Genetic testing of inherited thrombophilia

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Thesis mentor: Assist. Prof. Sanja Dević Pavlić, PhD

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LIST OF ABBREVIATIONS AND ACRONYMS

- 1q23-q25.1 – q arm of chromosome 1, band 23 to 25.1
- 1q24.2 – q arm of chromosome 1, region 24, sub-band 2
- 2q13-q14 – q arm of chromosome 2, band 13 to 14
- ACCP – American College of Chest Physicians
- aCGH - Array Comparative Genomic Hybridization
- APC – Activated Protein C
- aPTT - activated Partial Thromboplastin Time
- ASH – American Society of Haematology
- AS-PCR - Allele-Specific Polymerase Chain Reaction
- BSH – British Society of Haematology
- BMI – Body Mass Index
- C4b - Complement Component 4b
- COC – Combined Oral Contraceptive
- CVAD – Central venous access device
- DIC - Disseminated Intravascular Coagulation
- DNA – Desoxynucleinacid
- DVT – Deep Vein Thrombosis
- ELISA - enzyme-linked immunosorbent assay
- ESC - European Society of Cardiology
- FISH - Fluorescence In Situ Hybridization
- FVL - Faktor V Leiden
- GTG - G-banding with Trypsin and Giemsa
- HBS - heparin-binding-site
- HIV – Human Immunodeficiency Virus
- HRT – Hormonal Replacement Therapy
- IXa – activated Factor IX
- LA - Lupus Anticoagulant
- MLPA - Multiplex Ligation-dependent Probe Amplification
- mRNA– messenger Ribonucleic Acid
- MS - Mass Spectrometry
- MPN – Myeloproliferative Neoplasm

- NGS - Next-Generation Sequencing
- PC – Protein C
- PCD – Protein C deficiency
- PCR - Polymerase Chain Reaction
- PE - pleotrophic effects
- PGM – Prothrombin Gene Mutation
- PNH – Paroxysmal nocturnal Haemoglobinuria
- PROC – Protein C
- PROS1 – Protein S
- PSD – Protein S deficiency
- qPCR - Quantitative Polymerase Chain Reaction
- QF-PCR- Quantitative Fluorescence Polymerase Chain Reaction
- RFLP - Restriction Fragment Length Polymorphism
- RS - reactive site
- SERPINC1 - Serpin Family C Member 1
- TP-PCR - Triplet Repeat Primed Polymerase Chain Reaction
- USA – United States of America
- Va – activated Factor V
- VIIIa – activated Factor VIII
- VTE – Venous Thromboembolism
- Xa – activated Faktor X
- IIa – activated Faktor II

Introduction

Thrombophilia refers to a group of disorders characterised by an increase clotting tendency and pathological predisposition to develop thrombosis. These vascular occlusions caused by blood clots are formed in intact vessels, rather than in response to an injury. Deep vein thrombosis (DVT) occurring in the legs are the most common type. This thrombus can potentially detach from the vessel wall, travel with the bloodstream and become lodged in the lungs. There it can create a life-threatening venous thromboembolism (VTE) called pulmonary embolism (PE). Dysregulated blood clot formation can occur less frequently in heart chambers or arteries. Arterial occlusions can lead to infarctions in the area supplied by the blocked vessel (heart attack, cerebral infarction). Blood coagulation is a complex process influenced by acquired and hereditary factors resulting in multifactorial etiologies of thrombophilia (1). Inherited thrombophilia refers to conditions in which a genetic mutation influences the function or quantity of a factor involved in the blood coagulation cascade (2). The most common form of inherited thrombophilia is a mutation in the coagulation factor V gene, known as a factor V Leiden mutation, which results in hyperactivity of Va. The second most common form is a mutation in the prothrombin gene (factor II) which leads to increased production of prothrombin. In the case of antithrombin, protein C and protein S, the condition is due to a deficiency. Protein S, protein C and antithrombin III deficiencies appear with lower frequency in the population but have a higher thromboembolic risk. The genetic causes originate in the genes that code for these particular proteins. Furthermore the conditions can be classified as either loss of function or gain of function mutations (2,3). Acquired thrombophilia and acquired conditions that contribute to a hypercoagulable state, including Antiphospholipid syndrome, cancer, HIV, inflammation, estrogen containing medications, surgery, immobilization, smoking, obesity, advanced age, pregnancy, and postpartum period, will not be addressed specifically (4). Nevertheless, among the inherited thrombophilia, different risk profiles result from cumulative effects of different genetic mutations and acquired conditions. These multifactorial risk profiles are relevant to the question of the necessity of genetic testing and therapy selection (3).

Aims and Objectives

The primary aim of this thesis is to explore the genetic basis of inherited thrombophilia and the corresponding testing methods, with a particular focus on the Factor V Leiden mutation, Prothrombin mutation, Antithrombin III deficiency, Protein C deficiency, and Protein S deficiency. Conducting a comprehensive literature review on each of the five selected thrombophilia's will determine the prevalence rates, pathophysiological mechanisms, thrombosis risks, molecular basis of gene mutations and allow assessment of the effectiveness and limitations of current genetic testing methods for detecting these mutations. Additionally, the thesis will evaluate the sensitivity, specificity, and accuracy of various genetic testing techniques and investigate the practical challenges and ethical considerations in the implementation of genetic testing for inherited thrombophilia. Finally, different testing guidelines for specific clinical indications will be analysed and discussed. This will include reviewing of existing clinical guidelines and providing recommendations based on study findings. By addressing these objectives, this thesis aims to enhance the understanding of genetic factors underlying inherited thrombophilia and to improve clinical testing, management and outcomes for affected individuals and their families.

Literature Review

Genetic testing

Genetic testing is a diagnostic tool that analyses an individual's DNA to detect variations in genetic material. The testing process involves collecting a sample of blood, saliva, or other tissue, which is analysed in a specialized laboratory. The test results can guide healthcare decisions, influence lifestyle adjustments, and provide insight into familial health risks. Genetic testing is an integral component of establishing a genetic diagnosis, encompassing a comprehensive process that includes several stages (5):

1. Identification of the Indication: This crucial initial step focuses on the selection of appropriate patients based on specific clinical symptoms and the establishment of a preliminary diagnosis. Accurate patient selection is essential to ensure that the genetic test is relevant and beneficial.

2. Genetic Testing: This phase involves selecting the optimal testing method tailored to the clinical indication. It encompasses performing the genetic test and interpreting the results to confirm a genetic diagnosis. The success of this stage depends on the precise alignment of the test method with the diagnostic needs (6).

3. Aftercare: Following the genetic diagnosis, this stage is dedicated to providing detailed information about the condition. This includes informing the patient about the nature of the disorder, its potential progression, its inheritance and the possible effects on their future health. Comprehensive aftercare ensures that patients and their families are well equipped to manage the condition effectively, offering support and guidance for their ongoing health journey (6,7).

Despite its benefits, genetic testing also presents ethical, legal, and social challenges. Concerns about privacy, the potential for genetic discrimination, and the psychological impact of results necessitate careful consideration. For that reason, genetic counselling is essential during the entire process to help patient understand the medical, psychological, and familial implications of genetic test results and to enable them to make independent, informed decisions. Genetic counsellors are healthcare professionals who have extensive knowledge and experience in both medical genetics and patient counselling (8,9).Specifically related to thrombophilia testing, counsellors create an individualized risk profile. This includes a detailed family history of provoked or unprovoked VTEs and consideration of numerous factors such as medications (especially those containing estrogen), age, BMI, smoking status, and history of pregnancy loss. Patients receive tailored education on avoiding smoking, prolonged immobility, certain

medications, the potential use of anticoagulants, the benefits of weight loss, and the possible need for thromboprophylaxis during pregnancy and postpartum. Recommendations for preventive measures, such as switching to progestin-only hormonal contraceptives or wearing support/compression stockings during long-haul flights, can be provided individually (10).

Genetic Testing Methods

Based on the specific indication, genetic testing can be subdivided into several types: diagnostic testing, predictive testing (presymptomatic and predisposition), carrier testing, prenatal testing, population screening (reproductive and non-reproductive), and pharmacogenomic testing. In the context of investigating inherited thrombophilia's, only the diagnostic and predictive testing categories are relevant. Diagnostic testing confirms or exclude a specific working diagnosis in individuals presenting symptoms. Concerning inherited thrombophilia's, the manifestation of these symptoms signifies that the patient has already experienced a venous thromboembolism (VTE). On the other hand, predictive testing determines the likelihood of developing genetic conditions before symptoms emerge. Those entail conducting testing of individuals who have not yet experienced any thromboembolic events but have a positive family history of a specific thrombophilic gene disorder. Genetic testing employs several methods to analyse DNA, each of which is suitable for different diagnostic requirements (Figure 1) (5,11).

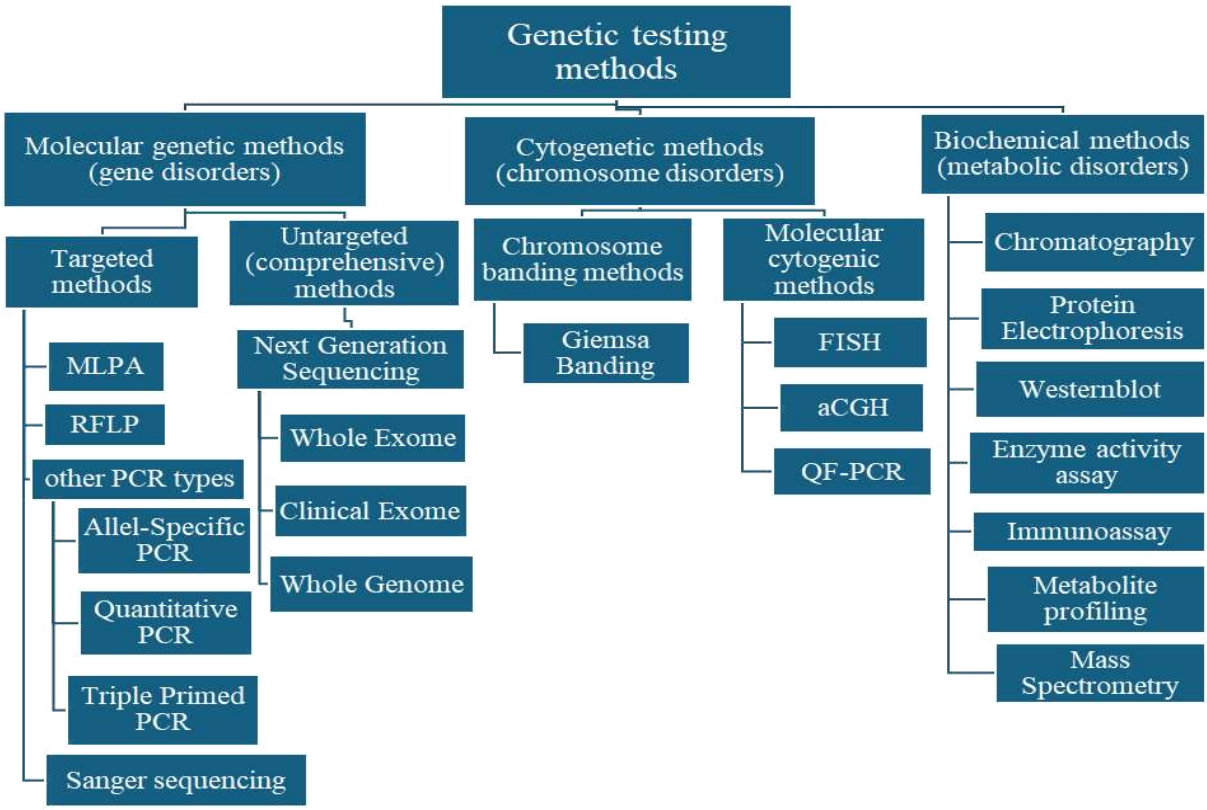

Figure 1: Hierarchical Classification of Genetic Testing Methods.

Molecular Genetic Methods, primarily used in the detection for gene disorders, can be subdivided into targeted and untargeted (comprehensive) methods. Targeted methods focus on specific genes to identify genetic variations. Most of them involve diverse types of polymerase chain reaction (PCR) for the amplification of specific DNA segments or can be performed by sequencing the specific genetic variations of interest. More precisely these methods include (12):

- MLPA (Multiplex Ligation-dependent Probe Amplification): Detects copy number changes in specific DNA sequences (5).
- RFLP (Restriction Fragment Length Polymorphism): Identifies genetic variations by cutting DNA into pieces with restriction enzymes, after PCR amplification has been performed (12).
- Other PCR types:
- AS-PCR (Allele-Specific Polymerase Chain Reaction): Selectively amplifies a specific allele or variant of a gene. Commonly used to detect single nucleotide mutations (5).
- qPCR (Quantitative Polymerase Chain Reaction): Enables real-time amplification of a specific DNA sequence (5).
- TP-PCR (Triple Primed PCR): Identifies expansions of triplet repeats within genes (5).
- Sanger Sequencing: Unveils the precise sequence of nucleotides, identifying mutations precisely (12).

Untargeted (comprehensive) methods cover larger regions of DNA for broader analysis, especially when it is unclear which gene is affected. They often involve sequencing large amounts of DNA using next-generation sequencing (NGS). While Sanger sequencing technology can also be used for untargeted analysis, it is more time-consuming and less practical for analysing larger regions of DNA. Depending on the number of genes sequenced, NGS can be divided into (12,13):

- Whole exome sequencing: performs sequencing of all exons of the genome (13).
- Clinical exome sequencing: performs sequencing of exons of specific genes (13).
- Whole genome sequencing: performs sequencing of the entire genomic DNA, including introns (13).

Cytogenetic Methods, mainly used for testing of chromosomal disorders, can be subdivided into chromosome banding methods and molecular cytogenic methods. Chromosome banding methods, commonly GTG Banding (G-banding using Trypsin and Giemsa), visualize chromosomes under a microscope, revealing large structural changes or abnormalities in

chromosome count. Molecular Cytogenetic Methods, on the other hand, are advanced methods for detailed chromosomal analysis which include (14):

- FISH (Fluorescence In Situ Hybridization): Utilizes fluorescent probes to identify and visualize the presence or absence of specific DNA sequences, allowing the precise localization of targeted regions on chromosomes (12).
- aCGH (Array Comparative Genomic Hybridization): Analyzes copy number variations across the genome (12).
- QF-PCR (Quantitative Fluorescence PCR): Quantifies DNA sequences to detect aneuploidies (5).

Biochemical methods are essential for diagnosing a variety of metabolic disorders by analyzing the activities of proteins and metabolites, rather than directly focusing on the DNA sequence. This offers valuable insights into the metabolic and functional impacts of genetic mutations. Biochemical methods include:

- Chromatography: Separates and analyzes mixture of components based on their different movement rates through a stationary and a mobile phase (15).
- Protein Electrophoresis: Divides proteins in a gel, based on their size and charge using an electric field, aiding in the diagnosis of protein disorders (16).
- Western Blot: Uses gel electrophoresis followed by transfer to a membrane and antibody staining to detect specific proteins in a sample (17).
- Enzyme Activity Assay: Quantifies the catalytic function of enzymes to diagnose disorders related to enzyme deficiencies or abnormalities (18).
- Immunoassay: Employs antibodies to specifically detect and measure the concentration of proteins or hormones (19).
- Metabolite Profiling: Identifies and quantifies metabolites in biological samples (e.g. blood, urine) to diagnose related disorders (20).
- MS (Mass Spectrometry): Measures the mass-to-charge ratio of ions to identify and quantify molecules in a sample, providing detailed molecular information (21).

Considering that inherited thrombophilia's are mainly gene disorders, molecular genetic methods or functional biochemical tests are used for their detection. Due to the rising incidence of VTEs, molecular genetic testing for hypercoagulability is a prevalent procedure in clinical genetics laboratories. FVL and Prothrombin related tests are frequently requested. Evaluating other significant inherited thrombophilia's (antithrombin, protein C, and protein S deficiency) typically involves functional tests to assess activity or antigen levels (22).

Interpreting the results of genetic testing

Accurate interpretation of genetic test results includes assessing their clinical significance and their impact on the patient's treatment. A positive result indicates the presence of a mutation or variant in the tested gene. A negative result, on the other hand, means that no mutations or variants are identified in the targeted gene. It is crucial to understand that a negative finding does not necessarily rule out the possibility of a genetic condition. Numerous factors can contribute to negative results, such as the sensitivity of the testing method, the presence of mutations in genes not covered by the test, or the impact of environmental factors on disease manifestation. The third result option is a detection of a variant of uncertain significance (VUS) (12). This classification refers to the identification of a genetic alteration whose impact on disease risk or pathogenicity remains ambiguous. VUS outcomes are challenging for both patients and clinicians, necessitating further investigation and potentially additional testing of relatives (23–25).

Inherited Thrombophilias

Inherited thrombophilias are genetic conditions that increase an individual's risk to form abnormal blood clots. These disorders are often due to mutations in genes involved in blood clotting, such as Factor V Leiden, Prothrombin G20210A mutation, Antithrombin III deficiency, Protein S deficiency and Protein C deficiency (Table 1). These genetic alterations may lead to recurrent venous thromboembolism (VTE), encompassing conditions such as deep vein thrombosis (DVT) and pulmonary embolism (PE). Although these conditions can be serious, many affected individuals remain asymptomatic and unaware of their status until they undergo genetic testing (1,22,26,27).

Table 1: The most common types of inherited thrombophilias and their genetic causes

Thrombophilia type	Inheritance type	gene	location	Sequence variant
Faktor V Mutation Leiden	AD	FV	1q24.2	Base substitution
Prothrombin G20210A mutation	AD	FII	11p11-q12	Base Substitution
Antithrombin III deficiency	AD	SERPINC1	1q23-q25.1	Many different
Protein S deficiency	AD	PROS1	3q11.2	Usually Point Mutations
Protein C deficiency	AD	PROC	2q13-q14	Many different

Different types of inherited thrombophilias are diagnosed using different combinations of genetic testing methods. The choice of the method depends on various factors, including the thrombophilia type, patient's clinical symptoms, personal and family history and the presence of other risk factors. Moreover, clinical guidelines and recommendations based on the latest research and consensus among specialists from professional organizations can influence the choice of testing as well. Commonly used genetic testing methods for diagnosis of specific types of thrombophilia are listed in the Table 2.

Table 2: Recommended testing methods for inherited thrombophilias

Thrombophilia type	Testing methods
Factor V Leiden	• Functional clotting assay to detect APC resistance • DNA based testing (PCR types, Sanger sequencing)
Prothrombin G20210A Mutation	• DNA based testing (PCR types, Sanger sequencing)
Antithrombin III deficiency	• Functional chromogenic assay • Antigen assay to distinguish between type I and type II
Protein S deficiency	• Functional clotting assay to detect type II • Antigenic assays detect free or total Protein S to distinguish between type I and type III
Protein C deficiency	• Functional clotting/ chromogenic assay • Antigenic assay to distinguish between type I and type II

Faktor V Mutation Leiden

Factor V Leiden Mutation (FVL) is the most common inherited thrombophilia. Its Inheritance follows an autosomal dominant pattern, meaning inheriting one copy from a parent can lead to the disorder. Therefore, mutations can be classified into heterozygous or homozygous. Heterozygous means that two different alleles were inherited: one allele has the genetic material for the wildtype version of factor V and the second allele carries the mutation. If the mutation is present on both alleles the individual is classified as homozygous (28). Heterozygosity occurs in approximately 3-8% of the general population in Europe and USA, but the prevalence varies between populations leading to large differences in the distribution. In Europe, the values vary from 15% in Greece to 3.3% in Spain. In the USA, 5.2% of Caucasians are affected, 1,2% of African Americans and only 0.45% of Asian Americans. The condition is rarely present in Africa, Southeast Asia, Japan and in indigenous populations of America and Australia. The frequency of homozygosity is significantly lower than 1% (22,29). Heterozygosity increases the lifetime risk of thrombosis by a factor of 7, while homozygosity increases the risk 80-fold (22).

The mutation alters a single nucleotide in chromosome one (1q24.2), resulting in an altered base triplet (1691G>A variant) and finally causing a missense mutation (Arg506Gln). The change in the protein sequence results in so-called APC resistance. Normally, activated protein C (APC) initiates several cleavages on activated Faktor V (Va), which leads to its inactivation. APC acts as a natural anticoagulant. The altered structure of Va in FVL leads to the elimination of the first cleavage site and inhibits its own degradation. Consequently, Factor V retains its pro-coagulant effect leading to an imbalance of anticoagulant and procoagulant influences, increasing the tendency to develop thromboses (30,31).

FVL mutation can be identified using functional biochemical assays or molecular genetic methods (Table 2). Both methods are qualitative and enable classification into heterozygous and homozygous cases (32). Functional clotting assays are performed with a one-step or two-step activated Partial Thromboplastin Time (aPTT) assay. The innovative one-step clotting test is conducted with diluted plasma combined with several reagents. This reagent mixture includes factor Xa to trigger coagulation, various coagulation factors at stable concentrations, and APC. Increased APC resistance in Factor V results in a shorter coagulation time extension. The coagulation time measurements can differentiate between wild-type (>70 seconds), heterozygous (40-60 seconds), and homozygous (<40 seconds) individuals. This assay is unaffected by coagulation factor deficiencies, heparin therapy, or lupus anticoagulant (LA)

(32,33). A disadvantage of coagulation testing is the more complex pre-analytical process, including sample preparation, storage, and transfer to the laboratory. Since the functional test focuses solely on APC resistance, it has the advantage of detecting resistance even when it is not caused by the FVL mutation. This broader focus also leads to less specificity than molecular genetic methods (31,34,35). In molecular genetic testing, the identification of the single-nucleotide substitutions relies on assays capable of detecting such alterations, with PCR and Sanger sequencing standing out as the predominant techniques in clinical settings. PCR is employed to amplify specific regions of the Factor V gene containing the mutation, enabling selective amplification. When combined with RFLP analysis, DNA fragments are subjected to restriction enzyme digestion, revealing specific recognition sites that indicate the presence of the mutation. Alternatively, Allele-Specific PCR selectively amplifies the allele with the mutation using specialized primers (36,37). Sanger DNA sequencing is regarded as the gold standard, though it is not the first line test due to its higher cost and longer turnaround time. It is frequently used to confirm results from other methods or to identify rare mutations. The individual laboratory selects the method by considering factors such as sample quality, method sensitivity and specificity, resource availability, and cost-effectiveness. Since DNA is stable, the pre-analytical procedures for these methods are uncomplicated (38).

Prothrombin G20210A mutation

Prothrombin related thrombophilia, also known as prothrombin G20210A mutation, is the second most common inherited thrombophilia following factor V Leiden and adheres to the same autosomal dominant inheritance pattern. In the variant carriers, a substitution located in the prothrombin (FII) gene on chromosome 11p11-q12, more precisely in the non-translated 3' region at nucleotide position 20210 causes the base guanine to be replaced by adenine (Table 1). As the mutation affects an intron region that codes for the polyadenylation of the mRNA and regulates gene expression, the amino acid sequence of prothrombin is not affected. A structurally normal protein is produced. Nevertheless, due to heightened translation activity, an excessive amount is consistently generated. Functionally prothrombin represents the precursor for thrombin, which subsequently converts fibrinogen into fibrin. Higher concentration of prothrombin in the plasma leads to an increased clotting tendency (39,40).

The epidemiology of the pathological variant is comparable to the factor V mutation. As with factor V, the highest heterozygosity rate is found in Europe, but the variation between the individual European countries is smaller. The prevalence in southern Europe is around 3% and

1.7% in the northern countries (39,41). In the USA, 2-5% of Caucasians are affected, 2,2% Hispanics and only 0-0,6% of Asian Americans. The genetic variant is also very rarely found in the African, Asian, and indigenous populations. Homozygosity is much scarcer with a frequency of 1:10000 (42). Heterozygosity increases the risk of DVT in adults by the factor 2-5. The risk of VTE is presumably higher in individuals with the homozygous variant but cannot be clearly predicted based on lacking data (22). Multiple reports of asymptomatic 20210G>A homozygotes, clearly emphasize the contribution of other genetic and acquired risk factors to thrombosis (43). Half of the thromboembolic episodes in individuals with prothrombin thrombophilia are triggered by additional risk factors. Especially factors like pregnancy and oral hormone contraception should be considered (44).

For diagnostic purposes, either molecular genetic testing methods or specific biochemical laboratory tests can be employed. Given its similarity to the Factor V mutation, which also involves a single substitution, identical genetic methodologies can be utilized. These encompass PCR followed by RFLP analysis or Sanger DNA sequencing (34) (Table 2). Additionally, biochemical laboratory assessments such as Prothrombin Time (PT) and aPTT may be performed to evaluate clotting functionality. PT measures the overall time taken for blood coagulation, with its prolongation potentially indicating thrombophilia, whereas aPTT measures the coagulation time with a focus on the intrinsic pathway of the cascade. Abnormalities may suggest clotting factor deficiency or dysfunction. Despite that laboratory tests offer valuable insights, they do not specifically confirm the presence of a prothrombin mutation. For a precise diagnosis molecular genetic testing methods are essential (45).

Antithrombin III deficiency

Antithrombin III deficiency is inherited in an autosomal dominant manner with variable penetrance and caused by mutations in the SERPINC1 gene, which are located on chromosome 1 (1q23-q25.1) (Table 1). The condition can result from various types of mutations, currently 399 different pathological gene variants are identified (3,46). They can be broadly categorized into two main types, each leading to different forms of the deficiency. Type I deficiency is a quantitative deficiency, indicated by lower plasma levels of antithrombin. This reduction results from either diminished production or accelerated breakdown of the antithrombin protein. Common causative mutations include nonsense mutations, frameshift mutations, and large deletions or insertions. Nonsense mutations introduce a premature stop codon, leading to early termination of transcription. Frameshift mutations occur when insertions or deletions of

nucleotides alter the reading frame. Finally, large deletions or insertions can significantly impair protein synthesis as well. Type II Antithrombin Deficiency involves qualitative defects leading to reduced activity at normal plasma concentration. The specific site of the structural abnormality is further classified into HBS (heparin-binding-site), RS (reactive site) and PE (pleiotropic effects). Typically, the protein's ability to inhibit thrombin and other clotting factors is affected which leads to an impaired anticoagulant activity. In this case, the sequence changes are usually minor, characterized as missense point mutations. The substitution of an amino acid, depending on its location, can result in effects ranging from mild to severe (47). The mechanism of action of antithrombin is based on the inhibition of the pro-coagulable factors IIa, Xa and IXa which leads to the prevention of a thrombus formation. Even though the normal activity range is 80-120%, an antithrombin level below 87% is considered a mild deficiency, because clinical cohort studies have shown that levels in the lower part of the normal range (<5th percentile) are still associated with a 1.6 -3.7 times higher likelihood of recurrent VTE. In the case of hereditary antithrombin deficiency, the activity is typically reduced to 40-60% of the normal level (48,49).

The prevalence of hereditary antithrombin deficiency is estimated at 0,02-0,17% of general population and is associated with a 5- to 50-fold increased risk for venous thrombosis (50). Homozygous type I antithrombin deficiency remains unreported in clinical cases, suggesting it may be incompatible with life. This finding aligns with research indicating that homozygous knockout of the SERPINC1 gene is lethal in mice (51). Nevertheless, individuals homozygous for type II mutations have been delineated, exhibiting severe venous thrombosis and an elevated prevalence of arterial thrombosis (52). In general, the risk of VTE appears to be higher for antithrombin deficiency than for protein C or protein S deficiency, FVL, or prothrombin G20210A. As in protein C and protein S deficiency, the first thrombotic event tends to present between the ages of 10 and 50 years (53). Acquired causes of antithrombin deficiency are significantly more common than hereditary causes, therefore excluding these factors is essential before proceeding with hereditary testing (46).

Diagnostically, there are two different types of assays: a chromogenic activity assay and an antigen immunoassay (Table 2). Since the antigen assay only measures the amount of antithrombin without providing information about its functionality, the activity assay should be performed as the first-line test. These activity assays utilize either thrombin-based or factor Xa-based chromogenic methods and are generally performed using commercial kits. In thrombin-based assays, patient plasma is mixed with excess thrombin and heparin, allowing endogenous

antithrombin to inhibit the thrombin. The remaining uninhibited thrombin cleaves a chromogenic substrate, releasing a colour-changing compound. The change in absorbance is inversely proportional to antithrombin activity. Factor Xa-based assays follow a similar procedure but use factor Xa instead of thrombin. Parameters like the type of assay and incubation time can affect the sensitivity and specificity of these tests. For antigen detection, methods like ELISA, immunoelectrophoresis or radial immunodiffusion can be used. This type of test can be employed to differentiate between antithrombin type I and type II following a reduced level result from the functional test. To minimize potential errors, blood plasma should be used as the sample. It is recommended to avoid serum, clotted plasma specimens, or plasma that has been stored in a freezer. If transient acquired factors are suspected, the test should be repeated after at least four weeks to ensure accurate results. Including testing for Protein C and Protein S can provide better context for interpreting the results. For patients younger than six months, age-specific reference values must be taken into account. In these cases, it may be beneficial to test the parents as well (19,34,50,54,55). While molecular genetic testing can be informative in certain cases, it is not the primary method for diagnosing antithrombin deficiency in clinical practice. Due to the variety of mutations that can cause antithrombin deficiency in the SERPINC1 gene, and because many of these mutations lack a well-established association with clinical significance, genetic testing and the selection of test methods are challenging, costly, and not available in many laboratories (46).

Protein S deficiency

A deficiency of active, anticoagulant protein S (activity below 60%) leads to reduced fibrinolytic activity. Protein S is a vitamin K-dependent protein which, as a cofactor of activated protein C, accelerates the inactivation of coagulation factors Va and VIIIa. Normally, 60% of protein S is present in a complex with the C4b binding protein and only the free fraction of protein S is available to the activated protein C as a cofactor. The deficiency is associated with an increased tendency to venous thrombosis specifically before the age of 40. The prevalence in Europe is less than 0.5%. More than 200 different Mutations are grouped into the following three classifications (56–58):

Type I: Quantitative defect leading to a reduction in total and free protein S, including protein S activity.

Type II: Qualitative defect leading to a decrease in protein S activity at normal concentration level.

Type III: Quantitative defect leading to a decrease in free protein S and its activity. However, the total concentration is within the normal range (57).

The severity of the clinical phenotype and the age of onset are influenced by the type and localization of mutations in the PROS1 gene (Table 1). Typically, these are point mutations. Deletions of individual exons or of the entire gene only account for approx. 2-5% of all mutations (56). Since the hereditary form is inherited in an autosomal dominant manner with variable penetrance, similar to the other thrombophilia's previously mentioned, individuals who are heterozygous have a 5-10-fold increased risk of experiencing a first VTE episode (26). The homozygous form is very rare and often leads to perinatal purpura fulminans or massive thrombosis with lethal outcome (59). Usually, these patients have a protein S activity of less than 5%. It is important to differentiate the hereditary form from an acquired deficiency.

Diagnostic guidelines for protein S deficiency are designed to ensure accurate and reliable diagnosis, considering the complexity of the condition and the influence of various factors on protein S levels. The primary diagnostic tools are biochemical laboratory tests (34) (Table 2).

1. Total Protein S Antigen Test: Used to detect Type I deficiency by measuring the total amount of protein S in the blood.
2. Free Protein S Antigen Test: Mainly used to detect Type III deficiency by measuring the active, unbound form of protein S.
3. Protein S Functional Activity Test: Used to detect Type II deficiency by assessing the functional activity of protein S in inhibiting clot formation (58).

It is crucial to avoid temporary conditions that can lead to false negative or false positive results. Clinicians should avoid testing during an acute thrombosis, pregnancy or while using oral contraceptives and ensure anticoagulant therapy is discontinued for at least two weeks before testing. Given the variability of protein S levels, confirmatory testing is often indicated to verify the initial findings. Repeating the tests after an interval can help ensure consistent and accurate results. In certain cases, genetic testing for mutations in the PROS1 gene can be useful, especially in patients with a strong family history, unexplained or recurrent VTEs at a young age, in women with recurrent pregnancy loss or with ambiguous biochemical test results. Considering that the genetic basis of the deficiency is primarily attributed to point mutations, targeted methods like PCR or Sanger sequencing are suitable for detection. Since some mutations may result from deletions, MLPA is also a valuable method to consider. Due to the significant costs and lengthy turnaround times associated with genetic testing, it is not routinely recommended (34,60).

Protein C deficiency

Protein C is a vitamin K-dependent protein which, in its active form, leads to neutralization of factors Va and VIIIa. Consequently, it acts as a physiological anticoagulant in plasma. Reduced concentration or activity due to a genetic or acquired cause predisposes to thromboembolic events. The hereditary protein deficiency is inherited in an autosomal dominant manner and based on defective expression of *PROC* (2q13-q14) gene, controlling Protein C production (61). Typically, these mutations manifest as either quantitative (type I) or functional (type II) Protein C Deficiency (PCD). In type I PCD, there is a simultaneous reduction in both protein C activity and antigen concentration, while type II PCD is characterized by decreased protein C activity alongside normal protein C antigen values (62). The majority of protein C mutations lead to type I deficiency, with type II deficiency accounting for approximately 15% of symptomatic cases (63).

The PROC gene, located on chromosome 2q14.3, consists of 9 exons encoding for a signal peptide, a propeptide, and a mature protein with various functional domains (64) (Table 1). Over 500 mutations scattered across the gene have been identified, contributing to inherited PCD by disrupting protein synthesis and/or function. These mutations encompass missense mutations, nonsense mutations, splice site mutations, deletions and insertions. Missense mutations within the PROC coding region, particularly in the signal peptide and propeptide regions, are the most prevalent (63). The progression of the condition is contingent upon the specific genotype and the protein C activity level of each individual, ranging from asymptomatic to the development of VTE or even acute, life-threatening complications (63,65). Monoallelic Mutations (activity level: 30-70%) have a prevalence of 1: 200 – 1:500 (66). Although these patients can remain asymptomatic until adulthood, they still face a 5- to 7-fold higher risk of developing a VTE. Severe deficiency (activity level: 0 – 30%) resulting from homozygous inheritance is estimated to occur in approximately 1: 500.000 to 1:750.000 births (67). A homozygous deficiency with undetectable Protein C concentrations usually causes neonatal purpura fulminans. This severe form of disseminated intravascular coagulation (DIC) manifests itself with ecchymoses and extensive venous and arterial thromboses, usually on the first day of life (68). Both sexes are equally affected. It is important to differentiate the hereditary form from an acquired protein deficiency (69).

Two principles for diagnostic laboratory tests are employed for assessing PCD: antigen and activity assays (either clot-based or chromogenic) (Table 2). These tests are conducted on plasma samples. The measurement of PC activity in a coagulation test assesses the PC's capacity

to inactivate Va and VIIIa. Some assays necessitate the pre-dilution of patient plasma with PC-deficient plasma to reduce the risk of potential disruptions caused by concurrent anomalies such as protein S deficiency. In the first step, an aPTT reagent is added to the mixture, triggering the contact activation of factors XII and XI (the intrinsic coagulation pathway). This initiates the coagulation process. Next, a rapid PC activator is introduced. This reagent, derived from the venom of the Southern copperhead snake (Agkistrodon contortrix contortrix), is known as Protac (70,71). Activated PC subsequently cleaves and inactivates factors Va and VIIIa, resulting in a prolonged clotting time. The lower the functional PC content in the plasma, the weaker its anticoagulant effect, leading to a shorter extension of the clotting time (aPTT). This can be quantified by reading the time against a standard curve and expressing it as a percentage of the normal value (72,73). Chromogenic PC activity assays, on the other hand, assess APC's ability to cleave a synthetic chromogenic substrate. A chromogenic substrate is a specialized reagent designed to assess enzyme activity. It typically comprises an oligopeptide attached to a chromogenic dye, such as para-nitroaniline. The oligopeptide acts as an analog for the enzyme's natural target site. Upon enzymatic cleavage, the dye is released, causing a measurable color shift. This shift can be accurately quantified photometrically, allowing the enzyme activity to be calculated against a standard calibration curve. The release of paranitroaniline provides a direct correlation to the concentration of protein C in the plasma sample. The assay is also divided into two steps. First, the same PC activator (Protac) is added to the sample. Then, the chromogenic substrate is introduced, followed by photometric analysis (74,75). Although reference intervals for both assays generally range from about 70% to 140% in adults, specific ranges may vary depending on the assay utilized and should be determined by each laboratory (62,72). In the antigen assay, antibodies (either monoclonal or polyclonal) targeting Protein C (PC) are employed to measure PC levels in a sample using immunoassay techniques, such as enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay. Due to the nature of these tests, they do not provide information about the functional capacity of the PC molecules, rendering them unsuitable as screening tests. Consequently, guidelines recommend conducting a functional test as the primary approach. Both types of functional tests exhibit high sensitivity. Due to the limitations associated with the coagulation assay, including its slightly lower specificity (93%) compared to the chromogenic assay (97%), the chromogenic assay is recommended as the preferred initial test (74). If this test is positive, the antigen test can subsequently distinguish between Type I and Type II PCD. Furthermore, PCD testing is generally not recommended during a thromboembolic event, trauma, or major surgery. In such cases, it is advised to wait at least three months before performing tests (72,76). Routine genetic

mutation analysis is not practically used, because the condition can be caused by a wide variety of different mutations.

Guidelines for genetic testing of inherited thrombophilia

Thrombophilia genetic testing should be conducted only when the results can be utilized to enhance or adjust patient management. Testing is recommended for assisting in secondary prevention, such as determining the duration of anticoagulation after a thrombotic event, and for hereditary conditions to support primary prevention in relatives of affected individuals. The guidance statements are established by carefully weighing the costs and benefits of the results for the patient (2,3,77). These guidelines are often provided by professional organizations and updated periodically based on new research and clinical evidence. As of now, the following organizations provide key guidelines that are widely recognized:

- British Society for Haematology (BSH)
- American Society of Hematology (ASH)
- European Society of Cardiology (ESC)
- Current guidelines from the American College of Chest Physicians (ACCP)

Guidelines for genetic testing include following clinical indications:

1) Unprovoked VTE
2) Provoked VTE
3) VTE at unusual site
4) VTE in Neonates
5) Relatives of VTE patient
6) Female relatives of VTE patient considering Combined Oral Contraceptives (COC) or Hormonal replacement therapy (HRT)
7) Female relatives of VTE patient planning pregnancy

Unprovoked VTE

Testing for heritable thrombophilia traits after a first episode of unprovoked VTE is not routinely recommended to guide treatment duration. Current guidelines from ACCP, BSH and ASH all recommend continuing indefinite anticoagulation after an unprovoked VTE. Since a negative thrombophilia result is not sufficient to justify stopping anticoagulation therapy,

testing is generally unnecessary. Careful decisions should be made on an individual basis, if the patient has a high risk profile for bleeding complications (2,3,77).

Provoked VTE

Testing for heritable thrombophilia traits after a first episode of provoked VTE is not routinely recommended to guide treatment duration. The BSH suggests an individual clinical decision regarding the continuation of anticoagulation therapy after completing primary treatment, while the ASH distinguishes between different provoking factors: if the VTE is provoked by a major non-surgical risk factor, occurs during pregnancy or postpartum, or is associated with COC, testing can be indicated (3,77).

VTE at unusual site

Testing for heritable thrombophilia traits after a first thrombotic episode at unusual site is not routinely recommended to guide treatment duration (3,77). Unusual sites for thrombosis include cerebral veins and sinuses, abdominal veins (portal vein, mesenteric vein), renal veins, upper extremity veins, ovarian veins, retinal veins, and splenic veins (78). ASH guidelines advocate for testing in young patients with VTE at unusual site, when it is considered unprovoked and when standard clinical guidelines recommend discontinuation of anticoagulant treatment. In the last case, a positive thrombophilia would indicate the need for the continuation of treatment. Nonetheless, if standard clinical guidelines already recommend extending anticoagulation, no testing is performed as it does not influence treatment decisions (77,79). The BSH further emphasizes the importance of testing for acquired genetic traits such as myeloproliferative neoplasm (MPN) and paroxysmal nocturnal haemoglobinuria (PNH) and for non-genetic acquired conditions like antiphospholipid syndrome (3).

VTE in Neonates

Testing for heritable thrombophilia traits after VTE in neonates is not routinely recommended to guide treatment choice and duration (3), except in scenarios characterized by unexplained multiple thrombotic events or purpura fulminans (80).

Relatives of VTE patient

Testing for heritable thrombophilia traits in asymptomatic first-degree relatives of a patient with history of VTE is not routinely recommended to determine the need of thromboprophylaxis. The goal of selecting which relatives should be tested depends on whether the test result would influence the recommendation for thromboprophylaxis, especially if these relatives are exposed to a minor provoking factor, such as planned surgery or immobilization. The guidelines do not recommend testing in a family history of heterozygous FVL or Prothrombin G20210A mutation but indicate selective testing in a family history of protein S deficiency (PSD), protein C deficiency (PCD), or antithrombin III deficiency (ATIID). Additionally, a hereditary thrombophilia testing panel is recommended when multiple family members, particularly at a young age, have developed VTE and their thrombophilia status is unclear (3,77).

Female relatives of VTE patient considering COC or HRT

Testing for heritable thrombophilia traits in asymptomatic female relatives of a patient with history of VTE, who consider the use of COC, or HRT is not routinely recommended to guide these treatment decisions. The goal of selecting which females should be tested depends on whether the test result could influence the treatment decisions regarding COC or HRT. (3,77) The guidelines strictly reject a general female population testing before the initiation of COC or HRT (3,77). Furthermore, there is no indication for pursuing a comprehensive thrombophilia panel test or selective testing, if the family history is determined to be FVL heterozygotic or PGM (low risk thrombophilia). Selective testing is suggested in women with a positive family history of VTE and known PCD, PSD or ATIIID, because in these cases a positive test result would lead to the recommendation to avoid COC or HRT treatment (Table 3) (77).

Table 3: The reduction in the number of VTE events per 1000 women per year linked to the avoidance of COC or HRT (estrogen-only or combined) in cases where a first-degree relative possesses one of the five known thrombophilia (77).

First degree relative	Avoid COC	Avoid Estrogen only HRT	Avoid combined HRT
FVL (heterozygotic)	↓4,57	↓2,2	↓5,92
PGM (heterozygotic)	↓4,38	↓1,36	↓3,64
PCD	↓13,84	↓4,92	↓13,28
PSD	↓10,49	↓3,92	↓10,53
ATIIID	↓19,39	↓6,45	↓17,35

Female relatives of VTE patient planning pregnancy

Testing for heritable thrombophilia traits in asymptomatic female relatives of a patient with history of VTE is not routinely recommended to determine the need of thromboprophylaxis during pregnancy and postpartum. Genetic testing should be conducted prior to pregnancy and only if it will impact management decisions. BSH guidelines recommend thromboprophylaxis in patients with previous VTE, homozygous FVL, homozygous PGM, ATIII deficiency, PSD, and PCD and recommend consequently testing if one of these traits is known in the family. In contrast, ASH guidelines highlight that the benefit of thromboprophylaxis and testing in patients with PSD or PCD is unclear due to low statistical certainty in the evidence of the effect. Both guidelines concur that if a patient has already experienced a VTE, further genetic testing is unnecessary, as the thrombophilia status does not influence the prophylactic approach (3,77).

Discussion

Genetic tests for thrombophilia are controversial, but frequently performed. It is crucial for clinicians to recognize that the implications of genetic testing may differ from other laboratory tests. Genetic testing requires highly skilled personnel, specialized laboratories, involves high costs and reveals information that is unchangeable and has implications for the individual and their family. There is greater potential for psychological impact, concerns about confidentiality and genetic discrimination. The restrictive criteria for testing, as previously discussed through the guidelines, are based on the current scientific research, but are limited due to incomplete data, studies with small sample sizes, short observation periods or the inability to demonstrate a significant benefit from genetic testing in many clinical scenarios. More research, in particular large studies comparing the impact of management strategies with or without thrombophilia testing, is urgently needed. As modern medicine favours the personalized approach, where treatment strategies are tailored to the molecular characteristics of both the individual patient and their disease, molecular technologies, especially genetic sequencing are becoming increasingly important. This has influenced the development of new guidelines and diagnostic algorithms. Since personalized medicine approaches often limit the indications for pharmacological treatments and are therefore not in the pharmaceutical industry's best interest, it is unlikely that drug manufacturers will sponsor or support research projects with this focus. For that reason, we need organizations such as the BSH or the ASH to enable independent researchers access to public funds, ensuring that these important and necessary projects have a chance to be financed. However, due to the limited resources of the healthcare system, clear guidelines specifying the selection of patients are absolutely necessary (3,77,81,82).

Studies have shown that the absolute risk of recurrence is significantly higher in patients with unprovoked VTE than in patients with provoked VTE. The 5-year recurrence risk can increase to 30% if anticoagulant therapy is discontinued after initial treatment (83,84). Consequently, genetic testing offers no benefit as treatment is continued regardless of the test results. One scenario in which testing might be considered is if the patient's adherence to therapy decreases and the patient is considering discontinuing therapy. If a positive test result could potentially persuade the patient to continue therapy, then conducting the test might be beneficial (2). After a provoked VTE, treatment decisions should be based on an individualized risk assessment that includes acquired factors, laboratory results (such as D-Dimer levels), clinical condition, age, BMI, lifestyle, and other medications. The emphasis is on considering these factors rather than relying on genetic testing (84–86). In a comprehensive case-control study and a separate

prospective study, no significant difference in the recurrence rate was found between individuals with and without hereditary thrombophilia. It is therefore recommended that all patients receive time-limited anticoagulation therapy, regardless of whether they have thrombophilia or not (27,87,88).

ASH distinguishes between different provoking factors. If surgery is the provoking factor, it is recommended to discontinue anticoagulant therapy after completion of primary treatment in all patients, as there is an increased risk of bleeding. They cite an observational meta-study showing that indefinite anticoagulant treatment following thrombophilia testing leads to an increase of 2-7 major bleeds per 1000 patients per year. However, if the VTE is provoked by a major non-surgical risk factor, occurs during pregnancy or postpartum, or is associated with combined oral contraceptives (COC), testing may be indicated. A positive test result would lead to a recommendation to continue anticoagulation therapy. This recommendation is supported by the high recurrence risk of VTE, which is 50 per 1000 patients in the first year after the initial event and is reduced by a factor 21 with indefinite anticoagulation in patients with thrombophilia. On the other hand, the risk of severe bleeding is only 5-15 per 1000 patients. The potential benefit of testing and the consequent therapy adjustment is significant enough to justify testing (2,77).

The definitive value of thrombophilia testing after a first thrombotic episode at unusual site remains unknown. However, if the only indication is the occurrence of thrombosis at unusual sites, testing is not recommended. Since in 85% of cases, a clear identifiable provoking factor exists, clinicians should consider testing in cases classified as unprovoked. Additionally, the occurrence of thrombosis in young patients also warrants consideration for testing (3,54).

Neonatal VTE is multifactorial, with clinical risk factors, particularly central venous access devices (CVADs), which have been identified as a risk factor in 90% of neonatal VTE and 50% of pediatric VTE cases, playing a more important role in the choice of treatment than hereditary thrombophilias (3,89–91). This assertion is supported by empirical evidence suggesting that VTE incidence is the same regardless of thrombophilia status (92). In contrast, another study highlighted a significant predisposition to VTE in neonates with thrombophilic traits. Data from the Italian registry showed that 33% of neonates who developed VTE within the first day of life had an identifiable thrombophilic trait (80). Despite this finding, testing is not justified, since the presence of a genetic factor has no influence on the type or duration of treatment. Except in cases of purpura fulminans, an urgent test for Protein C and Protein S deficiency is recommended, due to the association with severe (homozygous) deficiency of these proteins.

This information is invaluable as specific replacement treatments are available (PCD = PC concentrate, PSD = fresh frozen plasma) (93).

A family history of thrombosis is associated with an increased risk of VTE, even in the absence of an identifiable thrombophilia. Therefore, a negative thrombophilia screening result in an asymptomatic relative does not equate to a normal risk of VTE. Research indicates that individuals with a confirmed thrombophilia are twice as likely to engage in preventive measures during high-risk situations compared to those who test negative. Although non-carriers are aware of their increased risk, they often neglect preventive measures. In this context, comprehensive family screening for thrombophilia could be beneficial to identify carriers. However, it may be counterproductive for non-carriers, as those with negative results may develop a false sense of security, leading to a reduced emphasis on preventive measures and consequently an increased risk of VTE. This highlights the pivotal role of genetic counsellors, particularly for asymptomatic family members. Genetic counsellors should explain high-risk scenarios and provide guidance on behavioural and lifestyle modifications to effectively reduce these risks (2).

Female relatives of a VTE patient who are considering COC or HRT and are identified as thrombophilia-positive after testing experience minimal reduction in thrombotic events, which is considered disproportionate in terms of the cost and psychosocial impact of being labelled. Nonetheless, if there is a family history of low-risk thrombophilia, caution should still be exercised when prescribing COC and HRT, as the risk of thrombosis has been shown to be doubled with a positive family history, regardless of the individual's thrombophilia status (94). In these cases, genetic counselling is crucial as it provides valuable insight into individual risks and explores alternative prophylactic therapies, such as the utilization of the progestin only pill (95).

Pregnancy represents a significant acquired hypercoagulable state characterized by a 5-15 fold increased risk of VTE, which escalates to a 20-80 fold increase during the postpartum period (96–98). As a result, VTE accounts for one of the most common causes of mortality among pregnant women and women in the postpartum phase (99). Nevertheless, it is also crucial to weigh the risk of major bleeding, another major cause of mortality, against the risk of VTE in these women. Genetic testing should be performed prior to pregnancy and only if it will impact management decisions (3,77).

To summarize, it is important to carefully weigh the pros and cons of genetic testing for each individual patient. The main concern is the risk of either withholding anticoagulants from high-

risk patients, potentially increasing their likelihood of experiencing a thrombotic event, or administering anticoagulants to low-risk patients, thereby increasing their risk of bleeding. Other drawbacks include significant costs, inaccurate results due to improper timing of testing, misunderstanding of results by patients or healthcare providers, emotional distress and anxiety, and the possibility of genetic discrimination. On the other hand, potential benefits include patient satisfaction derived from identifying biological risk factors associated with thrombotic events, improved adherence to thromboprophylaxis among high-risk family members and prevention of further thromboembolic events (100).

Conclusion

Inherited thrombophilia increases the risk of a first venous thromboembolism (VTE) episode, particularly at a younger age. Genetic variations can lead to conditions such as Factor V Leiden (FVL), Prothrombin Gene Mutation (PGM), Protein S Deficiency (PSD), Protein C Deficiency (PCD), or Antithrombin III Deficiency (ATIIID). Each of these conditions has different prevalences and risk profiles, which are also influenced by whether an individual has a heterozygous or homozygous gene set. However, it is crucial for patients to understand that their risk of thrombosis is determined by a complex mix of genetic and acquired factors. Counseling these patients and their asymptomatic family members is pivotal in understanding the implications of genetic testing and how the results may or may not affect treatment or prophylaxis decisions. Adequate education of these patients is essential to avoid risk factors (such as combined oral contraceptives, hormone replacement therapy and long-distance travel) and to recognize the impact of lifestyle choices (such as obesity, immobilization, smoking and pregnancy). This knowledge enables them to make informed decisions based on their individual risk. It is also important to avoid overestimating the risk of thrombosis, as many asymptomatic individuals misjudge their likelihood of developing a VTE. (101) This misjudgement can have a significant impact on their mental health and lead to unnecessary worry and requests for testing or prophylactic measures. The aim is to provide accurate, personalized risk assessment to identify patients who would benefit significantly from testing and to reduce medically unnecessary thrombophilia testing (1,100).

Summary

Inherited thrombophilia refers to a predisposition to developing thrombosis due to genetic factors. Genetic testing is a valuable tool to identify at-risk individuals and support clinical decisions and prevention strategies. Several important genetic mutations are commonly associated and result in various types of thrombophilia. The type of mutation determines the approach to molecular genetic testing. Commonly used targeted methods include PCR, PCR modifications and Sanger sequencing.In FVL, the most common hereditary thrombophilia, there is a mutation in the F5 gene that causes resistance against APC and significantly increases the procoagulant state. In Prothrombin G20210A, the mutated F2 gene leads to increased prothrombin levels, a precursor protein in the coagulation cascade. Although Protein C, Protein S and Antithrombin III deficiencies are less common, they are nevertheless significant as they impair the natural anticoagulant pathways potentially leading to severe clinical conditions, especially in homozygotes. The indications for genetic testing depend on the scenario and are critical for personalized, proactive patient care to improve clinical outcomes. However, the benefits of testing must be weighed against limitations such as psychological impact, cost, ethical considerations and privacy concerns. Testing should be conducted within the framework of scientific guidelines and comprehensive genetic counselling to ensure informed decision making and appropriate use of genetic information and testing capacity.

Curriculum Vitae

Teresa Laura Risch was born on the 16th of June 1997 in Rüsselsheim, Germany. In 2015, she completed her secondary education with a general university entrance qualification (Abitur) of 1.7 at Dreieichgymnasium in Langen. Following this, from 2015 to 2018, she studied Biowissenschaften at Goethe University in Frankfurt am Main. In October 2018, she enrolled into medical studies at the University of Rijeka, with an expected graduation in July 2024. Teresa's clinical experience began during her biology studies with a nursing internship, followed by employment as a nursing assistant in the cardiac surgery department at the Kerckhoff Clinic in Bad Nauheim. She continued working there as a student employee during the first two years of her medical studies, dedicating her free time to gaining further clinical experience. Throughout the summers, Teresa undertook various voluntary clinical internships to broaden her medical knowledge and expand her clinical skills. In 2020, she interned in the anaesthesiology department at the Kerckhoff Clinic in Bad Nauheim. In 2021, she worked in the gynaecology department at the Elisabethen Hospital in Frankfurt am Main. In 2022, she gained experience in the interdisciplinary emergency department at the Sana Clinic in Offenbach. In the summer of 2023, Teresa took on a voluntary clinical research project in the anaesthesiology department at the Kerckhoff Clinic, in collaboration with the University of Gießen. This project, which she is undertaking alongside her studies, aims to result in a Doctor of Medicine (Dr. med) degree, expected to be completed by early 2025. As part of her studies, Teresa participated in two clinical exchange projects. During her fourth year, she completed an 8-week internal medicine block at the Red Cross Clinic in Lindenberg, Germany. In her fifth year, she engaged in a 4-week surgery block in the visceral surgery department at the Hospital in Lüdenscheid, Germany. After obtaining her medical degree in July 2024, Teresa plans to contribute to a medical volunteer project in Uganda. After this enriching experience, she will return to focus on completing her doctoral research project. Teresa Laura Risch has consistently pursued both academic and practical experiences throughout her education and clinical training, demonstrating a strong commitment to the medical field. Her proactive approach and diverse experiences make her a promising future medical professional.

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