] | 1997 | no pregnancy risk data |
| Middeldorp [53] | 2001-1 | Lacks requirements for VTE diagnosis |
| Noboa [54] | 2008 | No separate thrombophilia data |
| Pabinger [55] | 1994 | Other study on same dataset included |
| Pabinger [56] | 2000 | proband selection |
| Pabinger [57] | 1996 | proband selection |
| Pabinger [58] | 2005 | Recurrent VTE |
| Pabinger [59] | 2002 | Recurrent VTE |
| Pai [60] | 2011 | Various |
| Philipp [61] | 2014 | Lacks requirements for VTE diagnosis |
| Pomp [62] | 2008 | Lacks requirements for VTE diagnosis |
| Procare [63] | 2004 | no pregnancy data |
| Procare [64] | 2000 | no pregnancy data |
| Rodger [65] | 2015 | VTE prophylaxis |
| Rodger [66] | 2014 | Lacks requirements for VTE diagnosis |
| Rodger [67] | 2014-2 | Review |
| Rogenhofer [68] | 2014 | proband selection |
| Rouse [69] | 2003 | Review |
| Sabadell [70] | 2010 | proband selection |
| Said [71] | 2010 | Lacks requirements for VTE diagnosis |
| Samama [72] | 2004 | language |
| Samama [73] | 2003 | proband selection |
| Sanson [74] | 1999 | VTE prophylaxis |
| Selmeczi [75] | 2015 | VTE prophylaxis |
| Simioni [76] | 1999 | No separate thrombophilia data |
| Simpson [77] | 2001 | No separate thrombophilia data |
| Tormene [78] | 2012 | VTE prophylaxis |
| Trauscht [79] | 1992 | Lacks requirements for VTE diagnosis |
| Vavrinkova [80] | 2014 | VTE prophylaxis |
| Vicente [81] | 1994 | proband selection |
| Villani [82] | 2015 | Various |
| Villani [83] | 2012 | No pregnancy risk data |

# APPENDIX FIGURES

**Appendix Table 1: Study characteristics**

| 1st Author | Year | Study type | Study design | Family study | Ante/postpartum | Antithrombin | Protein C | Protein S | Heterozygous fVL | Homozygous fVL | Heterozygous fII | Homozygous fII | Compound fVL & fII | NOS score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bank [84] | 2004 | cohort | retrospective | + | + | - | - | - | - | - | + | - | - | 8 |
| Bucciarelli [85] | 1999 | cohort | retrospective | + | + | + | + | + | - | - | - | - | - | 6 |
| Clark [86] | 2008 | cohort | prospective | - | + | - | - | - | + | + | - | - | - | 8 |
| Cochery [87] | 2007 | cohort | prospective | - | - | - | - | - | + | + | + | + | - | 8 |
| Cordoba [88] | 2012 | cohort | retrospective | + | - | - | - | - | + | - | + | - | - | 9 |
| Couturaud [89] | 2008 | cohort | retrospective | + | + | - | - | - | + | - | - | - | - | 9 |
| Dilley [90] | 2000 | case-control | NA | - | | - | - | - | + | - | + | - | - | 9 |
| Dizon [91] | 2005 | cohort | prospective | - | - | - | - | - | + | - | - | - | - | 9 |
| Eichinger [92] | 1999 | cohort | prospective | - | - | - | - | - | + | - | - | - | - | 9 |
| Folkeringa [93] | 2007 | cohort | retrospective | + | + | + | + | + | - | - | - | - | - | 9 |
| Friederich [94] | 1996 | cohort | retrospective | + | + | + | + | + | - | - | - | - | - | 8 |
| Gerhardt [95] | 2016 | case-control | NA | - | | + | + | + | + | + | + | - | + | 8 |
| Grandone [96] | 1998 | case-control | NA | - | | + | - | + | + | - | + | + | + | 8 |
| Hammerova [97] | 2011 | cohort | prospective | - | - | - | - | - | + | - | - | - | - | 7 |
| Heit [98] | 2005 | cohort | retrospective | - | - | - | - | - | + | - | - | - | - | 7 |
| Jacobsen [99] | 2010 | case-control | NA | - | | - | - | - | + | + | + | + | + | 9 |
| Kjellberg [100] | 2010 | cohort | prospective | - | + | - | - | - | + | - | - | - | - | 9 |
| Klai [101] | 2012 | case-control | NA | - | | + | + | + | + | + | + | - | - | 7 |
| Lindqvist [102] | 1999 | cohort | prospective | - | - | - | - | - | + | + | - | - | - | 8 |
| Martinelli [103] | 2001 | cohort | retrospective | + | + | - | - | - | - | + | - | - | + | 9 |
| Martinelli [104] | 2002 | case-control | NA | - | | + | + | + | + | + | + | - | + | 8 |
| Martinelli [105] | 2008 | cohort | retrospective | + | + | - | - | - | + | - | + | - | + | 9 |
| Meglic [106] | 2003 | case-control | NA | - | | + | + | + | + | - | + | - | - | 8 |
| Middeldorp [107] | 1998 | cohort | retrospective | + | + | - | - | - | + | - | - | - | - | 9 |
| Middeldorp [108] | 2001 | cohort | prospective | + | - | - | - | - | + | - | - | - | - | 6 |
| Mitic [109] | 2011 | case-control | NA | - | | + | + | + | + | + | + | + | + | 6 |
| Murphy [110] | 2000 | cohort | prospective | - | - | - | - | - | + | - | - | - | - | 8 |
| Ogunyemi [111] | 2003 | case-control | NA | - | | - | + | - | + | - | + | - | - | 7 |
| Samama [112] | 1996 | cohort | retrospective | + | + | - | - | - | + | + | - | - | - | 7 |
| Simioni [113] | 2002 | cohort | prospective | + | + | - | - | - | + | - | - | - | - | 9 |
| Tam [114] | 2012 | case-control | NA | - | | + | + | + | - | - | - | - | - | 6 |
| Tormene [115] | 2001 | cohort | retrospective | + | + | - | - | - | + | + | - | - | + | 8 |
| Tormene [116] | 2007 | cohort | retrospective | + | + | - | - | - | - | - | + | - | - | 9 |
| Van Boven [117] | 1999 | cohort | retrospective | + | + | + | - | - | - | - | - | - | - | 8 |
| Vora [118] | 2007 | case-control | NA | - | | - | + | + | + | - | - | - | - | 8 |
| Yilmazer [119] | 2003 | case-control | NA | - | | - | - | - | + | + | + | - | - | 8 |

Study characteristics of the 43 studies included in the systematic review and meta-analysis: + indicates yes, - indicates no. NA: not applicable. Pregnancies, n refers to the number of pregnancies reported in individual studies, the total number of pregnancies is 41297. NOS score: Newcastle-Ottawa scale assessment score

**Appendix Table 2 and 3: Quality assessment of studies.**

Quality assessment was performed using the Newcastle-Ottawa scale (NOS). It was used for all cohort studies, including those that lack a non-carrier group. As can be appreciated from the items scored, cohort studies that lack a non-carrier group can only get a maximum score of 6.

**Appendix Table 2: Quality assessment of cohort studies**

| Study | Year | Selection of exposed cohort | Selection of non-exposed cohort | Ascertainment of exposure | Exclusion of the outcome at start | Comparability of cases and controls | Assessment of outcome | Adequate of follow-up length | Adequacy of follow up of cohorts | Quality |
|---|---|---|---|---|---|---|---|---|---|---|
| Bank [84] | 2004 | * | * | * | * | ** | **NO** | * | * | **8** |
| Bucciarelli [85] | 1999 | * | **NA** | * | * | **NA** | * | * | * | **6** |
| Clark [86] | 2008 | * | * | * | * | ** | **NO** | * | * | **8** |
| Cochery [87] | 2007 | * | * | * | * | ** | **NO** | * | * | **8** |
| Cordoba [88] | 2012 | * | * | * | * | ** | * | * | * | **9** |
| Couturaud [89] | 2008 | * | * | * | * | ** | * | * | * | **9** |
| Dizon [91] | 2005 | * | * | * | * | ** | * | * | * | **9** |
| Eichinger [92] | 1999 | * | * | * | * | ** | * | * | * | **9** |
| Folkeringa [93] | 2007 | * | * | * | * | ** | * | * | * | **9** |
| Friederich [94] | 1996 | * | * | * | * | ** | * | * | **NO** | **8** |
| Hammerova [97] | 2011 | * | * | * | * | * | **NO** | * | * | **7** |
| Heit [98] | 2005 | * | * | * | * | * | * | * | **NO** | **7** |
| Kjellberg [100] | 2010 | * | * | * | * | ** | * | * | * | **9** |
| Lindqvist [102] | 1999 | * | * | * | * | ** | NA | * | * | 8 |
| Martinelli [103] | 2001 | * | * | * | * | ** | * | * | * | **9** |
| Martinelli [105] | 2008 | * | * | * | * | ** | * | * | * | **9** |
| Middeldorp [107] | 1998 | * | * | * | * | ** | * | * | * | **9** |
| Middeldorp [108] | 2001 | * | **NA** | * | * | **NA** | * | * | * | **6** |
| Murphy [110] | 2000 | * | * | * | * | * | * | * | * | **8** |
| Samama [112] | 1996 | * | * | * | * | **NO** | * | * | * | **7** |
| Simioni [113] | 2002 | * | * | * | * | ** | * | * | * | **9** |
| Tormene [115] | 2001 | **NO** | * | * | * | ** | * | * | * | **8** |
| Tormene [116] | 2007 | * | * | * | * | ** | * | * | * | **9** |
| Van Boven [117] | 1999 | * | * | * | * | ** | **NO** | * | * | **8** |

* Indicates points awarded. NO denotes points not awarded. NA denotes that information was not available (leading to not awarding of points).

**Appendix Table 3: Quality assessment of case-control studies**

| Study | Year | Definition of cases | Representativeness of cases | Selection of controls | Definition of controls | Comparability of cases and controls | Ascertainment of Exposure | Same method of ascertainment | Non-response rate | Quality |
|---|---|---|---|---|---|---|---|---|---|---|
| Dilley [90] | 2000 | * | * | * | * | ** | * | * | * | 9 |
| Gerhardt [95] | 2016 | * | * | * | * | ** | * | * | NO | 8 |
| Grandone [96] | 1998 | * | * | * | * | ** | NO | * | * | 8 |
| Jacobsen [99] | 2010 | * | * | * | * | ** | * | * | NO | 8 |
| Klai [101] | 2012 | * | * | * | * | * | * | * | NO | 7 |
| Martinelli [104] | 2002 | * | * | * | * | ** | * | * | NO | 8 |
| Meglic [106] | 2003 | * | * | * | * | ** | * | * | NO | 8 |
| Mitic [109] | 2011 | NO | * | NO | * | ** | * | * | NO | 6 |
| Ogunyemi [111] | 2003 | * | * | * | * | * | * | * | NO | 7 |
| Tam [114] | 2012 | NO | * | * | * | * | * | * | NO | 6 |
| Vora [118] | 2007 | * | * | * | * | ** | * | * | NO | 8 |
| Yilmazer [119] | 2003 | * | * | * | * | ** | * | * | NO | 8 |

* Indicates points awarded. NO denotes points not awarded.



**Appendix Table 4: Recommendations for prophylaxis based on thresholds.**

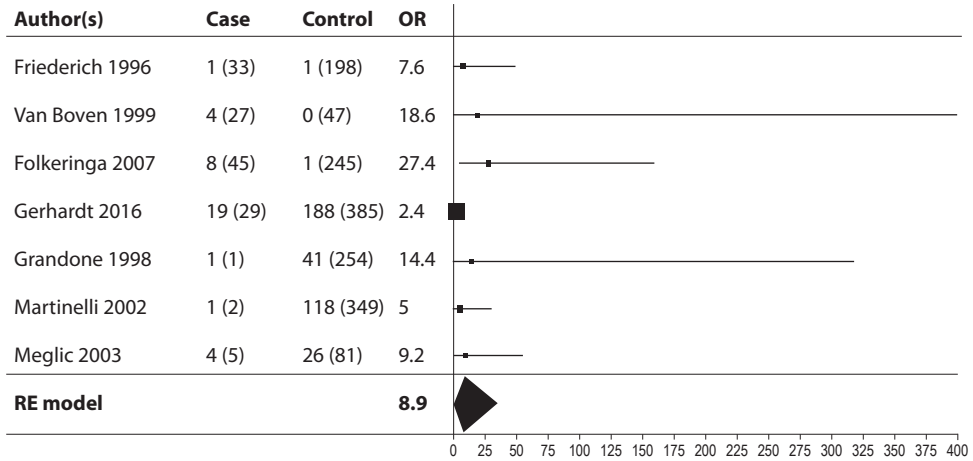
| Threshold | Family history | LMWH Antepartum | LMWH Postpartum |
|---|---|---|---|
| AR >1 | Yes | AT, PC, Hom FVL | AT, PC, PS, Hom FVL, FVL&FII, het FVL |
| | No | Hom FVL | Hom FVL |
| AR >3 | Yes | AT, PC, (Hom FVL?) | AT, PC, PS, (Hom FVL?) |
| | No | | |

Treatment thresholds and recommendations following from thresholds and family history. Based on expert-consensus the threshold chosen for treatment with low-molecular-weight heparin was 3%. This table shows this threshold of 3% is clearly more restrictive than the threshold of 1% in regard to whom should be treated. AR: Absolute risk, % of ante- or postpartum periods. Hom: homozygous. Het: heterozygous. LMWH: Low-molecular-weight heparin. The question mark in homozygous FVL reflects the following: The probability of antepartum VTE risk being >3% is 47%, and the probability of postpartum risk being >3% is 46%. In family studies the absolute risk estimates are higher than in non-family studies. Due to the limited data no ante- and postpartum analysis could be made for family studies on homozygous FVL could be made. Individual risk factors such as family history, but also other common risk factors for VTE may help guide clinical decision making.

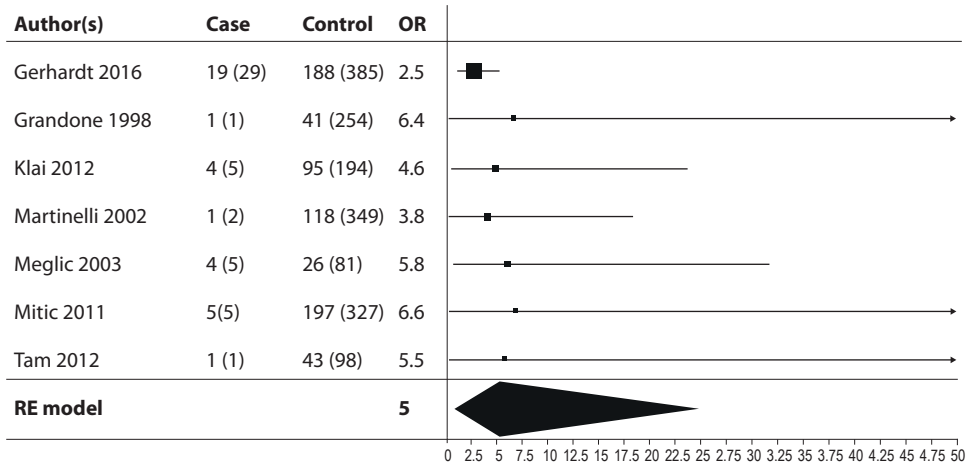
## RESULTS OF THE INDIVIDUAL STUDIES AND THEIR META-ANALYSES

## ANTITHROMBIN DEFICIENCY

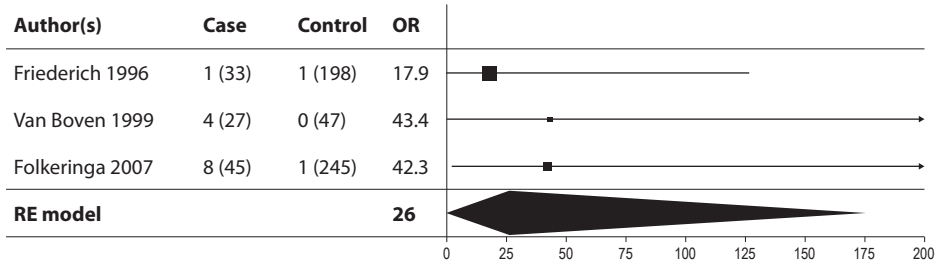
### Antithrombin deficiency: OR All studies



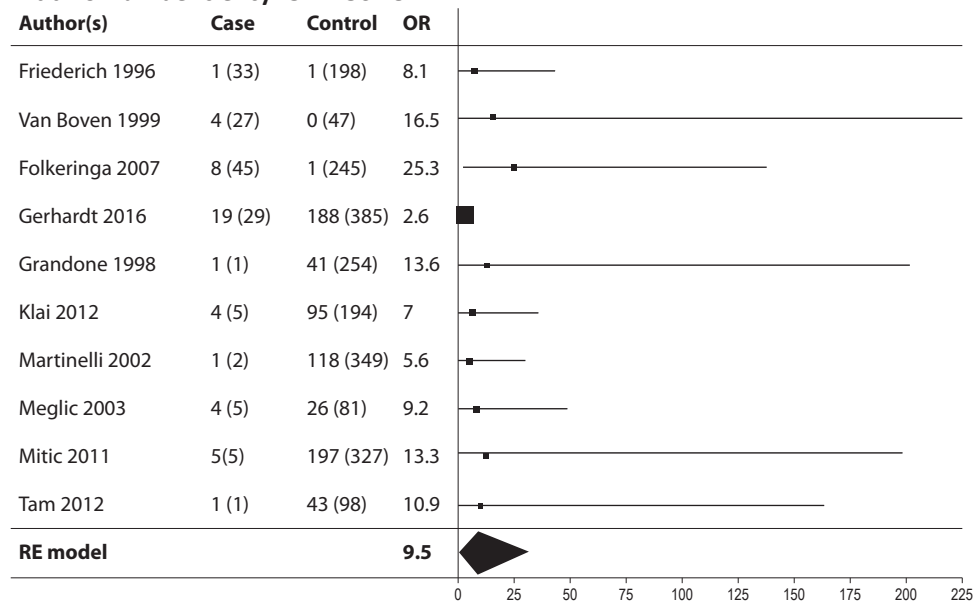

### Antithrombin deficiency: OR case-control studies



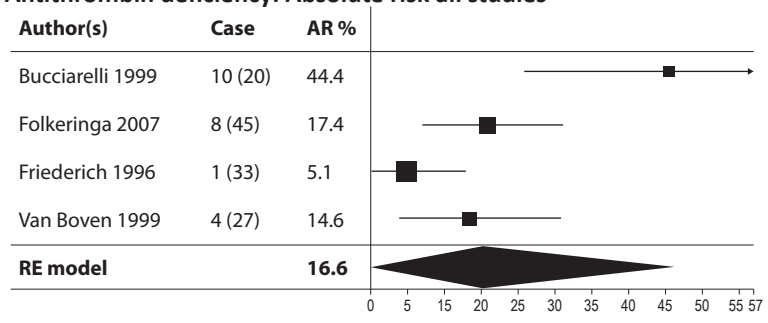

### Antithrombin deficiency: OR cohort studies



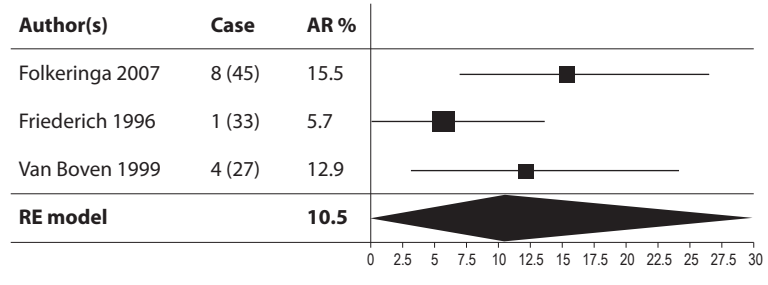

### Antithrombin deficiency: OR NOS ≥8




### Antithrombin deficiency: Absolute risk all studies



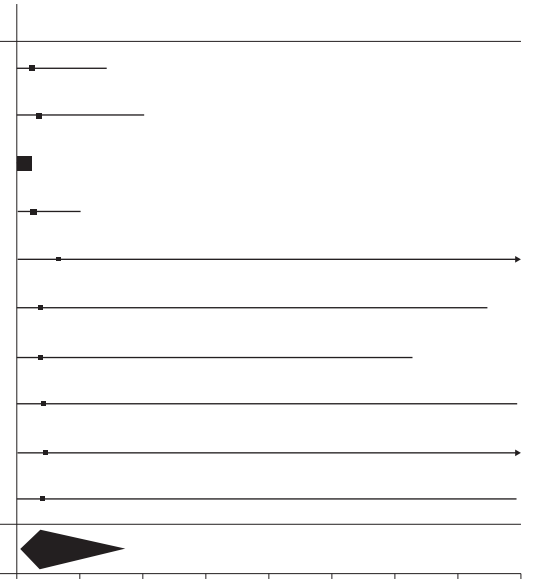

### Antithrombin deficiency: Absolute risk NOS ≥8



## PROTEIN C DEFICIENCY

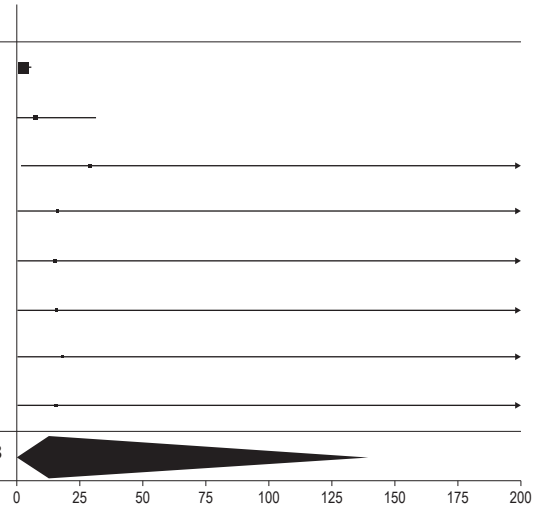
### Protein C deficiency: OR All studies

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Friederich 1996 | 1 (60) | 1 (198) | 6.5 |
| Folkeringa 2007 | 3 (63) | 1 (245) | 9.5 |
| Gerhardt 2016 | 19 (29) | 159 (350) | 3.1 |
| Klai 2012 | 11 (13) | 88 (186) | 7.3 |
| Martinelli 2002 | 6 (6) | 113 (345) | 17 |
| Meglic 2003 | 1 (1) | 29 (85) | 10.1 |
| Mitic 2011 | 2 (2) | 200 (330) | 9.9 |
| Ogunyemi 2003 | 2 (2) | 28 (58) | 10.7 |
| Tam 2012 | 3 (3) | 41 (96) | 12 |
| Vora 2007 | 1 (1) | 31 (131) | 10.9 |
| **RE model** | | | **9.3** |



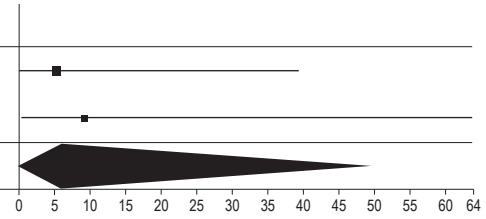

### Protein C deficiency: OR case-control studies

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Gerhardt 2016 | 19 (29) | 159 (350) | 2.9 |
| Klai 2012 | 11 (13) | 88 (186) | 7.7 |
| Martinelli 2002 | 6 (6) | 113 (345) | 29.1 |
| Meglic 2003 | 1 (1) | 29 (85) | 16.2 |
| Mitic 2011 | 2 (2) | 200 (330) | 15.2 |
| Ogunyemi 2003 | 2 (2) | 28 (58) | 15.9 |
| Tam 2012 | 3 (3) | 41 (96) | 18.4 |
| Vora 2007 | 1 (1) | 31 (131) | 15.8 |
| **RE model** | | | **12.3** |



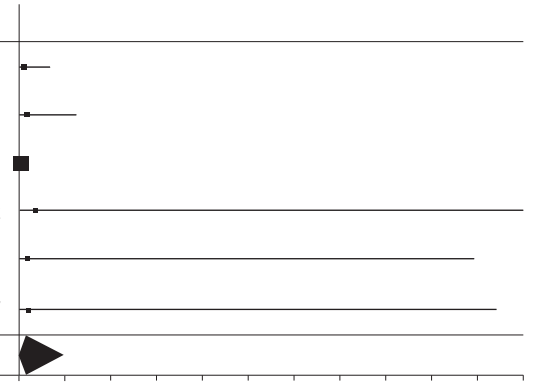

### Protein C deficiency: OR cohort studies

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Friederich 1996 | 1 (60) | 1 (198) | 5.2 |
| Folkeringa 2007 | 3 (63) | 1 (245) | 9.2 |
| **RE model** | | | **5.9** |



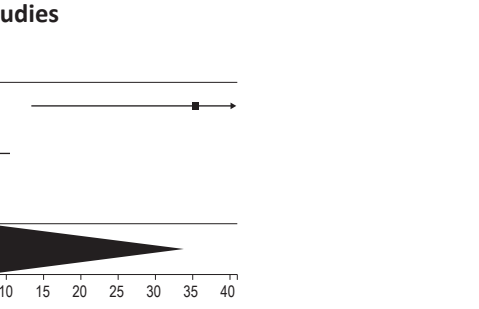

### Protein C deficiency: OR NOS ≥8

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Friederich 1996 | 1 (60) | 1 (198) | 5.5 |
| Folkeringa 2007 | 3 (63) | 1 (245) | 9.5 |
| Gerhardt 2016 | 19 (29) | 159 (350) | 2.8 |
| Martinelli 2002 | 6 (6) | 113 (345) | 18.2 |
| Meglic 2003 | 1 (1) | 29 (85) | 9.8 |
| Vora 2007 | 1 (1) | 31 (131) | 10.7 |
| **RE model** | | | **7.7** |



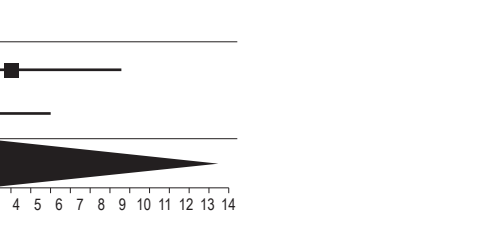

### Protein C deficiency: Absolute risk all studies

| Author(s) | Case | AR % |
|---|---|---|
| Bucciarelli 1999 | 6 (14) | 35.5 |
| Folkeringa 2007 | 3 (63) | 4.8 |
| Friederich 1996 | 1 (60) | 2.3 |
| **RE model** | | **7.8** |




### Protein C deficiency: Absolute risk NOS ≥8

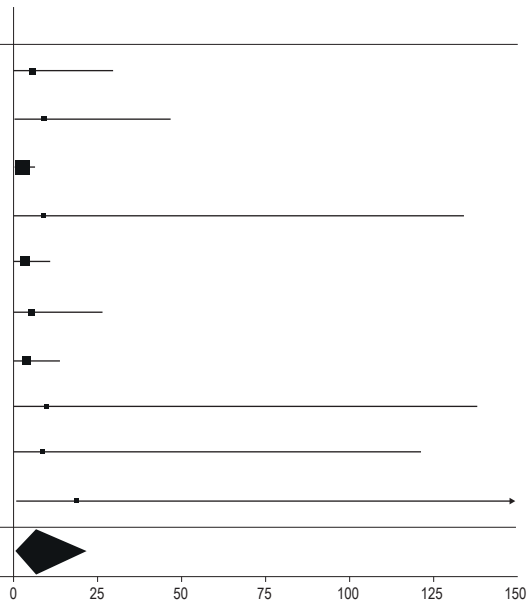
| Author(s) | Case | AR % |
|---|---|---|
| Folkeringa 2007 | 3 (63) | 3.7 |
| Friederich 1996 | 1 (60) | 1.8 |
| **RE model** | | **2.6** |



## PROTEIN S DEFICIENCY

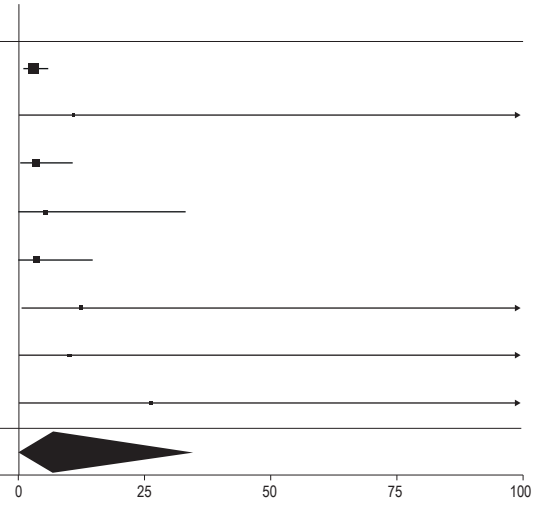
### Protein S deficiency: OR All studies

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Folkeringa 2007 | 1 (54) | 1 (245) | 6.1 |
| Friederich 1996 | 5 (76) | 1 (198) | 9.4 |
| Gerhardt 2016 | 19 (28) | 158 (349) | 3.3 |
| Grandone 1998 | 1(1) | 41 (254) | 9.4 |
| Klai 2012 | 7 (10) | 92 (189) | 4 |
| Martinelli 2002 | 2 (3) | 117 (348) | 5.8 |
| Meglic 2003 | 2 (4) | 28 (82) | 4.2 |
| Mitic 2011 | 7 (7) | 195 (325) | 10.4 |
| Tam 2012 | 2 (2) | 42 (97) | 9 |
| Vora 2007 | 7 (7) | 25 (125) | 19.2 |
| **RE model** | | | **7** |




### Protein S deficiency: OR case-control studies

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Gerhardt 2016 | 19 (28) | 158 (349) | 3 |
| Grandone 1998 | 1(1) | 41 (254) | 11 |
| Klai 2012 | 7 (10) | 92 (189) | 3.6 |
| Martinelli 2002 | 2 (3) | 117 (348) | 5.5 |
| Meglic 2003 | 2 (4) | 28 (82) | 3.7 |
| Mitic 2011 | 7 (7) | 195 (325) | 12.5 |
| Tam 2012 | 2 (2) | 42 (97) | 10.2 |
| Vora 2007 | 7 (7) | 25 (125) | 26.3 |
| **RE model** | | | **6.7** |



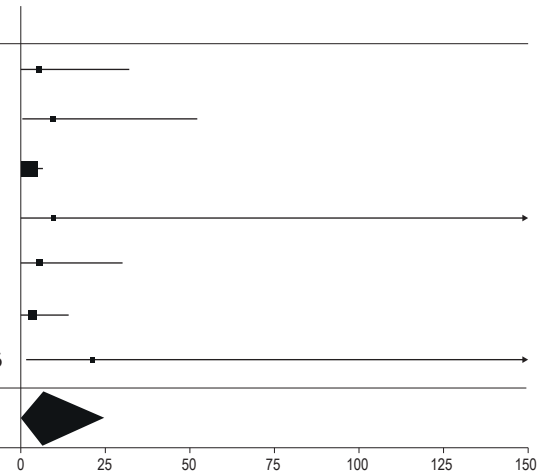



### Protein S deficiency: OR cohort studies

N=2, no forest-plot was constructed.

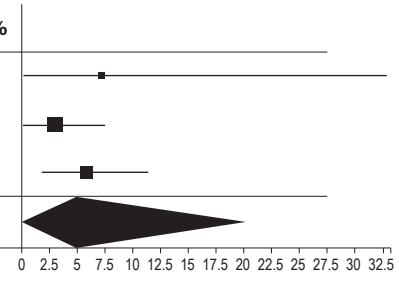
### Protein S deficiency: OR NOS ≥8

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Folkeringa 2007 | 1 (54) | 1 (245) | 5.9 |
| Friederich 1996 | 5 (76) | 1 (198) | 9.7 |
| Gerhardt 2016 | 19 (28) | 158 (349) | 3.1 |
| Grandone 1998 | 1(1) | 41 (254) | 10 |
| Martinelli 2002 | 2 (3) | 117 (348) | 5.7 |
| Meglic 2003 | 2 (4) | 28 (82) | 3.9 |
| Vora 2007 | 7 (7) | 25 (125) | 21.6 |
| **RE model** | | | **6.9** |



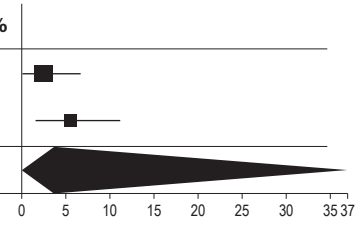

### Protein S deficiency: Absolute risk All studies

| Author(s) | Case | AR % |
|---|---|---|
| Bucciarelli 1999 | 1 (5) | 7.1 |
| Folkeringa 2007 | 1 (54) | 2.9 |
| Friederich 1996 | 5 (76) | 5.8 |
| **RE model** | | **4.8** |



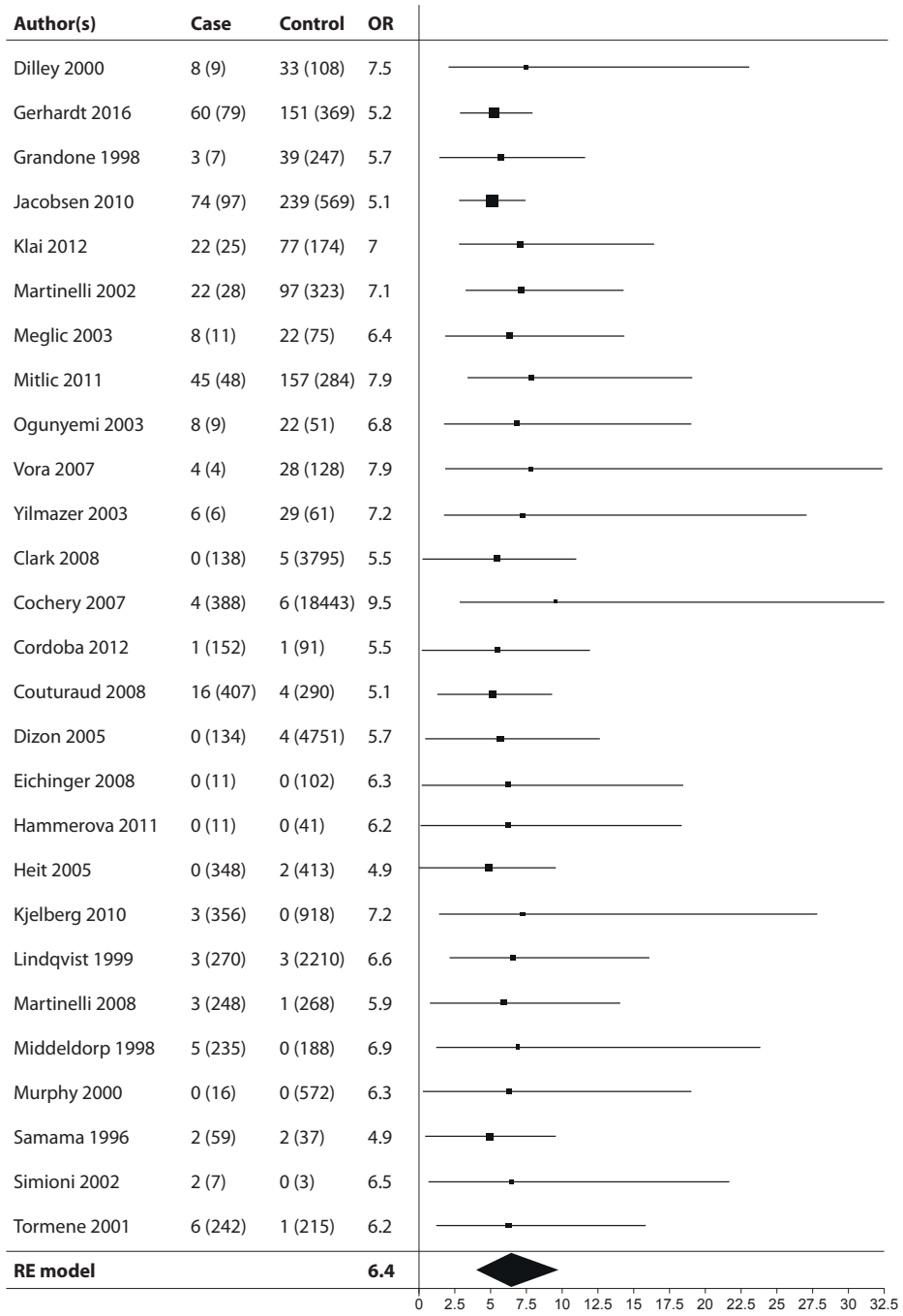

### Protein S deficiency: Absolute risk NOS ≥8

| Author(s) | Case | AR % |
|---|---|---|
| Folkeringa 2007 | 1 (54) | 2.3 |
| Friederich 1996 | 5 (76) | 5.5 |
| **RE model** | | **3.6** |

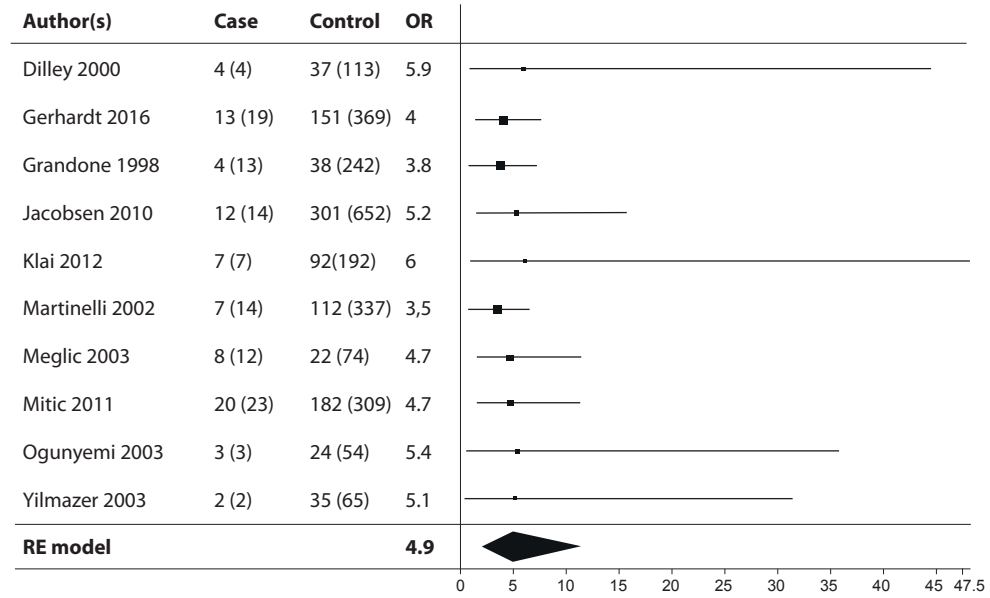


## HETEROZYGOUS FACTOR V LEIDEN MUTATION

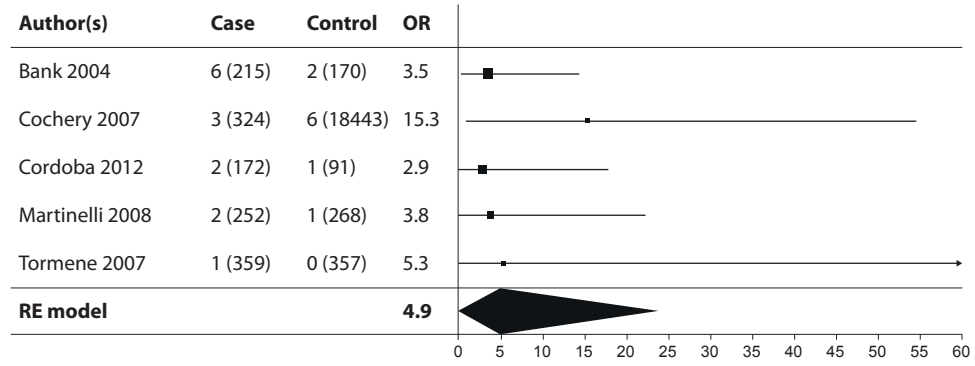
### Heterozygous Factor V Leiden mutation: OR All studies




### Heterozygous Factor V Leiden mutation: OR case-control studies

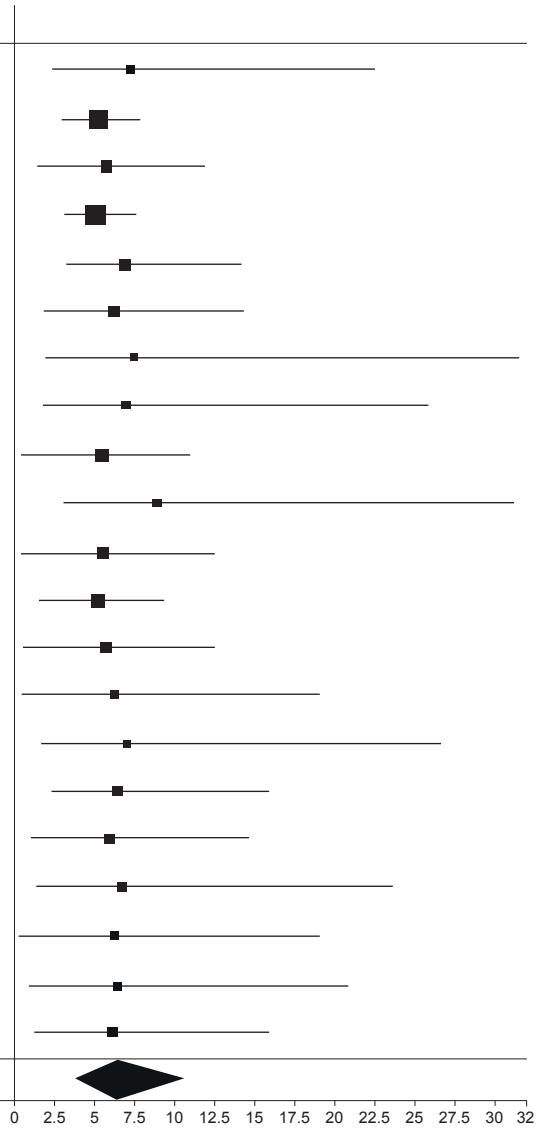



### Heterozygous Factor V Leiden mutation: OR cohort studies



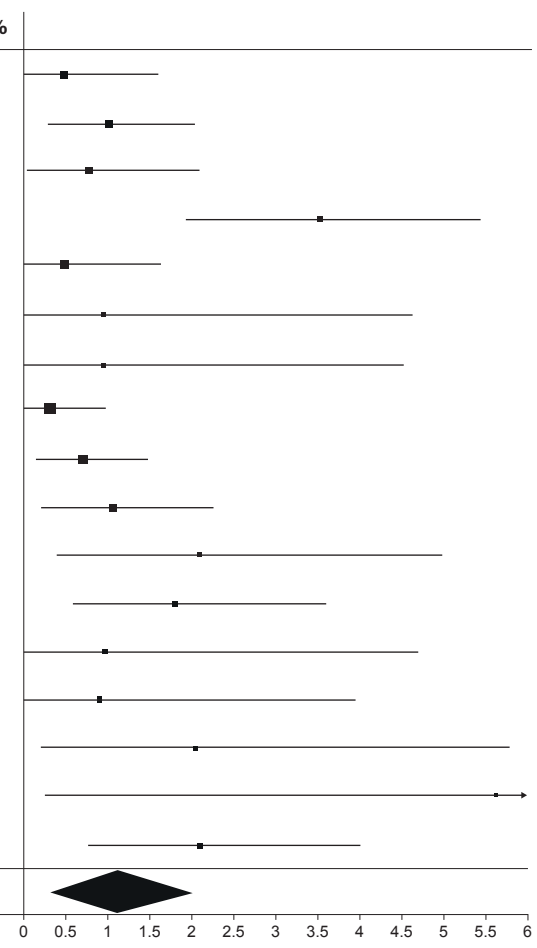
**Heterozygous Factor V Leiden mutation: OR NOS ≥8**

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Dilley 2000 | 8 (9) | 33 (108) | 7.3 |
| Gerhardt 2016 | 60 (79) | 151 (369) | 5.3 |
| Grandone 19983 | 3 (7) | 39 (247) | 5.8 |
| Jacobsen 2010 | 74 (97) | 239 (569) | 5.1 |
| Martinelli 2002 | 22 (28) | 97 (323) | 6.9 |
| Meglic 2003 | 8 (11) | 22 (75) | 6.3 |
| Vora 2007 | 4 (4) | 28 (128) | 7.5 |
| Yilmazer 2003 | 6 (6) | 29 (61) | 7 |
| Clark 2008 | 0 (138) | 5 (3795) | 5.5 |
| Cochery 2007 | 4 (388) | 6 (18443) | 8.9 |
| Cordoba 2012 | 1 (152) | 1 (91) | 5.6 |
| Couturaud 2008 | 16 (407) | 4 (290) | 5.2 |
| Dizon 2005 | 0 (134) | 4 (4751) | 5.7 |
| Eichinger 2008 | 0 (11) | 0 (102) | 6.3 |
| Kjelberg 2010 | 3 (356) | 0 (918) | 7.1 |
| Lindqvist 1999 | 3 (270) | 3 (2210) | 6.5 |
| Martinelli 2008 | 3 (248) | 1 (268) | 6 |
| Middeldorp 1998 | 5 (235) | 0 (188) | 6.8 |
| Murphy 2000 | 0 (16) | 0 (572) | 6.3 |
| Simioni 2002 | 2 (7) | 0 (3) | 6.5 |
| Tormene 2001 | 6 (242) | 1 (215) | 6.2 |
| **RE model** | | | **6.4** |

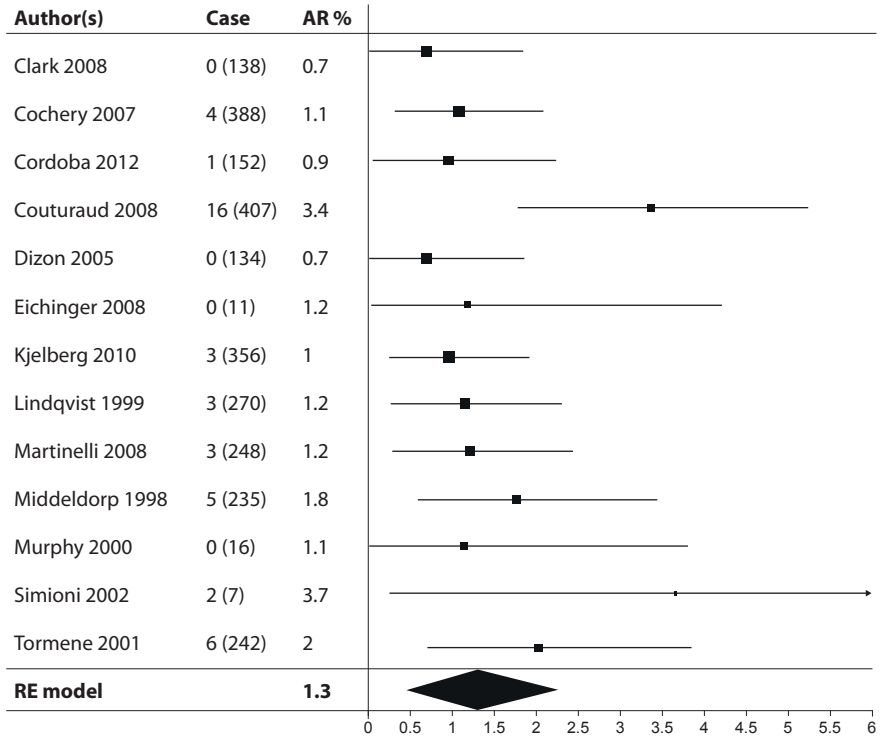



**Heterozygous Factor V Leiden mutation: Absolute risk all studies**

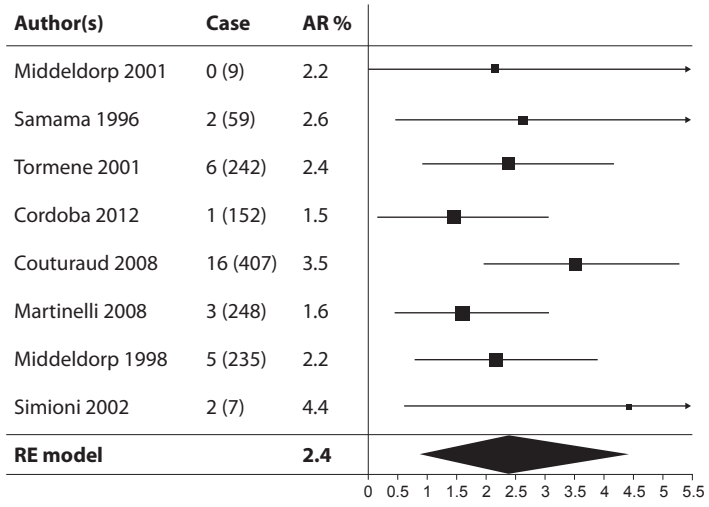
| Author(s) | Case | AR % |
|---|---|---|
| Clark 2008 | 0 (138) | 0.5 |
| Cochery 2007 | 4 (388) | 1 |
| Cordoba 2012 | 1 (152) | 0.8 |
| Couturaud 2008 | 16 (407) | 3.5 |
| Dizon 2005 | 0 (134) | 0.5 |
| Eichinger 2008 | 0 (11) | 0.9 |
| Hammerova 2011 | 0 (11) | 0.9 |
| Heit 2005 | 0 (348) | 0.3 |
| Kjelberg 2010 | 3 (472) | 0.7 |
| Lindqvist 1999 | 3 (270) | 1.1 |
| Martinelli 2008 | 3 (104) | 2.1 |
| Middeldorp 1998 | 5 (235) | 1.8 |
| Middeldorp 2001 | 0 (9) | 1 |
| Murphy 2000 | 0 (16) | 0.9 |
| Samama 1996 | 2 (59) | 2 |
| Simioni 2002 | 2 (7) | 5.6 |
| Tormene 2001 | 6 (242) | 2.1 |
| **RE model** | | **1.1** |



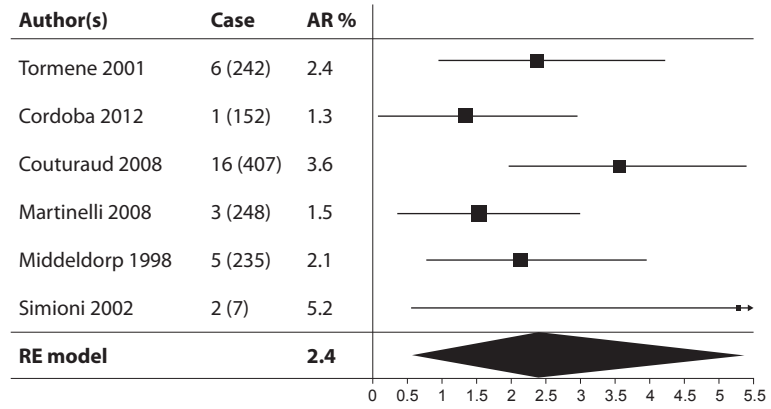
**Heterozygous Factor V Leiden mutation: Absolute risk all studies NOS ≥8**



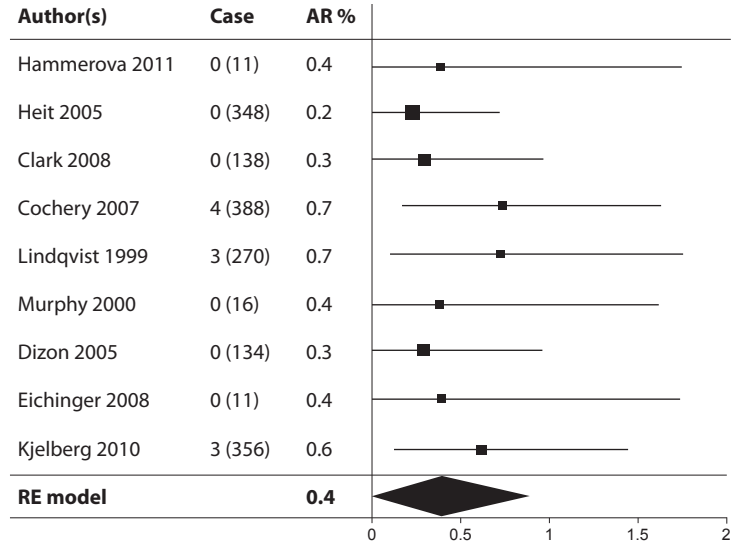

**Heterozygous Factor V Leiden mutation: Absolute risk family studies**



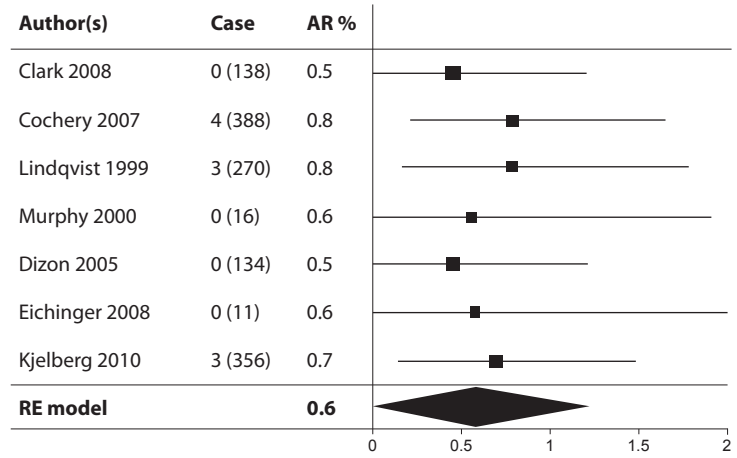

**Heterozygous Factor V Leiden mutation: Absolute risk family studies NOS ≥8**




**Heterozygous Factor V Leiden mutation: Absolute risk non-family studies**

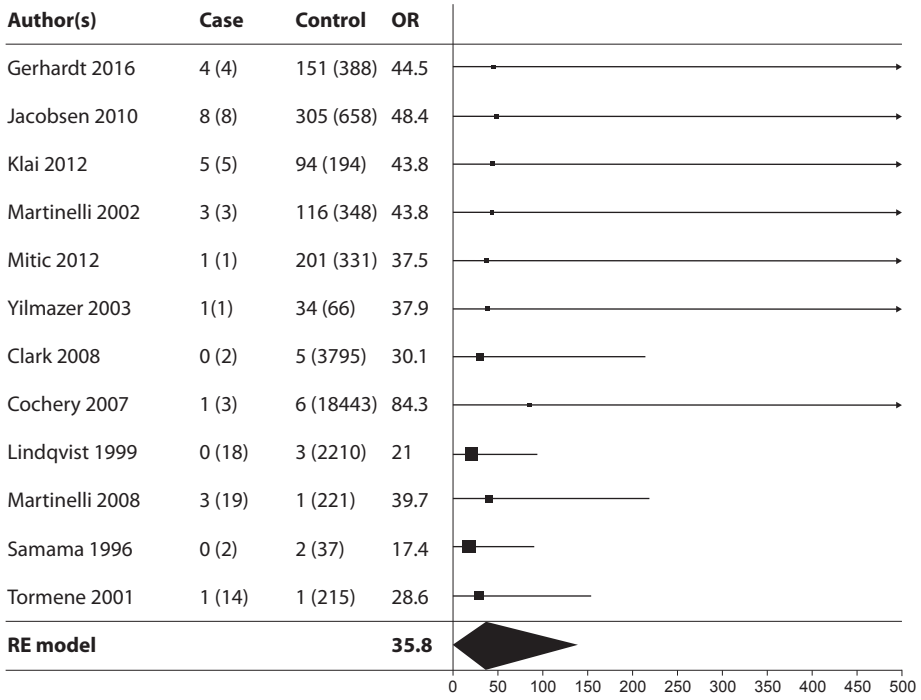



**Heterozygous Factor V Leiden mutation: Absolute risk non-family studies NOS ≥8**

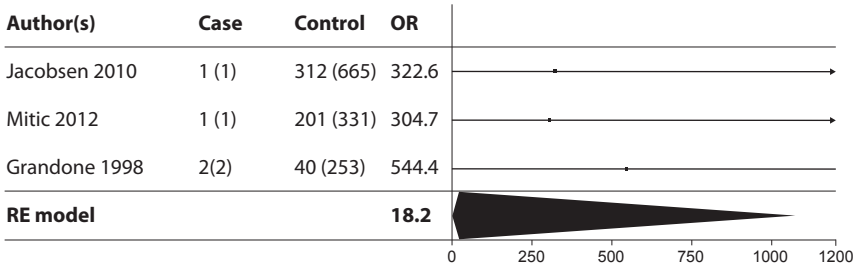


## HOMOZYGOUS FVL MUTATION

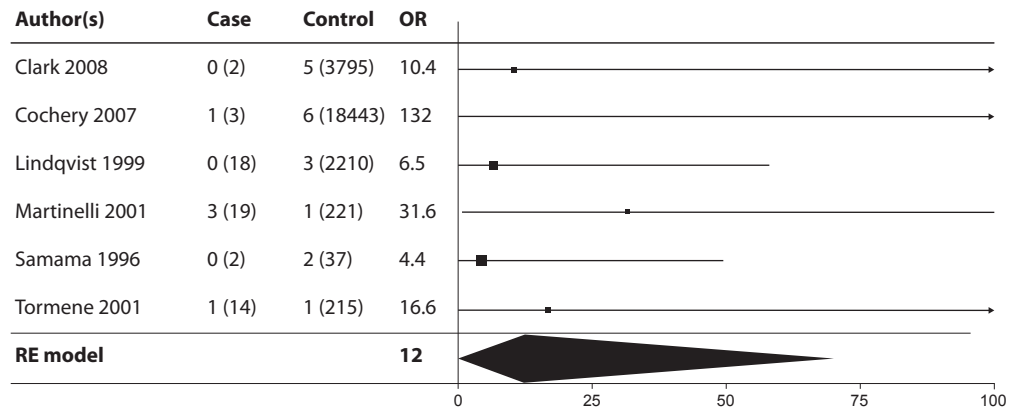
### Homozygous FVL mutation: OR All studies



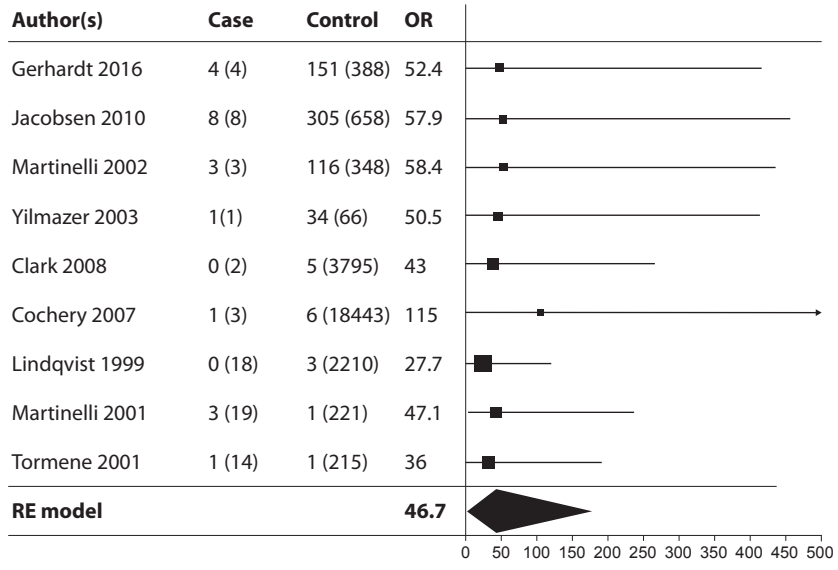

### Homozygous FVL mutation: OR case-control studies



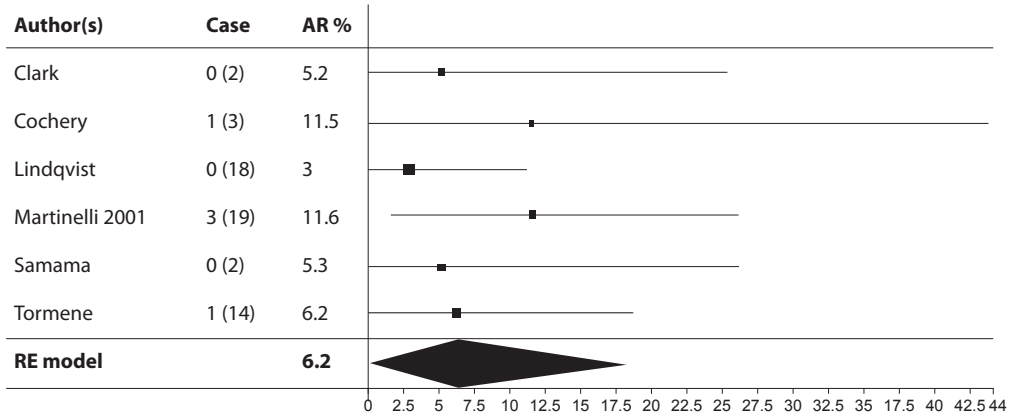

### Homozygous FVL mutation: OR cohort studies



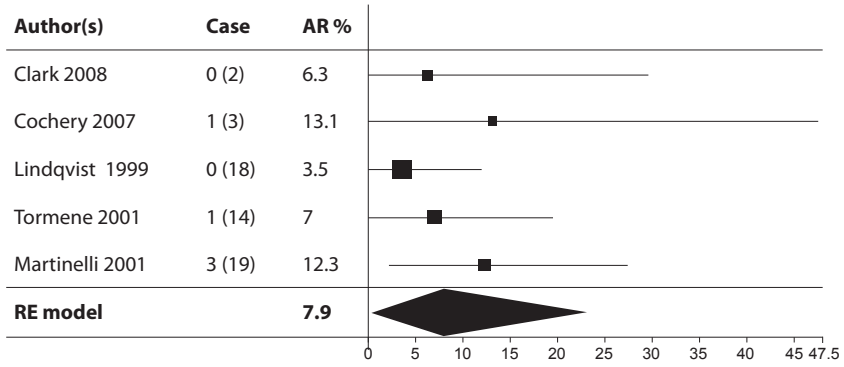

### Homozygous FVL mutation: OR NOS ≥8



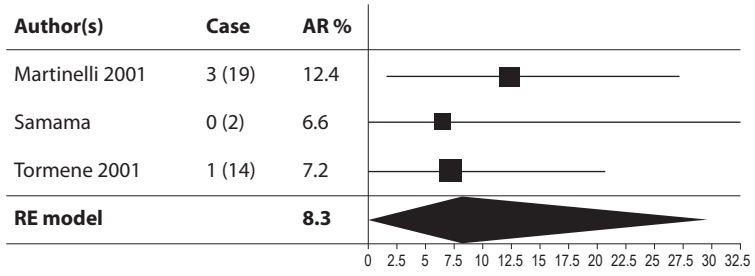
**Homozygous FVL mutation: Absolute risk all studies**



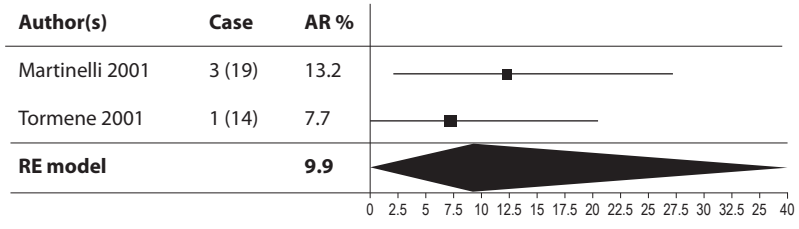

**Homozygous FVL mutation: Absolute risk all studies NOS ≥8**



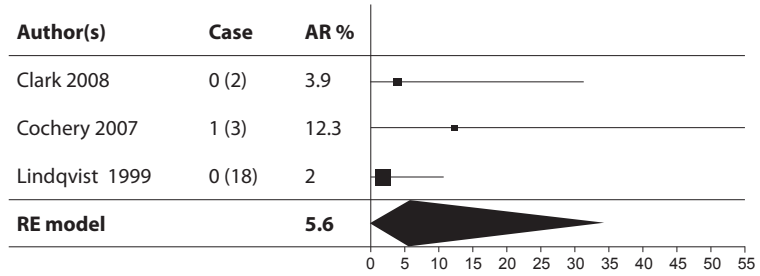

**Homozygous FVL mutation: Absolute risk family studies**



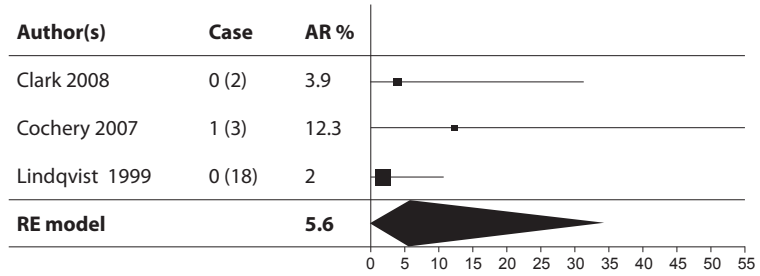

**Homozygous FVL mutation: Absolute risk family studies NOS ≥8**




**Homozygous FVL mutation: Absolute risk non-family studies**



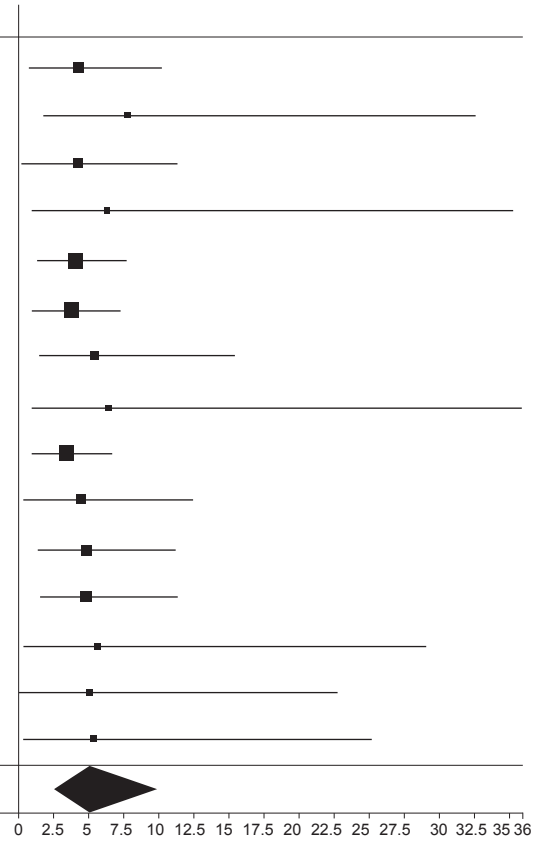

**Homozygous FVL mutation: Absolute risk non-family studies (only NOS ≥8)**



## HETEROZYGOUS PROTHROMBIN G20210A MUTATION

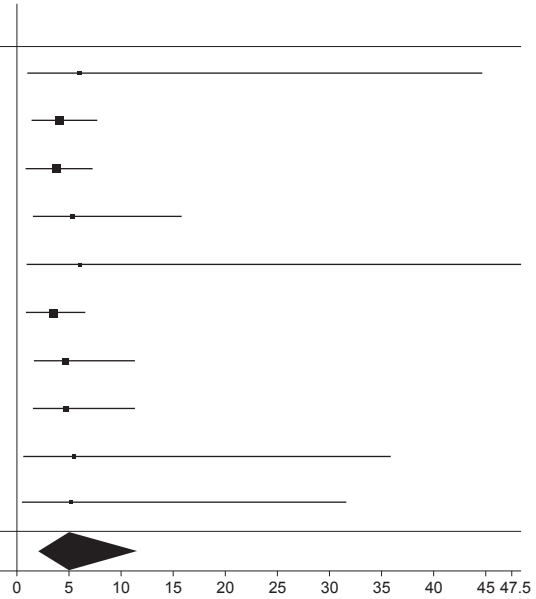
### Heterozygous prothrombin G20210A mutation: OR All studies

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Bank 2004 | 6 (215) | 2 (170) | 4.3 |
| Cochery 2007 | 3 (324) | 6 (18443) | 7.7 |
| Cordoba 2012 | 2 (172) | 1 (91) | 4.2 |
| Dilley 2000 | 4 (4) | 37 (113) | 6.3 |
| Gerhardt 2016 | 13 (19) | 151 (369) | 4.1 |
| Grandone 1998 | 4 (13) | 38 (242) | 3.8 |
| Jacobsen 2010 | 12 (14) | 301 (652) | 5.4 |
| Klai 2012 | 7 (7) | 92 (192) | 6.4 |
| Martinelli 2002 | 7 (14) | 112 (337) | 3.5 |
| Martinelli 2008 | 2 (252) | 1 (268) | 4.5 |
| Meglic 2003 | 8 (12) | 22 (74) | 4.8 |
| Mitlic 2011 | 20 (23) | 182 (309) | 4.9 |
| Ogunyemi 2003 | 3 (3) | 24 (54) | 5.7 |
| Tormene 2007 | 1 (359) | 0 (357) | 5.1 |
| Yilmazer 2003 | 2 (2) | 33 (65) | 5.3 |
| **RE model** | | | **5.1** |



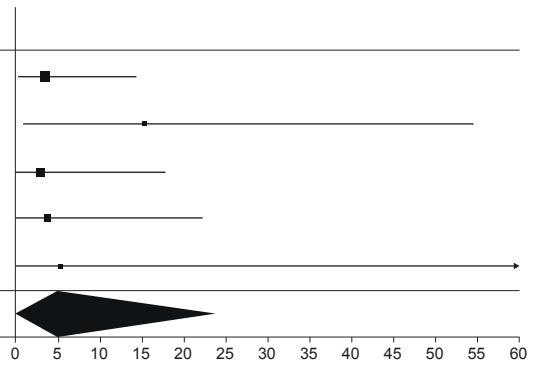

### Heterozygous prothrombin G20210A mutation: OR case-control studies

| Author(s) | Case | Control | OR |
|---|---|---|---|
| Dilley 2000 | 4 (4) | 37 (113) | 5.9 |
| Gerhardt 2016 | 13 (19) | 151 (369) | 4 |
| Grandone 1998 | 4 (13) | 38 (242) | 3.8 |
| Jacobsen 2010 | 12 (14) | 301 (652) | 5.2 |
| Klai 2012 | 7 (7) | 92 (192) | 6 |
| Martinelli 2002 | 7 (14) | 112 (337) | 3.5 |
| Meglic 2003 | 8 (12) | 22 (74) | 4.7 |
| Mitic | 20 (23) | 182 (309) | 4.7 |
| Ogunyemi 2003 | 3 (3) | 24 (54) | 5.4 |
| Yilmazer 2003 | 2 (2) | 33 (65) | 5.1 |
| **RE model** | | | **4.9** |

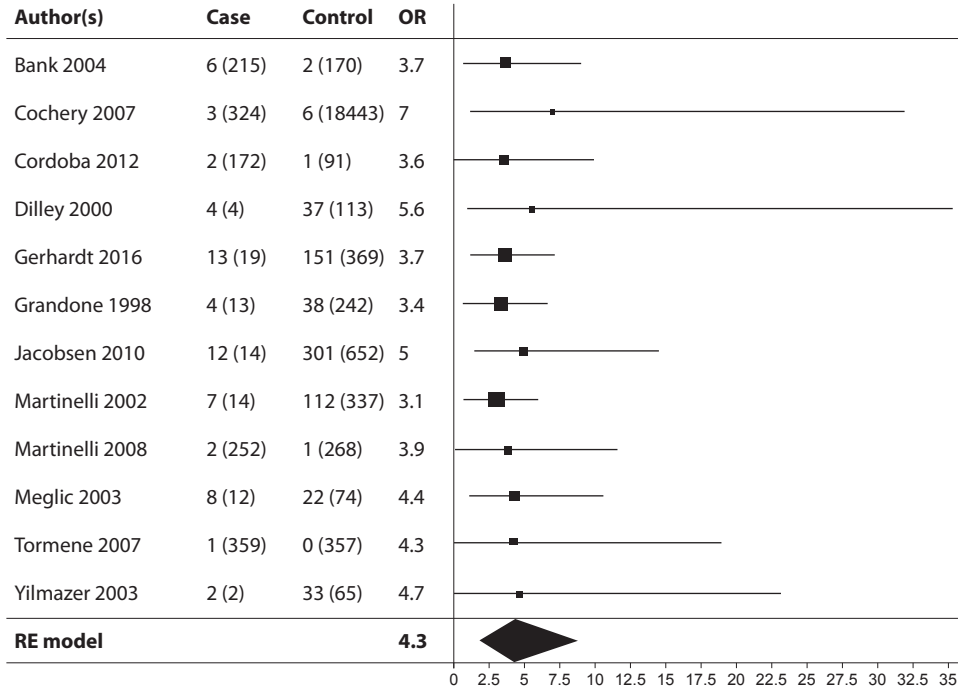



### Heterozygous prothrombin G20210A mutation: OR cohort studies

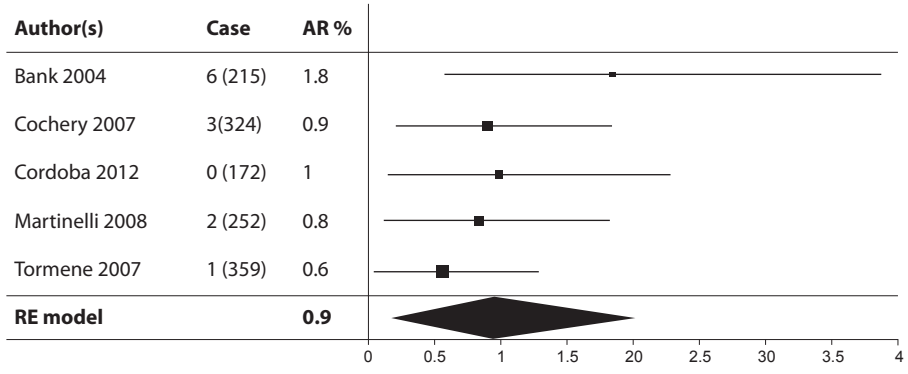
| Author(s) | Case | Control | OR |
|---|---|---|---|
| Bank 2004 | 6 (215) | 2 (170) | 3.5 |
| Cochery 2007 | 3 (324) | 6 (18443) | 15.3 |
| Cordoba 2012 | 2 (172) | 1 (91) | 2.9 |
| Martinelli 2008 | 2 (252) | 1 (268) | 3.8 |
| Tormene 2007 | 1 (359) | 0 (357) | 5.3 |
| **RE model** | | | **4.9** |



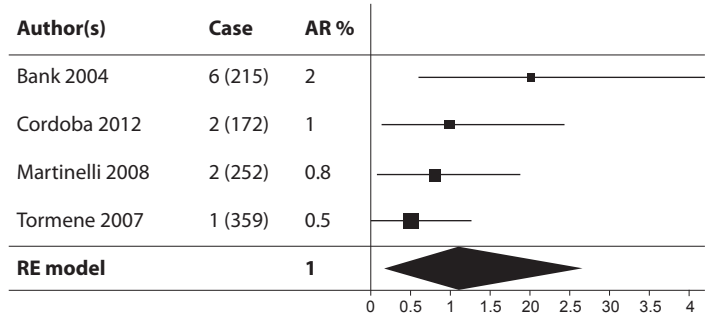
### Heterozygous prothrombin G20210A mutation: OR NOS ≥8



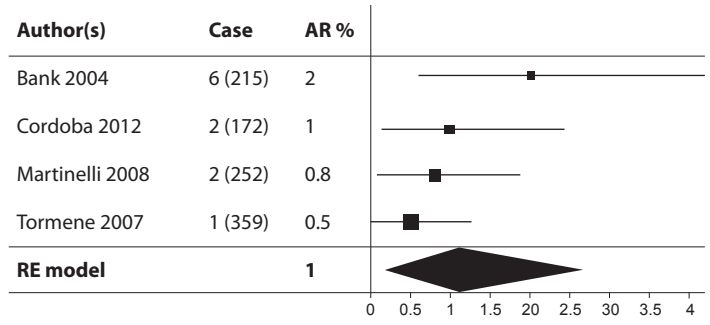

### Heterozygous prothrombin G20210A mutation: Absolute risk all studies




### Heterozygous prothrombin G20210A mutation: Absolute risk family studies




### Heterozygous prothrombin G20210A mutation: Absolute risk family studies NOS ≥8

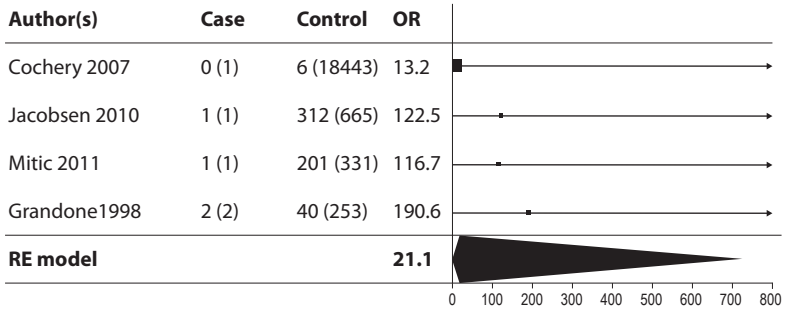



### Heterozygous prothrombin G20210A mutation: Absolute risk non-family studies

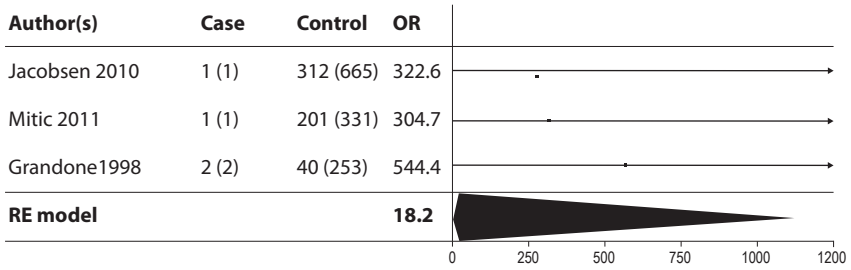
Only 1 non-family study, no forest-plot

## HOMOZYGOUS PROTHROMBIN G20210A MUTATION

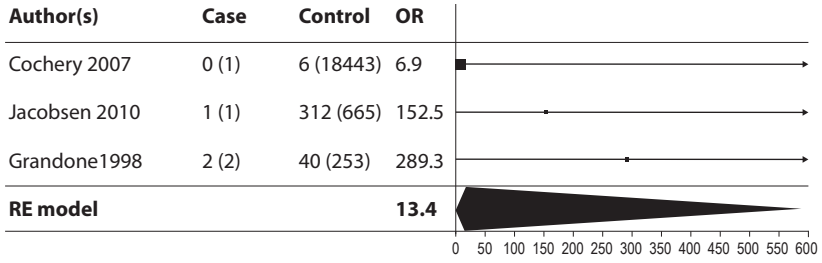
### Homozygous PROTHROMBIN G20210A mutation: OR All studies




### Homozygous PROTHROMBIN G20210A mutation: OR case-control studies




### Homozygous PROTHROMBIN G20210A mutation: OR NOS ≥8



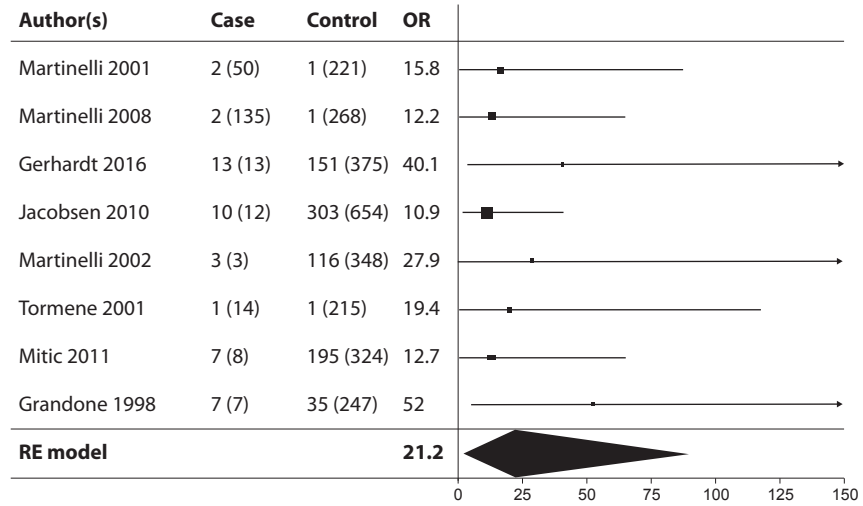

### Homozygous PROTHROMBIN G20210A mutation: Absolute risk all studies

Insufficient data (n=1)

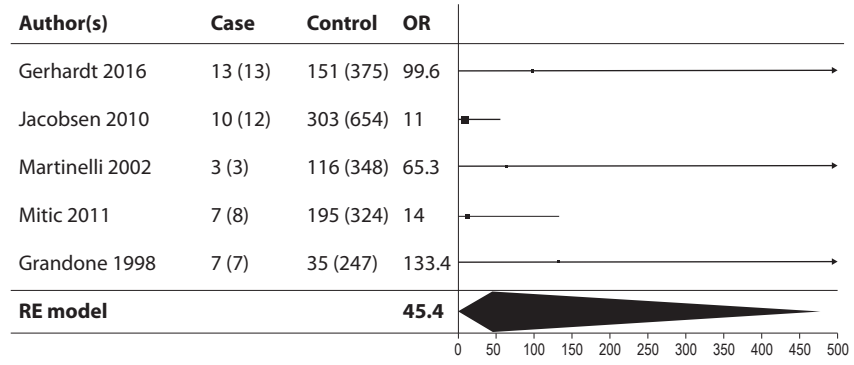


## COMPOUND HETEROZYGOUS FACTOR V LEIDEN & PROTHROMBIN G20210A MUTATION

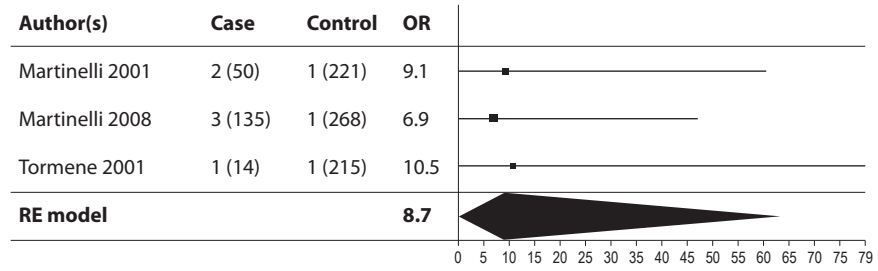
### Compound heterozygous Factor V Leiden & prothrombin G20210a mutation: OR All studies



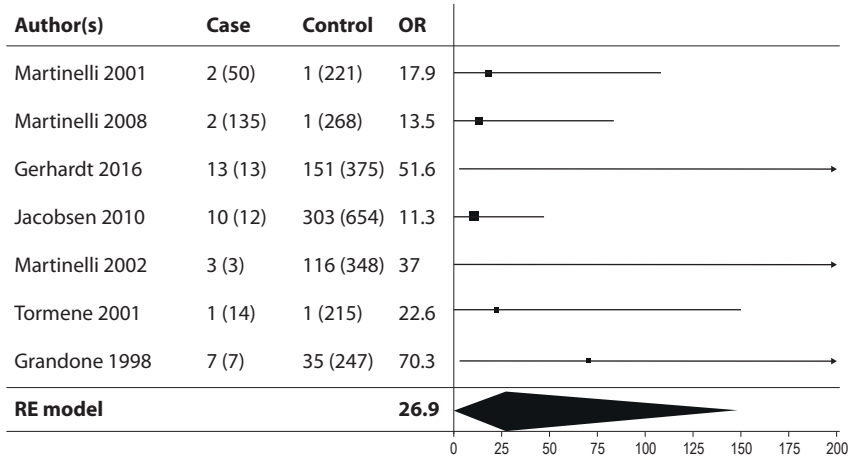

### Compound heterozygous Factor V Leiden & prothrombin G20210a mutation: OR case-control studies



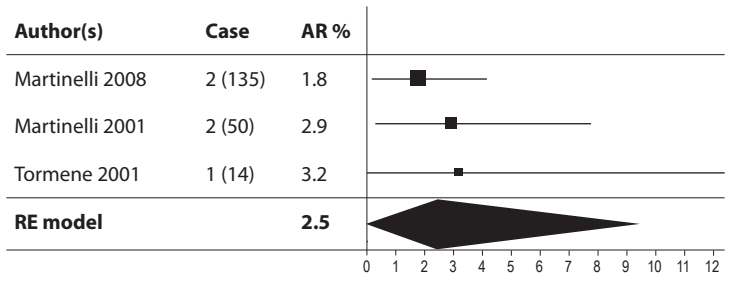

### Compound heterozygous Factor V Leiden & prothrombin G20210a mutation: OR cohort studies



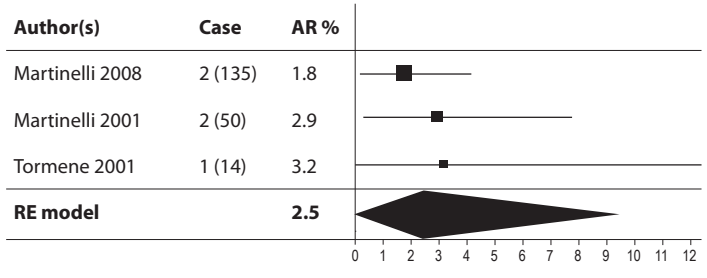
**Compound heterozygous Factor V Leiden & prothrombin G20210a mutation: OR NOS ≥8**




**Compound heterozygous Factor V Leiden & prothrombin G20210a mutation: Absolute risk all studies**

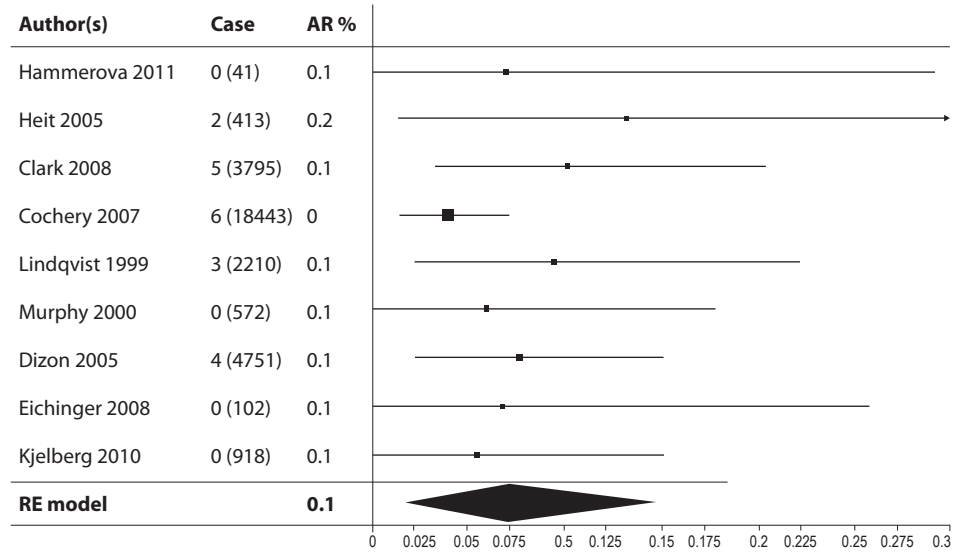



**Compound heterozygous Factor V Leiden & prothrombin G20210a mutation: Absolute risk NOS ≥8**

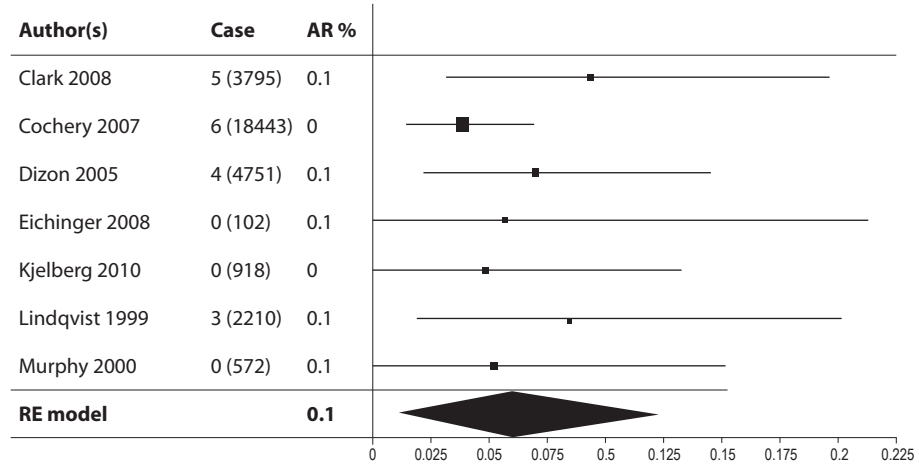



## NON-CARRIERS

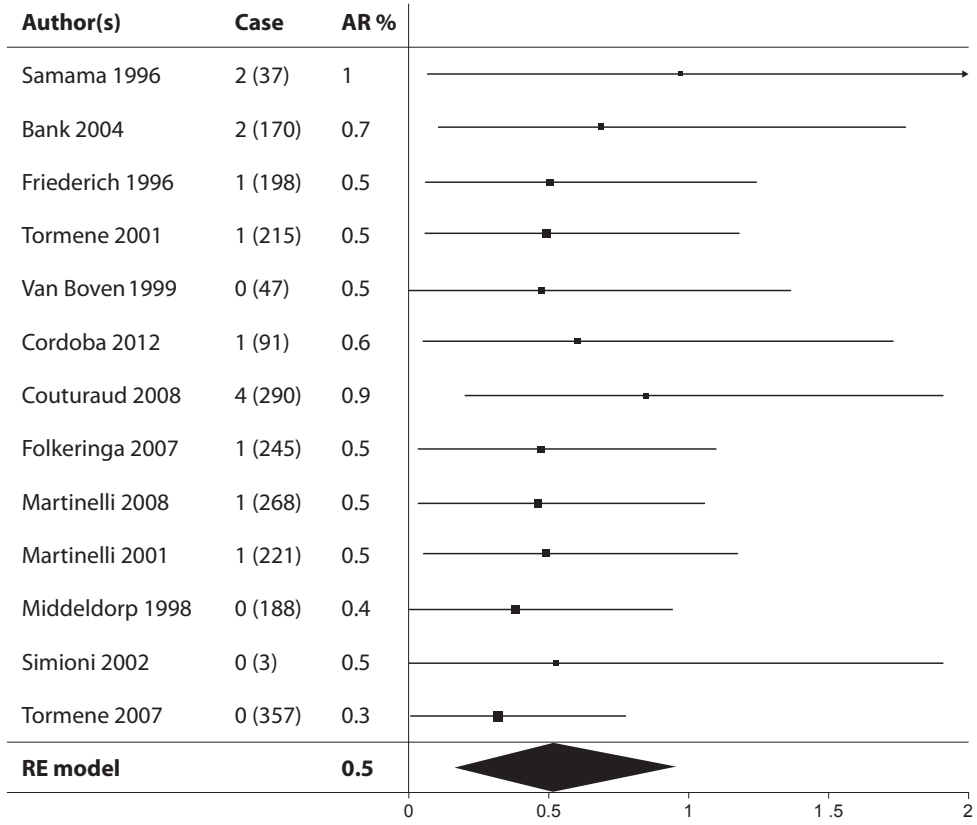
**Non-carriers: Absolute risk in non-family studies**



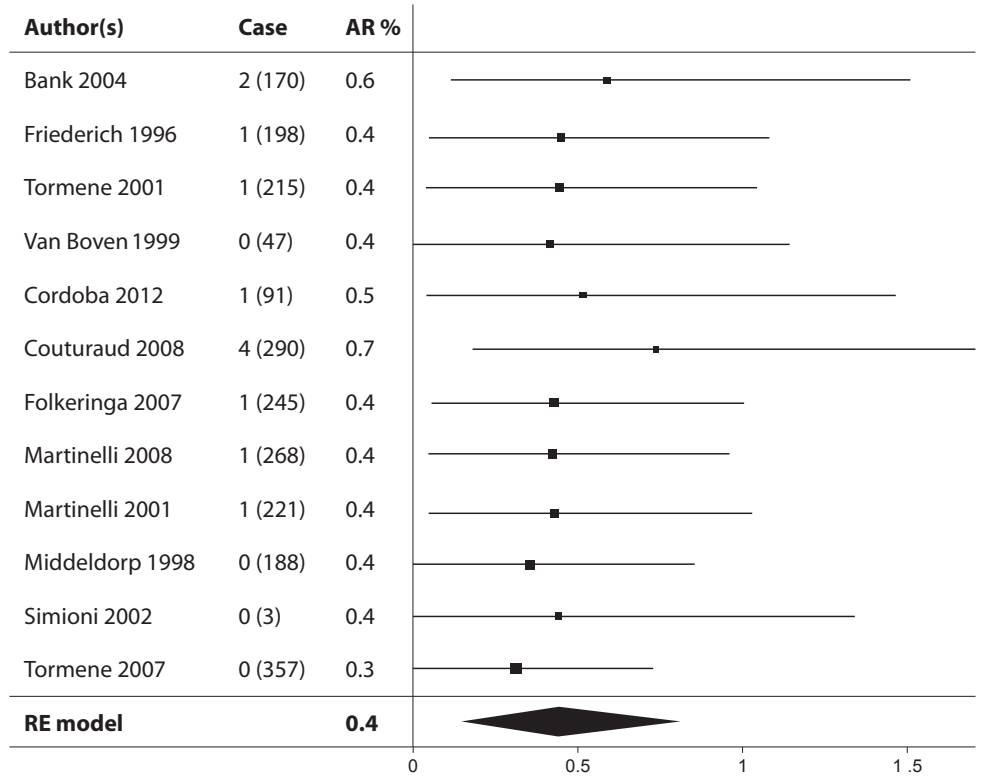

**Non-carriers: Absolute risk in non-family studies, NOS≥8**



**Non-carriers: Absolute risk in family studies**




**Non-carriers: Absolute risk in family studies, NOS≥8**



## REFERENCES


1. Jackman S. Bayesian analysis for the social sciences. . Vol. 846: Wiley & Sons; 2009
2. Adachi T, Hashiguchi K, Arai Y, Ohta H. Clinical study of venous thromboembolism during pregnancy and puerperium. *Semin Thromb Hemost*. 2001;27(2):149-154.
3. Allaart CF, Poort SR, Rosendaal FR, Reitsma PH, Bertina RM, Briet E. Increased risk of venous thrombosis in carriers of hereditary protein C deficiency defect. *LANCET*. 1993;341(8838):134-138.
4. Antovic A, Blombäck M, Bremme K, Van Rooijen M, He S. Increased hemostasis potential persists in women with previous thromboembolism with or without APC resistance. *J Thromb Haemost*. 2003;1(12):2531-2535.
5. Bergrem A, Jacobsen EM, Skjeldestad FE, Jacobsen AF, Skogstad M, Sandset PM. The association of antiphospholipid antibodies with pregnancy-related first time venous thrombosis--a population-based case-control study. *Thromb Res*. 2010;125(5):e222-227.
6. Bergrem A, Dahm AEA, Jacobsen AF, Sandvik L, Sandset PM. Differential haemostatic risk factors for pregnancy-related deep-vein thrombosis and pulmonary embolism: A population-based case-control study. *Thromb Haemost*. 2012;108(6):1165-1171.
7. Blanco-Molina A, Trujillo-Santos J, Criado J, et al. Venous thromboembolism during pregnancy or postpartum: Findings form the RIETE Registry. *Thromb Haemost*. 2007;97(2):186-190.
8. Bombeli T, Raddatz Mueller P, Fehr J. Evaluation of an optimal dose of low-molecular-weight heparin for thromboprophylaxis in pregnant women at risk of thrombosis using coagulation activation markers. *Haemostasis*. 2001;31(2):90-98.
9. Brouwer JLP, Veeger NJGM, Kluin-Nelemans HC, Van Der Meer J. The pathogenesis of venous thromboembolism: Evidence for multiple interrelated causes. *Ann Intern Med*. 2006;145(11):807-815.
10. Chopra N, Koren S, Greer WL, et al. Factor V Leiden, prothrombin gene mutation, and thrombosis risk in patients with antiphospholipid antibodies. *J Rheumatol*. 2002;29(8):1683-1688.
11. Conard J, Horellou MH, Van Dreden P, Lecompte T, Samama M. Thrombosis and pregnancy in congenital deficiencies in AT III, protein C or protein S: study of 78 women. *Thromb Haemost*. 1990;63(2):319-320.
12. Coppens M, van de Poel MH, Bank I, et al. A prospective cohort study on the absolute incidence of venous thromboembolism and arterial cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. *Blood*. 2006;108(8):2604-2607.
13. Coriu L, Copaciu E, Tulbure D, et al. Inherited thrombophilia in pregnant women with intrauterine growth restriction. *Medica*. 2014;9(4):351-355.
14. Couturaud F, Kearon C, Leroyer C, et al. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. *Thromb Haemost*. 2006;96(6):744-749.
15. Danilenko-Dixon DR, Heit JA, Silverstein MD. Risk factors for deep vein thrombosis and pulmonary embolism during pregnancy or post partum: a population-based, case-control study. *American journal of …*. 2001.
16. Dargaud Y, Rugeri L, Vergnes MC, et al. A risk score for the management of pregnant women with increased risk of venous thromboembolism: A multicentre prospective study. *Br J Haematol*. 2009;145(6):825-835.
17. Stefano VD, Leone G, Mastrangelo S. … of inherited thrombophilia: retrospective analysis and follow-up after diagnosis of 238 patients with congenital deficiency of antithrombin III, protein C, protein S. *Thrombosis and …*. 1994.
18. De Stefano V, Rossi E, Paciaroni K, et al. Different circumstances of the first venous thromboembolism among younger or older heterozygous carriers of the G20210A polymorphism in the prothrombin gene. *Haematologica*. 2003;88(1):61-66.
19. Stefano VD, Martinelli I, Rossi E, et al. The risk of recurrent venous thromboembolism in pregnancy and puerperium without antithrombotic prophylaxis. *Br J Haematol*. 2006;135(3):386-391.
20. DeSancho MT, Berlus N, Christos PJ, Rand J. Risk factors for clinical manifestations in carriers of Factor v Leiden and prothrombin gene mutations. *Blood Coagul Fibrinolysis*. 2010;21(1):11-15.
21. Dulíček P, Malý J, Šafářová M. Risk of thrombosis in patients homozygous and heterozygous for factor V Leiden in the East Bohemian region. *Clin Appl Thromb Hemost*. 2000;6(2):87-89.
22. Emmerich J, Alhenc-Gelas M, Aillaud MF, et al. Clinical features in 36 patients homozygous for the ARG 506 → GLN factor V mutation. *THROMB HAEMOST*. 1997;77(4):620-623.
23. Faioni EM, Razzari C, Martinelli I, Panzeri D, Franchi F, Mannucci PM. Resistance to activated protein C in unselected patients with arterial and venous thrombosis. *AM J HEMATOL*. 1997;55(2):59-64.
24. Finazzi G, Barbui T. Different Incidence of Venous Thrombosis in Patients with Inherited Deficiencies of Antithrombin-Iii, Protein-C and Protein-S. *Thromb Haemost*. 1994;71(1):15-18.
25. Gadelha T, Roldán V, Lecumberri R, et al. Clinical characteristics of patients with factor V Leiden or prothrombin G20210A and a first episode of venous thromboembolism. Findings from the RIETE Registry. *Thromb Res*. 2010;126(4):283-286.
26. Gat I, Dulitzki M, Schiff E, Sivan E, Simchen MJ. Peripartum thromboprophylaxis for homozygous and heterozygous FVL mutation carriers yields similar pregnancy outcome. *Isr Med Assoc J*. 2014;16(2):96-100.
27. Gerhardt A, Scharf RE, Beckmann MW, et al. Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium. *New Engl J Med*. 2000;342(6):374-380.
28. Gerhardt A, Scharf RE, Zotz RB. Effect of hemostatic risk factors on the individual probability of thrombosis during pregnancy and the puerperium. *Thromb Haemost*. 2003;90(1):77-85.
29. Grandone E, De Stefano V, Rossi E, Cappucci F, Colaizzo D, Margaglione M. Antithrombotic prophylaxis during pregnancy in women with deficiency of natural anticoagulants. *Blood Coagul Fibrinolysis*. 2008;19(3):226-230.
30. Hallak M, Senderowicz J, Cassel A, et al. Activated protein C resistance (factor V Leiden) associated with thrombosis in pregnancy. *AM J OBSTET GYNECOL*. 1997;176(4):889-893.
31. Hellgren M, Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thromboembolism associated with pregnancy and oral contraceptives. *AM J OBSTET GYNECOL*. 1995;173(1):210-213.
32. Hiltunen L, Rautanen A, Rasi V, et al. An unfavorable combination of factor V Leiden with age, weight, and blood group causes high risk of pregnancy-associated venous thrombosis-a population-based nested case-control study. *Thromb Res*. 2007;119(4):423-432.
33. Hirsch DR, Mikkola KM, Marks PW, et al. Pulmonary embolism and deep venous thrombosis during pregnancy or oral contraceptive use: Prevalence of factor V Leiden. *AM HEART J*. 1996;131(6):1145-1148.
34. Hvas AM, Ingerslev J, Salvig JD. Thrombophilia risk factors are associated with intrauterine foetal death and pregnancy-related venous thromboembolism. *Scand J Clin Lab Invest*. 2009;69(2):288-294.
35. Ilonczai P, Oláh Z, Selmeczi A, et al. Management and outcome of pregnancies in women with antithrombin deficiency: A single-center experience and review of literature. *Blood Coagul Fibrinolysis*. 2015;26(7):798-804.
36. Jacobsen AF, Skjeldestad FE, Sandset PM. Incidence and risk patterns of venous thromboembolism in pregnancy and puerperium—a register-based case-control study. *American journal of obstetrics …*. 2008.
37. James AH, Jamison MG, Brancazio LR, Myers ER. Venous thromboembolism during pregnancy and the postpartum period: Incidence, risk factors, and mortality. *Am J Obstet Gynecol*. 2006;194(5):1311-1315.

38. Khalafallah AA, Ibraheem ARO, Teo QY, et al. Review of management and outcomes in women with thrombophilia risk during pregnancy at a single institution. *ISRN Obstet Gynecol*. 2014;2014(1).
39. Knight M. Antenatal pulmonary embolism: Risk factors, management and outcomes. *BJOG Int J Obstet Gynaecol*. 2008;115(4):453-461.
40. Koster T, Rosendaal FR, Briet E, Meer FJVD. Protein C deficiency in a controlled series of unselected outpatients: an infrequent but clear risk factor for venous thrombosis (Leiden Thrombophilia Study)[see …. *Blood*. 1995.
41. Kovač M, Miković Ž, Mandić V, Radojković D, Đorđević V, Mitić G. Clinical characteristics of first venous thrombosis among women under and over 45 years of age. *Med Pregl*. 2014;67(9-10):328-333.
42. Laroche V, Delage R, Bazin S, et al. Risk of fetal loss and pregnancy-related venous thromboembolism in a large cohort of patients with protein C deficiency. *Blood*. 2001;98(11):272A-272A.
43. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. *Br J Haematol*. 2000;110(4):939-945.
44. Lenz B, Samardzija M, Drenjancevic D, Zibar D, Samardzija M, Milostic-Srb A. The investigation of hereditary and acquired thrombophilia risk factors in the development of complications in pregnancy in Croatian women. *J Matern -Fetal Neonatal Med*. 2016;29(2):264-269.
45. Lim MY, Deal AM, Kim S, et al. Thrombophilic Risk of Individuals with Rare Compound Factor V Leiden and Prothrombin G20210A Polymorphisms: An International Case-Series of 100 Individuals. *Eur J Haematol*. 2016.
46. Lindqvist P, Dahlbäck B, Maršál K. Thrombotic risk during pregnancy: a population study. *Obstetrics & Gynecology*. 1999.
47. Lindqvist PG, Kublikas M, Dahlbäck B. Individual risk assessment of thrombosis in pregnancy. *Acta Obstet Gynecol Scand*. 2002;81(5):412-416.
48. Linnemann B, Weingarz L, Schindewolf M, et al. Prevalence of established risk factors for venous thromboembolism according to age. *J Vasc Surg Venous Lymphatic Disord*. 2014;2(2):131-139.
49. Lykke JA, Bare LA, Olsen J, et al. Thrombophilias and adverse pregnancy outcomes: Results from the Danish National Birth Cohort. *J Thromb Haemost*. 2012;10(7):1320-1325.
50. Mahmoodi BK, Brouwer JLP, Ten Kate MK, et al. A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. *J Thromb Haemost*. 2010;8(6):1193-1200.
51. McColl MD, Ellison J, Reid F, Tait RC, Walker ID, Greer IA. Prothrombin 20210 G→A, MTHFR C677T mutations in women with venous thromboembolism associated with pregnancy. *Br J Obstet Gynaecol*. 2000;107(4):565-569.
52. McColl MD, Ramsay JE, Tait RC, et al. Risk factors for pregnancy associated venous thromboembolism. *THROMB HAEMOST*. 1997;78(4):1183-1188.
53. Middeldorp S, Libourel EJ, Hamulyák K, Van Meer JD, Büller HR. The risk of pregnancy-related venous thromboembolism in women who are homozygous for factor V Leiden. *Br J Haematol*. 2001;113(2):553-555.
54. Noboa S, Le Gal G, Lacut K, et al. Family history as a risk factor for venous thromboembolism. *Thromb Res*. 2008;122(5):624-629.
55. Pabinger I, Kyrie PA, Heistinger M, Eichinger S, Wittmann E, Lechner K. The risk of thromboembolism in asymptomatic patients with protein C and protein S deficiency: A prospective cohort study. *THROMB HAEMOST*. 1994;71(4):441-445.
56. Pabinger I, Nemes L, Rintelen C, et al. Pregnancy-associated risk for venous thromboembolism and pregnancy outcome in women homozygous for factor V Leiden. *Hematol J*. 2000;1(1):37-41.
57. Pabinger I, Schneider B. Thrombotic risk in hereditary antithrombin III, protein C, or protein S deficiency: A cooperative, retrospective study. *ARTERIOSCLER THROMB VASC BIOL*. 1996;16(6):742-748.





58. Pabinger I, Grafenhofer H, Kaider A, et al. Risk of pregnancy-associated recurrent venous thromboembolism in women with a history of venous thrombosis. *J Thromb Haemost*. 2005;3(5):949-954.
59. Pabinger I, Grafenhofer H, Kyrle PA, Quehenberger P. Temporary increase in the risk for recurrence during pregnancy in women with a history of venous thromboembolism. *Blood*. 2002.
60. Pai RP, Kangath RV, Brown TA. Saddle pulmonary thromboembolism with zero Wells' score. *Conn Med*. 2011;75(2):89-91.
61. Philipp CS, Faiz AS, Beckman MG, et al. Differences in thrombotic risk factors in black and white women with adverse pregnancy outcome. *Thromb Res*. 2014;133(1):108-111.
62. Pomp ER, Lenselink AM, Rosendaal FR, Doggen CJM. Pregnancy, the postpartum period and prothrombotic defects: Risk of venous thrombosis in the MEGA study. *J Thromb Haemost*. 2008;6(4):632-637.
63. Procare G. Risk of venous thromboembolism during pregnancy in homozygous carriers of the factor V Leiden mutation: are there any predictive factors? Vol. 2: ncbi.nlm.nih.gov; 2004.
64. Procare G. Comparison of thrombotic risk between 85 homozygotes and 481 heterozygotes carriers of the factor V Leiden mutation: retrospective analysis from the Procare Study. *Blood Coagul Fibrinolysis*. 2000;11(6):511-518.
65. Rodger MA, Phillips P, Kahn SR, James AH, Konkle BA. Low-molecular-weight heparin to prevent postpartum venous thromboembolism a pilot randomised placebo-controlled trial. *Thromb Haemost*. 2015;113(1):212-216.
66. Rodger MA, Hague WM, Kingdom J, et al. Antepartum dalteparin versus no antepartum dalteparin for the prevention of pregnancy complications in pregnant women with thrombophilia (TIPPS): A multinational open-label randomised trial. *Lancet*. 2014;384(9955):1673-1683.
67. Rodger M. Pregnancy and venous thromboembolism: 'TIPPS' for risk stratification. *Hematol-Am Soc Hematol Educ Program*. 2014:387-392.
68. Rogenhofer N, Bohlmann MK, Beuter-Winkler P, et al. Prevention, management and extent of adverse pregnancy outcomes in women with hereditary antithrombin deficiency. *Ann Hematol*. 2014;93(3):385-392.
69. Rouse DJ. The Factor V Leiden mutation does not increase the risk of pregnancy-related venous thromboembolism. *Evid -based Obstet Gynecol*. 2003;5(1):56.
70. Sabadell J, Casellas M, Alijotas-Reig J, Arellano-Rodrigo E, Cabero L. Inherited antithrombin deficiency and pregnancy: Maternal and fetal outcomes. *Eur J Obstet Gynecol Reprod Biol*. 2010;149(1):47-51.
71. Said JM, Higgins JR, Moses EK, et al. Inherited thrombophilia polymorphisms and pregnancy outcomes in nulliparous women. *Obstet Gynecol*. 2010;115(1):5-13.
72. Samama MM, Rached RA, Conard J, Horellou MH, Elalamy I. Pregnancy-associated venous thrombosis in women with hereditary heterozygous factor V Leiden and/or factor II gene mutations. *Bull Acad Natl Med*. 2004;188(8):1377-1393.
73. Samama MM, Rached RA, Horellou MH, et al. Pregnancy-associated venous thromboembolism (VTE) in combined heterozygous factor V Leiden (FVL) and prothrombin (FII) 20210 A mutation and in heterozygous FII single gene mutation alone. *Br J Haematol*. 2003;123(2):327-334.
74. Sanson BJ, Simioni P, Tormene D, Moia M. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. *Blood*. 1999.
75. Selmeczi A, Roach REJ, Móré C, et al. Thrombin generation and low-molecular-weight heparin prophylaxis in pregnant women with thrombophilia. *Thromb Haemost*. 2015;113(2):283-289.
76. Simioni P, Sanson BJ, Prandoni P, et al. Incidence of venous thromboembolism in families with inherited thrombophilia. *Thromb Haemost*. 1999;81(2):198-202.
77. Simpson EL, Lawrenson RA. Venous thromboembolism in pregnancy and the puerperium: incidence and additional risk factors from a London perinatal database. … *Journal of Obstetrics* …. 2001.

78. Tormene D, Grandone E, de Stefano V, et al. Obstetric complications and pregnancy-related venous thrombo - embolism: The effect of low-molecular-weight heparin on their prevention in carriers of factor V Leiden or prothrombin G20210A mutation. *Thromb Haemost*. 2012;107(3):477-484.

79. Trauscht-Van Horn JJ, Capeless EL, Easterling TR, Bovill EG. Pregnancy loss and thrombosis with protein C deficiency. *AM J OBSTET GYNECOL*. 1992;167(4 I):968-972.

80. Vavrinkova B, Binder T, Hadacova I, Hrachovinova I, Salaj P, Hruda M. Does asymptomatic carriage of FV Leiden and FII prothrombin mutations in heterozygous configuration pose an increased risk of thrombembolic complications in the course of pregnancy, labor and puerperium? *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2014;158(2):238-241.

81. Vicente V, Rodriguez C, Soto I, Fernandez M, Moraleda JM. Risk of thrombosis during pregnancy and post-partum in hereditary thrombophilia. *Am J Hematol*. 1994;46(2):151-152.

82. Villani M, Dentali F, Colaizzo D, et al. Pregnancy-related venous thrombosis: Comparison between spontaneous and ART conception in an Italian cohort. *BMJ Open*. 2015;5(10).

83. Villani M, Tiscia GL, Margaglione M, et al. Risk of obstetric and thromboembolic complications in family members of women with previous adverse obstetric outcomes carrying common inherited thombophilias. *J Thromb Haemost*. 2012;10(2):223-228.

84. Bank I, Libourel EJ, Middeldorp S, et al. Prothrombin 20210A mutation: A mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study. *Arch Intern Med*. 2004;164(17):1932-1937.

85. Bucciarelli P, Rosendaal FR, Tripodi A. Risk of Venous Thromboembolism and Clinical Manifestations in Carriers of Antithrombin, Protein C, Protein S Deficiency, or Activated Protein C Resistance. A Multicenter Collaborative Family Study. *ARTERIOSCLER THROMB VASC BIOL*. 1999;19:1026-1033.

86. Clark P, Walker ID, Govan L, Wu O, Greer IA. The GOAL study: A prospective examination of the impact of factor V Leiden and ABO(H) blood groups on haemorrhagic and thrombotic pregnancy outcomes. *Br J Haematol*. 2008;140(2):236-240.

87. Cochery-Nouvellon É, Mercier É, Lissalde-Lavigne G, et al. Homozygosity for the C46T polymorphism of the F12 gene is a risk factor for venous thrombosis during the first pregnancy. *J Thromb Haemost*. 2007;5(4):700-707.

88. Cordoba I, Pegenaute C, González-López TJ, et al. Risk of placenta-mediated pregnancy complications or pregnancy-related VTE in VTE-asymptomatic families of probands with VTE and heterozygosity for factor V Leiden or G20210 prothrombin mutation. *Eur J Haematol*. 2012;89(3):250-255.

89. Couturaud F, Leroyer C, Mottier D, Groupe dEtude de la Thrombose de Bretagne O. Risk factors and clinical presentation of venous thromboembolism according to the age of relatives of patients with factor V Leiden. *Thromb Haemost*. 2008;99(4):793-794.

90. Dilley A, Austin H, El-Jamil M, et al. Genetic factors associated with thrombosis in pregnancy in a United States population. *Am J Obstet Gynecol*. 2000;183(5):1271-1277.

91. Dizon-Townson D, Miller C, Sibai B, et al. The relationship of the factor V Leiden mutation and pregnancy outcomes for mother and fetus. *Obstet Gynecol*. 2005;106(3):517-524.

92. Eichinger S, Weltermann A, Philipp K, et al. Prospective evaluation of hemostatic system activation and thrombin potential in healthy pregnant women with and without factor V Leiden. *Thromb Haemost*. 1999;82(4):1232-1236.

93. Folkeringa N, Brouwer JLP, Korteweg FJ, Veeger NJGM, Erwich JJHM, Van Der Meer J. High risk of pregnancy-related venous thromboembolism in women with multiple thrombophilic defects. *Br J Haematol*. 2007;138(1):110-116.

94. Friederich PW, Sanson BJ, Simioni P, et al. Frequency of pregnancy-related venous thromboembolism in anticoagulant factor-deficient women: Implications for prophylaxis. *ANN INTERN MED*. 1996;125(12):955-960.



95. Gerhardt A, Scharf RE, Greer IA, Zotz RB. Hereditary risk factors of thrombophilia and probability of venous thromboembolism during pregnancy and the puerperium. *Blood*. 2016.

96. Grandone E, Margaglione M, Colaizzo D, et al. Genetic susceptibility to pregnancy-related venous thromboembolism: Roles of factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations. *Am J Obstet Gynecol*. 1998;179(5):1324-1328.

97. Hammerová L, Chabada J, Drobný J, Bátorová A. Factor V Leiden mutation and its impact on pregnancy complications. *Acta Medica (Hradec Kralove)*. 2011;54(3):117-121.

98. Heit JA, Sobell JL, Li H, Sommer SS. The incidence of venous thromboembolism among Factor V Leiden carriers: A community-based cohort study. *J Thromb Haemost*. 2005;3(2):305-311.

99. Jacobsen AF, Dahm A, Bergrem A, Jacobsen EM, Sandset PM. Risk of venous thrombosis in pregnancy among carriers of the factor V Leiden and the prothrombin gene G20210A polymorphisms. *J Thromb Haemost*. 2010;8(11):2443-2449.

100. Kjellberg U, Van Rooijen M, Bremme K, Hellgren M. Factor v Leiden mutation and pregnancy-related complications. *Am J Obstet Gynecol*. 2010;203(5):469.e461-469.e468.

101. Klai S, Fekih-Mrissa N, Sassi RB, Mrissa R, Rachdi R, Gritli N. Effects of prothrombotic markers and non-O blood group in maternal venous thromboembolism during pregnancy and postpartum. *Blood Coagul Fibrinolysis*. 2012;23(7):649-652.

102. Lindqvist PG, Svensson PJ, Maršál K, Grennert L, Luterkort M, Dahlbäck B. Activated protein C resistance (FV:Q506) and pregnancy. *Thromb Haemost*. 1999;81(4):532-537.

103. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannuccio Mannucci P. Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia. *Thromb Haemost*. 2001;86(3):800-803.

104. Martinelli I, De Stefano V, Taioli E, Paciaroni K, Rossi E, Mannucci PM. Inherited thrombophilia and first venous thromboembolism during pregnancy and puerperium. *Thromb Haemost*. 2002;87(5):791-795.

105. Martinelli I, Battaglioli T, De Stefano V, et al. The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A. *J Thromb Haemost*. 2008;6(3):494-498.

106. Meglič L, Stegnar M, Milanez T, et al. Factor V Leiden, prothrombin 20210G → A, methylenetetrahydrofolate reductase 677C → T and plasminogen activator inhibitor 4G/5G polymorphism in women with pregnancy-related venous thromboembolism. *Eur J Obstet Gynecol Reprod Biol*. 2003;111(2):157-163.

107. Middeldorp S, Henkens CMA, Koopman MMW, et al. The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. *Ann Intern Med*. 1998;128(1):15-20.

108. Middeldorp S, Meinardi JR, Koopman MMW, et al. A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. *Ann Intern Med*. 2001;135(5):322-327.

109. Mitic G, Kovac M, Jurisic D, et al. Clinical characteristics and type of thrombophilia in women with pregnancy-related venous thromboembolic disease. *Gynecol Obstet Invest*. 2011;72(2):103-108.

110. Murphy RP, Donoghue C, Nallen RJ, et al. Prospective evaluation of the risk conferred by factor V Leiden and thermolabile methylenetetrahydrofolate reductase polymorphisms in pregnancy. *Arterioscler Thromb Vasc Biol*. 2000;20(1):266-270.

111. Ogunyemi D, Cuellar F, Ku W, Arkel Y. Association between inherited thrombophilias, antiphospholipid antibodies, and lipoprotein A levels and venous thromboembolism in pregnancy. *Am J Perinatol*. 2003;20(1):17-23.

112. Samama MM, Simon D, Horellou MH, Trossaërt M, Elalamy I, Conard J. Diagnosis and clinical characteristics of inherited activated protein C resistance. *HAEMOSTASIS*. 1996;26(SUPPL. 4):315-330.

113. Simioni P, Tormene D, Prandoni P, Zerbinati P. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Vol. 99: Am Soc Hematology; 2002.

114. Tam WH, Ng MHL, Yiu AKW, Lau KM, Cheng GYM, Li CY. Thrombophilia among chinese women with venous thromboembolism during pregnancy. *Gynecol Obstet Invest*. 2012;73(3):183-188.

115. Tormene D, Simioni P, Prandoni P, et al. Factor V Leiden mutation and the risk of venous thromboembolism in pregnant women. *Haematologica*. 2001;86(12):1305-1309.

116. Tormene D, De Stefano V, Grandone E, et al. The G20210A prothrombin variant and the risk of venous thromboembolism or fetal loss in pregnant women: A family study. *J Thromb Haemost*. 2007;5(11):2193-2196.

117. Boven HHV, Vandenbroucke JP, Briet E. Gene-gene and gene-environment interactions determine risk of thrombosis in families with inherited antithrombin deficiency. Vol. 94: Am Soc Hematology; 1999.

118. Vora S, Ghosh K, Shetty S, Salvi V, Satoskar P. Deep venous thrombosis in the antenatal period in a large cohort of pregnancies from western India. *Thromb J*. 2007;5:5-9.

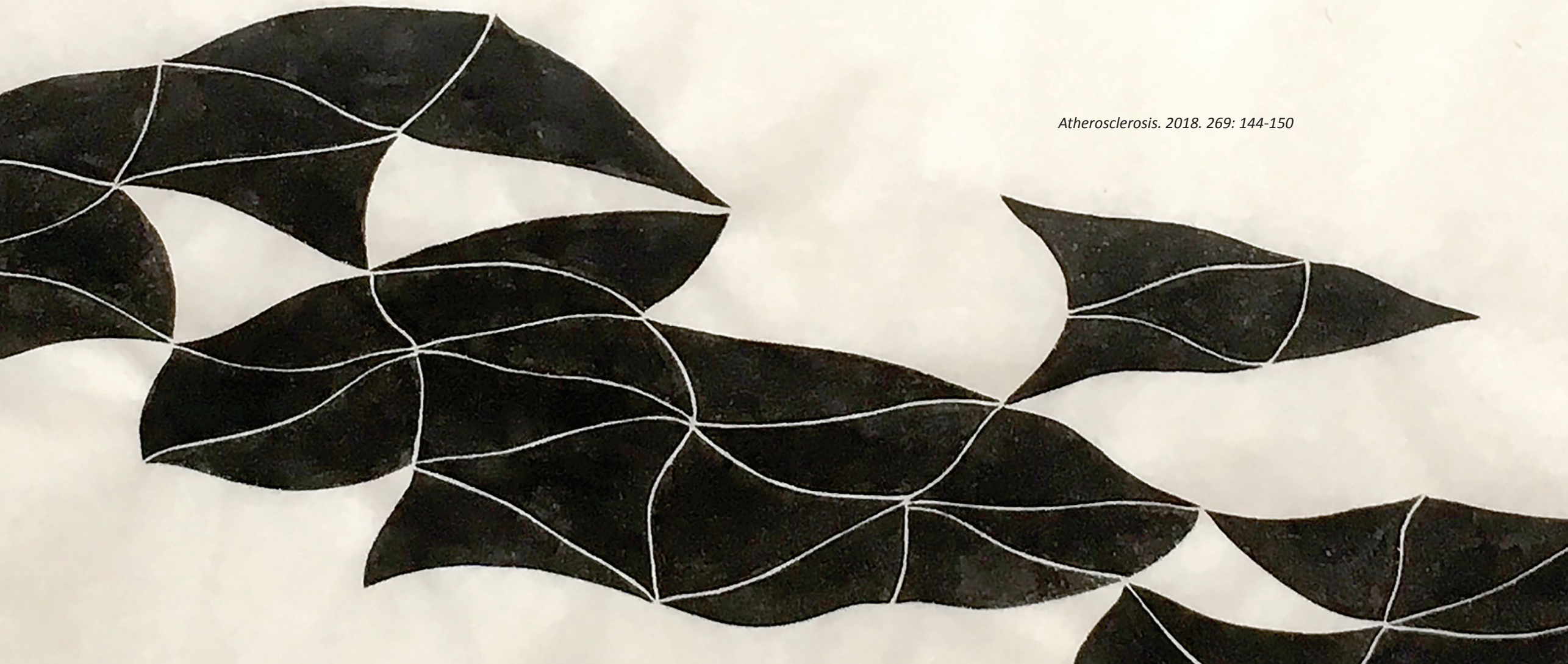
119. Yilmazer M, Kurtay G, Sonmezer M, Akar N. Factor V Leiden and prothrombin 20210 G-A mutations in controls and in patients with thromboembolic events during pregnancy or the puerperium. *Arch Gynecol Obstet*. 2003;268(4):304-308.

# 7

## ANTITHROMBIN LEVELS ARE ASSOCIATED WITH THE RISK OF FIRST AND RECURRENT ARTERIAL THROMBOEMBOLISM AT A YOUNG AGE

F.N. Croles, J.E. Van Loon, D.W.J. Dippel, M.P.M. De Maat, F.W.G. Leebeek

*Atherosclerosis. 2018. 269: 144-150*



## ABSTRACT

**Background and aims**

It is as yet unknown whether antithrombin levels are associated with arterial thromboembolism (ATE) at a young age. To investigate the association between antithrombin levels with premature ATE and with recurrent ATE we performed a case-control study and a subsequent nested cohort study of premature coronary heart disease (CHD) patients.

**Methods**

In the case-control study, we included 571 patients who had a recent premature ATE, including CHD and ischemic stroke (IS), and 461 healthy controls. The association between antithrombin levels (dichotomized: ≤median vs. >median) and ATE was investigated. Subsequently we studied the association between antithrombin levels and recurrent cardiac events, ATE or death in a nested cohort of 323 CHD patients.

**Results**

Low antithrombin levels (≤median, 1.04 IU/mL) are associated with an increased risk of ATE (OR 1.46; 95%CI:1.09-1.96), after adjustment for classical cardiovascular risk factors. This was observed in the subgroups of CHD patients (1.43; 1.01-2.02) and IS patients (1.48; 1.01-2.19). CHD patients with low antithrombin levels had a higher risk of recurrent cardiac events (HR 2.16, 95%CI:1.07-4.38). Especially in women with low antithrombin levels the risk of recurrent cardiac events was high (HR 5.97, 95%CI 1.31-27.13) as was the risk of recurrent ATE or death (HR 4.22, 95%CI 1.19-15.00).

**Conclusions:**

Individuals with relatively low antithrombin levels have an increased risk for ATE at a younger age. CHD patients with low antithrombin levels, especially women, have a higher risk of recurrent cardiac events.


## INTRODUCTION

Antithrombin is a strong inhibitor of blood coagulation through inactivation of mainly thrombin and factor Xa. The anticoagulant function of antithrombin in vivo is thought to be activated by heparan sulphate on the vascular endothelium[1]. It has been suggested that strongly reduced antithrombin levels as in hereditary antithrombin deficiency (around 50% of normal) are associated with arterial thrombotic events (ATE)[2]. Furthermore, a pooled analysis of four cohort studies showed that inherited thrombophilia, including antithrombin deficiency, increased the risk of ATE more pronouncedly in women than in men[3]. However it is as yet unknown whether slightly reduced levels are associated with ATE. The process of atherosclerosis is driven by traditional cardiovascular risk factors, and the occurrence of ATE is typically caused by rupture of atherosclerotic lesions and subsequent thrombus formation. A lack of inhibition of thrombus formation, for instance by low antithrombin levels, may cause acceleration of thrombus formation and increase of the risk of occlusion of blood vessels. This may cause an increased risk of ATE[3]. Hemostatic factors are thought to play a more important pathophysiological role in ATE in young patients than in older patients because in young individuals atherosclerosis is not as extensive as in elderly. In addition, in young patients with myocardial infarction normal coronary arteries are more often found than in older patients[4-6]. Indeed, levels of hemostatic factors such as fibrinogen, TAFI and ADAMTS13 have been associated with cardiovascular disease at a young age[7-9].

The mortality after myocardial infarction in young patients has been reported as high as 8% at 8 years after percutaneous coronary interventions[10]. Risk factors for mortality after a myocardial infarction include heart failure, ventricular arrhythmias, angina pectoris and re-infarction[11]. Finding more risk factors may allow for more precise identification of patients at risk for recurrence or death in those with documented coronary heart disease. We hypothesize that low-normal antithrombin levels are associated with both a first and with a recurrent ATE at a young age, and may differ between sexes, as this has been shown before in other thrombophilias[3]. Therefore we investigated the association of antithrombin levels with the risk of first and recurrent ATE at a young age, including a sex-specific analysis.

## PATIENTS AND METHODS

### Patients

We performed two related studies: First we performed a case-control study and subsequently we performed a follow-up (cohort-) study of the patients with coronary heart disease from the first study.

#### *Case-control study*

The 'Genetic risk factors for Arterial Thrombosis at a young age: the role of TAFI and other Coagulation factors (ATTAC) ' study is a single-center, case-control study investigating the role of coagulation factors on incidence of ATE at a young age. The design of the study and recruitment of patients and controls has been reported elsewhere in detail[7,8]. In short, cases were consecutive patients who presented with a first acute arterial ischemic event in our Center. Our center is a university hospital with a community function, located in the city centre. Our center employs no selection criteria of admission of cardiovascular patients, but young patients are overrepresented. Patients were men aged 45 years or younger or women aged 55 or younger. The case-control study originally consisted of three subgroups: Group CHD: coronary heart disease, including acute myocardial infarction and unstable angina pectoris (CHD); group IS: ischemic stroke(IS) or transient ischemic attack(TIA); and group PAD: peripheral arterial disease (PAD). Patients were included 1-3 months after the event, to avoid a possible acute phase response on plasma levels of the parameters investigated. Controls were neighbors or friends of the patients. They fulfilled the same age criteria but did not have a history of ATE.

#### *Follow-up study*

The 368 subjects included in the ATTAC study who presented with coronary heart disease were asked to participate in a follow-up study as reported elsewhere in more detail[9]. These subjects were followed at the outpatient clinic (and by telephone in case this was not possible) for recurrent cardiac events and for any recurrent cardiovascular event or all-cause mortality.

Participants were included in the study after written informed consent between October 2001 and June 2010. Both studies, the ATTAC study and the follow-up study, were approved by the institutional Medical Ethics Committee of the Erasmus MC, and both conform to the ethical guidelines of the 1975 declaration of Helsinki.

### Definitions

The definitions used in this study have been reported elsewhere[8]. In short, CHD was defined as acute myocardial infarction (AMI) or unstable angina pectoris (UAP). AMI was defined as typical chest pain, with elevated cardiac markers (CK MB/troponin T), and/or characteristic electrocardiographic findings. UAP was defined as typical chest pain while at rest. Transient ischemic attack (TIA) was defined as the acute onset of focal cerebral dysfunction, which could not be explained otherwise than by focal cerebral ischemia, as diagnosed by a neurologist. Symptoms had to be temporary and last less than 24 hours. Ischemic stroke (IS) was defined as the acute onset of focal cerebral dysfunction as a result of cerebral ischemia, with symptoms lasting longer than 24 hours. Brain imaging by CT-scan or MRI-scan had to be compatible with the diagnosis and was used to rule out intracerebral hemorrhage. Smoking status was defined as self-reported never, previous or current smoker. Hypercholesterolemia was originally defined as a subject-reported presence of hypercholesterolemia or the use of lipid lowering treatment on the day of the event. As most cases used cholesterol lowering medications at inclusion, cholesterol measurements were not representative of cholesterol levels before the event. Therefore in the case-control study cholesterol was not adjusted for in the analyses. In the follow-up study, as nearly all patients used lipid-lowering treatment, cholesterol levels were adjusted for in the analyses. Patients with a medical history of diabetes or using either oral anti-diabetic medication or insulin therapy on the day of the event were considered to have diabetes. Hypertension was defined by a systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg or the use of anti-hypertensive drugs.

In the follow-up study we used the endpoints recurrent cardiac event (defined as myocardial infarction or revascularization procedure) and any recurrent arterial thrombotic event or death (defined recurrent cardiac event, cerebrovascular event (IS or TIA) or all-cause mortality). At the time of inclusion in the study, direct acting oral anticoagulants were not yet registered in the Netherlands.

### Blood sampling

Blood was collected 1–3 months after the event in sodium citrate (final concentration 0.105M) using a Vacutainer System (Beckton, Dickinson and Company, Plymouth, UK) and centrifuged at 2000g for 10 min at 4°C. Plasma was additionally centrifuged for 10min at 20,000g for 10min at 4°C and stored in aliquots at −80°C. Technicians were not aware of the case–control status of the samples. Antithrombin activity measurements were performed using the factor Xa-based INNOVANCE® Antithrombin assay kit (Siemens). Measurements of samples were performed once, but AT values outside the reference range were performed in duplicate. Cholesterol and HDL were determined on Modular Analytics® (Roche Diagnostics, Mannheim, Germany).

### Statistical analysis

Descriptive statistics were used in both studies. The data are presented as means ± standard deviation.

#### *Case-control study*

To compare levels of the normally distributed risk factors between groups, ANOVA's were performed with adjustment for age and sex. We performed logistic regression for ATE with antithrombin activity as a continuous variable and as a binominal variable, using the predefined cut-off-levels of the lower limit of the reference range (≤0.80 U/mL) and the median antithrombin level of controls. Logistic regression analyses were adjusted for age, sex, BMI, smoking, family history, hypertension, diabetes mellitus. In addition, separate sex-specific analyses were performed, adjusted for the same cardiovascular risk factors.

#### *Follow-up study*

In the follow-up study, cumulative event-free survival curves were constructed using the Kaplan-Meier method. Comparisons in the Kaplan-Meier curves were performed using the Log-rank test. The median antithrombin level of the cohort of CHD patients was the predefined cut-off level. To determine by how much low antithrombin levels in this cohort increase the risk of ATE, Cox-regression analysis was performed with the median antithrombin levels after adjustment for age, BMI and sex, and after adjustment for age, BMI and sex, family history, cholesterol, HDL, hypertension, diabetes, and smoking (at inclusion).

All statistical analyses were performed using SPSS for Windows, version 21 (SPSS Inc., Chicago, IL, USA).



## RESULTS

#### *Case-control study*

The ATTAC study included a total of 1157 participants. From 76 participants (38 cases, 38 controls) plasma was no longer available, and 49 were PAD patients. This current study therefore included 1032 individuals, of whom 571 CHD and IS cases and 461 healthy controls. Forty-five percent of cases (257/620) and thirty-five percent of controls (162/461) were men. The mean age was 39.8, standard deviation (SD) ±7.8 for cases and 43.0 SD ±6.8 for controls. As expected, traditional risk factors were more prevalent in cases than in controls (Table 1).

When investigating classical risk factors for ATE using logistic regression, we found that age (OR 1.05, 95%CI:1.03-1.07), male sex (OR 2.08, 95%CI: 1.54-2.83), family history of cardiovascular disease (OR 2.25, 95%CI:1.66-3.05), hypertension (OR 3.98, 95%CI:2.54-6.24), and diabetes (OR 4.90, 95%CI: 2.17-10.89) were significantly associated with ATE. Hypercholesterolemia was associated with a lower risk of ATE (OR 0.65, 95%CI:0.43-0.96).

The median of antithrombin activity was similar across controls and subtypes of cases (in controls, CHD and IS: 1.04 U/mL). The distribution of antithrombin levels in the three groups is shown in figure 1. Since the distribution approached a normal distribution (even though the Kolmogorov-Smirnov test did not support a normal distribution) we performed parametric statistics, since this enables us to adjust for confounders and effect modifiers. Ten participants had antithrombin values ≤0.80 U/mL (lower limit of reference range). Logistic regression without adjustment for classical risk factors showed no predictive value of antithrombin activity as a continuous variable (OR1.53; 95%CI:0.48-4.89) or at the cut-offs of 0.80 U/ml (OR 1.21; 95%CI:0.34-4.33) and median antithrombin level (≤1,04 U/ml) (OR 1.12; 95%CI:0.87-1.43). However, when using antithrombin activity as a binary variable with predefined cut-off of the median antithrombin level, a 46% increased risk of ATE was found in the adjusted analysis for the participants with low antithrombin levels (OR 1.46; 95%CI:1.09-1.96). Similar results were found in the CHD group (OR 1.43; 95%CI:1.01-2.02) and in the IS group (OR 1.48; 95%CI: 1.01-2.19). In a separate sex-specific analysis no major differences were found between men and women. To investigate if the higher age at inclusion for women influenced results, women with the age of 46 or older were excluded. The adjusted OR was 1.55, 95%CI: 1.09-2.21. These results are summarized in Table 2.

**Table 1. Patient characteristics of the ATTAC case-control study on the risk of a first arterial thromboembolism.**

| | Controls (n=461) | Cases (n=571) | p-value |
|---|---|---|---|
| **Demographics** | | | |
| Age, mean (years) | 39.8 ±7.8 | 43.0 ±6.8 | <0.01 |
| Men, n (%) | 162 (35.1) | 257 (45.0) | <0.01 |
| Body mass index, mean (kg/m2 ±SD) | 25.7 ±4.5 | 27.0 ±4.7 | <0.01 |
| Index event | | | |
| UAP+AMI (n) | | 368 | |
| Stroke+TIA (n) | | 203 | |
| **Risk factors** | | | |
| Family history of ATE(%) | 22.6 | 51.5 | |
| Smoking (former+current)(%) | 52.5 | 75.7 | <0.01 |
| Hypertension(%) | 6.5 | 28.2 | 0.12 |
| Systolic blood pressure, mmHg, median ±SD | 123 ±16 | 122 ±21 | |
| Diastolic blood pressure, mmHg, median ±SD | 80 ±11 | 80 ±13 | |
| Diabetes(%) | 1.7 | 10.2 | <0.01 |
| Cholesterol, mean, ±SD | 5.17 ±0.94 | 4.29 ±0.96 | <0.01 |
| LDL, mean, ±SD | 3.17 ±0.89 | 2.51 ±0.85 | <0.01 |
| HDL, mean, ±SD | 1.52 ±0.43 | 1.29 ±0.42 | <0.01 |
| Antithrombin activity (U/mL, SD) | 1.04 ±0.10 | 1.04 ±0.11 | 0.77 |
| Antithrombin activity ≤0.80 U/mL | 4 | 6 | 0.47 |
| **Medication at study entry** | | | |
| Platelet function inhibitor | 5 | 541 | |
| Vitamin K antagonist | 4 | 47 | |
| B-blocker | 14 | 229 | |
| Ace-inhibitor or A2 antagonist | 12 | 293 | |
| Calcium blocker | 1 | 48 | |
| Diuretic | 7 | 72 | |
| Statin or fibrate | 9 | 483 | |

Abbreviations used in this table: n: Number. UAP: unstable angina pectoris. AMI: acute myocardial infarction. TIA: transient ischaemic attack. ATE: arterial thromboembolism. SD: standard deviation. LDL: low density lipids. HDL: High density lipids.



**Table 2. Antithrombin as risk factor for a first arterial thromboembolism in the ATTAC case-control study**

| | CHD+IS | CHD | IS |
|---|---|---|---|
| **Antithrombin continuous** | | | |
| Unadjusted | 1.53; 0.48-4.89 | | |
| Adjusted [a] | 0.46; 0.12-1.85 | 0.44; 0.09-2.28 | 0.58; 0.09-3.68 |
| **Antithrombin median or lower (≤1.04 U/mL)** | | | |
| Unadjusted | 1.12; 0.87-1.43 | | |
| Adjusted [a] | **1.46; 1.09-1.96** | **1.43; 1.01-2.02** | **1.48; 1.01-2.19** |
| Men, adjusted [a] | 1.38; 0.86-2.24 | 1.27; 0.76-2.13 | 1.97; 0.92-4.20 |
| Women, adjusted [a] | **1.46; 1.00-2.14** | 1.56; 0.97-2.52 | 1.33; 0.84-2.12 |
| Subjects aged <46, adjusted [a] | **1.55; 1.09-2.21** | | |
| **Antithrombin deficiency (≤0.80 U/mL)** | | | |
| Unadjusted | 1.21; 0.34-4.33 | | |
| Adjusted* | 1.52; 0.33-7.01 | 1.42; 0.19-10.54 | 2.06; 0.36-11.84 |

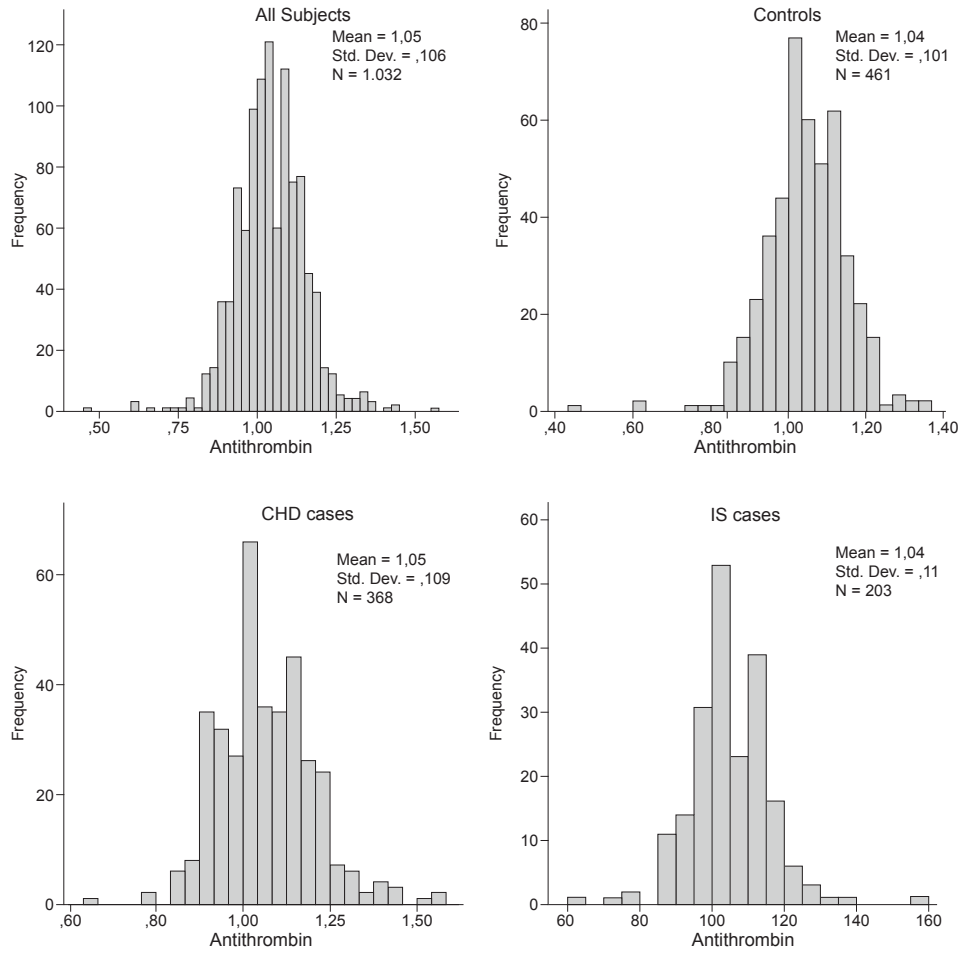
Abbreviations used in this table: CHD: Coronary heart disease. IS: Ischemic stroke. [a]: Adjusted for all cardiovascular risk factors.

### *Follow-up study*

Three-hundred-fifty-three subjects with coronary heart disease were entered the ATTAC follow-up study, of whom only 323 subjects could be evaluated, because no blood samples were available from 30 patients. Those subjects consisted of 181 men and 142 women, characteristics are summarized in supplemental table 1. At study entry, the median age was 42 years for men and 47 years for women. Seventy-one percent had single vessel disease. Most patients (94%) received a stent: 9% a bare metal stent, 85% a drug-eluting stent. No coronary artery bypass surgery was performed. Of all patients 99% used anti-platelet therapy, 94% used blood-pressure lowering drugs and 95% used statins. Median follow-up time was 3.6 years (range 0.05-9.36 years). Nine patients (3%) died and 37 (11%) had a recurrent arterial thrombotic event. Twenty-eight of the 181 men (15%) and 20 of the 142 women (14%) had a recurrent event or death. In the total group age, sex and BMI did not differ between patients with or without a recurrent event. Of the 142 women, 45 were postmenopausal, 97 were not. In postmenopausal women antithrombin levels were higher than in premenopausal women (mean in premenopausal women 1.01U/ml ±0.10, in postmenopausal women 1.07 ±0.11 ($p$=0.008).

Classical cardiovascular risk factors were not associated with recurrent events. A sex-specific analysis showed that diabetes was associated with recurrent events or death in men (HR 3.74, 95%CI 1.15-12.12). In women, none of the classical cardiovascular risk factors were associated with recurrence. Furthermore, menopausal state or use of oral contraceptives were not associated with recurrence. For a summary, see table 3.

**Figure 1: Distribution of antithrombin levels across categories in the ATTAC study.**




Distributions of antithrombin levels in all subjects (A), controls (B), coronary heart disease (CHD) patients (C) and in ischaemic stroke (IS) patients (D).

The median antithrombin level was 1.04 ±0.11 U/ml in the total CHD cohort, 1.05 ±0.11 U/ml in men and 1.02 ±0.11 U/ml in women (men vs. women, *p*=0.003).

In the total group of CHD patients, Kaplan-Meier analysis showed that patients with lower antithrombin levels, predefined as ≤median level of the cohort (≤1.04 U/mL) had a significantly higher risk of recurrent cardiac events(*p*=0.03). When stratified by sex this was observed only in women (*p*=0.021) but not in men (*p*=0.88) (Figure 2). Regarding recurrent ATE and death, a higher risk of events was found for participants with lower antithrombin levels (predefined as ≤median), but this was not significant (*p*=0.12). When stratified by sex, women with lower antithrombin levels had a significantly higher risk of events (*p*=0.012) than women with higher antithrombin levels, while no difference was seen in recurrent ATE or death between higher or lower antithrombin in men (*p*=0.82).

**Table 3. The risk of recurrent arterial events or death in the follow-up study. Traditional cardiovascular risk factors, median antithrombin and sex.**

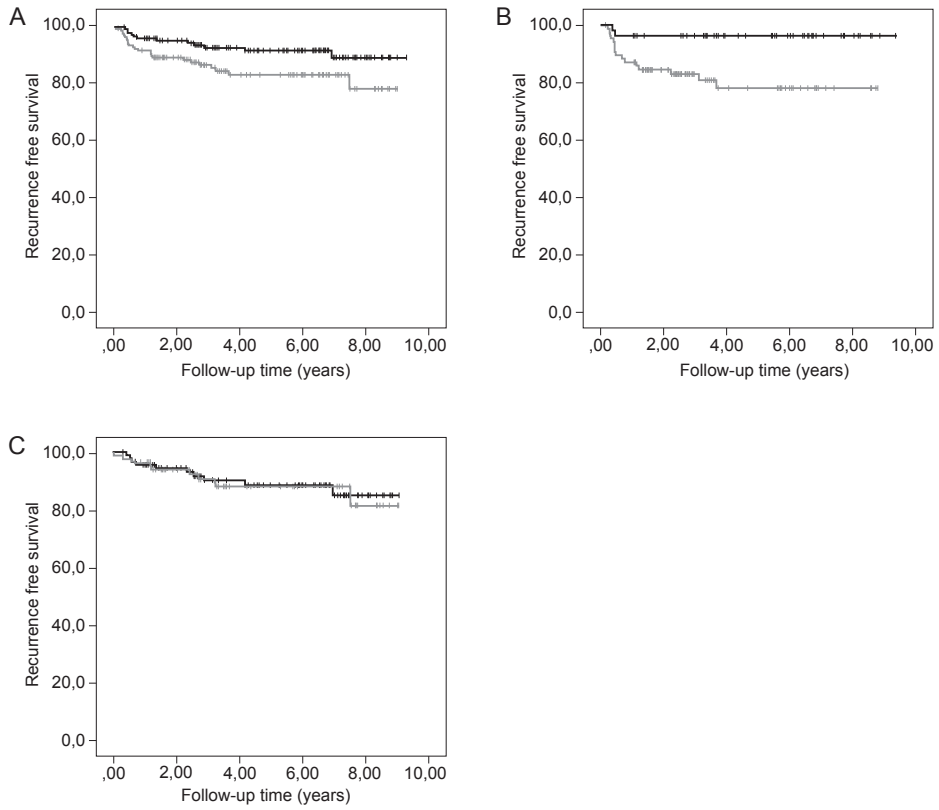
| | Any recurrent event HR (95%CI) | Men HR (95%CI) | Women HR (95%CI) |
|---|---|---|---|
| Age | 0.97 (0.92-1.03) | 0.95 (0.87-1.03) | 1.01 (0.94-1.10) |
| Female Sex | 1.05 (0.51-2.17) | | |
| Family history of cardiovascular disease | 1.04 (0.58-1.88) | 0.79 (0.35-1.80) | 1.33 (0.53-3.36) |
| Hypertension | 0.78 (0.36-1.67) | 0.83 (0.26-2.67) | 0.96 (0.33-2.83) |
| Diabetes | 1.02 (0.35-2.96) | 3.74 (1.15-12.12) | |
| Smoking, current | 0.92 (0.41-2.09) | 0.78 (0.23-2.58) | 0.36 (0.05-2.46) |
| Smoking, former | 0.62 (0.24-1.58) | 0.52 (0.17-1.56) | 1.83 (0.46-7.29) |
| Total cholesterol | 1.25 (0.88-1.78) | 1.30 (0.82-2.04) | 0.85 (0.43-1.68) |
| HDL | 1.10 (0.45-2.67) | 0.84 (0.23-3.06) | 2.58 (0.69-9.66) |
| BMI | 0.97 (0.90-1.03) | 0.93 (0.84-1.03) | 1.01 (0.92-1.12) |
| Median antithrombin (≤1.04 U/mL) | 1.67 (0.92-3.02) | 1.06 (0.48-2.35) | 4.22 (1.19-22.56) |

Cox regression analysis to assess the relationship between traditional cardiovascular risk factors and median antithrombin levels and the risk of recurrent arterial thrombotic events or death (all-cause). None of 19 women with diabetes had an event. Abbreviations used in this table: HR: Hazard ratio. HDL: High density lipids. BMI: body mass index.

In the total group of CHD patients, patients with lower (≤median) antithrombin levels had a higher chance of a recurrent cardiac event, with a HR of 2.11, 95%CI 1.06-4.21; after adjustment for age, BMI and sex: HR 2.12 95%CI 1.06-4.26; and adjustment for all risk factors: HR 2.16, 95%CI:1.07-4.38. Women with lower antithrombin levels had a higher rate of recurrent cardiac events: HR 6.01, 95%CI 1.38-26.24; after adjusting for age and BMI: HR 6.08, 95%CI 1.38-26.84; after adjusting for all risk factors: HR 5.97, 95%CI 1.31-27.13. In men, after adjusting for all risk factors no significant difference was found, HR 1.18, 95%CI 0.46-3.03.

For recurrent ATE and death in all CHD patients, no significantly different event free survival was observed for participants with lower antithrombin (adjusted for all factors: HR 1.67, 95%CI 0.92-3.02). After stratifying by sex, only women with lower antithrombin levels had a higher risk of recurrent ATE or death, with a HR of 4.2 (95%CI 1.25-14.69); after adjustment for age and BMI: HR 4.38 (95% CI 1.26-15.17); after adjustment for all cardiovascular factors: HR 4.22 (95%CI 1.19-15.00).

**Figure 2: recurrent cardiac event-free survival according to median antithrombin levels.**




Panel A: Recurrent cardiac event-free survival overall
Panel B: Recurrent cardiac event-free survival in women
Panel C: Recurrent cardiac event-free survival in men
Black bars: antithrombin level >median (1.04 U/mL)
Grey bars: antithrombin level ≤median (1.04 U/mL)



## DISCUSSION

The main finding of the case-control study is that reduced antithrombin levels (≤median) are associated with an increased risk for arterial thrombosis at a young age OR 1.46; 95%CI:1.09-1.96 when compared with individuals with high-normal (>median) antithrombin levels, after adjusting for major cardiovascular risk factors. This effect is most pronounced in women. In addition, low antithrombin levels are also associated with recurrent events in young individuals with CHD (HR 2.16, 95%CI:1.07-4.38).

Antithrombin deficiency is the first reported cause of inherited thrombophilia[12], and it is a rare but strong risk factor for venous thromboembolism (VTE). The risk of first VTE is increased up to 16-fold[13]. Furthermore, an inverse relationship between the risk of VTE and antithrombin levels within the normal range has been described; as compared with individuals with high-normal antithrombin levels, in individuals with antithrombin levels in the lower-normal range the risk of VTE is significantly increased[14]. Although venous and arterial thromboembolism are believed to be different disease entities, risk factors overlap. Inherited thrombophilia is not only associated with VTE, but also with an 1.7-fold increased risk of arterial thromboembolism (ATE), as shown in a combined analysis of four studies on thrombophilic families[3].

The finding that ≤median antithrombin levels are associated with an increased risk for arterial thrombosis at a young age is in part in line with previous studies. These other studies focused on inherited antithrombin deficiency and thus on much lower antithrombin levels (usually ≤0.80 U/ml). In a large prospective study in families with thrombophilia an up to 9-fold increased risk of ATE in individuals with inherited thrombophilia was observed[2]. In this study antithrombin deficient individuals had a 7-fold increased risk of ATE. More recently, a pooled analysis of four family studies by Mahmoodi et al showed that inherited thrombophilia overall is a risk factor for ATE[3]. This risk was even more pronounced in individuals below the age of 55 and in women. However, antithrombin deficiency by itself failed to show an association (HR 1.36, 95% CI 0.55-3.34), possibly due to a lack of power since only 72 antithrombin deficient participants were included in this analysis. Antithrombin levels were not associated with the incidence of ischemic stroke and of coronary heart disease in the ARIC study, a large, prospective population study. Of note, the individuals included in this study were older (age at inclusion 45-64) than in our study population, which included males aged ≤45 and females aged ≤55 years[15,16]. In the prospective Northwick Park Heart Study antithrombin levels and the association with ischemic heart disease were investigated in men only, aged 40-64. In this study individuals with antithrombin levels in the lowest tertile had a higher risk of death from arterial disease when compared to the middle tertile[17]. However, this was also observed for the highest tertile as compared to the middle tertile. The increased risk of ATE associated with low antithrombin levels in our large patient population with a first manifestation of ATE at an even younger age is therefore a novel and interesting finding.

In order to investigate whether antithrombin levels are associated with the risk of recurrent ATE, we subsequently followed 323 patients with CHD from our case-control study during a median of 3.6 years for recurrent events. The main finding is that lower antithrombin levels were associated with a higher risk of recurrence, with a HR of 2.16, 95%CI:1.07-4.38. The main contribution to the increased risk comes from women with lower (≤median) antithrombin levels. The women with lower antithrombin levels did not only have a high rate of recurrent cardiac events (HR 5.97, 95%CI 1.31-27.13), but also of recurrent ATE and death (HR 4.22, 95%CI 1.19-15.00) as compared to women with higher antithrombin levels. For men, having a ≤median antithrombin level was not a risk factor. This sex-specificity of lower antithrombin levels on recurrence risk of CHD has not been reported before, but gender specificity for thrombophilia as a risk factor for a first ATE has been reported before[3]. In IS patients, familial thrombophilia has been reported as risk factor for recurrent ATE, but antithrombin levels were not investigated[18]. Only one study has previously investigated the role of antithrombin levels on recurrent ATE in CHD patients. In a prospective cohort study by Pelkonen et al on acute coronary syndrome patients, patients who had a recurrent arterial event had lower antithrombin levels than patients who had no recurrence[19]. However, these subjects were mostly male, and substantially older (64 ± 10 years) than in our study (43.6 ± 5.8 years). No sex-specific analysis was performed in this study, and therefore it is difficult to compare the results of these studies. The pathogenetic explanation how lower antithrombin levels increase recurrence risk only in women has not yet been resolved. Many other gender differences in (risk factors of) coronary heart disease have been reported before[20].

Current concepts of the pathways involved in thrombus formation in acute coronary syndrome include both platelet activation and fibrin formation[21]. The latter pathway is controlled by antithrombin[1]. The hypothesis that lower antithrombin levels would increase the risk of (recurrent) cardiac events by tilting the hemostatic balance towards a more prothrombotic phenotype seems logical. When patients present with ATE, many of the risk factors causing the first arterial event such as hypercholesterolemia, diabetes, hypertension and possibly factors related to primary hemostasis will be actively managed by lifestyle adjustments or medication, as in our cohort. Therefore, other possible risk factors pertaining to inhibition of thrombin formation may become more apparent. Through these risk factors high-risk patients may be identified and potentially treated differently.

Our studies have limitations. The chosen definition of hypercholesterolemia at inclusion in the study, which includes the use of statins, irrespective of the reason why they were prescribed, did not allow for an adequate analysis of this in the case-control study. The different age-limit for men and women for inclusion in the study could have an effect on results, however, when excluding subjects (women) of age 46 or older, the effect of low antithrombin levels in the case-control-study was more pronounced, HR 1.55; 95%CI 1.09-2.21. The blood samples may have been influenced by post-event changes such as coagulation activation[22,23]. This was however not tested. Therefore the association of low antithrombin levels and ATE can result from the ATE itself through coagulation activation, or a low antithrombin levels may be a contributing cause of ATE. Post-event inflammation is unlikely to have played a role as hsCRP levels we low. At inclusion in the study and drawing of blood, 95% of patients used platelet function inhibitors and 8% vitamin K antagonists. Previous studies have shown that Vitamin K antagonists (warfarin) do not change antithrombin levels[24]. We are unaware of studies showing that platelet inhibiting drugs (clopidogrel, aspirin) influence antithrombin levels. None of the subjects used (low-molecular-weight-)heparin at the time of blood sampling.

Although our follow-up study of young patients with a first cardiac event is quite large for these type of patients, the number of recurrent events was still low. This limits the statistical power to detect risk factors. Therefore a rather crude cut-off of median antithrombin levels was chosen to investigate if there was any effect. If on a population level a more narrow cut-off is more predictive is unknown. This limits the use of antithrombin levels as a predictive tool in practice based on this study alone. The low statistical power may also explain that of the classical cardiovascular risk factors only diabetes in men was a significant risk factor for recurrence, while other classical cardiovascular risk factors were not. Even so, risk factors for first and recurrent events are not necessarily the same[25]. Despite this, the finding of a high risk in women with low antithrombin levels for ATE and recurrent ATE seems to be robust and in line with previous studies[3], but should be validated in further studies. Another limitation of the follow-up study is the lack of information on adherence to medications prescribed, as non-adherence has been shown to be an important risk factor for recurrent ATE[26].

In conclusion, we found that lower antithrombin levels increase the risk of a first arterial thrombotic event at a young age. Lower antithrombin levels are associated with a higher rate of recurrent cardiac events and with a higher rate of recurrent arterial thromboembolism or death in women.

**Conflicts of interest**

The authors declare no conflict of interest regarding this research. F.W.G. Leebeek is consultant for UniQure and Shire (fees go to the institution), received unrestricted research grants of CSL Behring and Baxalta (Shire), and travel support from Roche, all outside the submitted work.

**Financial support**

This work was supported by a MRACE grant of the Erasmus MC, Rotterdam, the Netherlands (F.W.G. Leebeek).

**Author contributions**

F.N. Croles performed analyses and wrote the manuscript. J.E. Van Loon performed analyses and critically revised the manuscript. D.W.J. Dippel designed the study and critically revised the manuscript. M.P.M. De Maat designed the study and critically revised the manuscript. F.W.G. Leebeek designed the study and wrote the manuscript.



## REFERENCES

1. Patnaik MM, Moll S. Inherited antithrombin deficiency: a review. *Haemophilia*. 2008;14(6):1229-1239.
2. Vossen CY, Rosendaal FR, Group ES. Risk of arterial thrombosis in carriers of familial thrombophilia. *J Thromb Haemost*. 2006;4(4):916-918.
3. Mahmoodi BK, Veeger NJ, Middeldorp S, et al. Interaction of Hereditary Thrombophilia and Traditional Cardiovascular Risk Factors on the Risk of Arterial Thromboembolism: Pooled Analysis of Four Family Cohort Studies. *Circ Cardiovasc Genet*. 2016;9(1):79-85.
4. Strong JP, Malcom GT, McMahan CA, et al. Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. *JAMA*. 1999;281(8):727-735.
5. Zimmerman FH, Cameron A, Fisher LD, Ng G. Myocardial infarction in young adults: angiographic characterization, risk factors and prognosis (Coronary Artery Surgery Study Registry). *J Am Coll Cardiol*. 1995;26(3):654-661.
6. Shah N, Kelly AM, Cox N, Wong C, Soon K. Myocardial Infarction in the "Young": Risk Factors, Presentation, Management and Prognosis. *Heart Lung Circ*. 2016;25(10):955-960.
7. Bongers TN, de Bruijne EL, Dippel DW, et al. Lower levels of ADAMTS13 are associated with cardiovascular disease in young patients. *Atherosclerosis*. 2009;207(1):250-254.
8. de Bruijne EL, Gils A, Guimaraes AH, et al. The role of thrombin activatable fibrinolysis inhibitor in arterial thrombosis at a young age: the ATTAC study. *J Thromb Haemost*. 2009;7(6):919-927.
9. van Loon JE, de Maat MP, Deckers JW, van Domburg RT, Leebeek FW. Prognostic markers in young patients with premature coronary heart disease. *Atherosclerosis*. 2012;224(1):213-217.
10. Waziri H, Jorgensen E, Kelbaek H, et al. Short and long-term survival after primary percutaneous coronary intervention in young patients with ST-elevation myocardial infarction. *Int J Cardiol*. 2016;203:697-701.
11. Fournier JA, Cabezon S, Cayuela A, Ballesteros SM, Cortacero JA, Diaz De La Llera LS. Long-term prognosis of patients having acute myocardial infarction when </=40 years of age. *Am J Cardiol*. 2004;94(8):989-992.
12. Egeberg O. Thrombophilia Caused by Inheritable Deficiency of Blood Antithrombin. *Scand J Clin Lab Invest*. 1965;17:92.
13. Di Minno MND, Ambrosino P, Ageno W, Rosendaal F, Di Minno G, Dentali F. Natural anticoagulants deficiency and the risk of venous thromboembolism: A meta-analysis of observational studies. *Thromb Res*. 2015;135(5):923-932.
14. Di Minno MN, Dentali F, Veglia F, Russolillo A, Tremoli E, Ageno W. Antithrombin levels and the risk of a first episode of venous thromboembolism: a case-control study. *Thromb Haemost*. 2013;109(1):167-169.
15. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*. 1997;96(4):1102-1108.
16. Folsom AR, Rosamond WD, Shahar E, et al. Prospective study of markers of hemostatic function with risk of ischemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Circulation*. 1999;100(7):736-742.
17. Meade TW, Cooper J, Miller GJ, Howarth DJ, Stirling Y. Antithrombin III and arterial disease. *Lancet*. 1991;338(8771):850-851.
18. Pezzini A, Grassi M, Lodigiani C, et al. Determinants of premature familial arterial thrombosis in patients with juvenile ischaemic stroke. The Italian Project on Stroke in Young Adults (IPSYS). *Thromb Haemost*. 2015;113(3):641-648.

19. Pelkonen KM, Wartiovaara-Kautto U, Nieminen MS, Ahonen K, Sinisalo J. Low normal level of protein C or of antithrombin increases risk for recurrent cardiovascular events. *Blood Coagul Fibrinolysis*. 2005;16(4):275-280.
20. Maas AH, Appelman YE. Gender differences in coronary heart disease. *Neth Heart J*. 2010;18(12):598-602.
21. Weitz JI. Insights into the role of thrombin in the pathogenesis of recurrent ischaemia after acute coronary syndrome. *Thromb Haemost*. 2014;112(5):924-931.
22. Figueras J, Monasterio Y, Lidon RM, Nieto E, Soler-Soler J. Thrombin formation and fibrinolytic activity in patients with acute myocardial infarction or unstable angina: in-hospital course and relationship with recurrent angina at rest. *J Am Coll Cardiol*. 2000;36(7):2036-2043.
23. Martinez-Sales V, Vila V, Reganon E, et al. Elevated thrombotic activity after myocardial infarction: A 2-year follow-up study. *Haemostasis*. 1998;28(6):301-306.
24. Sanfelippo MJ, Engel JM, Onitilo AA. Antithrombin levels are unaffected by warfarin use. *Arch Pathol Lab Med*. 2014;138(7):967-968.
25. Roach RE, Cannegieter SC, Lijfering WM. Differential risks in men and women for first and recurrent venous thrombosis: the role of genes and environment. *J Thromb Haemost*. 2014;12(10):1593-1600.
26. Pezzini A, Grassi M, Lodigiani C, et al. Predictors of long-term recurrent vascular events after ischemic stroke at young age: the Italian Project on Stroke in Young Adults. *Circulation*. 2014;129(16):1668-1676.



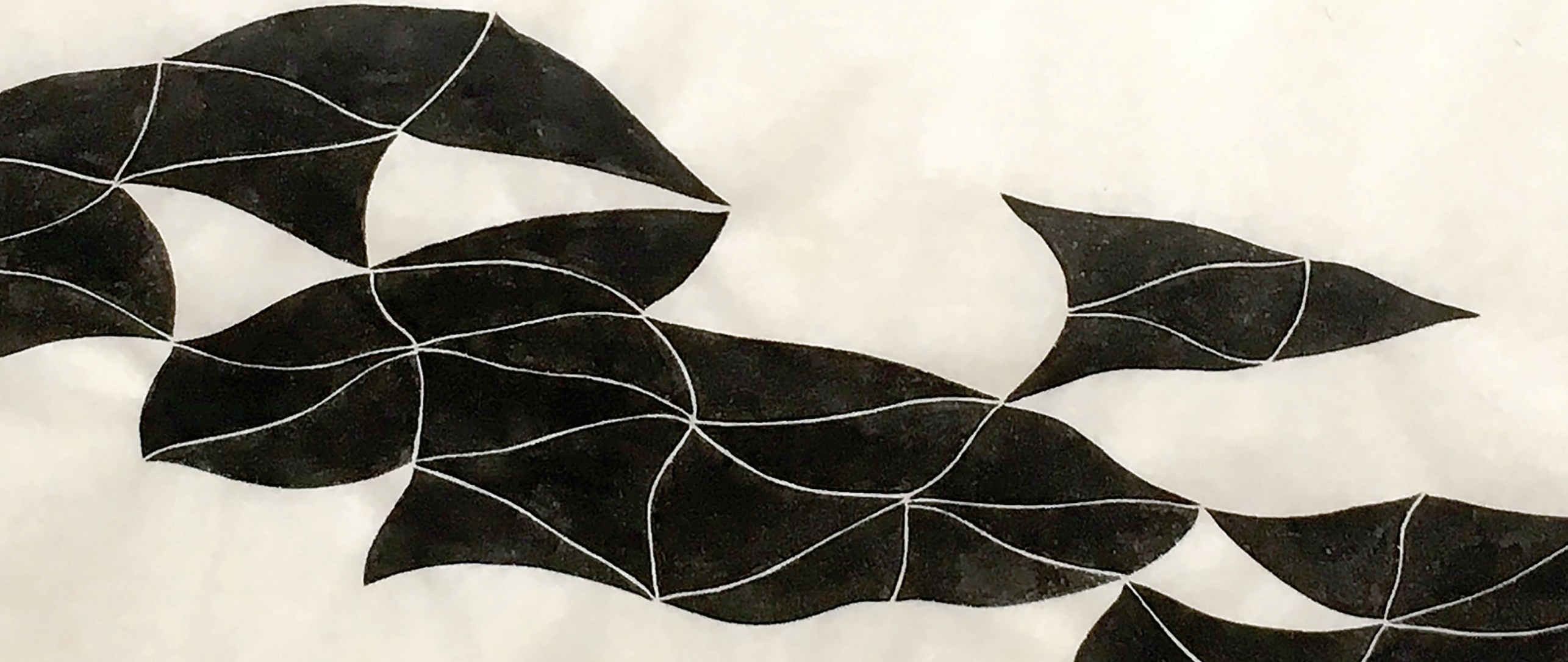

**Supplemental table 1. Patient characteristics of the cohort study on recurrence of arterial thrombotic events or death in young patients with cardiovascular disease.**

| | **Patients n=323** |
|---|---|
| **Demographics** | |
| Age, mean (years ±SD) | 43.7 ± 5.8 |
| Female sex, n (%) | 142 (44%) |
| Male sex, n (%) | 181 (56%) |
| **First Event** | |
| **Unstable angina pectoris, n (%)** | 52 (16%) |
| **Acute myocardial infarction, n (%)** | 271 (84%) |
| **Risk factors** | |
| Family history of cardiovascular disease, n (%) | 159 (49%) |
| Hypertension | 79 (24%) |
| Systolic blood pressure, median ±SD | 120 ±21 |
| Diastolic blood pressure, median ±SD | 80 ±12 |
| Diabetes | 32 (10%) |
| Smoking, current | 126 (39%) |
| Smoking, former | 147 (46%) |
| Total cholesterol (µmol/L ±SD), reference: 2.9-6.5 | 4.2 ± 0.9 |
| HDL (µmol/L), reference: >1.55 | 1.2 ± 0.4 |
| Hypercholesterolemia | 132 (41%) |
| BMI (kg/m2 ±SD) | 27.5 ± 4.6 |
| **Medication at inclusion** | |
| Antiplatelet drugs, n (%) | 319 (99%) |
| Anti-hypertensive drugs, n (%) | 303 (94%) |
| Statins, n (%) | 307 (95%) |
| **Laboratory markers** | |
| hsCRP, median, ±SD | 0.89 ±2.24 |
| Antithrombin, Median (U/mL, SD), reference 0.80-1.20 | 1.04 ±0.11 |

Abbreviations used in this table: SD: standard deviation. n: Number. BMI: body mass index.

# 8

# GENERAL DISCUSSION



## INTRODUCTION

Venous thrombosis (VTE) causes a considerable global burden of disease in terms of morbidity and mortality. Identifying groups of people who are at a high risk for venous thrombosis enables the implementation of targeted preventative strategies. Therefore the studies of thrombosis risk and identification of groups or individuals who are at a high risk of a first venous thrombosis is important. Known hereditary of acquired abnormalities in the blood that cause venous thrombosis are called thrombophilias. Guidelines regarding management of thrombosis risk in high risk situations such as pregnancy and surgery do take known thrombophilia into account. However, adequate absolute risk assessments in such situations are lacking. Moreover, patients who have suffered a venous thrombosis carry a high risk of new, recurrent venous thrombotic events. Current antithrombotic treatment strategies are mainly based on having had a temporary risk factor (provoked venous thrombosis) or having had an unprovoked venous thrombotic events. Individuals with temporary risk factors are usually prescribed antithrombotic treatment for a fixed duration, whereas for individuals with an unprovoked venous thrombotic event prolonged anticoagulant treatment is preferred, with regular assessment and weighing of both thrombotic risk and bleeding risk. In most recent guidelines, thrombophilia is generally not taken into account when deciding on duration of anticoagulant treatment.

In this thesis several studies on antithrombin deficiency as a risk factor for venous and arterial thrombosis are presented. The importance of antithrombin deficiency as a risk factor for thrombosis has been recognized from the first report by Egeberg[1] onward, but its rarity has left many questions regarding the risk of venous thrombosis debated for a long time. Relatively unexplored areas are the association of antithrombin with arterial thrombotic risk, and the exact function of antithrombin in vivo.

### Aim of the thesis

The aim of this thesis was to investigate inherited and acquired antithrombin deficiency, and its role in the pathogenesis of arterial and venous thrombosis. To determine the absolute risk of VTE associated with antithrombin deficiency, we performed a systematic review and Bayesian meta-analysis of previously reported studies. In contrast to the more commonly used classical meta-analysis, Bayesian meta-analysis enabled calculation of absolute risk estimates.

### Interpretation of results of Bayesian meta-analysis

For the correct interpretation of the Bayesian meta-analyses reported in this thesis, it is vital to have a basic understanding of how Bayesian meta-analysis works, and how the results should be interpreted. There are considerable differences between classical meta-analysis and Bayesian meta-analysis. We have explained these differences in the supplement of chapter 6.

In short, classical meta-analysis assumes that the estimated risk is in fact a fixed number. The 95% confidence intervals describes the region where the point-estimate of this fixed number would lie, should the measurements be repeated infinitely. In contrast, Bayesian statistics inference assumes that parameters in the model are not fixed values, but stochastic and have a distribution. The result of Bayesian hierarchical modelling is therefore a risk distribution, called a posterior distribution, of which certain features can be described.

Bayesian hierarchical modelling is a specific methodology used to combine results from multiple studies (e.g., meta-analysis) to obtain estimates[2]. Hierarchical modelling allows borrowing of information across studies, which means for larger studies we have good estimates, but for the smaller studies we may be able to borrow information from other studies to obtain more accurate estimates. The strength of borrowing depends on the homogeneity of these studies. Although in Bayesian statistics previous information about parameters can be used, in our two chapters we have not done this. Instead, we computed the posterior distributions (risk distributions) based on the available data from the studies only, to make sure these posterior distributions were data-driven only, and not based on assumptions. Parameter distributions are summarized by the median as a point estimate and the 95% area under the posterior distribution of the Parameter, i.e. credible intervals. This Bayesian term of credible interval should not be confused with the classical confidence interval. For example, in some analyses of odds ratios performed in our studies, the OR of 1 may fall in the 95% credible interval (95%CrI). If a classical confidence interval of odds ratios contains 1, we consider the point-estimate not to be statistically significant: we are not confident that the OR is greater than 1. Even if a credible interval contains 1, the probability of the OR being greater than 1 can still be over 99%. Therefore, if one accepts a 5% margin of error (as in a 95% confidence interval in classical statistics), this can be considered a "significantly" increased risk, even if 1 falls in the 95%CrI. In the supplement of chapter 6 we have described in more detail what risk distributions may look like, and what type of calculations can be performed.

**Should all meta-analyses be performed with Bayesian statistics?**

The first attempts of our meta-analyses were performed using the meta-analysis program Review Manager 5.3 (Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). With this program classical statistics analyses can be performed in a quite straightforward and fast manner. It generated results quickly, calculated heterogeneity and visualized results directly. It quickly revealed to us point estimates, and should the experiments be repeated infinitely, where the point-estimate would be with a confidence of 95%. However, upon closer inspection of the results, drawbacks of this method became apparent. Classical statistics meta-analysis only allowed calculation of relative risks, and several issues occurred while performing these analyses that led to doubts regarding the accuracy of the results: Only studies in which events occurred in the control groups an OR could be estimated. This means that studies with no events in the control group could not be used for analysis. This was a commonly encountered problem, influencing many of the analyses. On the other hand, in studies in which in the thrombophilia of interest no events occurred assumptions were made to generate an OR, which was sometimes larger with no events then when 1 event occurred. Review Manager software makes these corrections by replacing zeros by 0.5, thereby biasing results. This was a common problem in both the meta-analyses that we performed.



Bayesian analysis is conditional on the data and exact. It can be used to calculate both relative and absolute risks, and more complex questions can be answered. In terms of interpretation it is simpler than classical meta-analysis: the 95%CrI tells is where we believe the point estimate to be with a probability of 95%. The borrowing strength of Bayesian allows incorporation of studies with 0/100% events, and through borrowing information from other studies reliable point estimates for individual studies are made[3].

For many questions performing meta-analysis using classical statistics Bayesian analysis may not be as practical in its use as classical statistics, as the use of Bayesian analyses requires considerable computer power and experience with complex software[4]: The construction of posterior distributions that form the basis of our results of our study on pregnancy, thrombophilia and the risk of a first venous thrombosis required at least 140 million computer calculations. However, hierarchical modelling can be a very powerful research tool. It enables answering complex, clinically important questions that are unlikely to ever be resolved by clinical trials. An example of a relevant study with a complex study question is: "Which of 16 different treatment options is the best in relapsed/refractory multiple myeloma?" [5].

The answer to the question of if for all analyses Bayesian statistics should be used is no, but it should be used more often: for some questions regarding relative risks classical statistics will suffice, but Bayesian statistics has a wider use and provides more exact data. An important drawback is that it is much more complex and time-consuming to perform.

**Inherited antithrombin deficiency and the risk of first venous thrombosis**

We have investigated the risk of a first venous thrombosis in antithrombin deficiency. Using a systematic review, we have shown that in a Bayesian meta-analysis of 19 (case-control- and cohort-) studies with information on relative risks, the risk of venous thrombosis in 1,381 antithrombin deficient individuals as compared to 31,111 study controls is increased 14-fold, OR 14.0, 95% Credible interval (CrI) 5.5-29.0. The probability of the risk being higher than in controls was 100%. The absolute risk of first venous thrombosis overall was estimated at 1.2%/y (95%CrI: 0.8-1.7) in a Bayesian meta-analysis of 10 (cohort-) studies. However, risk estimates varied considerably between the 7 retrospective studies (annual incidence 1.0%, 95%CrI 0.6-1.5) and the 3 prospective cohort studies (annual incidence 2.3%, 95%CrI 0.2-6.5). The calculated annual incidence of VTE of 1.24% (95%CI 0.72-1.99%)

from our retrospective family study is in line with the risk estimate from our meta-analysis of retrospective studies (annual incidence 1.0, 95%CrI 0.6-1.5). The difference of absolute risk estimates between retrospective and prospective studies was the reason to perform a Bayesian regression meta-analysis where age at onset of VTE was a predictor in the model. It showed that the point estimate of annual incidence of a first VTE increased from about 1% at the age of 20, to about 3% by the age of 60.

In addition to inherited heterozygous antithrombin deficiency (with levels around 50%), mildly reduced antithrombin levels have also been associated with an increased risk of first venous thrombosis. As compared to individuals with antithrombin levels of >101 IU/dl, individuals with antithrombin levels between 100-91 IU/dl had a 1.25-fold increased risk, individuals with antithrombin levels of 81-90 IU/dl had a 1.65-fold increased risk and individuals with antithrombin levels between 70-80 had a 2.11-fold increased risk of a first venous thrombosis [6]. This is further evidence of the importance of antithrombin activity with respect to VTE risk in humans.

**Risk modifiers in inherited antithrombin deficiency**

We have attempted to investigate if specific features of the antithrombin deficiency itself modify the VTE risk, including subtypes of antithrombin deficiency, SERPINC1 mutations and β-antithrombin levels.

**Subtypes of antithrombin deficiency**

In our family study, we have investigated the impact of subtype on the risk of first venous thrombosis. In 29 family members with type I AT deficiency 14 first VTE occurred in 1044 observation years, corresponding with an annual incidence of 1.34% (95%CI 0.73-2.25%). In the 8 type II antithrombin-deficient family members 3 events in 323 observation years occurred (annual incidence 0.93%, 95%CI 0.19-2.71%). The difference in annual incidence was not statistically different (p=0.60), and both these annual incidences are high. No relevant difference in incidence of first VTE was found between family members with type I antithrombin deficiency as compared to type II antithrombin deficiency. Other studies on this subject have been performed previously: Mitsuguro et al. reported a 7.3-fold higher risk in type I antithrombin deficiency as compared to type II (no further subtyping was performed)[7]. Subsequently, Luxembourg et al. reported a significantly decreased VTE risk (HR 0.23) in subjects with type II heparin binding site (type IIHBS) antithrombin deficiency, but no clearly decreased risk in type II reactive site and type II pleiotropic effects [8]. Alhenc-Gelas et al. also reported a decreased risk for individuals with type IIHBS antithrombin deficiency as compared to type I (RR 0.28), but not for the other subtypes of type II[9]. In a more detailed analysis, specific type IIHBS mutations (e.g. type II HBS Basel) were reported to be at a lower risk than type I antithrombin deficiency, whereas others were not (e.g. type II HBS Toyama)[9]. In a sub-analysis of our systematic review on thrombosis risk of antithrombin deficiency, these three studies were meta-analysed to investigate the impact of antithrombin deficiency subtypes on the risk of venous thrombosis. In the meta-analysis of type I versus type II antithrombin deficiency, a remarkable difference was found between the relative risk estimates in the data of all subjects of these 3 studies (OR 9.3, 95%CrI: 0.0–30.9) and the relative risk estimates in the data obtained in family members only (from the latter 2 studies) (OR 2.6, 95%CrI: 0.0–19.5). Type II IIHBS may have a lower risk of VTE, but these risk estimates were all made in probands, and are therefore at risk for selection bias [8,9]. Considering the 14-fold increased VTE risk in all individuals with inherited antithrombin deficiency, and the relative small difference in type I vs type II antithrombin deficiency data from family members (OR 2.6), all individuals with antithrombin deficiency still have to be regarded as having a high risk for venous thrombosis, regardless of subtype. Treatment recommendations for antithrombin-deficient individuals should currently be considered as valid for all subtypes of antithrombin deficiency. A large prospective follow-up study of relatives could give a more definite answer regarding the usefulness of subtyping of antithrombin deficiency.

**SERPINC1 mutations**

We identified SERPINC1 mutations in 18/21 families with antithrombin deficiency. Unfortunately, our family study was too small to link specific mutations to the risk of VTE. Two studies by others have attempted to link SERPINC1 mutations to thrombosis risk in antithrombin-deficient individuals. The study by Luxembourg et al. grouped missense- and null mutations together [8]. This study showed that carriers of missense mutations showed a less severe VTE phenotype (HR 0.39, 95%CI 0.27-0.58) but possibly a more severe arterial thromboembolic event phenotype (HR 6.08, 95%CI 0.74-49.81), as compared to patients with null mutations. However, in this study both probands and relatives were assessed for thrombosis risk. This may bias the results of this study. A more recent study by Alhenc-Gelas et al investigated the impact of specific mutations in a cohort of 540 heterozygous carriers of SERPINC1 mutations [9]. They have reported results in only relatives separately. Only some type IIHBS-causing mutations were associated with a lower VTE risk. The mutation causing type IIHBS Padua showed a lower risk in family members as compared to type I antithrombin-deficient individuals (adjusted RR 0.12, 95%CI 0.03-0.48). These results suggest that large datasets on SERPINC1-mutations may enable the identification of specific subsets of individuals with a lower risk of VTE than others with antithrombin deficiency. Differential VTE risk of specific mutations within the same subtype of antithrombin deficiency has also been shown by others [10], indicating that analysis of SERPINC1 mutations may have a role in establishing VTE risk in antithrombin-deficient subjects in future. However, large datasets will be needed to use this kind of information reliably.

**Beta-antithrombin and the risk of first venous thrombosis**

Antithrombin is known to be activated by heparins, with heparin accelerating the moderate rates of the reactions of antithrombin with its targets several 1000-fold [11], and it is thought to be activated in vivo by heparin-like substances such as glycosaminoglycans on the vessel wall [12]. Antithrombin consists of two isoforms, 90% of circulating antithrombin being α-antithrombin and 10% β-antithrombin. The N-glycan at Asn135 is not present in β-antithrombin. β-antithrombin has a higher affinity for heparin [13,14], and binds better to the endothelium [15] and may therefore serve as the major inhibitor of coagulation in vivo. It had been reported that in 2 homozygous type IIHBS antithrombin deficient patients, the β-antithrombin-activity was completely retained and that, in contrast to α-antithrombin, β-antithrombin could be activated by heparin. As homozygous antithrombin deficiency is usually lethal in utero, this retained β-antithrombin-activity was thought to have caused an amelioration of the phenotype [16].

In a family study we were the first to investigate the impact of β-antithrombin activity levels in plasma and the risk of first venous thrombosis. We found that β-antithrombin activity in plasma was preserved in a proportion of subjects of all subtypes of antithrombin deficiency. Preserved β-antithrombin activity levels had no major impact on VTE risk in our study. The absolute annual risks of a first VTE in subjects with low or preserved β-antithrombin activity were both high (1.36% vs.0.79%, NS). To our knowledge no other clinical studies investigating the role of β-antithrombin and its relation to venous thrombosis have been performed. Given the results of our family study, a maintained interaction of β-antithrombin with heparin may not at all be important in vivo, as the interaction with heparin may be a bad predictor of the interaction between antithrombin and its supposed natural targets, the glycosaminoglycans in the vessel walls [12].

**Other ways to investigate thrombosis risk in antithrombin deficiency.**

As the hemostatic system is considered a delicate balance between procoagulant and anticoagulant factors, an appealing way of investigating the impact of antithrombin deficiency on the risk of thrombosis would be to assess the global influence of antithrombin deficiency in the hemostatic potential. This can be measured by the endogenous thrombin potential (ETP). This has recently been performed by Kovac et al., and in all antithrombin deficiency subtypes the ETP was indeed found to be increased [17].

**Risk of venous thrombosis in high-risk situations.**

As antithrombin deficiency is a severe inherited thrombophilia, this may have implications in terms of management aimed at the prevention of VTE. In several high-risk situations the knowledge of inherited thrombophilia can be used in the prevention of VTE, either by the use of antithrombotic prophylaxis, or avoiding high-risk situations. In the 2012 American College of Chest Physician's (ACCP) guidelines on prevention of VTE, thrombophilia including



antithrombin deficiency is taken into account: In non-surgical patients, the Padua prediction model is used [18]. In this model, four points or higher identifies high-risk patients for VTE, with absolute risk estimates of 11% per admission to hospital. Established thrombophilia counts as 3 points in this model, indicating that thrombophilia alone is not enough to establish a high risk (defined as ≥4 points) [19]. The Padua score is shown in table 1.

**Table 1: Padua prediction score [19]**

| Baseline features | Score |
|---|---|
| Active cancer | 3 |
| Previous VTE (with the exclusion of superficial vein thrombosis) | 3 |
| Reduced mobility | 3 |
| Already known thrombophilic condition | 3 |
| Recent (≤1 month) trauma and/or surgery | 2 |
| Elderly age (≥70 years) | 1 |
| Heart and/or respiratory failure | 1 |
| Acute myocardial infarction or ischemic stroke | 1 |
| Acute infection and/or rheumatologic disorder | 1 |
| Obesity (BMI ≥30) | 1 |
| Ongoing hormonal treatment | 1 |

High risk is defined as ≥4 points. ACCP guidelines use the Caprini risk assessment model for surgical patients [20]. Preventative measures (which may include thrombosis prophylaxis) with a Caprini score of ≥3 points. The Caprini score is shown in table 2. In this model, inherited thrombophilia is also assigned 3 points. However, total absolute risk is also assessed by the type of surgery performed [20]. Therefore, thrombophilia alone is considered an important risk modifier, but not the only factor that has to be taken into account when assessing thrombosis risk [21]. In the Dutch guidelines a simplified version of the Caprini score is used, but in agreement with ACCP guidelines known thrombophilia is an important risk factor that has to be taken into account, as well as the type of surgery [22]. In all these situations thrombosis prophylaxis is advised when risk is assessed as high. This way of risk-assessment reflects well the concept of venous thrombosis being a multicausal disease [23].

**Table 2. Caprini risk assessment model** [21]

| 1 Point | 2 Points | 3 Points | 5 Points |
|---|---|---|---|
| Age 41-60 y | Age 61-74 y | Age ≥75 y | Stroke (<1mo) |
| Minor surgery | Arthroscopic surgery | History of VTE | Elective arthroplasty |
| BMI ≥25 kg/m2 | Major open surgery (> 45 min) | Family history of VTE | Hip, pelvis, or leg fracture (<1mo) |
| Swollen legs | Laparoscopic surgery (> 45 min) | Factor V Leiden | |
| Pregnancy or postpartum | Malignancy | Prothrombin 20210A | |
| History of unexplained or recurrent spontaneous abortion | Confi ned to bed (>72 h) | Lupus anticoagulant | |
| Oral contraceptives or hormone replacement | Immobilizing plaster cast | Anticardiolipin antibodies | |
| Sepsis ( <1 mo) | Central venous access | Elevated serum homocysteine | |
| Serious lung disease, including pneumonia (<1 mo) | | Heparin-induced thrombocytopenia | |
| Abnormal pulmonary function | | Other congenital or acquired thrombophilia | |
| Acute myocardial infarction | | | |
| Congestive heart failure (<1 mo) | | | |
| History of infl ammatory bowel disease | | | |
| Medical patient at bed rest | | | |



In the situation of the use of combined oral contraceptives, a recent systematic review showed an important risk difference between women with or without high-risk thrombophilia [24]: In two studies, the annual incidence of venous thrombosis in oral contraceptive users with antithrombin, protein C or protein S deficiency was 4.3% (95% CI, 1.4-9.7) and 4.62% (95% CI, 2.5–7.9) vs. 0.48% (95% CI, 0.1–1.4) and 0.7% (95% CI, 0.0–3.7) in non-deficient women using oral contraceptives [25,26]. The practical consequence should be not to prescribe oral contraceptives to women with high-risk inherited thrombophilia. In our family study, women with known antithrombin deficiency had been advised to avoid using oral contraceptives. This policy may explain that in our family study the proportion of provoked VTE was lower in family members than in probands.

**Pregnancy, thrombophilia and the risk of a first venous thrombosis**

In contrast to surgical and non-surgical admissions, present Dutch guidelines do not assign a high risk of venous thrombosis to pregnancy in women with deficiencies of natural anticoagulants (antithrombin, protein C or protein S). In women with a deficiency of a natural coagulant without previous VTE, thrombosis prophylaxis would only be considered in the postpartum period, and only in women with a positive family history for venous thrombosis. The only thrombophilias considered to be at a very high risk were homozygous factor V Leiden and homozygous prothrombin G20210A mutations[22].The risk-estimates used in the creation of this guideline were based on the risk-estimates used in the ACCP guidelines on venous thrombosis, thrombophilia and pregnancy [27]. These in turn, are based on a 2006 systematic review [28] and a limited number of cohort studies. Given the paucity of data used, we performed a new systematic review and Bayesian analysis assessing relative and absolute risk estimates of pregnancy-associated venous thrombosis for women with/without thrombophilia without previous venous thrombosis. From 36 studies, the data on 5,994 women with thrombophilia, and 35,303 controls/non-carriers were meta-analysed to calculate the relative and absolute risks of pregnancy-associated venous thrombosis, according to family history, antepartum and postpartum period. After at least 140.000.000 computer calculations a transformed picture of pregnancy-associated venous thrombosis risk had emerged: first of all, relative risk estimates were all high for all thrombophilias: heterozygous prothrombin G20210A mutation OR 5.1, heterozygous factor V Leiden OR 6.4, protein S deficiency OR 7.0, protein C deficiency OR 9.3, antithrombin deficiency 9.5, homozygous prothrombin G20210A mutation OR 21.1, compound heterozygous factor V Leiden and prothrombin G20210A mutation OR 21.2 and finally homozygous factor V Leiden 35.8. These numbers however are put in an entirely new perspective by the absolute risk analyses that were performed on data from cohort studies: our results indicate that high-risk thrombophilias are antithrombin deficiency (antepartum: 7.3%, 95% credible interval 1.8% to 15.6%; post partum: 11.1%, 3.7% to 21.0%), protein C deficiency (antepartum: 3.2%, 0.6% to 8.2%; post partum: 5.4%, 0.9% to 13.8%), protein S deficiency (antepartum: 0.9%, 0.0%

to 3.7%; post partum: 4.2%; 0.7% to 9.4%), and homozygous factor V Leiden (antepartum: 2.8%, 0.0% to 8.6%; post partum: 2.8%, 0.0% to 8.8%). Absolute combined antepartum and postpartum risks for women with heterozygous factor V Leiden, heterozygous prothrombin G20210A mutations, or compound heterozygous factor V Leiden and prothrombin G20210A mutations were all below 3%. These risk-estimates were then related to recent guidelines, which set different thresholds for starting thrombosis prophylaxis, varying from 1-3% [18,22,27]. The latest guidelines set the threshold for thrombosis prophylaxis at the absolute risk of venous thrombosis at 3% antepartum and 3% postpartum [29]. Based on our risk estimates and the threshold of about 3% per risk period, we concluded that women with antithrombin, protein C, or protein S deficiency or with homozygous factor V Leiden should be considered for antepartum or postpartum thrombosis prophylaxis, or both. In contrast we concluded that women with heterozygous factor V Leiden, heterozygous prothrombin G20210A mutation, or compound heterozygous factor V Leiden and prothrombin G20210A mutation should generally not be prescribed thrombosis prophylaxis on the basis of thrombophilia and family history alone [30]. The risk estimates and recommendations and the differences with current Dutch [22] and 2012 ACCP guidelines [27] are summarized in Table 3.

The magnitude of risk-estimates differ considerably between relative and absolute risk-estimates on the risk of pregnancy-associated VTE [30]. This is most likely explained by the different absolute VTE risks in the control populations of these studies. For the deficiencies of antithrombin, protein C and protein S only family studies are available. This is not surprising given the rarity of these inherited defects. The controls in family cohort studies were found to have a 5-fold higher thrombosis risk as compared to non-family study controls. This may explain that the relative risk estimates in these high-risk thrombophilias were relatively low, whereas absolute risk estimates are considerably higher.

### Genetic background of antithrombin deficiency

In order to explain the inherited antithrombin deficiency we have assessed the presence of SERPINC1-gene mutations found in 21 families included in a family study. We found causal mutations in 18 of 21 families (86%), 5 of which had not been reported before. The percentage of identified causal mutations is similar to other studies by Caspers [31] (84%) and Luxembourg [8] (80%). These results indicate that in an important percentage of families with antithrombin deficiency, the cause of the antithrombin deficiency remains to be elucidated. The absence of SERPINC1 mutations can be explained by detection failure of SERPINC1 mutations, such by the presence of a duplication of SERPINC1 Exon 6 [32], by mutations of other genes or other processes. Upon the report on duplications of SERPINC1 exon 6 that can hardly be detected by current methods [32], we have performed the same analysis in our remaining 3 families in which no mutation was found. We found no duplications of exon 6 in these families. Mutations in the antithrombin-modulating gene LARGE have been associated



with antithrombin levels. The LARGE-gene has been proposed to modulate extracellular secretion of antithrombin and other proteins, including prothrombin [33]. In addition, specific microRNA's can potentially modulate antithrombin levels [34]. These are relatively unexplored areas and warrant further research.

**Table 3: Risk estimates of pregnancy-associated venous thromboembolism, and recommendations for thrombosis prophylaxis.**

| Thrombophilic defect | Odds ratio | Absolute risk of VTE antepartum, % pregnancies | Absolute risk of VTE postpartum , % pregnancies | Absolute risk of VTE antepartum and postpartum combined, % pregnancies | Antepartum /postpartum prophylaxis recommended? | ACCP /Dutch guideline recommendations |
|---|---|---|---|---|---|---|
| No defect, and no thrombophilia in family | | NA | NA | 0.1 | No/No | No/No |
| No defect, thrombophilia in family | | NA | NA | 0.5 | No/No | No/No |
| Antithrombin deficiency | 9.5 | 7.3 | 11.1 | 16.6 | Yes/Yes | No/Yes* |
| Protein C deficiency | 9.3 | 3.2 | 5.4 | 7.8 | Yes/Yes | No/Yes* |
| Protein S deficiency | 7.0 | 0.9 | 4.2 | 4.8 | No/yes | No/Yes* |
| Factor V Leiden, heterozygous | 6.4 | 0.4 | 2.0 | 1.1 | No/No | No/Yes* |
| Factor V Leiden, homozygous | 35.8 | 2.8 | 2.8 | 6.2 | Yes/Yes | Yes*/Yes |
| Prothrombin G20210A mutation heterozygous | 5.1 | 0.0 | 0.9 | 0.9 | No/No | No/Yes* |
| Prothrombin G20210A mutation homozygous | 21.1 | NA | NA | NA | NA/NA | Yes*/Yes |
| Compound heterozygous factor V Leiden & prothrombin G20210A mutation | 21.2 | NA | NA | 2.5 | No/No | No/Yes* |

This tables summarizes the findings of our systematic review and Bayesian meta-analysis. The odds ratios were estimated from data from both case-control and cohort studies. Absolute risk estimates were estimated from cohort study data. The final column shows the recommendations of Dutch 2016 and ACCP 2012 guidelines. NA: Not available. *: Only if family history is positive for VTE.

**Antithrombin and arterial thrombosis**

Inherited thrombophilia has been associated with a 1.7-fold increased risk of arterial thromboembolism in a large combined analysis of 1891 individuals from 4 family cohort studies on inherited thrombophilia [35]. Of these, 72 were antithrombin deficient. Antithrombin deficiency in this large cohort was not associated with an increased risk of arterial thrombosis (HR 1.36, 95% CI 0.55-3.34). Interestingly, in this large study there was a clear synergistic interaction of thrombophilia and classical cardiovascular risk factors. Furthermore, there was a stronger association in females and in younger individuals (<55y) as compared with men and older individuals [35]. We have shown the association of low (≤median) antithrombin levels with ATE at a young age (OR 1.46; 95%CI:1.09-1.96), and with recurrent cardiac events in young individuals with a myocardial infarction (HR 2.16, 95% CI:1.07-4.38). The risk of a first event was somewhat further increased in women (OR1.46; 95%CI: 1.00-2.14) than in men (OR1.38, 95%CI: 0.86-2.24), and was even stronger in women aged <46 (OR 1.55, 95%CI: 1.09-2.21). The risk of recurrent cardiac events was especially high in women with low antithrombin levels (HR 5.97, 95%CI 1.31-27.13), but not increased in men. These results seem to be in concordance with the results from Mahmoodi et al.: In men and in older individuals no association was found between thrombophilia and ATE, whereas an association was found in younger individuals (HR 2.74, 95%CI: 1.63-4.60) and in women (HR 2.60, 95%CI: 1.42-4.73) [35]. Although our novel findings are interesting, it is hard to put them into practice: a more narrow cut-off value of antithrombin levels may be more predictive than the median. Furthermore, no intervention studies have been performed to investigate whether additional anticoagulant therapy in young women with a myocardial infarction and low antithrombin levels better is useful in the prevention of a recurrent event.

**Practical consequences of establishing inherited antithrombin deficiency**

The consequences of establishing inherited antithrombin deficiency are many. We have shown that the risk of a first venous thrombosis is high. In high-risk settings for VTE such as hospital admissions and surgery, antithrombin deficiency, according to national [22] and international [18,20] guidelines should be taken into account when considering the risk of VTE and therefore thrombosis prophylaxis. We have also shown that in antithrombin-deficient women, the risk of a pregnancy-related first VTE is high, and that thrombosis prophylaxis should be considered: 16.6%/pregnancy, which is much higher than previous estimates of about 4.1%/pregnancy [36]. It has also been shown by others in our group that inherited antithrombin deficiency should be considered a contra-indication for oral contraceptives [24]. In addition, if an individual with antithrombin deficiency develops venous thrombosis, we have shown that the risk of recurrence is so high that long-term anticoagulant therapy should be considered (8.8%, 95% CrI, 4.6–14.1 as compared to non-antithrombin-deficient VTE patients: 4.3%, 95% CrI, 1.5–7.9).



**Optimal anticoagulant therapy?**

An important remaining question is what the optimal anticoagulant therapy is in antithrombin deficient individuals. The most commonly used classes of drugs to treat venous thrombosis are heparins, vitamin K antagonists (VKA) and direct acting oral anticoagulants (DOACs). When anticoagulant treatment is initiated for treatment of VTE, patients treated with VKA or the DOAC dabigatran or edoxaban are first given a short course of LMWH, usually 5-7 days. In certain subgroups of VTE patients, such as patients with cancer-associated VTE or pregnant VTE patients LMWH is given for 3-6 months, or even longer. The available data in literature come from subjects that have been treated with vitamin K antagonists (VKA) after a short course of low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH). Therefore, the only evidence-based long-term treatment for antithrombin-deficient individuals with VTE is treatment with VKA. We have shown the extent to which antithrombin levels are correlated with anti-Xa measurements after spiking with LMWH and UFH. In most antithrombin-deficient subjects anti-Xa measurements were too low. Although this is indirect (non-clinical) evidence, the efficacy of standard doses of these drugs in individuals with antithrombin-deficiency should be questioned. Could this mechanism be a cause for the high recurrence rates in individuals with inherited antithrombin deficiency? In cirrhosis patients with acquired antithrombin deficiency, it has been shown that drug levels of heparins are underestimated by using anti-Xa tests, but overestimated by APTT-based tests [37]. Similar results to our work in individuals with inherited antithrombin deficiency have been reported regarding heparins, antithrombin levels and anti-Xa levels in cirrhosis patients. This also raised concerns regarding the efficacy of heparins in cirrhosis patients with acquired antithrombin deficiency[38]. In children undergoing cardiopulmonary bypass a similar association between antithrombin levels and anti-Xa measurements was found. Worryingly, higher markers of coagulation activation were found in those children with lower minimal anti-Xa levels [39].

As for the use of DOAC's in individuals with inherited antithrombin deficiency specifically, only case-reports have reported efficacy in inherited antithrombin deficiency, providing no significant evidence [40-42]. As for thrombophilia overall, no impact of thrombophilia was found in a post-hoc analysis of three trials comparing the efficacy of dabigatran vs warfarin for the treatment of VTE [43]. To our knowledge, no such investigations have been reported for the other DOACs. As yet, data are lacking on the efficacy of DOACs in antithrombin-deficient individuals as compared to VKA. Their respective IIa or Xa activities do not depend on antithrombin. On theoretical grounds a DOAC not requiring initial treatment with LMWH (apixaban, rivaroxaban) may be preferred over DOACs requiring initial treatment with LMWH (dabigatran, edoxaban).

**Antithrombin testing**

There are three important issues to consider when deciding upon antithrombin testing: 1. Who should be tested for antithrombin deficiency? 2. What test should be used if one decided to test a patient? 3. What factors should be taken into account when testing for antithrombin deficiency?

Testing for inherited thrombophilia is controversial [44]. Although clinical guidelines are lacking in advice to either test or not test for thrombophilia [45], a recent review by Goodwin et al. recommended the following: "Not to test at the time of a VTE event", "not to test a patient who is receiving anticoagulant therapy" and "not to test if VTE is provoked by strong risk factors". On the other hand: Goodwin et al. did recommend to "consider testing in patients in whom VTE occurs at a young age in association with weak provoking factors or a strong family history of VTE or in patients who have recurrent VTE", and to identify goals of testing[44]. These goals may include clinical management of the patient, and management thrombosis risk of asymptomatic family members. When VTE is unprovoked, current guidelines already support prolonged use of anticoagulant therapy [22,45]. Therefore testing would not impact management. However, in provoked VTE, especially when the provoking factor is weak, the finding of antithrombin deficiency can be considered as an important factor favouring long-term treatment. Furthermore, especially by identifying antithrombin-deficient women of childbearing age important preventative measures such as avoidance of using oral contraceptives and prescription of thrombosis prophylaxis during pregnancy and puerperium may well prevent VTE in those women. An argument against *routine* antithrombin testing in VTE patients is that the population attributable risk of antithrombin levels is far too low [46]. Therefore, having a clinical suspicion for a severe thrombophilia before testing is advised [44]. This may be based on either family history, heparin resistance or other clinical factors.

Antithrombin testing should be performed by using tests of antithrombin function, given that a significant portion of antithrombin-deficient individuals have only qualitative defects, and not qualitative defects [8,9]. Commonly used antithrombin activity assays measure the capacity of antithrombin to either inhibit coagulation factor IIa or Xa in the presence of heparin. These tests vary widely in the dilution of plasma, incubation time with heparin and substrate [47]. Therefore more than one sort of test may be needed to enable detection of all forms of antithrombin deficiency [48,49].

When testing for antithrombin deficiency, it is important to realize that in individuals using DOACs antithrombin tests are affected: With higher levels of direct Xa inhibitors, antithrombin levels are overestimated using Xa-based antithrombin tests[50]. In these individuals a factor IIa-based test should be considered. Although no studies to our knowledge have been published investigating this, the converse could very well be true for individuals using dabigatran: factor IIa-based tests could overestimate antithrombin activity with relevant levels of dabigatran present in blood samples. In this case a factor Xa-based antithrombin test is likely to be more appropriate.



**Future directions**

Although we have found risk estimates for VTE for antithrombin-deficient subjects to be high risk estimates are still imprecise. To enable further fine-tuning of thrombosis risk in antithrombin-deficient individuals, large studies with genotyping of subjects with antithrombin deficiency may be the best way forward to identify and confirm low-risk genotypes[9]. Likewise, studies employing a more refined array of testing for the cause of antithrombin deficiency are needed to improve the detection rate of causal mutations. This could enable establishing thrombosis risk in those families with antithrombin deficiency in whom no SERPINC1 mutation has yet been identified [32].

In our systematic review and meta-analysis we have reported absolute risk estimates for pregnancy-associated VTE, incorporating information on 41.297 pregnancies. Whereas the risk estimates for the more prevalent but mild thrombophilias are quite clear, for the rare thrombophilias such as antithrombin deficiency there still is a lack of data: absolute risk estimates for antithrombin-deficient women are based on 125 pregnancies only. However, finding pregnant women with rare thrombophilias and establishing risk without thrombosis prophylaxis is hardly ethical given the magnitude of absolute risk estimates in some of them. One could argue that in fact efficacy data on thrombosis prophylaxis are lacking [51,52], and that therefore a large randomized trial should be performed to establish efficacy of prophylactic strategies, in the high-risk women identified in our systematic review of **chapter 6**. For pregnant women with a previous VTE event the Highlow study is being performed, but this study will not answer the question of efficacy of LMWH in *primary* prevention[53].

We have shown a reduced anti-Xa activity of heparins in antithrombin-deficient subjects, and even reductions in the normal range. These results underline the importance of investigation of antithrombin levels in studies in which heparins are used for a longer period of time. This could include trials regarding thrombosis prophylaxis in pregnant women, or trials investigating the use of LMWH in cancer-associated VTE. For example, the use of DOAC's has been shown to be more effective in cancer-associated VTE in terms of recurrence, but at the cost of an almost equally increased rate of major bleeding [54,55]. Should a reduced efficacy of LMWH be shown in subjects with reduced antithrombin levels, this knowledge may improve management: Risk modifiers such as antithrombin levels may shift the balance in favor of one treatment over the other. In pregnant women with antithrombin deficiency with VTE, prescription of antithrombin concentrates may be considered [56].

Our findings from the study regarding the risk of first and recurrent ATE at a young age are compelling and seem to be in line with other studies [35]. However, these data require confirmation of a large prospective trial. Should it become established that especially in women recurrence-risk is increased with relatively low antithrombin levels, these women may have to be treated differentially. Management studies should then be performed. Maybe, in such patients addition of low-dose DOAC's to aspirin may be more successful in the prevention of recurrent events [57]. New research is needed to solve these questions.

## REFERENCES


1. Egeberg O. Inherited fibrinogen abnormality causing thrombophilia. *Thromb Diath Haemorrh*. 1967;17(1-2):176-187.
2. Jackman S. Bayesian analysis for the social sciences. . Vol. 846: Wiley & Sons; 2009
3. Smith TC, Spiegelhalter DJ, Thomas A. Bayesian approaches to random-effects meta-analysis: a comparative study. *Stat Med*. 1995;14(24):2685-2699.
4. R_Core_Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Accessed July 1st 2016.
5. van Beurden-Tan CHY, Franken MG, Blommestein HM, Uyl-de Groot CA, Sonneveld P. Systematic Literature Review and Network Meta-Analysis of Treatment Outcomes in Relapsed and/or Refractory Multiple Myeloma. *J Clin Oncol*. 2017;35(12):1312-1319.
6. Di Minno MN, Dentali F, Veglia F, Russolillo A, Tremoli E, Ageno W. Antithrombin levels and the risk of a first episode of venous thromboembolism: a case-control study. *Thromb Haemost*. 2013;109(1):167-169.
7. Mitsuguro M, Sakata T, Okamoto A, et al. Usefulness of antithrombin deficiency phenotypes for risk assessment of venous thromboembolism: type I deficiency as a strong risk factor for venous thromboembolism. *Int J Hematol*. 2010;92(3):468-473.
8. Luxembourg B, Pavlova A, Geisen C, et al. Impact of the type of SERPINC1 mutation and subtype of antithrombin deficiency on the thrombotic phenotype in hereditary antithrombin deficiency. *Thromb Haemost*. 2014;111(2):249-257.
9. Alhenc-Gelas M, Plu-Bureau G, Hugon-Rodin J, Picard V, Horellou MH, Thrombophilia GsgoG. Thrombotic risk according to SERPINC1 genotype in a large cohort of subjects with antithrombin inherited deficiency. *Thromb Haemost*. 2017;117(6):1040-1051.
10. Gindele R, Selmeczi A, Olah Z, et al. Clinical and laboratory characteristics of antithrombin deficiencies: A large cohort study from a single diagnostic center. *Thromb Res*. 2017;160:119-128.
11. Olson ST, Bjork I, Bock SC. Identification of critical molecular interactions mediating heparin activation of antithrombin: implications for the design of improved heparin anticoagulants. *Trends Cardiovasc Med*. 2002;12(5):198-205.
12. Olson ST, Richard B, Izaguirre G, Schedin-Weiss S, Gettins PG. Molecular mechanisms of antithrombin-heparin regulation of blood clotting proteinases. A paradigm for understanding proteinase regulation by serpin family protein proteinase inhibitors. *Biochimie*. 2010;92(11):1587-1596.
13. McCoy AJ, Pei XY, Skinner R, Abrahams JP, Carrell RW. Structure of beta-antithrombin and the effect of glycosylation on antithrombin's heparin affinity and activity. *J Mol Biol*. 2003;326(3):823-833.
14. Picard V, Ersdal-Badju E, Bock SC. Partial glycosylation of antithrombin III asparagine-135 is caused by the serine in the third position of its N-glycosylation consensus sequence and is responsible for production of the beta-antithrombin III isoform with enhanced heparin affinity. *Biochemistry*. 1995;34(26):8433-8440.
15. Witmer MR, Hatton MW. Antithrombin III-beta associates more readily than antithrombin III-alpha with uninjured and de-endothelialized aortic wall in vitro and in vivo. *Arterioscler Thromb*. 1991;11(3):530-539.
16. Martinez-Martinez I, Navarro-Fernandez J, Ostergaard A, et al. Amelioration of the severity of heparin-binding antithrombin mutations by posttranslational mosaicism. *Blood*. 2012;120(4):900-904.
17. Kovac M, Mitic G, Lalic-Cosic S, et al. Evaluation of endogenous thrombin potential among patients with antithrombin deficiency. *Thromb Res*. 2018;166:50-53.
18. Kahn SR, Lim W, Dunn AS, et al. Prevention of VTE in nonsurgical patients: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e195S-e226S.
19. Barbar S, Noventa F, Rossetto V, et al. A risk assessment model for the identification of hospitalized medical patients at risk for venous thromboembolism: the Padua Prediction Score. *J Thromb Haemost*. 2010;8(11):2450-2457.
20. Gould MK, Garcia DA, Wren SM, et al. Prevention of VTE in nonorthopedic surgical patients: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e227S-e277S.
21. Caprini JA. Thrombosis risk assessment as a guide to quality patient care. *Dis Mon*. 2005;51(2-3):70-78.
22. Huisman MV, Bakx R, Coppens M, et al. Richtlijn antitrombotisch beleid. Veneuze trombo-embolie tijdens de zwangerschap en postpartum periode. *https://richtlijnendatabasenl/richtlijn/antitrombotisch_beleid/preventie_vte_in_de_verloskunde/trombo-embolie_bij_zwangerschap_en_postpartumhtml.* 2016; Accessed November 1st 2016.
23. Rosendaal FR. Venous thrombosis: a multicausal disease. *Lancet*. 1999;353(9159):1167-1173.
24. van Vlijmen EF, Wiewel-Verschueren S, Monster TB, Meijer K. Combined oral contraceptives, thrombophilia and the risk of venous thromboembolism: a systematic review and meta-analysis. *J Thromb Haemost*. 2016;14(7):1393-1403.
25. Simioni P, Sanson BJ, Prandoni P, et al. Incidence of venous thromboembolism in families with inherited thrombophilia. *Thromb Haemost*. 1999;81(2):198-202.
26. van Vlijmen EF, Brouwer JL, Veeger NJ, Eskes TK, de Graeff PA, van der Meer J. Oral contraceptives and the absolute risk of venous thromboembolism in women with single or multiple thrombophilic defects: results from a retrospective family cohort study. *Arch Intern Med*. 2007;167(3):282-289.
27. Bates SM, Greer IA, Middeldorp S, et al. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e691S-736S.
28. Robertson L, Wu O, Langhorne P, et al. Thrombophilia in pregnancy: A systematic review. *Br J Haematol*. 2006;132(2):171-196.
29. Bates SM, Middeldorp S, Rodger M, James AH, Greer I. Guidance for the treatment and prevention of obstetric-associated venous thromboembolism. *J Thromb Thrombolysis*. 2016;41(1):92-128.
30. Croles FN, Nasserinejad K, Duvekot JJ, Kruip MJ, Meijer K, Leebeek FW. Pregnancy, thrombophilia, and the risk of a first venous thrombosis: systematic review and bayesian meta-analysis. *BMJ*. 2017;359:j4452.
31. Caspers M, Pavlova A, Driesen J, et al. Deficiencies of antithrombin, protein C and protein S - practical experience in genetic analysis of a large patient cohort. *Thromb Haemost*. 2012;108(2):247-257.
32. de la Morena-Barrio B, de la Morena-Barrio ME, Padilla J, et al. Identification of a New Mechanism of Antithrombin Deficiency Hardly Detected by Current Methods: Duplication of SERPINC1 Exon 6. *Thromb Haemost*. 2018.
33. de la Morena-Barrio ME, Buil A, Anton AI, et al. Identification of antithrombin-modulating genes. Role of LARGE, a gene encoding a bifunctional glycosyltransferase, in the secretion of proteins? *PLoS One*. 2013;8(5):e64998.
34. Teruel R, Corral J, Perez-Andreu V, Martinez-Martinez I, Vicente V, Martinez C. Potential role of miRNAs in developmental haemostasis. *PLoS One*. 2011;6(3):e17648.
35. Mahmoodi BK, Veeger NJ, Middeldorp S, et al. Interaction of Hereditary Thrombophilia and Traditional Cardiovascular Risk Factors on the Risk of Arterial Thromboembolism: Pooled Analysis of Four Family Cohort Studies. *Circ Cardiovasc Genet*. 2016;9(1):79-85.
36. Middeldorp S, van Hylckama Vlieg A. Does thrombophilia testing help in the clinical management of patients? *Br J Haematol*. 2008;143(3):321-335.

37. Lisman T, Violi F. Cirrhosis as a risk factor for venous thrombosis. *Thromb Haemost*. 2017;117(1):3-5.
38. Potze W, Arshad F, Adelmeijer J, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. *Br J Haematol*. 2013;163(5):666-673.
39. Manlhiot C, Gruenwald CE, Holtby HM, et al. Challenges with heparin-based anticoagulation during cardioplmonary bypass in children: Impact of low antithrombin activity. *J Thorac Cardiovasc Surg*. 2016;151(2):444-450.
40. Kawano H, Maemura K. Edoxaban Was Effective for the Treatment of Deep Vein Thrombosis and Pulmonary Thromboembolism in a Cancer Patient with Antithrombin III Deficiency. *Intern Med*. 2016;55(22):3285-3289.
41. Van Bruwaene L, Huisman A, Urbanus RT, Versluys B. Heparin-resistant Thrombosis Due to Homozygous Antithrombin Deficiency Treated With Rivaroxaban: A Case Report. *J Pediatr Hematol Oncol*. 2016;38(8):658-660.
42. Kawai H, Matsushita H, Kawada H, Ogawa Y, Ando K. The Successful Prevention of Thromboembolism Using Rivaroxaban in a Patient with Antithrombin Deficiency during the Perioperative Period. *Intern Med*. 2017;56(17):2339-2342.
43. Goldhaber SZ, Eriksson H, Kakkar A, et al. Efficacy of dabigatran versus warfarin in patients with acute venous thromboembolism in the presence of thrombophilia: Findings from RE-COVER(R), RE-COVER II, and RE-MEDY. *Vasc Med*. 2016;21(6):506-514.
44. Goodwin AJ, Adcock DM. Thrombophilia Testing and Venous Thrombosis. *N Engl J Med*. 2017;377(23):2297-2298.
45. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. *Chest*. 2016;149(2):315-352.
46. Sokol J, Timp JF, le Cessie S, et al. Mild antithrombin deficiency and risk of recurrent venous thromboembolism: results from the MEGA follow-up study. *J Thromb Haemost*. 2018;16(4):680-688.
47. Corral J, Vicente V. Puzzling questions on antithrombin: Diagnostic limitations and real incidence in venous and arterial thrombosis. *Thromb Res*. 2015;135(6):1047-1048.
48. Kristensen SR, Rasmussen B, Pedersen S, Bathum L. Detecting antithrombin deficiency may be a difficult task--more than one test is necessary. *J Thromb Haemost*. 2007;5(3):617-618.
49. Javela K, Engelbarth S, Hiltunen L, Mustonen P, Puurunen M. Great discrepancy in antithrombin activity measured using five commercially available functional assays. *Thromb Res*. 2013;132(1):132-137.
50. Ruhl H, Reda S, Muller J, Oldenburg J, Potzsch B. Activated Factor X-Based versus Thrombin-Based Antithrombin Testing in Thrombophilia Workup in the DOAC Era. *Thromb Haemost*. 2018;118(2):381-387.
51. Bain E, Wilson A, Tooher R, Gates S, Davis LJ, Middleton P. Prophylaxis for venous thromboembolic disease in pregnancy and the early postnatal period. *Cochrane Database of Systematic Reviews*. 2014(2).
52. Roeters van Lennep JE, Meijer E, Klumper FJ, Middeldorp JM, Bloemenkamp KW, Middeldorp S. Prophylaxis with low-dose low-molecular-weight heparin during pregnancy and postpartum: is it effective? *J Thromb Haemost*. 2011;9(3):473-480.
53. Bleker SM, Buchmuller A, Chauleur C, et al. Low-molecular-weight heparin to prevent recurrent venous thromboembolism in pregnancy: Rationale and design of the Highlow study, a randomised trial of two doses. *Thromb Res*. 2016;144:62-68.
54. Raskob GE, van Es N, Verhamme P, et al. Edoxaban for the Treatment of Cancer-Associated Venous Thromboembolism. *N Engl J Med*. 2018;378(7):615-624.
55. Li A, Garcia DA, Lyman GH, Carrier M. Direct oral anticoagulant (DOAC) versus low-molecular-weight heparin (LMWH) for treatment of cancer associated thrombosis (CAT): A systematic review and meta-analysis. *Thromb Res*. 2018.

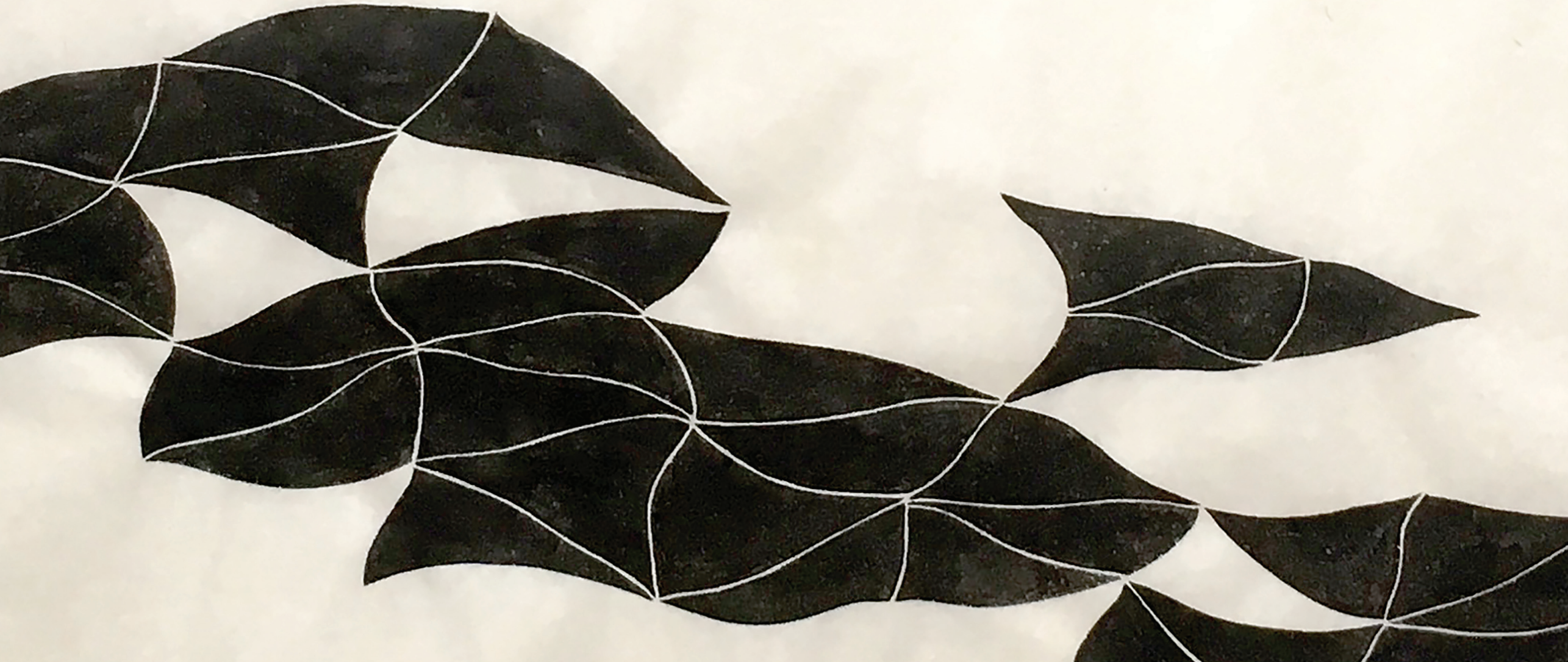




56. James AH, Bates SM, Bauer KA, et al. Management of hereditary antithrombin deficiency in pregnancy. *Thromb Res*. 2017;157:41-45.
57. Eikelboom JW, Connolly SJ, Bosch J, et al. Rivaroxaban with or without Aspirin in Stable Cardiovascular Disease. *N Engl J Med*. 2017;377(14):1319-1330.

# 9

## SUMMARY



## SUMMARY

Antithrombin deficiency is an inherited, heterozygous disorder that was first described in 1965 by Egeberg. It is associated with an increased risk of venous thrombosis. The main objective of this thesis was to understand how the risk of arterial and venous thrombosis associates with low levels of or deficiency of the natural anticoagulant antithrombin.

In **chapter 1** we give a general introduction on the coagulant an anticoagulant systems, thrombosis and on antithrombin.

In **chapter 2** we report a systematic review and Bayesian meta-analysis of the risk of first and recurrent venous thrombosis associated with hereditary antithrombin deficiency. We found that in individuals with antithrombin deficiency the risk of a first VTE is increased 14-fold as compared to study controls (OR 14.0, 95% Credible interval (CrI) 5.5-29.0). In absolute terms, annual VTE risk in antithrombin deficient individuals was found to be 1.2% (95%CrI 0.8-1.7%), versus 0.07% (95%CrI 0.01-0.14%) in non-antithrombin-deficient controls. Thrombosis risk increased with age in antithrombin deficient individuals. The risk of recurrent venous thrombosis in antithrombin-deficient VTE patients was also increased (OR 2.1; probability of OR being >1, 88.4%) in a mix of patients with short-term or long-term anticoagulant therapy. As for absolute risk estimates, we found considerably higher recurrence risks in antithrombin deficient VTE patients as compared to non-antithrombin-deficient VTE patients. Without anticoagulant therapy the annual recurrence risk was higher in antithrombin-deficient VTE patients than in non-antithrombin-deficient VTE patients, 8.8% vs 4.3%, with the probability of recurrence risk being higher in antithrombin deficient patients of 95%. In antithrombin-deficient VTE patients on anticoagulant therapy the annual risk of recurrence was 2.6%, versus 0.7% in non-antithrombin-deficient VTE patients. In the meta-analysis of 3 studies focusing on the impact of subtypes of antithrombin deficiency on the risk of first VTE, a higher risk was found in type I antithrombin deficiency as compared to type II antithrombin deficiency. However, these data are hampered by proband selection, considerably influencing VTE risk estimates: with proband selection OR 9.3 (95%CrI 0.0-30.9), without proband selection OR 2.6 (95%CrI 0.0-19.5). Therefore, no clear “Low-risk” form of antithrombin deficiency could be established. We concluded that based on the high annual recurrence risk of antithrombin deficient individuals, antithrombin deficient patients with VTE require long-term anticoagulant therapy.

In **chapter 3** we present a study investigating the role of isoforms of antithrombin in 21 Dutch antithrombin deficient families. Antithrombin consists of two isoforms, 90% of circulating antithrombin being α-antithrombin and 10% β-antithrombin. We found that β-antithrombin levels were decreased in most individuals with type I and type IIPE antithrombin but normal

levels were found in all subtypes of antithrombin deficiency. The annual incidence of VTE in antithrombin-deficient family members was 1.24%, 95%CI:0.72-1.99%. In individuals with low β-antithrombin levels the annual incidence of VTE was 1.36% (95%CI:0.76-2.25%) and in individuals with normal β-antithrombin levels the annual incidence of VTE was 0.79% (95%CI:0.10-2.77). The annual incidence of recurrence was 3.1% (95%CI 0.9-7.1%) in antithrombin-deficient VTE patients. Duration of anticoagulation had an impact on recurrence risk: In family members annual recurrence with fixed duration was 10% (95%CI:2.1-29.2%), with indefinite duration 1.5% (95%CI:0.2-5.4%), p<0.05. Beta-antithrombin levels were not associated with the risk for first or recurrent VTE in antithrombin deficient subjects. We conclude that our study population is a typical high-risk antithrombin-deficient population, with risk estimates comparing quite well to our systematic review. Both antithrombin-deficient subjects with low and normal plasma β-antithrombin activity levels had high risks of first and recurrent VTE. This suggests that β-antithrombin is not as important for thrombosis risk as was previously reported.

In **chapter 4** we present a study investigating the relation between the anti-Xa activity and antithrombin and β-antithrombin activity in plasma samples of the study population reported in **chapter 3**, that were spiked with either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). Anti-Xa measurements in the expected range (0.6-1.0 IU/mL) after UFH spiking were found in all 17 non-deficient subjects and in 1/22 antithrombin-deficient subjects. Expected anti-Xa measurements after LMWH spiking were found in all 17 non-deficient subjects and in 8/34 antithrombin-deficient subjects. Antithrombin activity was correlated with anti-Xa activity of UFH (R= 0.77) and of LMWH (R=0.66). Mixing studies of pooled normal plasma and antithrombin deficient plasma showed a clear relation between antithrombin levels and anti-Xa measurements: anti-Xa recovery was reduced with antithrombin levels below 100%. Subjects with inherited antithrombin deficiency treated with UFH or LMWH may thus be undertreated, which may result in a further increased risk of (recurrent) venous thromboembolism.

In **chapter 5** we describe our investigations of the molecular background of antithrombin deficiency in the 21 Dutch families described in **chapter 3**, focusing on mutations in the SERPINC1 gene, which codes for the antithrombin molecule. In this analysis mutations were detected by sequencing all 7 exons of the SERPINC1 gene and its regulatory regions, and by using multiplex ligation-dependent probe amplification. In 86% of the families a detrimental SERPINC1 mutation that segregated in the family was found. The percentage of families having a SERPINC1 mutation found in this study is in agreement with percentages found in literature. Of the 13 different mutations found in these 21 families, 5 had not been described before. These included 3 missense mutations, 1 splice site variant and 1 insertion. How these variants cause antithrombin deficiency is described in this chapter. Of the 8 previously



reported mutations, we found that the p.Ala126Gly mutation was associated with a type IIPE phenotype, whereas it had previously been described to be associated with a type I phenotype.

In **chapter 6** we report our findings of a systematic review and Bayesian meta-analysis to analyze the absolute risk of pregnancy-associated VTE in relation to thrombophilia. Thirty-six studies were included in the analyses. All thrombophilias were found to increase the risk of pregnancy-associated VTE. Regarding absolute risks of pregnancy associated VTE, high risk thrombophilias were antithrombin deficiency (antepartum: 7.3%, 95%CrI 1.8-15.6%; post partum: 11.1%, 3.7-21.0%), protein C deficiency (antepartum: 3.2%, 0.6%-8.2%; post partum: 5.4%, 0.9%-13.8%), protein S deficiency (antepartum: 0.9%, 0.0%-3.7%; post partum: 4.2%; 0.7%-9.4%), and homozygous factor V Leiden (antepartum: 2.8%, 0.0%-8.6%; post partum: 2.8%, 0.0%-8.8%). Absolute combined antepartum and postpartum risks for women with heterozygous factor V Leiden, heterozygous prothrombin G20210A mutations, or compound heterozygous factor V Leiden and prothrombin G20210A mutations were all below 3%. As these findings were considerably different than the estimates in the 2012 ACCP guidelines, we recommended to consider these data for future guidelines on pregnancy-associated VTE risk. With expert opinion favoring thrombosis prophylaxis with absolute risk estimates ≥3%, this led us to concluded that women with antithrombin, protein C, or protein S deficiency or with homozygous factor V Leiden should be considered for antepartum or postpartum thrombosis prophylaxis, or both. Furthermore, women with heterozygous factor V Leiden, heterozygous prothrombin G20210A mutation, or compound heterozygous factor V Leiden and prothrombin G20210A mutation should not be prescribed thrombosis prophylaxis on the basis of thrombophilia and family history alone.

In **chapter 7** we describe our study on antithrombin levels and arterial thromboembolism. Arterial thromboembolism (ATE), including acute myocardial infarction and ischemic stroke, is a multifactorial disease, in which arteriosclerotic lesions develop over time. Rupture of these lesions leads to subsequent thrombus formation, which may lead to obstruction of blood vessels. We hypothesized that a lack of inhibition of thrombus formation (e.g. through a deficiency of antithrombin), may cause acceleration of thrombus formation, and hence increase the risk of blood vessel occlusion. As in young people atherosclerosis is not as extensive as in the elderly, it is thought that in young patients with ATE hemostatic factors play a more important pathophysiological role. We investigated if antithrombin levels were associated with ATE at a young age, and found that relatively low antithrombin levels (≤median levels) are associated with an increased risk of ATE (OR 1.46; 95%CI:1.09-1.96). We also investigated if low antithrombin levels were associated with recurrent ATE in young patients with a previous myocardial infarction. In all patients, patients with low antithrombin levels had a higher risk of recurrent arterial thromboembolism, HR 2.16, 95%CI:1.07-

4.38. Especially in women with low antithrombin levels this increased risk of recurrent cardiac events was pronounced, HR 5.97, 95%CI 1.31-27.13. We concluded that that lower antithrombin levels are associated with an increased risk of a first arterial thrombotic event at a young age, and that lower antithrombin levels are associated with a higher rate of recurrent cardiac events, especially in women.

In **Chapter 8** we discuss the findings of the studies described in this thesis and we give suggestions for further studies and for improvements of guidelines.



## NEDERLANDSE SAMENVATTING

Antitrombine deficiëntie is een autosomaal dominante erfelijke afwijking die voor het eerst door Egeberg beschreven werd in 1965. Deze afwijking is geassocieerd met een verhoogd risico op veneuze trombose. Het hoofddoel van dit proefschrift was om inzicht te krijgen in hoe het risico op arteriële en veneuze trombose samenhangt met lage spiegels of deficiëntie van antitrombine.

In **hoofdstuk 1** geven we een overzicht van de bloedstolling, trombose en antitrombine.

In **hoofdstuk 2** beschrijven we een systematische review met Bayesiaanse meta-analyse over hoe het risico op een eerste of recidief veneuze trombose geassocieerd is met antitrombine deficiëntie. We hebben gevonden dat in mensen met antitrombine deficiëntie het risico op veneuze trombose 14-voudig verhoogd is in vergelijking tot mensen zonder deze afwijking (OR 14.0, 95% Credible interval (CrI) 5.5-29.0). We hebben het absoluut risico op veneuze trombose in mensen met antitrombine deficiëntie ingeschat op 1.2% (95%CrI 0.8-1.7%), en 0.07% (95%CrI 0.01-0.14%) in mensen zonder deze afwijking. Het risico op trombose nam toe met de leeftijd in individuen met antitrombine deficiëntie. Ook het risico op recidief trombose was verhoogd in een patiënten populatie waarin mensen deels kortdurend en deels langdurig behandeld werden met antistolling (OR 2.1; de waarschijnlijkheid op een verhoogd risico ofwel OR >1 was 88.4%). Bij de berekening van het jaarlijks absolute risico op een recidief veneuze trombose bij patiënten met veneuze trombose waarbij de antistolling gestopt was vonden we aanzienlijk hogere recidief risico's bij antitrombine-deficiënte patiënten dan patiënten zonder antitrombine deficiëntie: 8.8% vs 4.3%, met een waarschijnlijkheid dat het risico hoger was in antitrombine-deficiënte patiënten van 95%. Het jaarlijks recidief risico bij patiënten met langdurige antistolling was 2.6% in antitrombine-deficiënte patiënten en 0.7% in niet-antitrombine-deficiënte patiënten. In de meta-analyse naar het effect van subtype van antitrombine deficiëntie werd gevonden dat type I antitrombine deficiëntie met een hoger risico op een eerste veneuze trombose was geassocieerd dan type II. Deze berekeningen werden echter sterk beïnvloed door selectie van index patiënten: Met selectie van index patiënten was de OR 9.3 (95%CrI 0.0-30.9). Zonder selectie van index patiënten was de lager: OR2.6 (95%CrI 0.0-19.5). Daarom kon geen duidelijke laag-risico vorm van antitrombine deficiëntie worden vastgesteld. We concludeerden dat gezien het hoge absolute risico op een recidief veneuze trombose, antitrombine deficiënte patiënten met veneuze trombose langdurig met antistolling behandeld moeten worden.

In **hoofdstuk 3** presenteren we een studie naar de rol van de isovormen van antitrombine in 21 Nederlandse families met antitrombine deficiëntie. Antitrombine in het bloed bestaat

uit twee isovormen: 90% α-antitrombine en 10 β-antitrombine. We hebben gevonden dat in de meeste type I en type II PE antitrombine-deficiënte deelnemers β-antitrombine activiteit verlaagd was, hoewel normale β-antitrombine activiteit werd gevonden in alle subtypes van antitrombine deficiëntie. Het jaarlijks risico op een veneuze trombose bij familieleden van index-patiënten was 1.24%, 95%CI:0.72-1.99%. In familieleden met een lage β-antitrombine activiteit was het jaarlijks risico op veneuze trombose 1.36% (95%CI:0.76-2.25%), in familieleden met een normale β-antitrombine activiteit was dat 0.79% (95%CI:0.10-2.77). Het jaarlijks recidief risico was 3.1% (95%CI 0.9-7.1%) in antitrombine-deficiënte patiënten met veneuze trombose. De duur van antistollingsbehandeling had een grote invloed op het jaarlijks recidief risico: met antistollingsbehandeling van beperkte duur was dat 10% (95%CI:2.1-29.2%), met langdurige antistollingsbehandeling was dat 1.5% (95%CI:0.2-5.4%), $p<0.05$. Beta-antitrombine activiteit was niet geassocieerd met het risico op eerste en veneuze trombose in antitrombine deficiënte deelnemers aan het onderzoek. We concluderen dat we een typische antitrombine-deficiënte populatie hebben onderzocht, waarvan de gevonden risico's goed overeenkomen met de risico's die we in onze systematische review hebben gevonden. Deelnemers met lage en normale β-antitrombine activiteit hadden hoge risico's op een eerste en recidief veneuze trombose. Dit suggereert dat β-antitrombine activiteit niet zo belangrijk zijn als eerder werd gerapporteerd.

In **hoofdstuk 4** rapporteren we een studie naar de relatie tussen anti-Xa activiteit en antitrombine- en β-antitrombine activiteit in plasma monsters waaraan ongefractioneerde heparine of laag-moleculair-gewichtsheparine (LMWH) was toegevoegd. In monsters van deelnemers met normaal antitrombine waren alle anti-Xa activiteit metingen op het verwachte niveau, terwijl dit maar bij 1/22 samples van antitrombine-deficiënte deelnemers zo was na toevoeging van ongefractioneerde heparine. Na toevoeging van LMWH aan de samples waren alle anti-Xa activiteit metingen normaal bij de niet-antitrombine-deficiënten, en slechts 8/34 metingen normaal bij antitrombine-deficiënten. De antitrombine activiteit was geassocieerd met de anti-Xa activiteit van ongefractioneerde heparine (R= 0.77) en van LMWH (R=0.66). Mengproeven van normaal-pool plasma met antitrombine-deficiënt plasma toonden een duidelijke relatie tussen de antitrombine activiteit en de anti-Xa metingen: de anti-Xa nam af bij antitrombine activiteit van onder de 100%. Derhalve zouden antitrombine-deficiënte patiënten onder behandeld kunnen worden met standaard doseringen van ongefractioneerde heparine of LMWH. Dit zou het risico op veneuze trombose verder kunnen verhogen.

In **hoofdstuk 5** beschrijven ons onderzoek naar de moleculaire achtergrond van de antitrombine deficiëntie van de 21 Nederlandse families die we in **hoofdstuk 3** beschreven. Daarbij hebben we ons gericht op SERPINC1-gen, dat codeert voor het antitrombine molecuul. We zochten naar mutaties door middel van het sequensen van alle 7 exonen



van het SERPINC1 gen, de regulerende regio's van dit gen, en door middel van de multiplex ligation-dependent probe amplification techniek. Daarmee werd in 86% van de families een veroorzakende SERPINC1 mutatie gevonden. Van de 13 mutaties die gevonden werden, waren er 5 niet eerder beschreven. Hieronder waren 3 missense mutaties, 1 splice site variant, en 1 insertie. Hoe deze verschillende mutaties antitrombine deficiëntie veroorzaken wordt in dit hoofdstuk beschreven. Van één van de 8 eerder beschreven mutaties vonden we dat de p.Ala126Gly mutatie niet met een zoals eerder beschreven type I maar met een type IIPE fenotype was geassocieerd.

In **hoofdstuk 6** presenteren we onze bevindingen van een systematische review en Bayesiaanse meta-analyse naar de invloed van trombofilie op het absolute risico op zwangerschaps-gerelateerde veneuze trombose. Zesendertig studies werden in deze analyses geïncludeerd. Alle onderzochte trombofilieën bleken het risico op zwangerschaps-gerelateerde veneuze trombose te verhogen. Trombofilieën met een hoog trombose risico waren antitrombine deficiëntie (antepartum: 7.3%, 95%CrI 1.8-15.6%; postpartum: 11.1%, 3.7-21.0%), proteïne C deficiëntie (antepartum: 3.2%, 0.6%-8.2%; postpartum: 5.4%, 0.9%-13.8%), proteïne S deficiëntie (antepartum: 0.9%, 0.0%-3.7%; postpartum: 4.2%; 0.7%-9.4%), en de homozygote factor V Leiden mutatie (antepartum: 2.8%, 0.0%-8.6%; post partum: 2.8%, 0.0%-8.8%). Van de overige trombofilieën (heterozygote protrombine G20210A mutatie, heterozygote factor V Leiden mutatie en gecombineerde heterozygotie van de protrombine G20210A en factor V Leiden mutatie) waren de absolute risico's op veneuze trombose van de gecombineerde ante- en postpartum periode allen onder de 3% Deze bevindingen verschilden behoorlijk van de vigerende ACCP richtlijnen uit 2012. Daarom adviseren we de bovenstaande gegevens voor toekomstige richtlijnen te gebruiken. Experts adviseren om trombose profylaxe te geven bij een absoluut risico op veneuze trombose van ≥3% per antepartum of postpartum periode. Daarom concludeerden wij dat bij vrouwen met antitrombine, proteïne C of proteïne S deficiëntie, of homozygote factor V Leiden mutaties het geven van tromboseprofylaxe moet worden overwogen de postpartum en/of antepartum periode. Bij vrouwen met heterozygote protrombine G20210A mutatie, heterozygote factor V Leiden mutatie en gecombineerde heterozygote protrombine G20210A en factor V Leiden mutatie hoeft geen tromboseprofylaxe meer overwogen te worden op basis van deze afwijking en de familieanamnese.

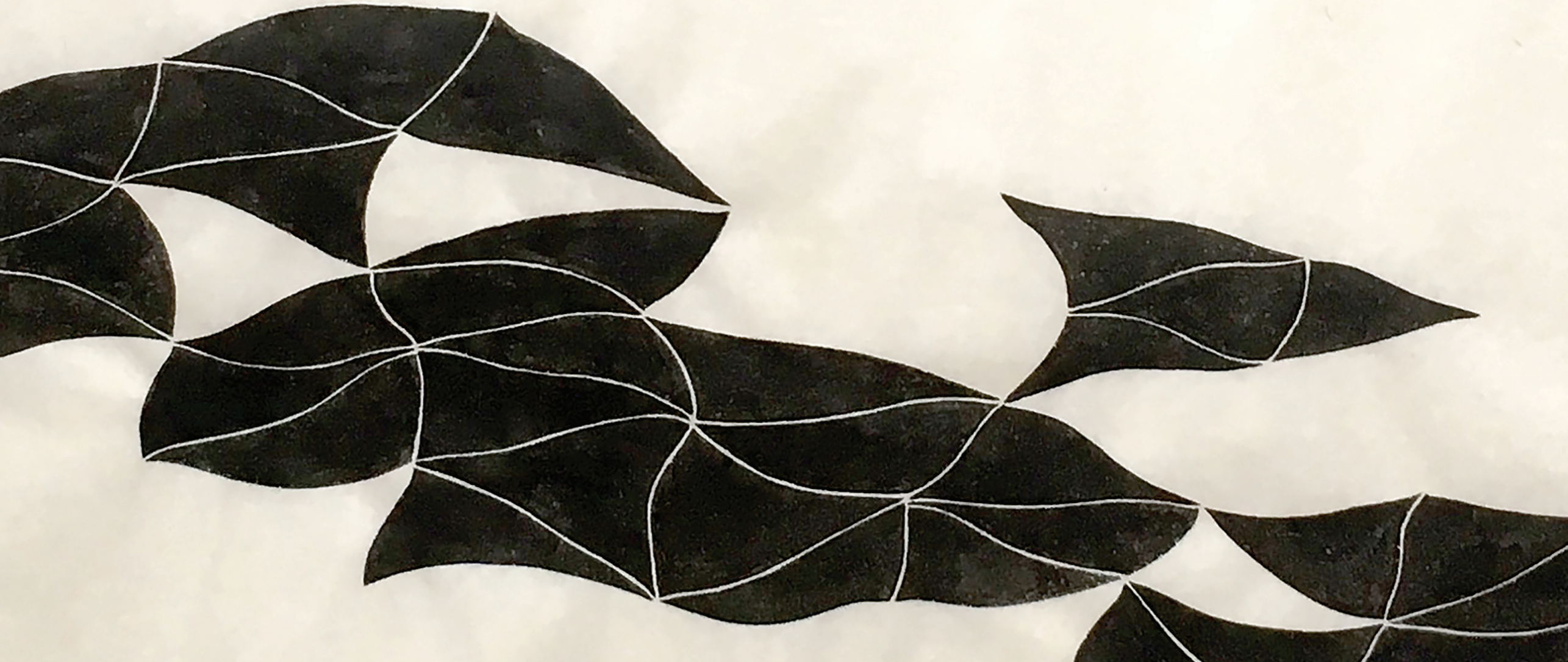
In **hoofdstuk 7** onderzoeken we of antitrombine activiteit is geassocieerd met arteriële trombose. Arteriële trombose, waaronder hartinfarcten en cerebrovasculaire trombose, is een multifactoriële ziekte, waarbij slagaderverkalking zich in verloop van tijd ontwikkelt tot plaques. Bij ruptuur van deze plaques wordt de bloedstolling geactiveerd, waarbij bloedstolsels bloedvaten kunnen verstoppen. We bedachten de hypothese dat een gebrek aan remming van de bloedstolling tot geaccelereerde stolselvorming kan leiden,

en op die manier het risico op arteriële trombose zou kunnen verhogen. Omdat in jonge mensen minder slagaderverkalkingen hebben dan ouderen mensen, is de gedachte dat juist afwijkingen in de bloedstolling juist bij jonge mensen een grotere rol speelt in de ontwikkeling van arteriële trombose. We vonden dat een relatief lage antitrombine activiteit (≤mediane activiteit) geassocieerd waren met een toegenomen risico op arteriële trombose (OR 1.46; 95%CI:1.09-1.96). We onderzochten ook of in jonge patiënten met een eerder hartinfarct een relatief lage antitrombine activiteit (≤mediane activiteit) geassocieerd was met een recidief van een arteriële trombose. Onder alle patiënten met een myocardinfarct hadden diegenen met een ≤mediane antitrombine activiteit een hoger risico op een recidief arteriële trombose, HR 2.16, 95%CI:1.07-4.38. Vooral bij vrouwen met een lage antitrombine activiteit was dit risico verhoogd, HR 5.97, 95%CI 1.31-27.13. We concludeerden dat het hebben van een lage antitrombine-activiteit geassocieerd is met een verhoogd risico op arteriële trombose op jonge leeftijd, en dat na een hartinfarct op jonge leeftijd het hebben van een lage antitrombine-activiteit geassocieerd is met een hoger risico op een recidief hartinfarct, vooral bij vrouwen.

In **hoofdstuk 8** hebben we onze bevindingen bediscussieerd, en doen we aanbevelingen voor verder onderzoek en aanpassingen van vigerende richtlijnen.

# A

## APPENDICES



## LIST OF PUBLICATIONS


1. Croles N, Smorenburg CH, van Groeningen CJ, Giaccone G, Boven E. FOLFOX3 in heavily pretreated patients with metastatic colorectal cancer. *Neth J Med*. 2004;62(7):242-245.
2. Croles FN, Brasse BP, Duisenberg-van Essenberg M, Baars HF, Schweitzer CM. [Connection between hypertension and myocardial infarction and chewing of khat leaves] Verband tussen hypertensie en myocardinfarct en het kauwen van qatbladeren. *Ned Tijdschr Geneeskd*. 2009;153(1-2):38-43.
3. Van der Hoeve EP, Croles FN, Wismeijer D. [Severe periodontal disease as the cause of a fever of unknown origin] Ernstige paradontitis als oorzaak voor onbegrepen koorts. *Ned Tijdschr Tandheelkd*. 2010;117(12):620-622.
4. Croles FN, Hebeda KM, Kats-Ugurlu G, Hermans JJ, Bonenkamp HJ, van der Velden WJ. Splenic gas as a result of a non-Hodgkin's lymphoma in a patient with common variable immunodeficiency. *J Clin Oncol*. 2011;29(23):e666-667.
5. Croles FN, Beerenhout CH, Mulder AB, Meijer K, Nijziel MR. [AL amyloidosis-associated factor X deficiency] Factor X-deficientie bij AL-amyloidose. *Ned Tijdschr Geneeskd*. 2014;158(6):A6678.
6. Verbeek DE, Waanders F, Mulder AB, Bierman WF, Croles FN. Quiz page may 2014: acute kidney injury with red urine. *Am J Kidney Dis*. 2014;63(5):A21-24.
7. Mulder R, Croles FN, Lukens MV, Meijer K, Mulder AB. A novel c.290G>A mutation in the Factor 11 gene in a Dutch Caucasian family with a Factor XI deficiency. *Thromb Res*. 2015;135(4):767-769.
8. Stolmeijer TM, van der Berg AP, Koeze J, et al. Interplay of co-inherited diseases can turn benign syndromes in a deadly combination: haemoglobinopathy and bilirubin transport disorder. *Neth J Med*. 2015;73(5):247-252.
9. Mulder R, Croles FN, Mulder AB, Huntington JA, Meijer K, Lukens MV. SERPINC1 gene mutations in antithrombin deficiency. *Br J Haematol*. 2017;178(2):279-285.
10. Croles FN, Nasserinejad K, Duvekot JJ, Kruip MJ, Meijer K, Leebeek FW. Pregnancy, thrombophilia, and the risk of a first venous thrombosis: systematic review and bayesian meta-analysis. *BMJ*. 2017;359:j4452.
11. Croles FN, Van Loon JE, Dippel DWJ, De Maat MPM, Leebeek FWG. Antithrombin levels are associated with the risk of first and recurrent arterial thromboembolism at a young age. *Atherosclerosis*. 2018;269:144-150.
12. Croles FN, Borjas-Howard J, Nasserinejad K, Leebeek FWG, Meijer K. Risk of Venous Thrombosis in Antithrombin Deficiency: A Systematic Review and Bayesian Meta-analysis. *Semin Thromb Hemost*. 2018;44(4):315-326.
13. Croles FN, Mulder R, Mulder AB, Lukens MV, Meijer K. beta-Antithrombin, subtype of antithrombin deficiency and the risk of venous thromboembolism in hereditary antithrombin deficiency: A family cohort study. *Thromb Res*. 2018;168:47-52.

## DANKWOORD

Dit proefschrift is tot stand gekomen met de hulp van velen. De eerste aanzet werd gegeven in Groningen, waar ik met behulp van **Karina Meijer** en **André Mulder** de Groninger Antitrombine Studie mocht initiëren (afgekort als GRAS, *niet* GAS). Een onderzoek waarbij ik vele families met antitrombine deficiëntie heb mogen zien, die vanuit heel het land naar het verre Groningen afreisden om te participeren. Heel bijzonder! Ik wil **René Mulder** en **Michaël Lukens** danken voor de steun, input en het uitvoeren van een belangrijk deel van het laboratoriumonderzoek. **Jaime Borjas-Howard** wil ik graag bedanken voor zijn inzet als tweede reviewer van de antitrombine review. **Karina**, ik dank je hartelijk voor het vertrouwen en de steun die je me hebt gegeven om het project op te kunnen starten en af te ronden. Je hebt me veel ruimte gelaten om onderzoek te doen en richting te geven, en geholpen waar dat nodig was! Het was goed om na mijn verhuizing naar Rotterdam telefonisch, per mail en tijdens congressen van gedachten te wisselen over het onderzoek.

Bij mijn verhuizing naar Rotterdam in 2015 was het vanaf de eerste gesprekken met **Frank Leebeek** duidelijk dat er voldoende ruimte zou zijn om verder te gaan met het promotieonderzoek. Ik heb ondanks initiële tegenslagen wat betreft publicaties bij mijn komst naar Rotterdam ontzettend veel vertrouwen gekregen om het onderzoek uit te kunnen breiden om tot een volwaardig proefschrift te kunnen komen. Frank, in de discussies ben je ongelofelijk veelzijdig en creatief en je bent ook een fijn mens om mee samen te werken! Je bent goed in het in staat stellen van andere mensen om te excelleren. Een van de beste herinneringen van de afgelopen jaren is hoe je binnen enkele minuten na binnenkomst van de acceptatie van de zwangerschapsreview in *BMJ* juichend mijn kamer binnenkwam! **Marieke Kruip** was je overigens al voor. Marieke, een van jouw goede adviezen is geweest om contact op te nemen met **Wichor Bramer**. Zijn hulp met de zoek strategieën was ontzettend effectief, heeft maanden werk uitgespaard en heeft de reproduceerbaarheid van de reviews enorm vergroot. **Hans Duvekot** wil ik graag danken voor zijn inzet als 2e reviewer van de zwangerschapsreview. Het was een fijne samenwerking die er nog steeds is op het gebied van hemofilie en hemoglobinopathie.

Een enorme boost voor de systematic reviews is de betrokkenheid van **Kazem Nasserinejad** geweest! Kazem, door jou ben ik geïntroduceerd in de Bayesiaanse statistiek. We namen tijdens de circa miljard berekeningen die we samen hebben uitgevoerd (ja, er moest ook wel eens wat opnieuw gedaan worden!) de tijd om goed te kunnen sparren over wat precies de klinische vraag was en hoe het antwoord daarop het best berekend en gegeven kon worden. Dit heeft mij een heel nieuw denkraam opgeleverd, en was voor de artikelen van een belangrijke toegevoegde waarde. Ik vond onze samenwerking inspirerend. Het was het heel fijn om met jou samen te werken!

Ook een speciaal woord van dank aan **Moniek de Maat**. We hebben de afgelopen jaren veel gespard over onderzoek en over patiënten. Ook jij bent in mijn ogen erg goed in staat

anderen te laten excelleren en daar van te genieten. Ik ben daarom blij en vereerd dat mijn verdediging een van de eerste gelegenheden is waarbij je als hoogleraar plaats mag nemen achter de tafel! Ook de praktische hulp van **Debbie Priem-Visser** die me geholpen heeft de bepalingen uit te voeren mag genoemd. Dank daarvoor.

De afgelopen jaren zijn de research besprekingen van de **hemostasegroep** van grote inspiratie geweest. De vele onderzoekers die mooi werk verrichten op allerlei terreinen binnen de trombose en hemostase die op hoog niveau presteren, met eigen ideeën mooie publicaties weten te maken! Van velen heb ik hulp gehad, van praktisch advies hoe welke computerprogramma's te gebruiken tot ideeën voor onderwijs en presentaties.

Speciale dank gaat uit naar **Hiroe Okubo**. Zij heeft de omslag van dit proefschrift heeft ontworpen, en het Erasmus MC verrijkt door een gulle schenking van haar kunstwerken. Hiroe, het contact met jou en Jos voelde als enorm wederzijds inspirerend! Je hebt ondanks een verhuizing naar Japan een omslag gemaakt waarop voor de goede kijker *veel meer* te zien is dan zwart en wit. Het is een weergave van de complexiteit van onderzoek doen en de verrijking en diepgang die het oplevert als je het doet. Het is niet alleen een verzameling van zwart en wit. Het zwart is niet overal even zwart, het wit is niet overal even wit. Er zit een vorm aan het samenspel van zwart en wit dat een driedimensionaal beeld oplevert dat de lezer zelf mag invullen. Maar wie goed kijkt ziet dat deze ruimtelijke afbeelding staat getekend op een vel papier, dat door de inkt van de afbeelding zelf *ook* vervormd is van een tweedimensionaal vlak tot een driedimensionaal vlak. Daarom stond je erop dat dat aspect van het papier zichtbaar moest blijven op de kaft. Dat past beter bij een proefschrift: Onderzoek doen creëert door steeds op een andere wijze te kijken naar een bestaande realiteit steeds weer een nieuwe vorm aan de werkelijkheid. Laat de lezer van dit proefschrift de vorm en realiteit maar weer veranderen door het openslaan daarvan! Hiroe, je multidimensionale afbeeldingen zullen nog lang een bron van verwondering en inspiratie voor me blijven!

Graag bedank ik **Egied Simons** voor de hulp bij de vormgeving van dit proefschrift.

Speciale dank aan **prof. dr. J.A. Lisman**, **prof. dr. M.V. Huisman** en **prof. dr. ir. H. Boersma** voor het plaatsnemen in de kleine commissie.

Veel steun heb ik gehad van mijn **familie en vrienden**, wiens interesse en aandacht tot grote steun is geweest. Mijn **vader** heeft zelfs alle eerdere proefschriften en stellingen van familieleden opgediept, en was een geïnteresseerd lezer van al mijn stukken. Als laatste maar zeker niet als minste zou ik mijn lieve echtgenote **Loes** en mijn kinderen **Maurits, Frederik** en **Francine** willen noemen: Van Loes heb ik al die tijd veel steun gehad om aan dit traject te beginnen en het vol te houden. Maar misschien is de belangrijkste rol wel van mijn gezin het voorzien van de nodige afleiding hiervan. Of het nu vakanties zijn in de bergen, klimmen of uit eten met Loes, naar het voetbal van Maurits gaan, met Frederik in de tuin werken of een rondje fietsen met Francine, al deze momenten zorgen voor een eindeloze bron aan energie!

## CURRICULUM VITAE

Ik, Frederik Nanne Croles, werd op 9 oktober 1979 geboren te ’s-Hertogenbosch. In 1998 slaagde ik cum laude aan het Gymnasium Beekvliet te Sint-Michielsgestel. Ik startte in 1999 met de studie geneeskunde aan de Vrije Universiteit te Amsterdam, en was daarnaast actief binnen het studentencorps aan de Vrije Universiteit, en binnen de Universitaire Studentenraad van de Vrije universiteit. Na mijn eerste wetenschappelijke publicatie in 2004 en na afronden van mijn coschappen in 2005 behaalde ik het doctoraal geneeskunde. Aansluitend werd ik toegelaten tot de opleiding interne geneeskunde van het UMC St. Radboud te Nijmegen. Als AIOS heb ik gewerkt in het TweeSteden ziekenhuis te Tilburg, het Jeroen Bosch Ziekenhuis te ’s-Hertogenbosch en het UMC St. Radboud te Nijmegen. In het UMC St. Radboud voltooide ik de opleiding interne geneeskunde en de subspecialisatie hematologie in 2011. Nadien kreeg ik mijn eerste baan als hematoloog in het UMC Groningen, met het aandachtsgebied trombose en hemostase. In 2012 startte ik daar met medisch wetenschappelijk onderzoek, genaamd Groninger Antitrombine Studie. Het doel was een wetenschappelijke promotie. Ik werd vanaf de start begeleid door prof. Karina Meijer. In 2015 verhuisde ik met mijn gezin naar Bergschenhoek, voor een nieuwe baan in het Erasmus MC. Daar heb ik als hematoloog gewerkt, met als aandachtsgebieden trombose en hemostase en hemoglobinopathie. Prof. Frank Leebeek werd daar mijn nieuwe promotor naast prof. Karina Meijer, en onder zijn leiding kon ik een aantal extra hoofdstukken schrijven. Deze nieuwe samenwerking heeft mede geleid tot het proefschrift dat nu voor u ligt.

# PHD PORTFOLIO

## SUMMARY OF PHD TRAINING AND TEACHING ACTIVITIES

| | |
|---|---|
| Name PhD student: F.N. Croles | PhD period: dec 2011- 2018 |
| Erasmus MC Department: Hematology | Promotor(s): Prof. dr. F.W.G. Leebeek, |
| Research School | Prof. dr. K. Meijer |

**1. PhD training**

| | Year | Workload (ECTS) |
|---|---|---|
| **Research skills** | | |
| • Basis course on regulations and organization for clinical investigators (BROK, UMCG 2013, Erasmus MC 2018) | 2013, 2018 | 3.0 |
| **In-depth courses (e.g. Research school, Medical Training)** | | |
| • London haemophilia preceptorship course | 2014 | 0.9 |
| • 1x NTVH course on thrombosis and haemostasis | 2015 | 1.0 |
| • Haemophilia Academy | 2015 | 1.4 |
| **Presentations** | | |
| • Presentation NVVH | 2012 | 0.5 |
| • Presentation 4e casuistiek symposium | 2013 | 0.5 |
| • Presentations Rotterdamse nascholing hematologie | 2015, 2016, 2017 | 1.5 |
| • Poster presentations: 1x ECTH, 1x ISTH, 1x London preceptorship course, 1x haemophilia academy | 2016, 2017 | 1.2 |
| **Conferences and symposia** | | |
| • ISTH Amsterdam 2013, Berlin 2017 | 2013, 2017 | 3.6 |
| • 3x NVTH | 2015, 2017, 2018 | 1.8 |
| • 3x ASH | 2011, 2016 | 3.6 |
| • WFH | 2018 | 1.2 |
| • EHA | 2013, 2014, 2015, 2018 | 4.8 |
| • Dutch Hematology Congres 2013 (3d), 2014(3d), 2016 (1d), 2018 (3d) | 2013, 2014, 2016, 2018 | 3.0 |
| • ECTH | 2016 | 0.9 |
| • Regionale nascholing hematologie | 2015, 2016, 2017 | 0.9 |
| • Van Creveld Symposium | 2017 | 0.3 |
| • Internistendagen | 2013, 2015 | 1.2 |
| • Klinische dag NNVH | 2011, 2012, 2013 | 0.9 |
| • AMSTOL symposium | 2012 | 0.3 |
| • Update hemostase | 2017 | 0.6 |
| • Nijmegen symposium rare bleeding disorders | 2015 | 0.3 |

| | | |
|---|---|---|
| **Seminars and workshops** | | |
| • COEUR symposium lecture | 2018 | 0.5 |
| **Didactic skills** | | |
| • Teach the teacher training | 2018 | 0.6 |
| **Other** | | |
| **2. Teaching activities** | | |
| **Lecturing** | | |
| • Lecture for midwives (anemia, hemoglobinpathies) | 2018 | 0.1 |
| • Lecture for nursing specialists, physician assistants | 2017 | 0.1 |
| • Lecture for gynaecology residents | 2017 | 0.1 |
| • Lecture for internal medicine residents | 2015, 2017 | 0.4 |
| • Lecture for medical students | 2016 | 0.2 |
| **Supervision of students** | | |
| • Systematic review 2nd year medical students during elective course on blood coagulation | 2017, 2018 | 0.5 |
| **Other** | | |
| • Weekly departmental research lecture attendance and presentations | 2015-2018 | 9.0 |
| **Total** | | 44.9 |