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Prothrombotic Single Nucleotide Polymorphisms and Risk of Myocardial Infarction: A Narrative Review of the Literature
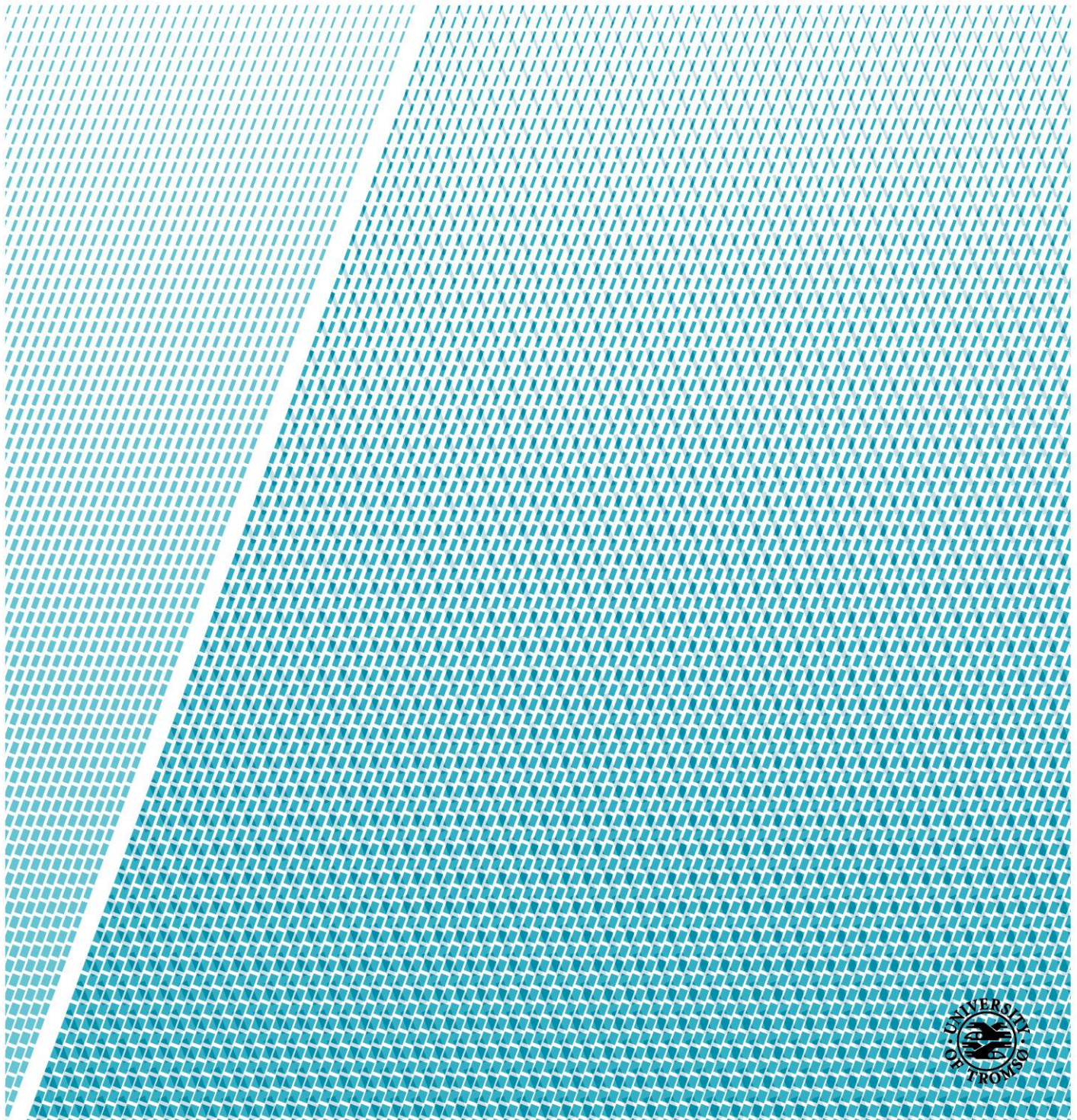
Master thesis, MED-3950

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Preface

The aim of this master thesis is to create an overview of the available studies regarding the association between a set of prothrombotic single nucleotide polymorphisms and risk of myocardial infarction.

My interest in clinical epidemiology and risk factors for cardiovascular diseases started in 2016, when I was accepted to the integrated research program at the medical studies in Tromsø. I had a full year of research in 2017/18 as a part of K.G Jebsen – Thrombosis Research and Expertise Center (TREC). So far, my work has led to two publications as a first author; "*Myocardial Infarction as a Transient Risk Factor for Incident Venous Thromboembolism: Results from a Population-Based Case-Crossover Study*" and "*Myocardial infarction, prothrombotic genotypes and venous thrombosis risk: The Tromsø Study*".

The writing of this thesis has given me the opportunity to learn more about the field of genetics in thrombosis. I am certain this work has given me valuable knowledge and skills that I will take advantage of in my future scientific and clinical work. The work of this thesis started during the autumn semester 2018 and was completed from March to September 2020, in the time designated to the master thesis.

I would like to thank my supervisor, Professor Sigrid Kufaas Brækkan, for good help and constructive feedback. In addition to introducing me to the research jungle and to the field of cardiovascular diseases, she always helps me with methodological considerations in epidemiology, scientific writing, and proofreading of manuscripts.

Finally, I am really grateful and privileged to be part of the TREC-team.

Joakim Knutsen Sejrup

24.08.20

Contents

Summary

Background: Environmental risk factors for myocardial infarction (MI) have been extensively investigated. In addition, family history of MI is an important risk factor for the disease. Several prothrombotic genotypes are well-established risk factors for venous thrombosis. However, the role of prothrombotic genotypes as risk factors for arterial thrombosis is less emphasized.

Aim: To create a general overview of the existing literature on the role of non-O/O blood type, Prothrombin G20210A and Factor V Leiden; the main genetic determinants of thrombophilia, as risk factors for incident MI.

Methods: A narrative literature review of studies published after 2005 was performed. The biomedical databases Medline and Embase were used in a structured literature search regarding the association between non-O/O blood type, Prothrombin G20210A and Factor V Leiden as risk factors for MI. Relevant MeSH terms for exposure and outcome were combined in the Ovid search platform; a common search software for the two databases.

Results: Several meta-analyses have been published during the recent years and report modest effects of non-O blood type (ORs ranging from 1.1 to 1.3), Prothrombin G20210A (ORs around 1.4) and Factor V Leiden (ORs ranging from 1.2 to 1.9) on the risk of MI. For Prothrombin G20210A, the adjusted OR for ST-elevation myocardial infarction (STEMI) for carriers versus non-carriers of the risk allele was 2.2 (95% CI: 1.1-4.3) among subjects <35 years. Moreover, when compared to wild genotype carriers, Factor V Leiden heterozygous or homozygous mutant carriers were more likely associated with a trend towards more severe coronary artery disease (CAD) (OR 1.85; 95% CI 1.26-2.72 and OR 3.70; 95% CI 1.71-8.00).

Conclusion: In conclusion, the existing literature supports an association between non-O blood type, Prothrombin G20210A and Factor V Leiden and risk of MI. The Prothrombin G20210A and Factor V Leiden variants seem to be associated with premature adverse events and more severe CAD.

Abbreviations

CVD – Cardiovascular disease

CHD – Coronary heart disease

CAD – Coronary artery disease

IHD – Ischemic heart disease

STEMI – ST-elevation myocardial infarction

MI – Myocardial infarction

IS – Ischemic stroke

SNP – Single nucleotide polymorphism

vWF – von Willebrand factor

MMP – Matrix metalloproteinase

ADAMTS13 – a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13

LDL – Low-density lipoprotein

HDL – High-density lipoprotein

BMI – Body mass index

PAI-1 – Plasminogen activator inhibitor-1

GPIIIa / GPIa/IIa – Glycoprotein IIIa / Glycoprotein Ia/IIa

CI – Confidence interval

IR – Incidence rate

HR – Hazard ratio

OR – Odds ratio

WHO – World Health Organization

Introduction

The term cardiovascular disease (CVD) constitutes all conditions affecting the heart and/or blood vessels (1). Arterial CVD is a more narrow term that leaves out diseases related to the venous system, heart valves and the hearts electrical conduction system. Coronary heart disease (CHD), or coronary arterial disease (CAD), comprise diseases affecting the coronary vessels only, and the most common CHD is **ischemic heart disease** (IHD), with a spectrum of clinical manifestations ranging from stable angina pectoris to fulminant ST-elevation myocardial infarction (STEMI). In pathophysiological terms, this spectrum is described by a gradual narrowing of the coronary vessels due to atherosclerosis and atherothrombosis, ultimately leading to necrosis of the cardiomyocytes when the balance between oxygen supply and demand becomes disrupted (myocardial ischemia) (2).

Environmental risk factors for myocardial infarction (MI) have been extensively investigated, where hypertension, obesity, diabetes mellitus and dyslipidemia have demonstrated to be important contributors to disease risk (3, 4). Additionally, several lines of evidence have shown that CHD is heritable, and family history of MI has been reported to be an important risk factor (5). Several prothrombotic genotypes are well-known and strong risk factors for venous thrombosis (6), but their role as risk factors for arterial CVD has received less focus.

Atherosclerosis

The term **atherosclerosis** is derived from the ancient greek **athera**, meaning gruel, and **sclerosis**, meaning hardening, and is a hardening of an artery specifically due to an atheromatous plaque (7). The process of atherosclerosis (atherogenesis) starts early in life in most individuals and progresses at different rates depending on both environmental and genetic risk factors (8, 9). Predilection sites for atherosclerotic plaques are bifurcations (e.g. the carotid bifurcation) or at ostia of exiting vessels (e.g. the coronary arteries) (10, 11). The prevalence of carotid atherosclerosis increased across 6-year intervals, from 14.1% among 42 year olds, to 32.0% in 48 year olds, 67.7% in 54 year olds and ultimately 81.9% among 60 year olds (12).

The understanding of atherosclerosis has improved considerably during the past decades (2, 7, 13). Previously considered a cholesterol storage disease, we now view atherosclerosis as an inflammatory disease (14, 15) encompassing a complex interplay between arterial vessel wall components (**the solid state**) and circulating blood components (**the fluid phase**) (Illustration 1). Normal haemostasis is maintained by a delicate equilibrium between prothrombotic and

antithrombotic processes in the endothelium and fluid phase. However, the endothelial lining of the blood vessel wall is in contact with blood components that may alter the expression of proinflammatory and prothrombotic molecules (16). Activation of a defensive response in the endothelium is common for all risk factors included in atherogenesis. For instance, proinflammatory cytokines from excess adipose tissue and vasoconstrictor hormones associated with hypertension (e.g. angiotensin II), induce expression of adhesion molecules that promote leukocyte adhesion and transmigration (17). In addition, variants of the galectin-2-binding protein gene which encode monocyte-binding proteins on the surface of smooth muscle cells in the atherosclerotic lesion have been shown to be associated with MI (18), and knockdown of the gene in experimental studies suppressed activation of NFkB, which is a central mediator of inflammation.
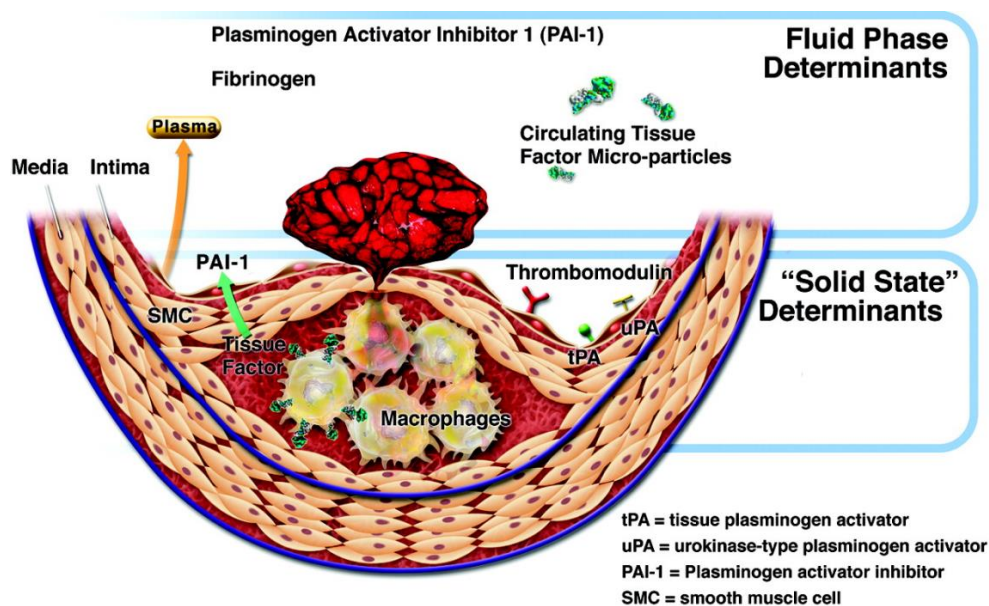
Illustration 1. Determinants of thrombosis in coronary atherosclerotic plaques (17). Gradual narrowing of the coronary vessel ultimately leads to necrosis of the cardiomyocytes when balance between oxygen supply and demand becomes disrupted.

The plaque usually grows in the intima zone of the coronary vessels and initially comprises triacylglycerols, fatty acids and oxidized LDL-particles (19). Oxidized LDL is an important chemoattractant molecule that induces the expression of so-called VCAMs (Vascular cell adhesion molecules) on the endothelial surface (16). Monocytes and lymphocytes adhere to the VCAM-1-molecules and transmigrate to the subendothelial space. Macrophages devour oxidized LDL-particles by means of scavenger receptors and form foam cells (14). At this point in the atherosclerotic development, the lesion is called a **fatty streak**. These lesions are

clinically silent. Increase in reactive oxygen species also inactivates nitric oxide (endothelium-derived relaxing factor), an inherently important vasodilator (20). Reduced vasodilation increases shear stress against the vessel wall, further damaging the endothelium. The next step in the development is migration of smooth muscle cells and fibroblasts to the intima zone and deposition of extracellular matrix including collagen and neutrophil extracellular traps (NETs) in the lesion (21) (Illustration 2).
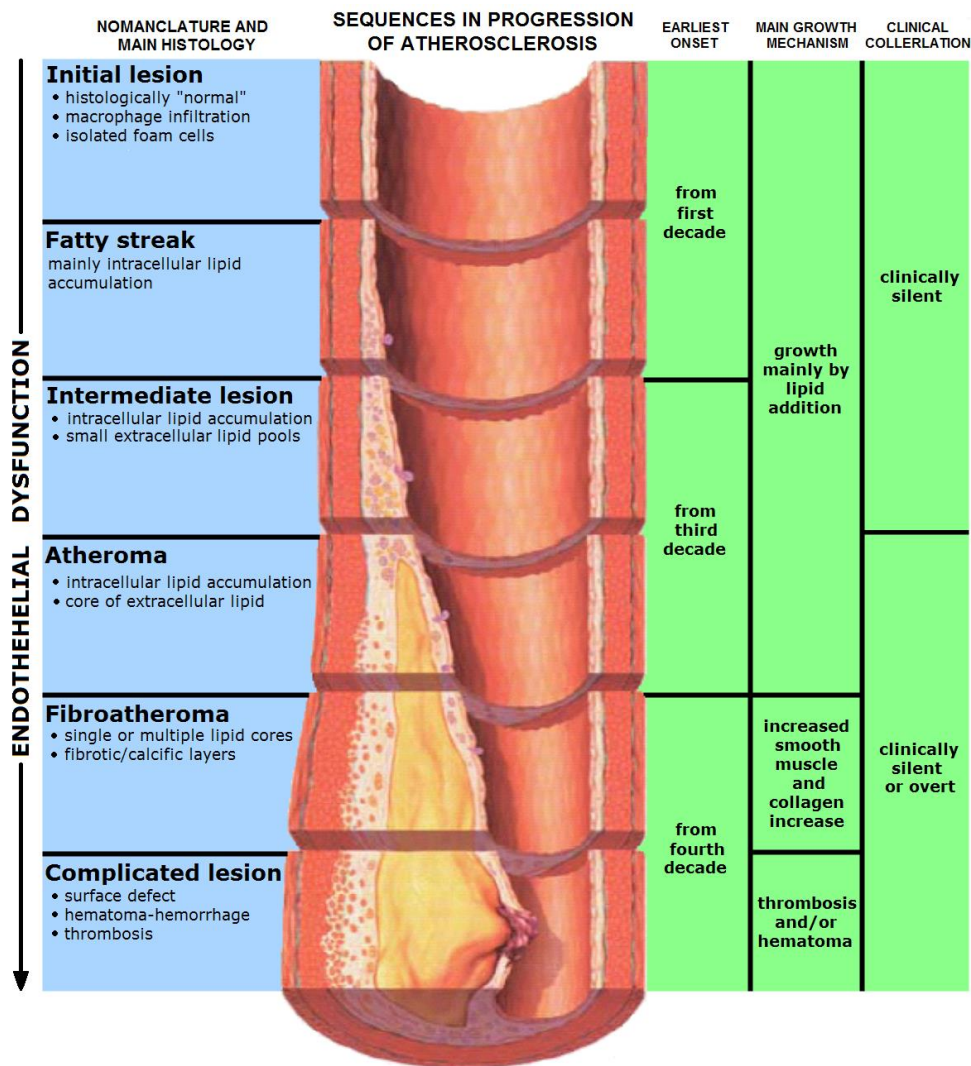

Illustration 2. Atherosclerotic plaque development. From a fatty streak, a harmless lesion usually seen at young age, the vulnerable and potentially fatal atherosclerotic plaque normally takes decades to develop. (https://upload.wikimedia.org/wikipedia/commons/9/9a/Endo_dysfunction_Athero.PNG)

Myocardial infarction - atherothrombosis

The pathophysiological mechanism leading to an MI is in the majority of cases related to the rupture of atherosclerotic plaques (type I MI) with subsequent exposure of collagen, von Willebrand factor (vWF) and tissue factor to circulating platelets, coagulation factors, immune cells, and other blood components (22, 23). The macrophages inside the plaque produce enzymes (MMPs or matrix metalloproteinase) that degenerate the fibrous cap responsible for the tensile strength of the plaque, predisposing it to rupture (24). When the plaque ruptures, platelets adhere through glycoreceptor-mediated binding, aggregate, activate, and release secondary aggregators like thromboxane A_2, ADP and serotonin, which results in a rapidly growing thrombus (primary hemostasis).

The coagulation cascade, which is triggered by the presence of tissue factor expressed by macrophages and smooth muscle cells, generates fibrin (secondary hemostasis) (Illustration 3). Primary and secondary hemostatic pathways reinforce each other, as thrombin generation amplifies activation of platelets and other cells in the lesion (17). Further, these processes ultimately produces a thrombus that reduces blood flow to the downstream myocardial muscle (Illustration 4). The impaired balance between O_2 supply- and demand leads to myocardial damage and ultimately necrosis. The arterial clots are platelet rich, and are often referred to as **white clots** (25).
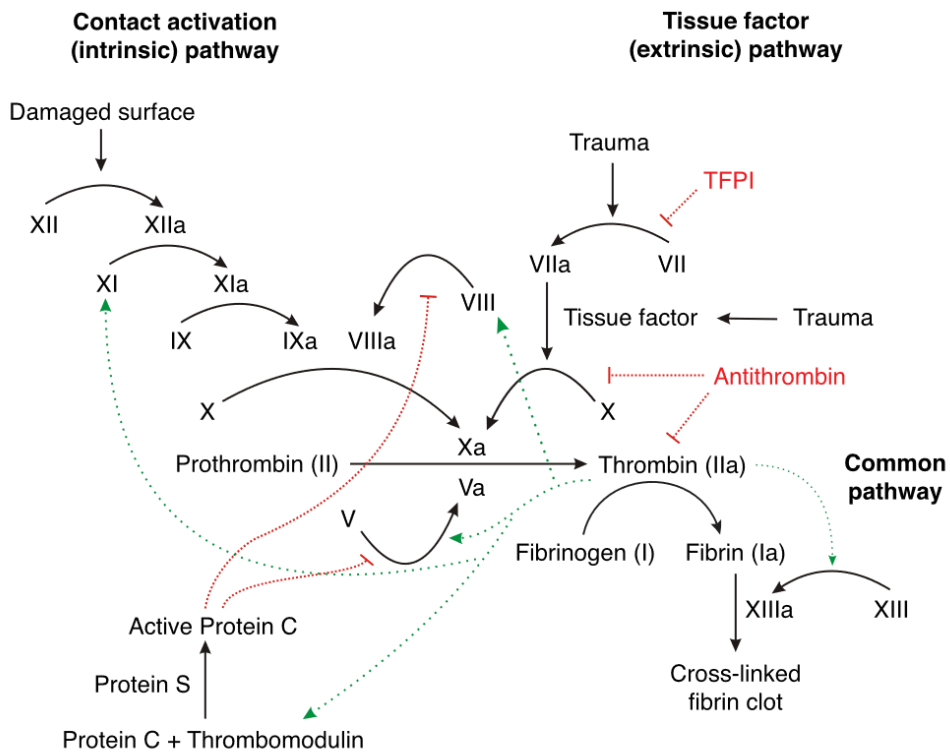
Illustration 3. The coagulation cascade shows an intricate balance between pro- and anti-coagulant mechanisms. Green arrows indicate activation and red arrows indicate inhibition. End product: fibrin (clot) (https://upload.wikimedia.org/wikipedia/commons/b/b6/Coagulation_full.svg)
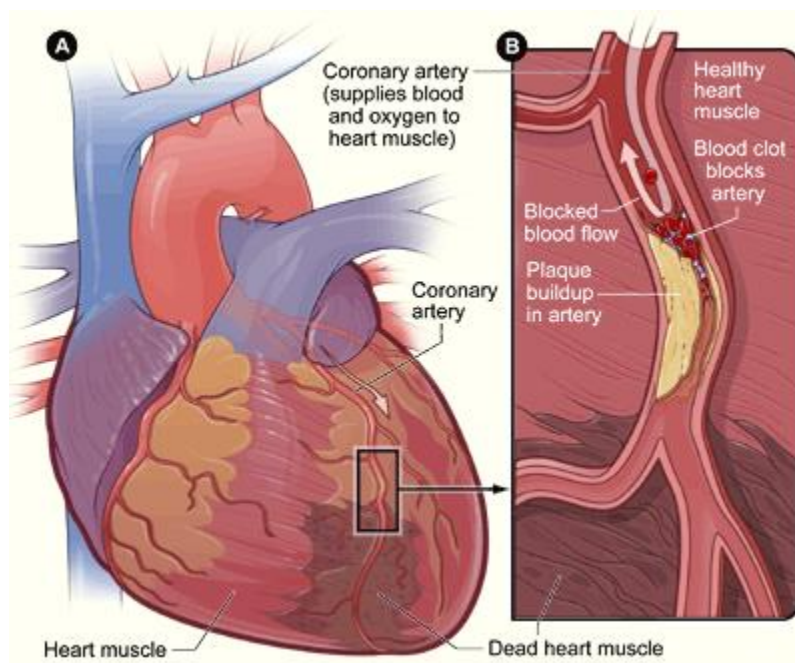

Illustration 4. The left ascending artery occluded by a thrombus on top of a ruptured atherosclerotic plaque (https://upload.wikimedia.org/wikipedia/commons/0/03/Heart_attack-NIH.gif)

Epidemiology

The prevalence of CHD is increasing worldwide and regional differences exist (1). MI is the most prevalent CHD with a prevalence of 2.8% (4.0% for men and 1.8% for women) in US adults ≥20 years of age (26). The incidence of CHD in the Western world is decreasing (27, 28) despite an aging population and increase in the prevalence of cardiometabolic risk factors such as diabetes mellitus, obesity and hypertension (29). Data from the Worcester Heart Attack Study have reported incidence rates for acute MI of 277 and 209 pr. 100,000 PYs in 1975 and 2005, respectively (28). A report from the Tromsø study showed an age- and sex adjusted incidence decline of first ever MI of 3% each year from 1994 to 2010 (30). The CVD mortality has decreased with approximately 30% in the European Union countries from 1985-89 (139/100,000) to 2000-04 (93/100,000) for both sexes (31). Nevertheless, CHD is the leading cause of morbidity and mortality globally. CVD is responsible for about 51% of deaths in women and 42% of deaths in men, with CHD contributing to about half of these deaths (32).

Pathophysiological basis of thrombosis

In 1862, Rudolph Virchow presented a triad of pathophysiological alterations in thrombosis, consisting of changes in the composition of blood (hypercoagulability), blood flow (stasis) and changes in the endothelium lining the innermost layer of the vessel wall (endothelial dysfunction) (33) (Illustration 4). The risk factors presented below all contribute to the thrombosis process through one or more of the components of Virchow's triad.
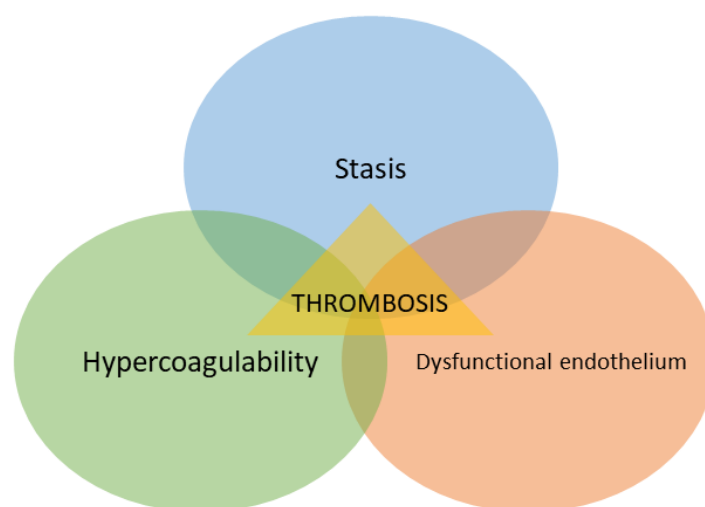
Illustration 5. Virchow's triad. A triad of pathophysiological alterations in thrombosis, consisting of stasis, hypercoagulability and endothelial dysfunction

Risk factors

The Framingham study was initiated in 1947 and became the first prospective cohort study investigating risk factors for CHD (3). The first conclusion from the study was that **hypertension** increases the risk of CHD, and that systolic blood pressure is superior to diastolic blood pressure in predicting CVD. Additionally, the Framingham investigators found that **diabetes mellitus** increases CVD mortality and that HDL cholesterol is associated with lower CHD rate (3). From a large modern, multinational case-control study comprising 15,152 cases and 14,820 age- and sex matched controls it was reported that abnormal lipids (**dyslipidemia**), **smoking**, hypertension, diabetes, abdominal **obesity**, psychosocial factors, consumption of fruits, vegetables and alcohol, and regular physical activity account for most of the risk of MI worldwide (4). Moreover, **family history** of MI is an independent risk factor for MI and the risk is particularly pronounced if the relatives are of first-degree or young (5, 34, 35). A family history of CHD also increases the risk of coronary artery calcification (36) and cardiovascular mortality (37, 38).

In addition, results from the Framingham study illustrate that blood type status may contribute to the risk of arterial CVD (39) as **non-O blood type** conferred increased risk of intermittent claudication or peripheral arterial disease.

Thrombophilia

Thrombophilia is the term used to describe a tendency to increased blood clotting, and can be either inherited or acquired (40, 41). Single nucleotide polymorphisms (SNPs) are variants of certain DNA segments appearing in the population at different frequencies (42), and several SNPs associated with increased blood clotting (prothrombotic genotypes) have been identified (40, 43, 44). The prothrombotic genotypes enhance thrombosis tendency as fluid phase determinants by contributing to thrombus formation through the hypercoagulability component of Virchow's triad through one of the two mechanisms: **Gain-of-function** or **loss-of-function** mutations.

Gain-of-function is caused by mutations in genes that code for natural procoagulant proteins, which lead to an up-regulation of the *concentration* (e.g. non-O blood type and Prothrombin G20210A) or *activity* of a normal protein, or *impaired down-regulation* of a normal protein (e.g. Factor V Leiden). Loss-of-function or inactivation mutations result in the gene product having less or no function (e.g. antithrombin deficiency).

Prothrombotic genotypes

The **ABO locus** has three allelic forms- A, B and O, which give rise to six possible genotypes and four possible phenotypes (Illustration 6) (45). Blood group O is the most common phenotype in most populations with a frequency of approximately 40% among Caucasians. Further, the rs8176719 in *ABO* is the most frequent prothrombotic genotype and differentiates non-O from O blood types. The variant encodes either A or B (heterozygous) or A, B or AB (homozygous). Non-O blood type is present in 60 to 70% of the population (46) and is correlated with higher levels of circulating factor VIII and vWF in plasma (45, 47). Non-O blood type is associated with a 2.1-fold increased risk of venous thrombosis (46).

Blood Group	Antigen(s) present on the red blood cells	Antibodies present in the serum	Genotype(s)
A	A antigen	Anti-B	AA or AO
B	B antigen	Anti-A	BB or BO
AB	A antigen and B antigen	None	AB
O	None	Anti-A and anti-B	OO

Illustration 6. *The relationship between blood group (phenotype), antigen(s) present on the red cells, antibodies in serum and corresponding genotype (s) (45).*

The **Prothrombin G20210A** polymorphism is present in 2-4% of the population and is the second most common cause of inherited thrombophilia (47). The variant is a single base mutation (G/A nucleotide substitution) in the untranslated region of the gene promoter, which causes an overproduction of prothrombin by altering mRNA expression efficiently. In heterozygous subjects the prothrombin concentration is elevated up to 30%, while the concentration is increased up to 70% in homozygous individuals (48). The G20210A variant in the prothrombin gene is associated with a 2.5-fold increased risk of venous thrombosis (49).

In the Caucasian population, **Factor V Leiden** is found in up to 8%, while in Asian, African and indigenous Australian populations, the mutation is rare (47, 50). The variant is caused by a single point missense mutation in the factor V gene, which eliminates one of the three activated protein C (APC) cleavage sites, and causes the majority of APC resistance (50). Activated Factor V does not act as an enzyme but as a co-factor in the coagulation cascade. Protein C is one of the natural anticoagulant proteins circulating in plasma that cleaves and,

thereby inactivates procoagulant Factor Va and VIIIa resulting in a downregulated thrombin generation (Illustration 3) (50). Heterozygous carriers of FV Leiden have a 2-3 fold increased risk of venous thrombosis, whereas homozygous carriers have a 15-20 fold increased risk of venous thrombosis (51).

Aim of the thesis

The aim of the present thesis was to create a general overview of the existing literature on the role of non-O/O blood type, Prothrombin G20210A and Factor V Leiden; the main genetic determinants of thrombophilia, as risk factors for incident MI.

Methods

A structured literature search was performed to retrieve original epidemiological articles regarding the association between non-O/O blood type, Prothrombin G20210A and Factor V Leiden and risk of MI. In order to ensure comprehensive acquisition of papers, the bibliography of included articles were manually screened to identify additional relevant studies (cross-referencing). A thorough overview of the databases, search strategy and data extraction will be presented in the following section.

Databases

The biomedical databases **Medline** and **Embase** were searched for original scientific papers applying the **Ovid** search software. Both Medline and Embase cover biomedical literature, including articles within the fields of clinical medicine, odontology, nursing, veterinary medicine, and other areas related to life science. Further, Embase have an additional focus on pharmacology and medical devices. There is an overlap between articles found in each database. Medline is a subset of PubMed, which is the online version of Index Medicus produced by the US National Library of Medicine. In addition, PubMed also holds articles yet to be published. Medline indexes approximately 5,600 journal titles, while Embase indexes more than 8,500. Moreover, Medline and Embase contain over 22 and 31 million records, respectively.

Search strategy

The search platform Ovid was used to standardize searches in both databases in the *Advanced Search* mode (Illustration 7). MeSH (Medical subject heading)-terms were used in both the

Medline and Embase search as all MeSH terms are mapped to Embase's own thesaurus Emtree. All records in Medline are indexed with MeSH-terms. In order to find articles on the association between the SNPs and MI, multiple suitable MeSH-terms were chosen. Before the search was performed, several essential articles were selected to see if the chosen terms would identify these articles. Furthermore, suitable subheadings in these articles, which would help limit the search to epidemiological articles, were chosen.

The structured searches in Medline and Embase are presented in Supplementary Table 1-6. For MI, the following MeSH-terms were entered: *myocardial infarct* / cardiovascular stroke* / heart attack* / acute coronary syndrome* / coronary occlusion* / coronary thrombos* / coronary artery stenos* / coronary stenos**. For *ABO*, the following MeSH-terms were used: *ABO-blood / non-O blood / rs8176719*. MeSH-terms for *F2* applied were: *hyperprothrombinemia / rs1799963 / prothrombin G20210A / prothrombin G20210A mutation* / factor II mutation* / F2 / factor II / blood coagulation factor II / coagulation factor II / differentiation reversal factor / factor II, coagulation / II, coagulation factor*. Ultimately, MeSH-terms combined for *F5* were: *FV leiden / rs6025 / F5 / factor V leiden thrombophilia / thrombophilia due to factor V leiden / hereditary resistance to activated protein c / thrombophilia V / protein c cofactor deficiency / apc resistance / resistance, apc*. Additional terms considered relevant were *genotype*, *risk factor* and *cohort studies, meta-analysis* and *case-control studies*.

For each separate search, an "adjacent-term" (*adj3*) was added. This term includes articles where the two words *myocardial* and *infarction(s)* are mentioned in the title and/or abstract with maximum three words in between (Supplementary Table 1-6, #10). Subheadings added were "Blood", "Complications", "Epidemiology", "Etiology", "Genetics", "Pathology", "Physiology", "Physiopathology" and "Prevention and Control". After the systematic searches were performed, citations and references were imported to EndNote X8 (Thompson Reuters) to help manage and organize the literature.
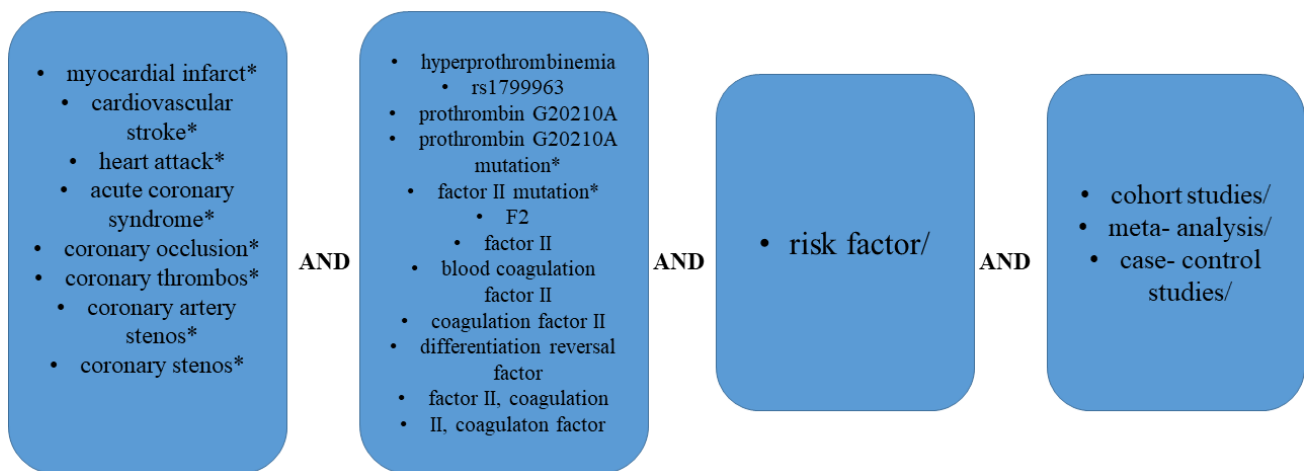
Illustration 7. Example of a box-model of the literature search in the Ovid-platform (see Supplementary Table 1-6 for the complete search phrases)

Furthermore, a modified version of the PRISMA flow chart for each search is shown in Figure 1-3. This is a four-component diagram applied in systematic reviews to ensure transparent reporting and a clear overview of what is planned, performed and found in a review. The diagram shows the numbers of identified articles, excluded and included studies from the structured search.

Inclusion/exclusion criteria

Searches were limited to English language and humans. Additionally, the search was restricted to articles published after the year 2005 to the current date. Studies included were meta-analyses, and observational studies, i.e. cohort studies and case-control studies. Consequently, randomized-controlled trials (RCTs), case-reports, comments and notes were excluded. The last search was performed on May 1st 2020 and studies published beyond this date were not included.

Extraction of data

The following data were extracted to be presented in a pre-defined table: author(s) and year of publication, study design, number of study participants, main results and conclusions (Results Table 1-5).

GRADE

The Grading of recommendation, assessment, development and evaluation (GRADE) system is a method applied by systematic reviewers and guideline developers to assess the quality of evidence (52). The method classifies quality of evidence in one of four levels (high (4+), moderate (3+), low (2+) or very low (1+)) and offers two grades of recommendation – "strong" and "weak" (53). The GRADE approach was used to assess the quality of some important articles (n=4) in the field, and chosen in collaboration with my supervisor. In this thesis, we applied the step-by-step approach used for GRADE-evaluation presented in Journal of Evidence-Based Medicine (Supplementary Figure) (52), and "recommendations" were left out. This method was used because the articles were observational studies where an association rather than an intervention was investigated, and in which a recommendation is not applicable (54).

Results

Twenty-five full-text papers from the structured search are included in the thesis. Results from the literature search are presented in Results Table 1-5. The GRADE tables are listed at the end of the thesis in Supplementary material.

ABO-blood group (*ABO*)

Articles published during the previous 10-15 years suggest that non-O blood types confer a higher risk of MI than group O. Results from a meta-analysis conducted by Wu and colleagues (55) in 2006 included 22 studies (5 longitudinal), and yielded pooled odds ratios of 1.25 (95% CI: 1.14-1.36) for MI and 1.14 (95% CI: 1.01-1.27) for peripheral vascular disease, which confirmed the impression of a linkage between arterial CVD and non-O blood group status. Furthermore, separate analyses showed that results from retrospective studies yielded higher risk estimates than results from prospective studies (OR 1.33, 95% CI: 1.21-1.46 versus OR 1.01, 95% CI: 0.84-1.23). A more recent meta-analysis from 2016 (56) included 17 studies and 225 810 study participants, and showed that the risk of CAD was significantly higher in blood group A with an OR of 1.14 (95% CI 1.00-1.10). The risk was significantly lower in blood group O compared to non-O blood type (OR 0.85, 95% CI: 0.78-0.94). In separate analyses, there was a higher incidence of CAD in blood group A compared to non-A

in case-control studies (OR 1.14, 95% CI: 1.04-1.26), while there was no difference between the same blood groups in cohort studies.

In a third meta-analysis from 2015 (57) with 174 945 participants the authors found a 14% increase in CAD incidence in non-O blood groups compared to O-blood group (OR/HR 1.14, 95% CI: 1.04-1.25) in multivariable adjusted logistic and Cox proportional hazards regressions models. Additionally, non-O blood group was associated with a 16% increase in MI risk. The study concluded that non-O blood group may be an independent risk factor for both CAD and MI. However, results from a case-control study by Tanis et al (58) showed that the effect of non-O blood group on MI risk disappeared after adjustment for factor VIII and vWF. In the unadjusted model, the OR for MI for blood group non-O versus O was 1.6 (95% CI: 1.1-2.3).

Carpeggiani et al conducted a cohort study (59) of 4 901 patients examined for coronary heart disease with coronary angiography and followed them for a maximum of 10 years. Blood type non-O was associated with presence of coronary atherosclerosis and was found to be a powerful predictor of cardiac mortality in patients aged <65 years (HR 1.53, 95% CI: 1.06-2.21). In another large cohort (60) of 62 073 women and 27 482 men from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) 6.27% of the CHD cases were attributable to non-O blood group.

Sari and colleagues conducted a case-control study (61) of 476 patients with STEMI and 203 age-and sex matched controls and found that the distribution of ABO blood groups in cases versus controls was essentially similar (A in 43.1% versus 44.3%, B in 15.1% versus 15.3%, AB in 10.7% versus 12.3% and O in 31.1% versus 28.1%). In another case-control study, non-O blood group was an independent risk factor for the presence and severity of CAD in hypertensive patients with poor blood pressure control (62).

Prothrombin G20210A (*F2*)

Zheng Ye and colleagues published a large meta-analysis involving 66 155 cases and 91 307 controls in the Lancet in 2006 (63), where they investigated the relationship between the *F2* G20210A and FV Leiden variants and risk of CHD. They reported a per-allele relative risk for CHD of the Prothrombin G20210A of 1.31 (95% CI: 1.12-1.52). A replication study comprising 1 880 cases (1 680 men and 210 women) and an equal number of controls was performed in 2010 (64) and did not reach statistical significance (OR 1.32, 95% CI: 0.96-

1.80) with regard to MI risk conferred by *F2* exposure. Still, the risk estimates were essentially similar. Additionally, a validation study from 2007 (65) evaluated 85 genetic susceptibility variants for MI, where the Prothrombin G20210A variant yielded an OR of 0.92 (95% CI: 0.52-1.64) in the regression model.

Furthermore, results from a meta-analysis conducted in 2017 (66) with 14 611 MI cases and 84 358 controls yielded an OR of 1.41 (95% CIs: 1.16-1.72, 1.15-1.72) in both the heterozygous and homozygous model, and showed that MI relative risk conferred by *F2* was even higher in subjects ≤55 years. Moreover, variation in the prothrombin gene both at the level of haplotypes and SNPs was demonstrated to be associated with MI in a case-control study of postmenopausal women by Hindorff et al (67). The OR associated with the G20210A polymorphism was 2.2 (95% CI: 1.1-4.7) in a fully adjusted model, and the risk was not influenced by use of hormone replacement therapy.

Results from the Danish population-based prospective Copenhagen General Population- and Copenhagen City Heart studies showed that carriers of the Prothrombin G20210A allele have a 1.3-fold (95% CI 1.0-1.7) increased risk of MI (2 708 events in total) (68). The combination of the prothrombin allele and *F5* or *ABO* blood type did not confer an increased risk of MI that exceeded the sum of the individual risk components. Another study by Martinelli et al (69) evaluated ten common genetic polymorphisms including the Prothrombin G20210A, variants in the fibrinogen gamma gene, PAI-1 (inhibitor of plasminogen activator), GPIIIa (platelet receptor for collagen and vWF), GPIa/IIa (platelet receptor for collagen), P2RY12 H1/H2 (platelet receptor for ADP) and ultimately two variants in the FVII- and FV genes, respectively. No consistent association was found when each polymorphism was considered separately (including both the Prothrombin G20210A and Factor V Leiden). However, those with ≥8 alleles had an increased risk of MI (OR 2.49, 95% CI: 1.03-6.01) (69).

Moreover, three small case-control studies could not demonstrate an association between *F2* and CAD (70) or MI (71, 72). However, Slama et al (73) showed that the presence of *F2* in combination with one or more of the traditional metabolic risk factors may have a stronger effect on MI risk. Rallidis et al (74) further showed that the *F2* polymorphism is associated with an increased risk of premature STEMI (OR 2.2 95% CI: 1.1-4.3), and that the risk increases markedly when smoking is present (OR 22.0 95% CI: 9.2-66.5).

Factor V Leiden (*F5*)

A meta-analysis of 41 case-control studies from 2010 (75) comprising 7 790 cases and 19 276 healthy controls found a significantly higher frequency of *F5* among MI cases than controls, yielding an OR of 1.6 (95% CI: 1.98-4.44). Results from the meta-analysis by Zheng et al (63) yielded a per-allele relative risk for CHD for FVL of 1.17 (95% CI: 1.08-1.28). The validation study by Mannucci et al (64) demonstrated that *F5* was associated with an increased MI risk (OR 1.66, 95% CI: 1.15-2.38). Furthermore, in a case-control study comprising 1 083 cases with angiographically confirmed luminal stenosis of ≥50% and 511 controls, Boroumand and colleagues (76) showed that possessing one or two copies of the risk variant was more likely associated with a trend towards higher vessel score, which indicates a more severe CAD.

On the contrary, in three case-control studies from Venezuela (77), Croatia (78), and Costa Rica (72) an association between Factor V Leiden and risk of MI could not be demonstrated. Celik (70) and Linnemann et al (71) could not demonstrate an association in two additional case-control studies with 129 and 40 cases, and 107 and 993 control subjects, respectively. Results from the cohort study based on the Danish Copenhagen General Population- and Copenhagen City Heart studies (79) yielded an adjusted HR of 1.0 (95% CI: 0.9-1.2) for *F5* as risk factor for MI in a multivariable adjusted Cox model. A case-control study from Tunis (73) showed similar results (OR 1.55, 95% CI: 0.58-4.12). However, when combined with one or more of the traditional cardiometabolic risk factors a stronger effect on MI risk conferred by *F5* may be expected (73).

General discussion

In the present thesis, the role of *ABO*, *F2* and *F5* single nucleotide polymorphisms as risk factors for MI was summarized in the form of a narrative literature review. Results from the structured search support an association between *ABO, F2* and *F5* as risk factors for MI. Several meta-analyses have been published during the recent years and report modest effects of non-O blood type (ORs ranging from 1.1 to 1.3), *F2* (ORs around 1.4) and *F5* (ORs ranging from 1.2 to 1.9) on the risk of MI. The *F2* and *F5* variants appeared to be associated with premature adverse events and more severe CAD.

The acute manifestation of IHD is an MI, which is a complex trait or phenotype. The disease develops through the interaction between multiple environmental and genetic risk factors,

none of which can cause disease solely by themselves. In the study by Martinelli et al (69) no consistent association was found when each polymorphism was considered separately. However, when several alleles (≥8) were combined, an increased risk of MI was observed (OR 2.49, 95% CI: 1.03-6.01). The results suggest that the combination of SNPs may help to predict MI in patients with severe CAD. In addition, the joint presence of a high genetic predisposition score and hypertension conferred a higher risk of CHD (80). Studies also suggest that the genetic risk profile differs between STEMI- and non-STEMI events (81). STEMI patients more often possess the gain-of-function variants *5A5A* or *5A6A* encoding MMP-3, resulting in higher titers of MMP-3 in serum. This predispose to development of less connective tissue and a thin fibrous cap in the atherosclerotic lesion (81) and could be an explanation why these patients are prone to develop fulminant ST-elevation MI.

The combination of genetic and environmental risk factors may be essential in the pathogenesis, as studies indicate that *F5* may contribute to MI risk in those with other traditional cardiovascular risk factors (50, 82). For instance, heterozygous FVL individuals who smoked had a 30-fold increased risk of MI compared with subjects with neither risk factor (50), and had an approximately 2-fold increased risk of premature MI (83). Additionally, a pooled analysis of four family cohorts reported a stronger association between thrombophilia and arterial thrombosis in the presence of traditional cardiovascular risk factors, especially diabetes mellitus (82).

Traditional modifiable atherosclerotic risk factors, including hypertension, dyslipidemia and diabetes mellitus influenced the association between family history of MI and incident MI by weakening the risk estimates in a multivariable adjusted regression model (34). This indicates that genetic predisposition to hypertension, dyslipidemia or diabetes rather than prothrombotic genotypes could determine the association between family history of MI and risk of incident MI. Further, family history of MI performed a synergistic effect with age, BMI, and total cholesterol on the risk of MI.

Several potential pathophysiological alterations support that hypercoagulability is a potential risk factor for arterial thrombosis (84). The biological mechanism of the relationship between non-O blood group and thrombosis has been studied thoroughly (85), and its major determinants are increased levels of vWF and coagulation factor VIII in plasma (56). In fact, non-O blood groups have the lowest expression of O antigen and generates the highest vWF and FVIII-levels. It has been shown in laboratory studies that the glycosylated vWF in non-O blood group protects it from degradation by ADAMTS13 (*von Willebrand factor-cleaving*

protease) (86). Moreover, the carbohydrate erythrocyte antigen encoded by the SNP is also found on platelets and vascular endothelium, which may modulate the function of the respective cells. For instance, *ABO* antigens are expressed on platelet membrane proteins, including GPIIb, which is a subunit of the fibrinogen receptor (86). Additionally, a lower aPTT ratio in non-O has been recognized (79), and recent studies show that increased serum-lipids may mediate the relationship between blood type A and CAD (56).

The mechanism of the relationship between Prothrombin G20210A and thrombosis is increased expression of the prothrombin molecule, which is a precursor of thrombin, a key enzyme in the coagulation cascade that generates fibrin. Moreover, activated Factor V interacts with activated factor X as a coenzyme in the reaction where prothrombin is converted into thrombin. Factor V Leiden is resistant to degradation by APC; hence, increased blood clotting is the result. The factor V Leiden subjects have more circulating substrate available for the growing thrombus in the coronary circulation, which could potentially be a mechanistic explanation why the polymorphism appear to be associated with more severe CAD.

Clinical perspective

Several scores have been developed during the recent decades to help physicians to stratify patients into low, moderate and high risk of cardiovascular disease, and to guide clinical decision making regarding antihypertensive, lipid lowering and antidiabetic treatment (87). However, the therapeutic consequences of the presence of thrombophilia remains controversial, and a thrombophilia workup is usually not considered in case of arterial thrombosis. Only in rare cases, such as in the setting of antiphospholipid syndrome (APS) with high titers of anti-cardiolipin antibodies, guidelines recommend indefinite anticoagulation treatment with Warfarin after an unprovoked MI (88). These patients are often screened for APS for other reasons (i.e. prolonged aPTT or thrombocytopenia) if the syndrome was unrecognized prior to the acute MI event.

However, the findings provided from the present thesis do not support routine thrombophilia screening of *ABO*, *F2* and *F5* polymorphisms in MI patients due to the fact that the contribution to disease development seems to be small. Hence, the cost of routine screening probably outweighs the benefit.

Methodological considerations

The main strength of the present thesis was a thorough and structured literature search covering high quality papers from top rated journals. Additionally, previous knowledge on the field of interest is an advantage when performing a structured search. The search can be made more broad or narrow if you know what the existing literature have discussed. However, due to the limited time frame of the thesis, the search was restricted to papers published after 2005, and a targeted approach was used in order to obtain a feasible number of papers to screen. A targeted search approach was considered adequate, as the aim was to perform a narrative review. In addition, crosscheck of references was made in order to identify important papers that could potentially have been missed in the literature search. Some of the articles were missing online full-texts and non-English language articles were excluded, which may have led to selection bias in the thesis. However, it seems unlikely that the latter would lead to selection bias as the vast majority of papers covered by the search were from English language journals. It is unlikely that the few non-English papers would demonstrate considerable different results and change the conclusions of this review.

The studies included in this thesis have some methodological issues, which will be discussed in the following paragraphs.

Firstly, results from the meta-analyses included in the present thesis illustrate that retrospective case-control studies tend to find an association, while prospective cohort studies fail to find an association between non-O blood type and MI. **Reverse causation** is not an issue of concern in genetic case-control studies because the temporal sequence between exposure and outcome is always known. Nevertheless, selection of control subjects for uncommon polymorphisms in the population could overestimate the effect in case-control studies. Further, it could be that we measure a survival advantage of non-O blood group, a paradoxical phenomenon called **survivorship bias**. This phenomenon has been described in well-known prognostic studies, where we see an association between active smoking and lower mortality rates after MI (89, 90). It could be that the individuals with non-O blood group have the best prognosis shortly after the acute event, and thereby are selected into the case-group.

Secondly, the **relative risk** of MI conferred by *F2* was higher in subjects ≤55 years in the large meta-analysis by Li et al (66). We know that the relative risk depends on the baseline risk of MI in the population we study. The baseline risk in the population <55 years is lower

than in the older population. Hence, the **absolute risk** increase by *F2* exposure could be similar in the younger and older subjects.

Lastly, **misclassification bias** comes about when the information collected about study participants is erroneous and information about exposure and/or disease status therefore becomes misclassified (91). The errors that arise when cases and controls are incorrectly or imprecisely diagnosed may lead to misclassification bias (92). Non-differential misclassification of the outcome is present when the misclassification is independent of the exposure, and most often result in underestimation of the true association (91). The implementation of more sensitive methods for diagnosing MI (troponins and/or CK-MB) has probably resulted in a higher detection rate in the most recent studies. On the contrary, some of the studies reported only CK-MB as part of the MI definition and several of the studies also included patients with coronary artery disease/stenosis without information on whether they had experienced an acute MI or not (64). The heterogeneity in the outcome definitions between studies most likely lead to misclassification of outcome and underestimation of the true association. The studies also apply different genotyping strategies (63). However, the classification of genetic exposures are considered precise (93).

Conclusion

In conclusion, the existing literature supports an association between non-O blood type, Prothrombin G20210A and Factor V Leiden and risk of MI. Prothrombin G20210A and Factor V Leiden variants seem to be associated with premature adverse events and more severe CAD.

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Tables and Figures

Results Table 1. Articles regarding the association between non-O/O blood type and risk of myocardial infarction (MI).

1st Author	Study design	Number of participants	Main results	Conclusion(s)
O. Wu, 2008 (55)	Meta-analysis.	22 studies with MI as outcome. Five (5) of the studies were conducted prospectively.	Non-O blood group yielded pooled ORs of 1.25 (95% CI 1.14-1.36), 1.03 (95% CI: 0.89-1.19), 1.45 (95% CI: 1.35-1.56), 1.14 (95% CI: 1.01-1.27) and 1.79 (95% CI: 1.56-2.05) for MI, angina, peripheral vascular disease, cerebral ischemia of arterial origin and venous thromboembolism, respectively. Separate analyses on MI showed that retrospective studies are associated with a greater risk estimate than prospective studies (OR 1.33, 95% CI: 1.21-1.46 versus OR 1.01, 95% CI: 0.84-1.23).	The study confirms the historical impression of a linkage between some vascular disorders and non-O blood group status.
Z. Chen, 2016 (56)	Meta-analysis.	17 studies, covering 225 810 study participants.	The risk of CAD was significantly higher in blood group A compared with non-A (OR 1.14, 95% CI 1.03-1.26) and lower in blood group O compared to non-O (OR 0.85, 95% CI 0.78-0.94). Similar results was observed for MI cases. An increase in CAD incidence was observed in subjects in blood group A in case-control studies (OR 1.14 95% CI: 1.04-1.26), while there was no difference between blood group A and non-A in cohort studies.	The study indicates that both blood group A and non-O are risk factors for CAD and MI.
H. Takagi, 2015 (57)	Meta-analysis.	Ten (10) studies with 174 945 participants (21 570 cases) were identified and included. Eight (8) studies reporting data on acute	Non-O blood group was associated with a 14% increase in CAD incidence relative to O blood group (OR/HR 1.14, 95% CI: 1.04-1.25). Non-O blood group was associated with a 16% increase in MI risk (OR/HR 1.16, 95% CI: 1.02-1.31). When data was stratified on study design and analyzed	The study concludes that non-O blood group appears to be an independent risk factor for CAD and MI.

		MI were added in a separate model.	separately, non-O blood group was associated with an increase in CAD risk in both cross-sectional and cohort studies but no increase in CAD incidence in case-control studies was observed (OR/HR 1.02, 95% CI 0.62-1.68).	
B. Tanis, 2006 (58)	Case-control study.	200 women with MI before the age of 49 were compared with 626 control subjects from the Risk of Arterial Thrombosis In Relation with Oral contraceptive use (RATIO) study.	Mean levels of factor VIII activity, vWF antigen and factor IX activity were higher in cases (133, 134, 132 IU/dL) than controls (111, 107, 120 IU/dL). Mean levels of factor XI were equal in cases (114 IU/dL) and controls (113 IU/dL). The OR for MI for blood group non-O versus O was 1.6 (95% CI: 1.1-2.3). Adjustment for factor VIII and vWF reduced the ORs (1.3, 95% CI 0.9-1.9 versus 1.2 95% CI: 0.9-1.8) markedly.	Non-O blood group, high vWF, factor VIII and factor IX levels are associated with an increased risk of MI in young women, while high factor XI levels are not. The effect of blood group on MI risk disappeared after adjustment for factor VIII and vWF.
C. Carpeggiani, 2010 (59)	Cohort study.	4901 patients examined for ischemic heart disease with coronary angiography were blood typed and followed for a maximum of 10 years.	A significant association was found between non-O blood group and family history of ischemic heart disease, hypercholesterolemia and presence of coronary atherosclerosis. Higher prevalence of A and B alleles was found in patients with MI. Group non-O was a powerful predictor of cardiac mortality in patients aged <65 years (HR 1.53, 95% CI: 1.06-2.21).	Group non-O is associated with increased mortality in patients with ischemic heart disease.
M. He, 2012 (60)	Cohort study.	62 073 women and 27 482 men from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) were enrolled.	In the NHS and HPFS 2 055 and 2 015 participants developed CHD, respectively. ABO blood group was significantly associated with the risk of CHD in both women and men. Those with blood group A, B or AB were more likely to develop CHD (adjusted HRs of 1.06 (95% CI: 0.99-1.15), 1.15 (1.04-1.26) and 1.23 (1.11-1.36), respectively) compared to participants with O blood type. Overall, 6.27% of the CHD cases were attributable to non-O blood group.	The authors conclude that ABO blood group is significantly associated with CHD risk. Moreover, those with blood group O have moderately lower risk compared to other blood groups.

I. Sari, 2008 (61)	Case-control study.	476 patients with acute ST-elevation MI and 203 age- and sex matched healthy subjects.	The distribution of ABO blood groups in cases versus controls was A in 43.1% versus 44.3%, B in 15.1% versus 15.3%, AB in 10.7% versus 12.3% and O in 31.1% versus 28.1%. The frequency of cardiovascular risk factors was similar in patients with different blood groups.	The authors conclude that ABO blood group distribution among patients with MI was similar with control subjects. The relationship between ABO blood group distribution, cardiovascular risk factors, CAD and MI needs to be evaluated with large-scale prospective studies.
B. Zhou, 2017 (62)	Case-control study.	2708 patients with primary hypertension (HTN) were enrolled and underwent coronary angiography (CAG) due to angina-like chest pain. The population was divided into two groups: HTN with CAD (2185) and HTN without CAD (523).	The percentage of A blood group was statistically higher and O blood group was significantly lower in HTN with CAD compared to HTN without CAD. The same effect was observed for non-O versus O-blood group. The percentage of angiography-proven CAD was higher in A blood group than that in non-A blood group after adjustment for confounding factors (OR 1.42, 95% CI: 1.0-2.0).	A blood group was an independent risk factor for the presence and severity of CAD in hypertensive patients with poor blood pressure control. However, in patients with good blood pressure control A blood group was not associated with CAD.

MI, Myocardial infarction; CAD, Coronary artery disease; vWF, von Willebrand Factor; CHD, Coronary heart disease

Results Table 2. Articles regarding the association between Prothrombin G20210A and risk of myocardial infarction (MI).

1st Author	Study design	Number of participants	Main results	Conclusion(s)
C. Li, 2017 (66)	Meta-analysis.	34 studies, covering 14 611 MI cases and 84 358 controls.	A statistical significant association was observed between Prothrombin G20210A polymorphism and MI in the allele model (OR 1.43; 95% CI 1.18-1.72), heterozygote model (OR 1.41; 95% CI 1.16-1.72) and dominant model (OR 1.41; 95% CI 1.15-1.72). A significant association was found in a subpopulation younger than 55 years.	In conclusion, the meta-analysis demonstrated that Prothrombin G20210A polymorphism may represent a risk factor for MI in an age-related manner.
L. Hindorff, 2006 (67)	Case-control study.	273 cases and 788 controls were recruited from Washington State Caucasian women from the Group Health Cooperative (GHC) (1995-1999).	Prothrombin haplotypes were associated with MI (with minimal adjustment, p=0.056; with full adjustment, p=0.034) and similar among users and nonusers of hormone therapy. The OR associated with the G20210A polymorphism was 2.2 (95% CI: 1.1-4.7) in the fully adjusted model.	The results suggest that common genetic variants in the prothrombin gene, including the G20210A variant, are associated with MI in postmenopausal women.

Results Table 3. Articles regarding the association between Factor V Leiden and risk of myocardial infarction (MI).

1st Author	Study design	Number of participants	Main results	Conclusion(s)
M. Dowaidar, 2010 (75)	Meta-analysis.	41 studies, covering 7790 cases and 19 276 controls.	The overall frequency of FVL mutation in MI cases was 6.8%, which was significantly higher than among controls (1.3%), yielding an OR of 1.6 (95% CI 1.98-4.44).	The study concludes that there is a definite risk related to the carriage of FVL mutation among MI cases. This should have a potential impact on the genetic counseling of all family members of affected cases for proper prophylaxis.
M. Boroumand, 2013 (76)	Case-control.	1 083 cases having angiographic evidence of atherosclerosis with ≥50% luminal stenosis, and 320 controls with no luminal stenosis and 191 controls with <50% luminal stenosis were enrolled.	FVL was found to be independently associated with the occurrence of CAD (p=0.020). As compared to wild genotype, heterozygote or homozygote mutant genotype were more likely associated with a trend towards more severe CAD (OR 1.85; 95% CI 1.26-2.72 and OR 3.70; 95% CI 1.71-8.00).	The study concludes that the FVL mutation is a significant determinant of CAD risk. FVL is associated with increasing CAD severity. The findings do not support routine screening for FVL; however, screening for this mutation in selected patients may improve risk stratification.
C. Pestana, 2009 (77)	Case-control study.	437 cases (208 with DVT, 175 with MI and 54 with stroke) and 134 control subjects were enrolled from a Venezuelan population.	FVL was associated with a fourfold increase in the risk of DVT (OR 4.24, 95% CI: 1.35-14.79). No relation was observed between the presence of FVL and the risk of MI or stroke.	The authors conclude that a clear association between the FVL mutation and DVT was demonstrated. These findings are in agreement with those found in other populations with different ethnic backgrounds. The FV Leiden mutation was not associated with an increased risk of MI or stroke.

Results Table 4. Articles evaluating the impact of non-O/O blood type, Prothrombin G20210A and Factor V Leiden on the risk of myocardial infarction (MI).

1st Author	Study design	Number of participants	Main results	Conclusion(s)
I. Jukic, 2013 (78)	Case-control study.	182 cases with acute MI and 236 healthy controls with no family history of CAD or MI.	No statistically significant difference in the frequency of non-O and O genotypes between cases and controls was observed (OR 1.41, 95% CI: 0.94-2.11). A significantly higher OR in the O1A1 and O1A2 genotypes versus OO carriers (OR 1.66, 95% CI: 1.03-2.68 and OR 2.57, 95% CI: 1.01-6.55) was observed. Results from analyses on heterozygote carriers of FV Leiden and prothrombin G20210A did not indicate an increased risk of MI.	The authors conclude that the genetic prothrombotic factors are not associated with an increased MI risk.
B. Sode, 2013 (79)	Cohort study.	66 001 study participants (2708 MI events) from two (2) Danish population-based studies were genotyped for *ABO*, *FVL* and *F2*.	The multivariable adjusted HR for MI was 1.1 (95% CI 1.0-1.1) among individuals with non-O blood type. Among those with *FVL* the associated adjusted HR was 1.0 (95% CI 0.9-1.2). For *F2*, the adjusted HR was 1.3 (95% CI 1.0-1.7) for heterozygote individuals. Combinations of ABO blood type and FV Leiden or prothrombin G20210A genotypes were not associated with a consistent increase in MI risk.	*ABO*, *FVL* and *F2* either evaluated separately or in combination, were not consistently associated with MI.

Results Table 5. Articles evaluating the impact of Prothrombin G20210A and Factor V Leiden on the risk of myocardial infarction (MI).

1st Author	Study design	Number of participants	Main results	Conclusion(s)
Zheng Ye, 2006 (63)	Meta-analysis.	191 studies in relation to factor V Leiden, factor VII G10976A, Prothrombin G20210A, plasminogen activator inhibitor-1 (PAI-1), and three platelet glycoprotein (GP) receptor variants, involving 66 155 coronary disease cases and 91 307 controls, were included.	The per-allele relative risks (RR) for coronary disease of FV Leiden and Prothrombin G20210A were 1.17 (95% CI: 1.08-1.28) and 1.31 (1.12-1.52), respectively.	The FV Leiden and Prothrombin G20210A gene variants, both of which increase circulating thrombin generation, might each be moderately associated with the risk of coronary heart disease.
M. Mannucci, 2010 (64)	Case-control study.	1 880 MI cases and an equal number of controls were enrolled.	The minor A allele of FV Leiden (2.6% frequency in cases and 1.7% in controls) was associated with an increased risk of MI in the fully adjusted model (OR 1.66, 95% CI: 1.15-2.38). The positive association with MI for the minor allele A of *F2* G20210A (2.5% frequency in cases and 1.9% in controls) did not reach statistical significance (OR 1.32, 95% CI: 0.96-1.80).	The authors conclude that in a large cohort of young patients with acute MI the gain-of-function variant FV Leiden was associated with an increased MI risk. The findings on the *F2* variant confirmed the previously reported results. However, the association was not statistically significant. These findings support the important role of hypercoagulability in the pathogenesis of MI in the young.
T. Morgan, 2007 (65)	Case-control study.	811 cases and 650 age- and sex-matched controls from 2 Kansas City	Of the 85 variants tested, only 1 risk genotype (-455 promoter variant in beta-fibrinogen) was nominally statistically significant. The	In conclusion, the results provide no support for the hypothesis that any of the 85 genetic variants tested is a

		University-affiliated hospitals were enrolled.	reported ORs for Prothrombin G20210A and FV Leiden was 0.92 (95% CI: 0.52-1.64) and 0.93 (0.58-1.50), respectively.	susceptibility factor for acute coronary syndrome. The results emphasize the need for robust replication of putative genetic risk factors before introduction to clinical care.
N. Martinelli, 2008 (69)	Case-control study.	In total, 804 subjects were included. 489 had angiographically proven severe CAD, with or without MI (n=307; n=182; respectively).	The prevalence of MI increased linearly with an increasing number of prothrombotic alleles. In a multiple logistic regression model, the numbers of the same alleles remained significantly associated with MI after adjustment for traditional cardiometabolic risk factors. Those with ≥8 alleles had an increased risk of MI (OR 2.49, 95% CI: 1.03-6.01). No consistent association was found when each polymorphism was considered separately.	The combination of prothrombotic polymorphisms may help to predict MI in patients with severe CAD.
M. Celik, 2008 (70)	Case-control study.	129 MI cases and 107 control subjects under the age of 45 were included.	A statistically significant difference in terms of obesity, smoking, triglyceride, total cholesterol, high-density lipoprotein, very-low density lipoprotein, family history, hypertension, diabetes and left ventricular hypertrophy was detected between cases and controls. Ten patients (7.8%) and four controls (3.7%) had heterozygote FV Leiden mutation (OR 1.26, 95% CI: 0.84-6.89). Homozygous Prothrombin G20210A was detected in one patient (1.1%) (OR 1.65, 95% CI: 0.73-5.98).	The authors conclude that the difference was not statistically significant in terms of carriage of thrombophilic mutations.
B. Linnemann, 2008 (71)	Case-control study.	1081 patients (40 MI- and 41 stroke cases and 993 controls) with a	A higher rate of lupus anticoagulant in MI patients was observed, with an adjusted OR of 3.3 (95% CI: 0.84-12.8). No difference in any	The authors conclude that the inherited thrombophilias do not seem

|				
		previous VTE registered in the MAISTHRO (MAin-ISar-THROmbosis) database were analyzed with regard to arterial thrombotic events and contributing risk factors.	other tested thrombophilia was observed in patients with MI relative to those without.	to substantially increase the risk of arterial thrombosis.
L. Salazar-Sanchez, 2006 (72)	Case-control study.	186 MI cases and 201 age- and sex-matched controls were enrolled.	High fibrinogen levels was an important risk factor and interaction with smoking was detected. Mainly, the genotype 34LeuLeu of FXIII showed significant protective effect, (OR 0.32, 95% CI: 0.13-0.80) while the other polymorphisms showed no significant difference between cases and controls. Carriers of FVII (OR 2.75, 95% CI: 1.07-7.02) and FXIII (OR 4.20, 95% CI: 2.03-8.67) polymorphisms showed an interaction with fibrinogen.	The authors conclude that important interaction between the common risk factors and the polymorphisms (FVII;FXIII) in the development of MI was detected.
D. Slama, 2013 (73)	Case-control study.	100 MI cases hospitalized in the Principal Military Hospital of Tunis and 200 control subjects with no history of MI were enrolled.	FV Leiden prevalence was higher in MI patients (9%) than in control subjects (6%), which yielded an OR of 1.55 (95% CI: 0.58-4.12), whereas the prevalence of Prothrombin G20210A was 3% and 2.5% in cases and controls, respectively (OR 1.21, 95% CI: 0.22-5.94). The results indicate that presence of FV Leiden or Prothrombin G20210A and one or more of the metabolic risk factors increased the risk of MI.	The authors conclude that FV Leiden and the Prothrombin G20210A variant contributed weakly to the risk of MI. However, combined with one or more of the traditional cardiometabolic risk factors a stronger effect on MI risk may be expected.
L. Rallidis, 2017 (74)	Case-control study.	255 cases who survived a STEMI ≤35 years of age (224 men) and 400	The Prothrombin G20210A polymorphism was more prevalent among cases than controls (7.4% versus 3.5%). The adjusted	The Prothrombin G20210A gene polymorphism is associated with an increased risk of premature STEMI,

			healthy age- and sex-matched controls.	OR for STEMI for carriers versus non-carriers of the risk allele was 2.2 (95% CI: 1.1-4.3). The risk was increased by 22-fold (95% CI: 9.2-66.5) when the *F2* variant was present in combination with smoking. A similar effect could be observed for the combination of *F2* and hypercholesterolemia, hypertension, diabetes mellitus or obesity. There was no difference in the prevalence of FV Leiden between cases and controls.	and the risk increases markedly when smoking is present.
P. de Moerloose, 2007 (94)	Narrative review.		The study aimed to review the link between Factor V Leiden, Prothrombin G20210A, as well as deficiencies of antithrombin, protein C, and protein S, and risk of arterial CVDs.	Overall, the association between these genetic disorders and the three main arterial complications (MI, ischemic stroke, and peripheral arterial disease) is modest. However, when the arterial event occurs in young individuals, inherited thrombophilia seem to play a role, particularly when associated with smoking or oral contraceptive use.	The authors conclude that thrombophilia tests may be informative in a very restricted population with arterial events. Anticoagulation rather than antiplatelet therapy may be preferable in these patients.

Figure 1. Modified PRISMA flow diagram showing the process of the structured search concerning non-O/O blood type as risk factor for myocardial infarction (MI).
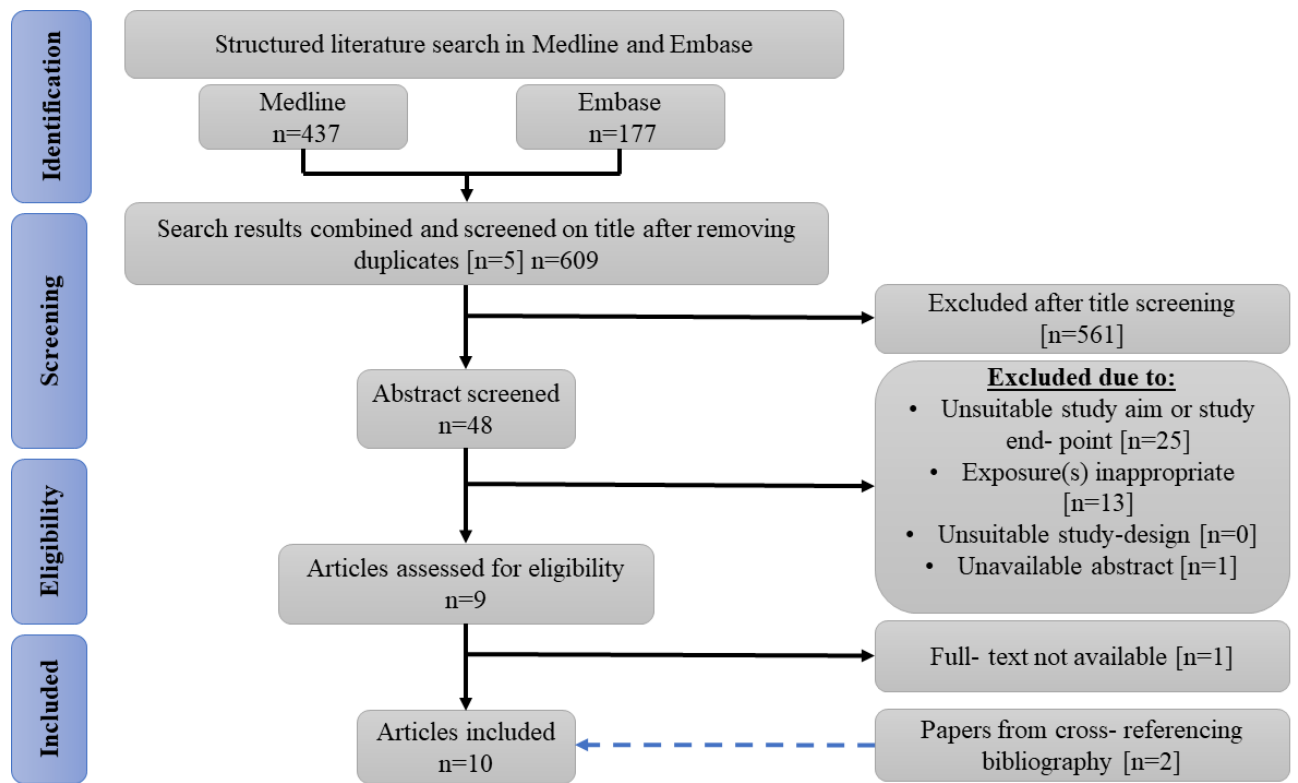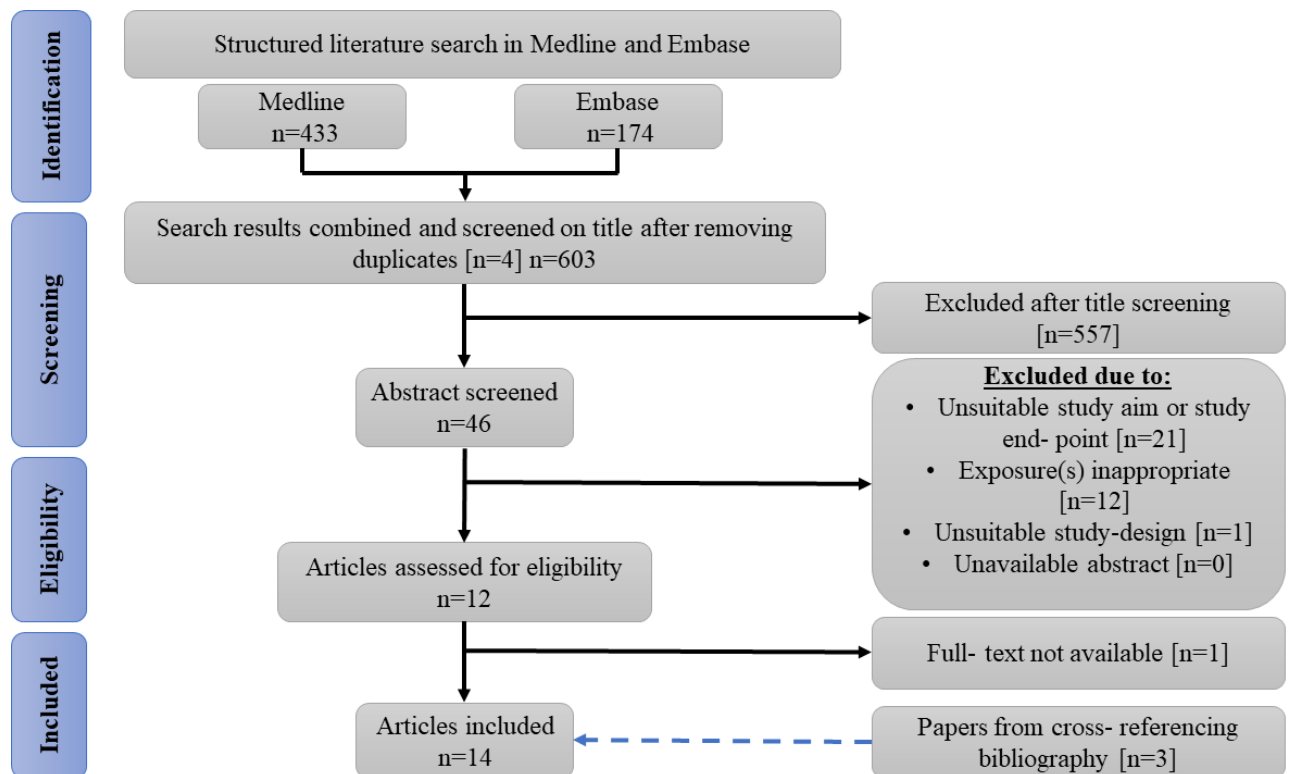
Figure 2. Modified PRISMA flow diagram showing the process of the structured search concerning Prothrombin G20210A as risk factor for myocardial infarction (MI).
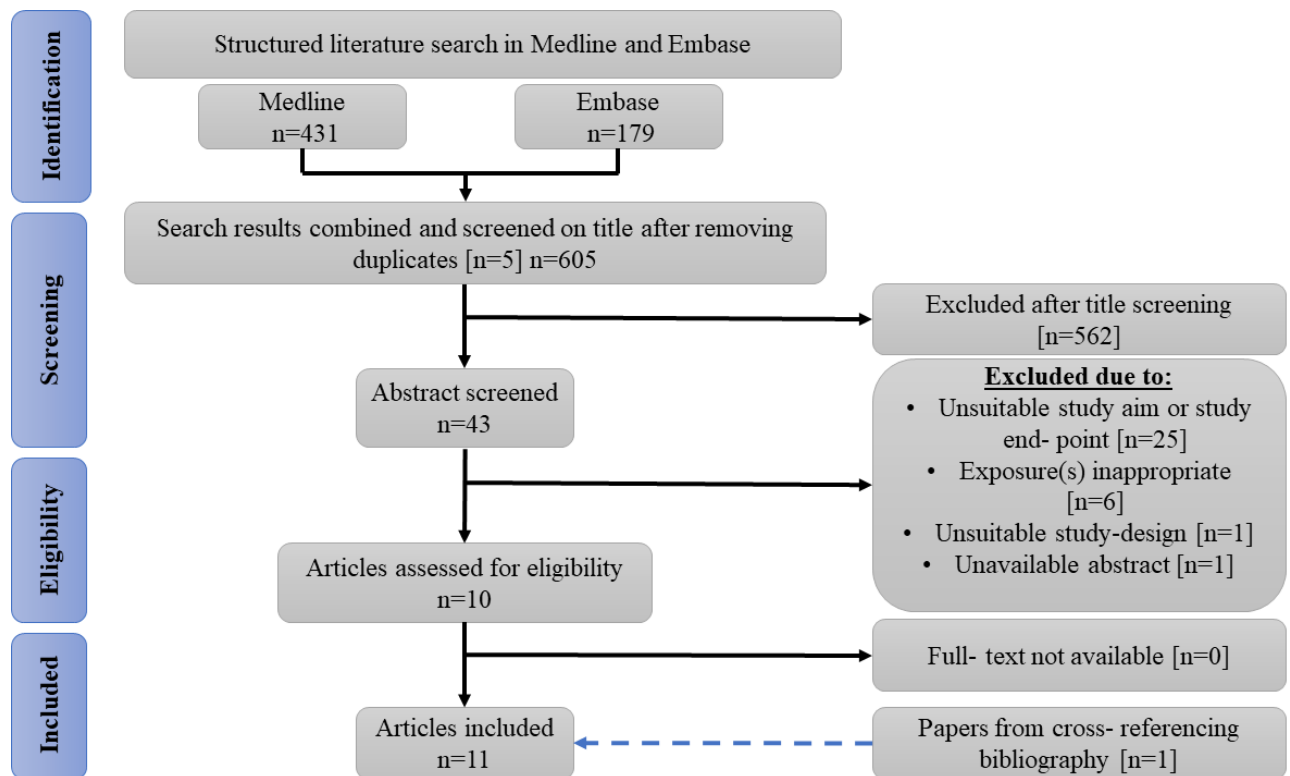
Figure 3. Modified PRISMA flow diagram showing the process of the structured search concerning Factor V Leiden as risk factor for myocardial infarction (MI).

Supplementary material

Supplementary Table 1. Structured literature search in Ovid Medline for papers regarding the association between non-O/O blood type and risk of myocardial infarction (MI).

#	Search	Text results	Type
1	exp myocardial infarction/ bl,co,ep,et,ge,pa,ph,pp,pc [Blood, Complications, Epidemiology, Etiology, Genetics, Pathology, Physiology, Physiopathology, Prevention & Control].	108 921	Advanced
2	myocardial infarct*.ab,ti,kw.	196 033	Advanced
3	cardiovascular stroke*.ab,ti,kw.	42	Advanced
4	heart attack*.ab,ti,kw.	5 488	Advanced
5	acute coronary syndrome*.ab,ti,kw.	31 634	Advanced
6	coronary occlusion*ab,ti,kw.	6 704	Advanced
7	coronary thrombos*.ab,ti,kw.	2 038	Advanced
8	coronary artery stenos*.ab,ti,kw.	5 117	Advanced
9	coronary stenos*.ab,ti,kw.	6 351	Advanced
10	(myocardial adj3 infarct*).ti,ab.	192 809	Advanced
11	or/1-10	262 209	Advanced
12	exp genotype/	406 244	Advanced
13	ABO Blood. ti, ab, kw.	3 588	Advanced
14	Non-O blood. ti, ab, kw.	228	Advanced
15	rs8176719.ti,ab,kw.	20	Advanced
16	or/12-15	409 535	Advanced
17	exp risk factor/	813 977	Advanced
18	exp cohort studies/mt[Methods]	205	Advanced
19	exp meta-analysis/	114 087	Advanced
20	exp case-control studies/	1 074 301	Advanced
21	or/18-20	1 180 201	Advanced
22	11 and 16 and 17 and 21	604	Advanced
23	Limit 22 to (english language and humans and yr= «2005-2020»)	437	Advanced

Supplementary Table 2. Structured literature search in Ovid Embase for papers regarding the association between non-O/O blood type and risk of myocardial infarction (MI).

#	Search	Text results	Type
1	exp myocardial infarction/ co,ep,et,pa,pc [Complications, Epidemiology, Etiology, Pathology, Prevention & Control].	54 769	Advanced
2	myocardial infarct*.ab,ti,kw.	279 764	Advanced
3	cardiovascular stroke*.ab,ti,kw.	74	Advanced
4	heart attack*.ab,ti,kw.	8000	Advanced
5	acute coronary syndrome*.ab,ti,kw.	57 207	Advanced
6	coronary occlusion*ab,ti,kw.	8 220	Advanced
7	coronary thrombos*.ab,ti,kw.	2 177	Advanced
8	coronary artery stenos*.ab,ti,kw.	8 016	Advanced
9	coronary stenos*.ab,ti,kw.	10 352	Advanced
10	(myocardial adj3 infarct*).ti,ab.	273 764	Advanced
11	or/1-10	361 867	Advanced
12	exp genotype/	438 852	Advanced
13	ABO Blood. ti, ab, kw.	4 877	Advanced
14	Non-O blood. ti, ab, kw.	418	Advanced
15	rs8176719.ti,ab,kw.	61	Advanced
16	or/12-15	443 284	Advanced
17	exp risk factor/	1 022 891	Advanced
18	exp cohort studies/mt[Methods]	572 734	Advanced
19	exp meta-analysis/	186 045	Advanced
20	exp case-control studies/	172 793	Advanced
21	or/18-20	903 096	Advanced
22	11 and 16 and 17 and 21	233	Advanced
23	Limit 22 to (english language and humans and yr= «2005-2020»)	177	Advanced

Supplementary Table 3. Structured literature search in Ovid Medline for papers regarding the association between Prothrombin G20210A and risk of myocardial infarction (MI).

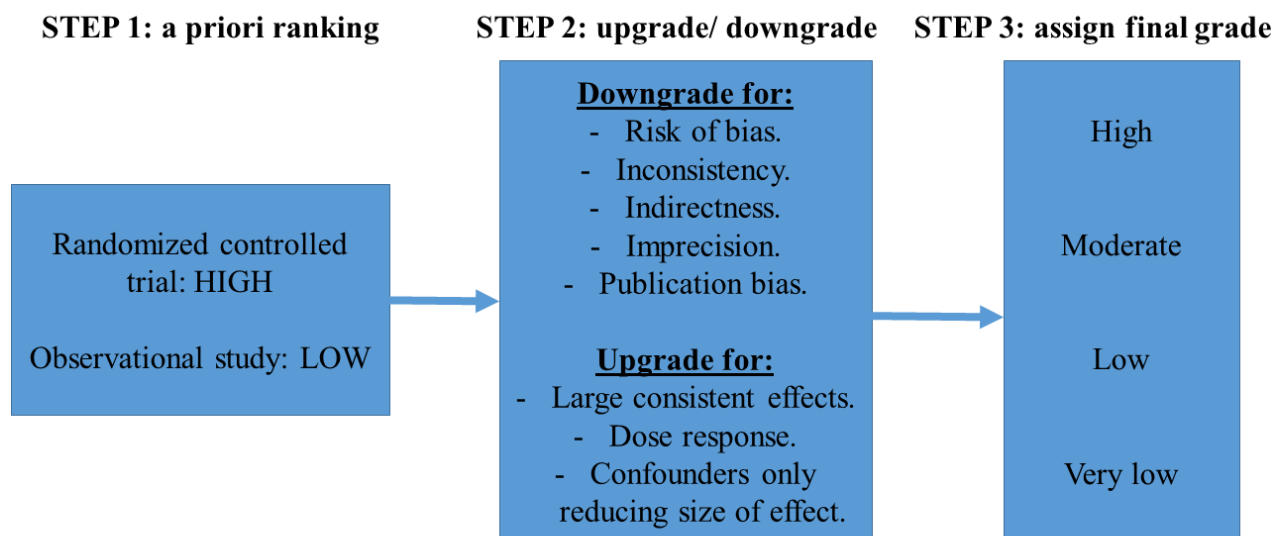| #	Search	Text results	Type
1	exp myocardial infarction/ bl,co,ep,et,ge,pa,ph,pp,pc [Blood, Complications, Epidemiology, Etiology, Genetics, Pathology, Physiology, Physiopathology, Prevention & Control].	108 982	Advanced
2	myocardial infarct*.ab,ti,kw.	193 626	Advanced
3	cardiovascular stroke*.ab,ti,kw.	42	Advanced
4	heart attack*.ab,ti,kw.	5 391	Advanced
5	acute coronary syndrome*.ab,ti,kw.	31 045	Advanced
6	coronary occlusion*ab,ti,kw.	6 671	Advanced
7	coronary thrombos*.ab,ti,kw.	2 030	Advanced
8	coronary artery stenos*.ab,ti,kw.	5 072	Advanced
9	coronary stenos*.ab,ti,kw.	6 262	Advanced
10	(myocardial adj3 infarct*).ti,ab.	190 439	Advanced
11	or/1-10	259 262	Advanced
12	exp genotype/	406 620	Advanced
13	hyperprothrombinemia.ab,ti,kw.	24	Advanced
14	rs1799963.ab,ti,kw.	53	Advanced
15	prothrombin G20210A.ab,ti,kw.	940	
16	prothrombin G20210A mutation*.ab,ti,kw.	421	
17	factor II mutation*.ab,ti,kw.	39	
18	F2.ab,ti,kw.	26 024	Advanced
19	factor II.ab,ti,kw.	4 360	Advanced
20	blood coagulation factor II.ab,ti,kw.	4	Advanced
21	coagulation factor II.ab,ti,kw.	113	Advanced
22	differentiation reversal factor.ab,ti,kw.	2	Advanced
23	factor II, coagulation.ab,ti,kw.	5	Advanced
24	II, coagulation factor.ab,ti,kw.	3	Advanced
25	or/12-24	435 124	Advanced
26	exp risk factor/	814 890	Advanced
27	exp cohort studies/mt[Methods]	205	Advanced
28	exp meta-analysis/	114 354	Advanced
29	exp case-control studies/	1 075 843	Advanced
30	or/27-29	1 181 991	Advanced
31	11 and 25 and 26 and 30	598	Advanced
32	Limit 28 to (english language and humans and yr= «2005-2020»)	431	Advanced

Supplementary Table 4. Structured literature search in Ovid Embase for papers regarding the association between Prothrombin G20210A and risk of myocardial infarction (MI).

#	Search	Text results	Type
1	exp myocardial infarction/co,ep,et,pa,pc [Complications, Epidemiology, Etiology, Pathology, Prevention & Control].	54 733	Advanced
2	myocardial infarct*.ab,ti,kw.	280 171	Advanced
3	cardiovascular stroke*.ab,ti,kw.	74	Advanced
4	heart attack*.ab,ti,kw.	8 012	Advanced
5	acute coronary syndrome*.ab,ti,kw.	57 324	Advanced
6	coronary occlusion*ab,ti,kw.	8 221	Advanced
7	coronary thrombos*.ab,ti,kw.	2 177	Advanced
8	coronary artery stenos*.ab,ti,kw.	8 026	Advanced
9	coronary stenos*.ab,ti,kw.	10 356	Advanced
10	(myocardial adj3 infarct*).ti,ab.	274 149	Advanced
11	or/1-10	362 347	Advanced
12	exp genotype/	439 752	Advanced
13	hyperprothrombinemia.ab,ti,kw.	22	Advanced
14	rs1799963.ab,ti,kw.	124	Advanced
15	prothrombin G20210A.ab,ti,kw.	1 601	Advanced
16	prothrombin G20210A mutation*.ab,ti,kw.	693	Advanced
17	factor II mutation*.ab,ti,kw.	74	Advanced
18	F2.ab,ti,kw.	30 639	Advanced
19	factor II.ab,ti,kw.	5 531	Advanced
20	blood coagulation factor II.ab,ti,kw.	8	Advanced
21	coagulation factor II.ab,ti,kw.	160	Advanced
22	differentiation reversal factor.ab,ti,kw.	2	Advanced
23	factor II, coagulation.ab,ti,kw.	6	Advanced
24	II, coagulation factor.ab,ti,kw.	4	Advanced
25	or/12-24	473 581	Advanced
26	exp risk factor/	1 025 051	Advanced
27	exp cohort studies/mt[Methods]	576 974	Advanced
28	exp meta-analysis/	187 078	Advanced
29	exp case-control studies/	173 317	Advanced
30	or/27-29	908 665	Advanced
31	11 and 25 and 26 and 30	239	Advanced
32	Limit 28 to (english language and humans and yr= «2005-2020»)	179	Advanced

Supplementary Table 5. Structured literature search in Ovid Medline for papers regarding the association between Factor V Leiden and risk of myocardial infarction (MI).

#	Search	Text results	Type
1	exp myocardial infarction/ bl,co,ep,et,ge,pa,ph,pp,pc [Blood, Complications, Epidemiology, Etiology, Genetics, Pathology, Physiology, Physiopathology, Prevention & Control].	109 050	Advanced
2	myocardial infarct*.ab,ti,kw.	193 795	Advanced
3	cardiovascular stroke*.ab,ti,kw.	42	Advanced
4	heart attack*.ab,ti,kw.	5 401	Advanced
5	acute coronary syndrome*.ab,ti,kw.	31 092	Advanced
6	coronary occlusion*.ab,ti,kw.	6 672	Advanced
7	coronary thrombos*.ab,ti,kw.	2 030	Advanced
8	coronary artery stenos*.ab,ti,kw.	5 078	Advanced
9	coronary stenos*.ab,ti,kw.	6 262	Advanced
10	(myocardial adj3 infarct*).ti,ab.	190 608	Advanced
11	or/1-10	259 496	Advanced
12	exp genotype/	406 971	Advanced
13	FV leiden.ti,ab,kw.	473	Advanced
14	rs6025.ti,ab,kw.	68	Advanced
15	F5.ti,ab,kw.	2 598	Advanced
16	factor V leiden thrombophilia.ti,ab,kw.	25	Advanced
17	thrombophilia due to factor V leiden.ti,ab,kw.	2	Advanced
18	hereditary resistance to activated protein c.ti,ab,kw.	10	Advanced
19	thrombophilia V.ab,ti,kw.	2	Advanced
20	protein c cofactor deficiency.ab,ti,kw.	1	Advanced
21	apc resistance.ti,ab,kw.	712	Advanced
22	resistance, apc.ti,ab,kw.	86	
23	or/12-22	410 198	Advanced
24	exp risk factor/	815 707	Advanced
25	exp cohort studies/	205	Advanced
26	exp meta-analysis/	114 661	Advanced
27	exp case-control studies/	1 077 174	Advanced
28	or/25-27	1 183 618	Advanced
29	11 and 23 and 24 and 28	600	Advanced
30	Limit 30 (english language and humans and yr= «2005-2020»)	433	Advanced

Supplementary Table 6. Structured literature search in Ovid Embase for papers regarding the association between Factor V Leiden and risk of myocardial infarction (MI).

#	Search	Text results	Type
1	exp myocardial infarction/ co,ep,et,pa,pc [Complications, Epidemiology, Etiology, Pathology, Prevention & Control].	54 732	Advanced
2	myocardial infarct*.ab,ti,kw.	280 037	Advanced
3	cardiovascular stroke*.ab,ti,kw.	74	Advanced
4	heart attack*.ab,ti,kw.	8 004	Advanced
5	acute coronary syndrome*.ab,ti,kw.	57 287	Advanced
6	coronary occlusion*ab,ti,kw.	8 220	Advanced
7	coronary thrombos*.ab,ti,kw.	2 177	Advanced
8	coronary artery stenos*.ab,ti,kw.	8 020	Advanced
9	coronary stenos*.ab,ti,kw.	10 354	Advanced
10	(myocardial adj3 infarct*).ti,ab.	274 019	Advanced
11	or/1-10	362 179	Advanced
12	exp genotype/	439 441	Advanced
13	FV leiden.ti,ab,kw.	979	Advanced
14	rs6025.ti,ab,kw.	157	Advanced
15	F5.ti,ab,kw.	3 907	Advanced
16	factor V leiden thrombophilia.ti,ab,kw.	29	Advanced
17	thrombophilia due to factor V leiden.ti,ab,kw.	7	Advanced
18	hereditary resistance to activated protein c.ti,ab,kw.	9	Advanced
19	thrombophilia V.ab,ti,kw.	3	Advanced
20	protein c cofactor deficiency.ab,ti,kw.	1	Advanced
21	apc resistance.ti,ab,kw.	1 069	Advanced
22	resistance, apc.ti,ab,kw.	120	Advanced
23	or/12-22	444 740	Advanced
24	exp risk factor/	1 024 544	Advanced
25	exp cohort studies/	575 835	Advanced
26	exp meta-analysis/	186 702	Advanced
27	exp case-control studies/	173 226	Advanced
28	or/25-27	907 121	Advanced
29	11 and 23 and 24 and 28	233	Advanced
30	Limit 30 (english language and humans and yr= «2005-2020»)	174	Advanced

STEP 1: a priori ranking	STEP 2: upgrade/ downgrade	STEP 3: assign final grade
Randomized controlled trial: HIGH Observational study: LOW	**Downgrade for:** - Risk of bias. - Inconsistency. - Indirectness. - Imprecision. - Publication bias. **Upgrade for:** - Large consistent effects. - Dose response. - Confounders only reducing size of effect.	High Moderate Low Very low

Supplementary Figure. The step-by-step approach in the GRADE system (52)

GRADE tables

Reference: Yusuf, Hawken, Ounpuu, et al. **Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case control study**			**Study design: Case-control**
			Quality of evidence MODERATE
Aim	**Material and methods**	**Results**	**Discussion/comments**
To investigate the association between nine potentially modifiable risk factors and risk of myocardial infarction in different geographical regions, ethnic groups and in men and women.	**Data foundation:** A national coordinator selected centres within every country on the basis of feasibility. To identify first cases of myocardial infarction, all patients admitted to the coronary care unit or equivalent cardiology ward, presenting within 24h of symptom onset, were screened.	**Main findings** 15 152 cases and 14 820 controls were recruited. Smoking (OR 2.87 for current vs never), raised ApoB/ApoA1-ratio (OR 3.25 for top vs lowest quintile), history of hypertension (OR 1.92), diabetes (OR 2.37), abdominal obesity (OR 1.12 for top vs lowest tertile), psychosocial factors (OR 2.67), daily consumption of fruits and vegetables (OR 0.70), regular alcohol consumption (OR 0.91), and regular physical acitivity (OR 0.86), were all significantly related to acute myocardial infarction.	**Authors discussion** The ApoB/ApoA1-ratio was the most important risk factor in all geographical regions. Therefore, a substantial modification of its population distribution is important for wordwide reduction of myocardial infarction, including both population-based strategies and targeted treatments.
Conclusion	**Inclusion criteria:** <u>Cases</u> were eligible if they had characteristic symptoms plus electrocardiogram changes indicative of a new myocardial infarction. <u>Controls</u> were age- and sex matched per case. Controls had no previous diagnosis of heart disease or history of exertional chest pain. Both hospital-based controls (58%) and community-based controls (36%) were recruited, in addition to undocumented source controls (3%) and controls from the WHO MONICA study.	The combined effect of all nine risk factors yielded an OR of 333.7 (99% CI 230.2-483.9) and a PAR of 90.4% (99% CI 88.1-92.4), suggesting that these risk factors account for most of the risk of acute myocardial infarction in the study population.	**Strengths**
Abnormal lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, consumption of fruits, vegetables, and alcohol, and regular physical activity account for most of the risk of myocardial infarction worldwide in both sexes and at all ages in all regions.			- The large size of the study provide high power and precision in estimates both overall and i subgroups. - A dose-response effect can be observed when adding risk factors in the regression model. - Consistent results for different ethnic groups and geographical locations are reported.
Country	**Exposures:** Smoking, ApoB/ApoA1-ratio, history of hypertension, diabetes, abdominal obesity, psychosocial factors, daily consumption of fruits and vegetables, regular consumption of alcohol and regular physical acitivity.		**Limitations**
Study participants were recruited from 262 centres from 52 countries in Asia, Europe, the Middle East, Africa, Australia, North America and South America.	**Outcome:** First lifetime myocardial infarction. **Validation of exposure and outcome:** Structured questionnaires and physical examination were undertaken in the same manner in cases and controls.		- Case-control studies are unable to explore temporal sequence and causation. - Case-control studies are prone to selection bias. Control subjects were not recruited from the general population, but were hospitalized for something else than myocardial infarction.
Year data collection		Similar odds ratio were recorded in subgroup-analyses for men and women separately. However, the increased risk associated with hypertension and diabetes, and the protective effect of exercise and alcohol, seemed to be greater in women than in men.	- Some of the exposures were measured with high accuracy (e.g. smoking), whereas others were based on history and therefore ascertained with some error (e.g. diabetes and hypertension).
February 1999-March 2003.	**Statistical methods:** Estimated ORs and CIs were calculated using unconditional logistic regression, and their population attributable risk (PAR) were calculated.	Moreover, the study reports consistent and statistical significant excess risk of myocardial infarction in most regions of the world and in every ethnic groups.	

Study design: Case-control

Quality of evidence | Very low.

Aim	Material and methods	Results	Discussion/comments
To investigate the relation between family history of ischemic heart disease (IHD) and risk of acute non-fatal myocardial infarction (MI).	**Data foundation:** Cases with a first episode of nonfatal acute MI (AMI) admitted to a network of general hospitals in the area were recruited to the study. **Inclusion criteria:** Cases of AMI were eligible if they were coded according to the ICD-9 (International Classification of Diseases). Controls were recruited from the same area, admitted to the same hospitals for acute conditions in the same time period as cases. However, they had no history of IHD. **Exclusion criteria:** Subjects admitted to hospital for cardio- and cerebrovascular, neoplastic, metabolic, and recognized smoking- or alcohol related conditions were not eligible as controls.	**Main findings** 507 cases and 478 controls were recruited. The overall odds ratio for those having ≥1 first-degree relatives with IHD was 2.1, and 3.8 for ≥ 2 relatives. Furthermore, the OR for those with an affected parent or sibling were similar. The OR were also similar across strata of sex, and selected AMI risk factors, which were risk factors also in those with a positive family history. The OR was 5.3 for subjects with more than one first-degree relative with IHD, and at least one of them diagnosed below age 55 years.	**Authors discussion** In this study, the age range in cases and controls is rather wide (25-79y), and it is possible that mechanisms for AMI differ in younger and older cases. A small proportion of the population with a strong family history of IHD appears to be at very high risk of AMI. The authors point out that this subgroup analysis is based on small numbers, and that this may be a chance finding.

Conclusion
Family history of IHD in first-degree relatives is an independent risk factor for acute MI. Consequently, for these subjects it is particularly important to reduce exposure to environmental risk factors.

Country
Milan, Italy.

Year data collection
Between 1995 and 1999.

Exposures: Family history of acute MI, education level, smoking status, body mass index, diabetes, hypertension, total cholesterol levels.
Outcome: First lifetime acute myocardial infarction.
Validation of exposure and outcome: Interviews conducted using a structured questionnaire including personal and socio-demographic characteristics, anthropometric variables, physical activity, smoking and other lifestyle habits. In addition, a problem-oriented medical history was performed.
Statistical methods: Estimated odds ratios (ORs) and confidence intervals (CIs) were calculated using unconditional logistic regression.

	Total	
	OR	(95% CI)
Family history of IHD		
No	1[b]	
Yes	2.1	(1.5–2.8)
Number of affected relatives		
1	1.7	(1.3–2.4)
≥2	3.8	(2.1–6.9)
Affected relative		
Mother	2.1	(1.2–3.4)
Father	2.1	(1.4–3.0)
Sibling(s)	2.0	(1.3–3.1)

	Cases: controls	OR	95% CI
No family history	313:370	1[b]	
One relative, age ≥ 55	80:48	2.0	(1.3–3.1)
One relative, age < 55	35:24	1.4	(0.8–2.5)
≥2 relatives, age ≥55	17:9	1.9	(0.8–4.4)
≥2 relatives, age <55	26:6	5.3	(2.0–14)

Strengths
- Controls recruited from the same time period as cases.

Limitations
- The outcome variable was based on the ICD-classification code for AMI and not validated using strict criteria for IHD by an end-point committee. Hence, considerable misclassification of outcome is possible.
- The exposure variables are based on interviews. Recall-bias may lead to misclassification of exposure. Additionally, it is not clear how information regarding s-cholesterol levels were obtained.
- Case-control studies are unable to explore temporal sequence and causation.
- Case-control studies are prone to selection bias. Control subjects were not recruited from the general population, but were hospitalized for something else than MI.

Reference: Lind, Enga, Mathiesen et al. **Family History of Myocardial Infarction and Cause-Specific Risk of Myocardial Infarction and Venous Thromboembolism: The Tromsø Study**			**Study design:** Population-based cohort study
			Quality of evidence — MODERATE

Aim	Material and methods	Results	Discussion/comments
The study aimed to determine the risks of myocardial infarction (MI) and venous thromboembolism (VTE) by family history of MI (FHMI) using a cause-specific model and to explore whether atherosclerotic risk factors could explain the association between FHMI and VTE.	**Data foundation:** Study participants recruited from the fourth (1994-1995) and fifth (2001-2002) surveys of the Tromsø Study. A total of 27 806 subjects (26 957 from Tromsø 4 and 849 unique subjects from Tormsø 5 that did not participate in Tromsø 4) aged 25-97y participated in ≥1 of the surveys. **Exclusion criteria:** Subjects who did not consent to medical research (n=222); subjects not officially registered as inhabitants of the municipality of Tromsø at baseline (n=45); subjects with VTE (n=57) or MI (n=686) before baseline; and subjects with missing values of body mass index (BMI), systolic or diastolic blood pressure (BP), triglycerides, total cholesterol, high-density lipoproteins (HDL), smoking, diabetes or family history of MI before the age of 60 years (n=5 394) were excluded.	**Main findings:** 1 311 subjects with a validated diagnosis of incident MI and 428 subjects with an incident VTE event during a median of 15.8 years of follow-up were identified. FHMI was associated with a 52% increased risk of MI (adj. HR 1.52, 95% CI: 1.35-1.70) and a 26% increased risk of VTE (adj. HR 1.26, 95% CI: 1.02-1.55). The risk estimates by status of FHMI were highest for unprovoked deep vein thrombosis (adj. HR 1.69, 95% CI: 1.12-2.56), and the risk increased with increasing number of affected relatives. Modifiable atherosclerotic risk factors slightly altered the association between FHMI and MI, but had a negligible effect on the association between FHMI and VTE. The population ARs of MI and VTE by FHMI were 19% and 13%, respectively.	**Authors discussion:** The modifiable atherosclerotic risk factors are affected by both genetic and lifestyle factors. In the present study, such risk factors influenced the relationship between FHMI and MI by weakening the risk estimates in the multivariable adjusted model. Hence, environmental factors are likely to contribute to the increased risk of MI associated with FHMI. Conversely, the same atherosclerotic risk factors only modestly affected the risk of VTE by FHMI. The authors conclude that these findings indicate that atherosclerotic risk factors do not explain the observed association between FHMI and VTE and that the effect of FHMI on VTE risk has to be mediated through different mechanisms.

Conclusion

FHMI was associated with increased risk of both MI and VTE. The presence of atherosclerotic risk factors slightly diluted the association between FHMI and MI. Apparently, the association between FHMI and VTE applied to unprovoked deep vein thrombosis and was not explained by modifiable atherosclerotic risk factors.

Country

Tromsø, Norway.

Year data collection

1994-2001.

Exposure: Family history of MI (before the age of 60y).
Adjustment variables: Age, sex, BMI, systolic/diastolic BP, total cholesterol, HDL, triglycerides, diabetes mellitus, smoking.
Outcome: MI, VTE.

Validation of exposure and outcome: Exposure information was collected by self-administered questionnaires, blood samples, and physical examination. The outcomes were validated by an end-point committee by searching medical records using strict diagnostic criteria..

Statistical methods: Cox proportional hazard regression models were used to estimate crude and multivariable adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for MI, unprovoked and provoked VTE, DVT and PE by family history of MI. Population attributable risks (AR%) by FHMI were calculated.

Strengths:
- The prospective design.
- Long and complete follow-up.
- Large numbers of participants from a general population with a high attendance rate.

Limitations:
- Self-reported FHMI. Over- and under-reporting are possible.
- Regression dilution bias, as modifiable atherosclerotic risk factors may change over time (e.g. smoking, diabetes mellitus, hypertension, hypercholesterolemia).
- Residual confouding cannot be ruled out.

Reference: Siegerink, Maino, Algra, Rosendaal. Hypercoagulability and the risk of myocardial infarction and ischemic stroke in young women.			Study design: Case-control
			Quality of evidence — Low.

Aim	Material and methods	Results	Discussion/comments
To investigate the relation between markers of a hypercoagulable state and risk of myocardial infarction (MI) and ischemic stroke (IS).	**Data foundation:** The Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) study. **Inclusion criteria:** <u>Cases</u> were women aged 19-49 years who were diagnosed with an MI or non-cardioembolic IS in one of the 16 Dutch participating hospitals. <u>Controls</u> were women free from arterial thrombotic disease recruited through random digit dialing, which was frequency matched on age, year of event, and area of residence of all case groups. **Exposures:** A set of 29 prothrombotic factors were assessed.	**Main findings** 380 cases (205 MIs, 175 ISs) and 638 controls were recruited. 22 of the 29 (72%) prothrombotic risk factors had a relative odds ratio >1; for 12 (41%) it was >2; and for 5 (17%) it was >2.75. The five risk factors with the largest difference in assocations were high levels of FXI and FXII, kallikrein, the presence of lupus anticoagulans, and a genetic variation in the FXIII gene. 4 of the prothrombotic factors (14%) yielded a PAF>0.1 in the MI analyses. The impact of the same factors on IS incidence was greater: 14 (48%) of the prothrombotic factors yielded a PAF>0.1 in the IS analyses.	**Authors discussion** Results from this study suggest that, in young women, prothrombotic risk factors have a stronger impact on IS than MI risk. Up to 20-30% of the IS incidence may be attributed to the prothrombotic factors studies. The authors point out that further studies are needed in order to investigate the role of hypercoagulability in subtypes of IS, especially the concept of *paradoxical embolism* through a patent foramen ovale.
Conclusion			
Prothrombotic factors are associated with both MI- and IS risk. However, the same factors investigated have a stronger impact on IS than MI risk.			**Strengths** - Controls matched on age, time period and area of residence of all case groups. - Random digit dialing as a method for recruiting controls. Reduces the risk of non-response bias, therefore, enhancing external validity. - The outcome variables was based on the ICD-classification code for acute MI and IS and validated by using strict criteria.
Country	**Outcome:** First lifetime acute MI and IS. **Validation of exposure and outcome:** Citrate plasma samples were collected and analyzed. Interviews were conducted using a structured questionnaire including personal and socio-demographic characteristics, anthropometric variables, physical activity, smoking and other lifestyle habits.		
The Netherlands.			
Year data collection			**Limitations** - The investigators was not able to distinguish between subtypes of IS. - The confounding variables are based on interviews. Recall-bias may lead to misclassification. - Case-control studies are unable to explore temporal sequence and causation. Hence, reverse causation is an issue.
Between 1990 and 1995.	MIs were found by searching hospital databases using relevant ICD-codes for acute MI and validated with the following criteria; 1) presence of symptoms 2) elevated cardiac-enzymes level and 3) electrocardiographic changes (Tanis et al., 2001). ISs was diagnosed on the basis of medical history, neurological examination and CT/MRI scans (Kemmeren et al., 2002). **Statistical methods:** Estimated odds ratios (ORs) and confidence intervals (CIs) were calculated using unconditional logistic regression. Population attributable fractions (PAFs) were ultimately calculated.	 PAF from MI represented by *blue square*, whereas the ischemic stroke results are denoted by *red dots*.	