NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL BIOLOGY
MOLECULAR MEDICINE PROGRAM

CYP2C19 POLYMORPHISMS IN PATIENTS WITH THROMBOSIS-ASSOCIATED MISCARRIAGE IN NORTHERN CYPRUS

M.Sc. THESIS

Abdulwahab Muhammad MUSTAPHA

Nicosia
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Approval

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Declaration

I hereby declare that all information, documents, analysis, and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also declare that, as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Abdulwahab MUHAMMAD MUSTAPHA

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ABDULWAHAB MUHAMMAD MUSTAPHA

ABSTRACT

CYP2C19 POLYMORPHISMS IN PATIENTS WITH THROMBOSIS-ASSOCIATED MISCARRIAGE IN NORTHERN CYPRUS

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Introduction: Miscarriage, particularly when recurrent or associated with thrombosis, is a significant reproductive health issue. Thrombophilia, an increased tendency towards blood clotting, is a known risk factor. While established genetic thrombophilia are often investigated, exploring other potential genetic contributors is important. The *CYP2C19* gene encodes an enzyme involved in metabolizing various compounds; common polymorphisms in this gene (*CYP2C19*2*, *3*, *17*) significantly alter its activity. While well-studied for their impact on drug metabolism (e.g., clopidogrel), the frequency and potential relevance of these polymorphisms in specific populations experiencing thrombosis-related pregnancy complications, independent of drug exposure, require investigation. Data on *CYP2C19* allele frequencies in the TRNC population, particularly in this context, are limited. We aimed to determine the allele and genotype frequencies of the functionally significant *CYP2C19* polymorphisms *2 (c.681G>A), *3 (c.636G>A), and *17 (c.-806C>T) in women residing in the TRNC with a history of thrombosis-associated miscarriage.

Methodology: Women with a history of thrombosis-associated miscarriage, of Turkish Cypriot ethnicity, and meeting age criteria (16-42 years) were recruited. Following informed consent, genomic DNA was extracted from peripheral blood samples using a commercial kit. Genotyping for the *CYP2C19*2*, *3*, and *17* variants was performed using validated real-time PCR 5' nuclease assays. Final sample sizes for analysis were N=37 for *3 and N=42 for *2 and *17, following initial recruitment and exclusions based on sample quality/quantity. Hardy-Weinberg Equilibrium (HWE) was assessed for each polymorphism using the Pearson Chi-Square test ($p < 0.05$ significance threshold).

Results: The calculated allele frequencies in this cohort were: *CYP2C19*2* (loss-of-function) = 0.38 (38%); *CYP2C19*3* (loss-of-function) = 0.40 (40%); and *CYP2C19*17* (gain-of-function) = 0.44 (44%). Genotype distributions for *CYP2C19*3* ($p=0.827$) and *17* ($p=0.520$) were found to

be in HWE. However, the genotype distribution for *CYP2C19*2* showed a significant deviation from HWE (p=0.0113) in this specific group.

Conclusion: This study provides the first assessment of *CYP2C19*2*, *3, and *17 allele frequencies in women with thrombosis-associated miscarriage in Northern Cyprus. The findings reveal a high prevalence of both loss-of-function (**2* and **3*) and gain-of-function (**17*) alleles within this cohort, suggesting significant inter-individual variability in potential CYP2C19 enzyme activity. While these results establish important baseline genetic data, they do not, in isolation, confirm a direct causal link between these polymorphisms and miscarriage risk. The deviation from HWE for *CYP2C19*2* warrants further investigation. Future larger case-control studies are necessary to explore potential associations and the clinical relevance of *CYP2C19* variants in the context of thrombosis-related pregnancy complications in this population.

Keywords: Recurrent Miscarriage, CYP2C19, Polymorphism, Thrombophilia, Allele Frequency, Northern Cyprus, Genetic Variation, Recurrent Pregnancy Loss

List of Abbreviations

APS: Antiphospholipid Syndrome

CAT: cancer-associated thrombosis

DVT: Deep vein thrombosis

EDTA: Ethylenediaminetetraacetic acid

GOF: Gain-of-function

HWE: Hardy-Weinberg Equilibrium

HIT: heparin-induced thrombocytopenia

IM: Intermediate metabolizer

LMWH: Low-molecular-weight heparin

LOF: Loss-of-function

PE: Pulmonary embolism

PTS: Post-thrombotic syndrome

PGT: Genetic Testing

SNP: Nucleotide Polymorphism

TRNC: Turkish Republic of Northern Cyprus

UM: Ultra-rapid metabolizer

VTE: Venous thromboembolism

Table of Contents

List of Tables

List of Figures

CHAPTER I

INTRODUCTION

Miscarriage, a common complication of early pregnancy, has multiple underlying causes, including chromosomal abnormalities, uterine abnormalities, hormonal imbalances, and thrombophilia, a condition characterized by abnormal blood coagulation (Magnus et al., 2019). Thrombophilia significantly increases the risk of thrombosis, or excessive blood clot formation, which can obstruct blood flow and lead to serious complications, including deep vein thrombosis (DVT), pulmonary embolism (PE), stroke, and adverse pregnancy outcomes (Wen et al., 2023). While blood clot formation is a normal physiological response to prevent excessive bleeding, an imbalance in clotting factors in thrombophilia leads to hypercoagulability, heightening the risk of thromboembolic event (Ahangari et al., 2019).

Pregnancy itself is a hypercoagulable state, increasing the risk of venous thromboembolism (VTE), including DVT and PE. Studies indicate that pregnant women are four to five times more likely to experience thromboembolism compared to non-pregnant individuals due to venous stasis, hypercoagulability, and endothelial injury (Nana et al., 2021). This increased clotting tendency poses a substantial maternal health risk, as PE remains one of the leading causes of maternal mortality in developed countries (Sedano-Balbás et al., 2011). Moreover, women who develop DVT may suffer from long-term complications such as post-thrombotic syndrome (PTS), which can further impact maternal health and future pregnancies. Proactive management strategies, including anticoagulation therapy, are crucial for women with known thrombophilic conditions or previous thrombotic events to mitigate these risks (Ahangari et al., 2019). The relationship between thrombophilia and pregnancy loss highlights the importance of genetic screening and personalized medical interventions (Coulam et al., 2006).

Cytochrome P450 2C19 (CYP2C19) is a crucial enzyme in the metabolism of various drugs, including clopidogrel, proton pump inhibitors, selective serotonin reuptake inhibitors, anticonvulsants, and some chemotherapeutic agents (Saydam et al., 2017). It belongs to the cytochrome P450 superfamily and is responsible for phase I drug metabolism. CYP2C19 catalyzes

oxidation reactions, converting lipophilic compounds into hydrophilic metabolites for elimination. CYP2C19 functions include product activation, drug detoxification and clearance, and synthesis of endogenous compounds (Sukprasong et al., 2021).

The CYP2C19 gene is highly polymorphic, with major allelic variants classified into loss-of-function, gain-of-function, and normal function alleles. Clopidogrel metabolism is highly dependent on CYP2C19 activity, as it is required for its conversion into an active metabolite. The combination of inherited alleles determines an individual's drug metabolism capacity(Shin et al., 2012). CYP2C19 polymorphisms are categorized into poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultra-rapid metabolizers (Tiroch et al., 2010). Poor Metabolizers (PM): Typically carry two loss-of-function (LOF) alleles (e.g., *2/*2, *3/*3, *2/*3). The *2 and *3 alleles are the most common LOF alleles. Intermediate Metabolizers (IM): Typically carry one functional allele and one LOF allele (e.g., *1/*2, *1/*3). The *1 allele is the normal function (wild-type) allele. Normal Metabolizers (NM): (Often referred to as Extensive Metabolizers - EM) Typically carry two functional alleles (e.g., *1/*1). Rapid Metabolizers (RM) / Ultra-Rapid Metabolizers (UM): Carry at least one increased function allele, most commonly 17, without any LOF alleles (e.g., *1/*17, *17/*17). The presence of *17 can sometimes override a LOF allele depending on specific guidelines (e.g. *2/*17 might be classified as NM or IM). Individuals carrying two LOF alleles have severely impaired Clopidogrel activation, leading to increased risk of stent thrombosis and recurrent miscarriage (RM) (Christofolini et al., 2015). Carrying two LOF alleles defines the Poor Metabolizer phenotype. This is strongly and consistently associated with impaired Clopidogrel activation and a significantly increased risk of Major Adverse Cardiovascular Events (MACE), particularly stent thrombosis, after procedures like Percutaneous Coronary Intervention (PCI) (El Ghannudi et al., 2010).

PROBLEM STATEMENT

Miscarriage, particularly when recurrent or associated with thrombotic events, represents a significant reproductive health challenge. Thrombophilia, an increased tendency to form blood clots, is a known risk factor for such pregnancy complications. While the genetic basis of thrombophilia is complex and involves multiple factors, exploring novel potential genetic contributors is crucial for better understanding and risk assessment.

The cytochrome P450 2C19 (CYP2C19) enzyme plays a well-established role in metabolizing various compounds, including some medications. Genetic variations (polymorphisms) in the *CYP2C19* gene can significantly alter enzyme activity. While extensively studied for their impact on drug response (like Clopidogrel), the potential role of these polymorphisms in influencing underlying physiological processes related to thrombosis or pregnancy maintenance, independent of drug exposure, is less understood. Investigating whether *CYP2C19* variants are associated with susceptibility to thrombosis-related pregnancy complications is therefore warranted.

In the Turkish Republic of Northern Cyprus (TRNC), there is limited data on the prevalence of *CYP2C19* polymorphisms within the general population, and specifically among women who have experienced thrombosis-associated miscarriage. Characterizing the distribution of these genetic variants in this specific patient group is an essential first step. Understanding the allelic frequencies could provide insights into whether certain *CYP2C19* genotypes are overrepresented in women with this condition, potentially indicating a role for this gene in the pathophysiology of thrombosis-associated miscarriage.

This research aims to address this knowledge gap by analyzing the allelic frequencies of clinically relevant *CYP2C19* polymorphisms in women with a history of thrombosis-associated miscarriage in the TRNC. The findings will contribute to understanding the genetic landscape of this population and explore a potential association between *CYP2C19* variants and the risk of miscarriage linked to thrombotic events.

AIM AND OBJECTIVE OF THIS STUDY

Genetic factors are known to contribute to thrombophilia and adverse pregnancy outcomes. While the role of *CYP2C19* polymorphisms in drug metabolism is well-established, their potential direct influence on thrombotic risk or pregnancy complications, independent of medication, warrants investigation. Understanding the prevalence of these variants in specific high-risk groups is a crucial first step.

This study aims:

1. To determine the allele and genotype frequencies of clinically relevant *CYP2C19* polymorphisms (specifically *2, *3, and *17) in women residing in the TRNC with a history of thrombosis-associated miscarriage.

2. To explore a potential association between these *CYP2C19* variants and the susceptibility to experiencing thrombosis-associated miscarriage in this specific population.

HYPOTHESIS OF THIS STUDY

The frequencies of specific *CYP2C19* polymorphisms (*2, *3, *17) in women with thrombosis-associated miscarriage in the TRNC may differ significantly from those observed in general or reference populations, suggesting a potential association between these genetic variants and the predisposition to this condition.

CHAPTER II

Epidemiology and Etiology of Thrombosis

Thrombosis is defined as the pathological formation of a blood clot (thrombus) within a blood vessel (artery or vein) or heart chamber, which obstructs normal blood flow. This process occurs due to the inappropriate activation of the coagulation cascade, leading to the conversion of fibrinogen into fibrin. The fibrin mesh traps blood cells, forming a solid clot that can cause ischemia (reduced blood supply) locally or detach and travel as an embolus to distant sites (Coulam et al., 2006).

The incidence and prevalence of thrombosis vary based on population demographics, risk factors, and genetic predisposition. According to the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), venous thromboembolism (VTE) affects 1 to 2 per 1,000 individuals annually, while arterial thrombosis, including stroke and myocardial infarction, is the leading cause of global mortality(Zhang et al., 2024). Up to 10 million cases of VTE occur worldwide each year, with mortality rates ranging between 10% and 30% within 30 days of diagnosis (Hamulyák et al., 2021). While specific epidemiological data for the TRNC are limited, thrombosis is recognized as an important health concern within the region, influenced by general risk factors prevalent globally.

The etiology of thrombosis is multifactorial, involving an interplay of genetic predisposition and acquired risk factors (components of Virchow's triad encompass endothelial damage, turbulent blood flow, and an elevated state of coagulability).

Key acquired risk factors include increasing age, major surgery or trauma, hospitalization and prolonged immobility, malignancy, obesity, smoking, and the use of certain medications like estrogen-containing contraceptives (Previtali et al., 2011). Crucially, pregnancy and the postpartum period are well-established hypercoagulable states, significantly increasing the risk of VTE in women of reproductive age. Inherited factors, collectively known as thrombophilia, also contribute substantially to thrombosis risk. These include common genetic variants like Factor V Leiden and the Prothrombin G20210A mutation, as well as rarer deficiencies in natural anticoagulants, predisposing individuals to thrombotic events, sometimes manifesting in specific situations like pregnancy (Alsheef et al., 2020).

Risk Factors for Thrombosis

Thrombosis development is typically influenced by a combination of acquired and inherited risk factors, often conceptualized through Virchow's triad (endothelial injury, stasis of blood flow, and hypercoagulability).

Acquired risk factors

Numerous acquired conditions and situations can increase the risk of thrombosis. These include:

- **Immobility and Stasis:** Prolonged bed rest, hospitalization, long-distance travel, and limb paralysis reduce blood flow, promoting clot formation (Lichota et al., 2020).
- **Tissue Injury/Inflammation:** Major surgery, significant trauma, infections (sepsis), inflammatory conditions (like inflammatory bowel disease), and malignancy trigger coagulation pathways (Liu et al., 2021).
- **Medical Conditions:** Chronic diseases such as heart failure, nephrotic syndrome, certain autoimmune disorders, myeloproliferative neoplasms, and obesity are associated with increased thrombotic risk (Leiva et al., 2022)
- **Medications and Hormones:** Estrogen-containing therapies (oral contraceptives, hormone replacement therapy), selective estrogen receptor modulators (SERMs), and certain chemotherapy agents can elevate risk (Neilson et al., 2010). Heparin-induced thrombocytopenia (HIT), an immune reaction to heparin, paradoxically increases clotting risk (I. Ahmed et al., 2007).
- **Indwelling Devices:** Central venous catheters and peripherally inserted central catheter (PICC) lines can cause endothelial injury and alter blood flow, predisposing to thrombosis (Neilson et al., 2010).
- **Antiphospholipid Syndrome (APS):** APS is a significant autoimmune cause of acquired thrombophilia, characterized by the presence of specific autoantibodies (lupus anticoagulant, anti-cardiolipin antibodies, anti-β2 glycoprotein-I antibodies). It is strongly linked to both recurrent arterial/venous thrombosis and adverse pregnancy outcomes, including RM. Diagnosis often involves clinical criteria (thrombosis or specific pregnancy morbidity) combined with persistently positive antibody tests (Sedano-Balbás et al., 2011).

Inherited risk factors

Inheritable thrombophilia is not linked to early pregnancy loss but are significantly correlated with still births after 20 weeks of gestation (Ahangari et al., 2019). Following a duration of 22 weeks, it was substantially associated with isolated and repeated fetal loss. Genetically, inherited thrombophilia follows an autosomal dominant pattern with variable penetrance in women with unexplained RM (Liu et al., 2021).

The genetic causes of thrombophilia include the G20210A prothrombin gene mutation increases prothrombin levels, increasing the risk of venous thrombosis, placental complications, and potentially impairing placental circulation, leading to miscarriages or stillbirth (Ahangari et al., 2019). Prothrombin gene mutant subtypes are linked to non-recurrent late fetal loss, recurrent fetal loss before 18 to 25 weeks, and recurrent first-trimester fetal loss.

Factor V Leiden (FVL) mutation causes resistance to activated protein C and results in prolonged clotting activity. Heterozygous carriers have a 5- to 7-fold increased risk of thrombosis, while homozygous carriers have up to an 80-fold increased risk (Chen et al., 2024). After 19 weeks, factor V Leiden is linked to non-recurrent fetal loss. (Al-Allawi et al., 2014). Protein C deficiency, a natural anticoagulant, leads to excess clotting and increases thrombosis risk, particularly in pregnancy, due to its degradation of factors Va and VIIIa. While mutated activated protein C resistance and protein S insufficiency, which are frequently acquired during pregnancy or inherited, are linked to recurrent fetal loss after 22 weeks and recurrent fetal loss in the first trimester, they increase the risk of deep vein thrombosis and obstetric complications(Patil et al., 2015).

Genetic predisposition plays a crucial role in thrombotic risk. Inherited thrombophilia typically follows an autosomal dominant pattern with variable penetrance, meaning not everyone with the genetic trait will develop thrombosis (Khan et al., 2006). Although there is dispute in the research about the link between hereditary thrombophilia and early recurrent pregnancy loss, their link to later pregnancy complications, such as stillbirth or fetal loss after 20-22 weeks, is more clearly established (Alecsandru et al., 2021). Key inherited risk factors include:

- **Factor V Leiden (FVL) Mutation (G1691A):** This common mutation causes resistance to activated protein C (APC), a natural anticoagulant, leading to prolonged clotting activity.

Heterozygous carriers face a 5- to 7-fold increased VTE risk, while homozygous risk is significantly higher (up to 80-fold) (Chen et al., 2024). FVL has been associated with late fetal loss (Al-Allawi et al., 2014, Ahangari et al., 2019).

- **Prothrombin Gene Mutation (G20210A):** This mutation leads to elevated prothrombin levels, increasing the propensity for clot formation and the risk of VTE. It has been implicated in placental complications and various patterns of fetal loss, potentially including recurrent first-trimester loss in some studies, although its role in early loss remains debated (Kupferminc, 2003).

- **Protein C and Protein S Deficiencies:** Proteins C and S are natural anticoagulants that regulate factors Va and VIIIa. Inherited deficiencies impair this regulation, leading to a hypercoagulable state and increased risk of VTE. These deficiencies have been associated with obstetric complications, particularly later fetal loss (Patil et al., 2015, Ahangari et al., 2019).

- **Antithrombin Deficiency:** Antithrombin is another crucial natural anticoagulant. Its deficiency, though rare, significantly increases thrombosis risk (Marco-Rico et al., 2023).

Pathophysiology of Thrombosis

Thrombosis occurs due to a disruption in the balance between coagulation and fibrinolysis, which can be categorized under Virchow's Triad, describing the three major factors that contribute to clot formation: endothelial injury, hypercoagulability, and venous stasis (Kumar et al., 2010, Bagot et al., 2008).

1. **Endothelial Injury/Dysfunction:** Under normal conditions, the vascular endothelium provides a crucial anticoagulant and antiplatelet surface. It expresses molecules like thrombomodulin (which activates the Protein C anticoagulant pathway) and releases substances (e.g., prostacyclin, nitric oxide) that inhibit platelet aggregation and promote vasodilation (Kumar et al., 2010, Boeldt et al., 2017). However, injury or activation of the endothelium disrupts this protective barrier. Damage can expose underlying procoagulant tissue factor and von Willebrand factor, initiating the coagulation cascade and facilitating platelet adhesion and aggregation (Posthuma et al., 2015). Endothelial dysfunction, often preceding overt injury, involves a shift towards a procoagulant, pro-inflammatory state

with reduced anticoagulant properties. Common causes of endothelial injury or dysfunction include physical trauma, surgery, inflammation (e.g., vasculitis, sepsis), hypertension, turbulent blood flow (e.g., at atherosclerotic plaques), metabolic factors (e.g., diabetes, hyperlipidemia), and toxins (e.g., cigarette smoke) (Bagot et al., 2008,Stone et al., 2017).

2. **Alterations in Blood Flow (Stasis and Turbulence)**: Normal laminar blood flow helps maintain endothelial health and prevents the accumulation of procoagulant factors. Disruptions in flow significantly contribute to thrombosis (Chiu et al., 2011).

Stasis (Slow Blood Flow): Venous stasis allows activated clotting factors to accumulate locally, overwhelming natural inhibitors. It also increases the contact time between platelets and the endothelium, promoting adhesion (Cines et al., 1998). Stasis is common in situations like prolonged immobility (e.g., bed rest, long travel), paralysis, heart failure, varicose veins, and external compression of veins (e.g., by pregnancy or tumors) (Kumar et al., 2010).

Turbulence: While stasis is key in venous thrombosis, turbulent flow (disordered flow with eddy currents), often occurring at sites of arterial narrowing (atherosclerosis) or around damaged heart valves, can directly cause endothelial injury/activation and enhance platelet aggregation (Vincelj et al., 2002, Bagot et al., 2008).

3. **Hypercoagulability (Thrombophilia):** This refers to an increased tendency of the blood to clot due to alterations in the constituents of the blood itself. Hypercoagulability can arise from inherited conditions (e.g., Factor V Leiden, prothrombin gene mutation, deficiencies of Protein C, S, or antithrombin) or acquired states (e.g., malignancy, pregnancy, estrogen therapy, inflammatory diseases, antiphospholipid syndrome) (Chan et al., 2010). In these states, the balance is tipped towards excessive coagulation due to increased levels/activity of procoagulant factors or decreased levels/activity of natural anticoagulants or fibrinolytic components (Palta et al., 2014).

These three elements of Virchow's Triad are interconnected and often coexist, synergistically increasing the risk of thrombosis (Kumar et al., 2010). For instance, stasis can promote endothelial dysfunction, and inflammation can cause both endothelial injury and a hypercoagulable state **(Table 1)**.

Table 1: Description of the three major factors that contribute to clot formation according to Virchow's Triad concept

Factor	Description	Example Conditions
Endothelial Injury	Damage or dysfunction of the blood vessel lining promotes clot formation.	Hypertension, Atherosclerosis, Trauma, Sepsis
Hypercoagulability	A higher risk of blood clotting as a result of inherited or acquired diseases.	Thrombophilia, Cancer, Pregnancy, APS
Alterations in blood flow	Slow blood flow increases clot risk, especially in the deep veins of the legs.	Bed rest, Prolonged immobilization, Heart failure

Increased Risk of Thrombosis During Pregnancy and Postpartum

Pregnancy induces significant physiological changes that create a temporary hypercoagulable state, primarily viewed as an evolutionary adaptation to minimize blood loss during childbirth (Dimitriadis et al., 2020). However, this prothrombotic shift markedly increases the risk of venous thromboembolism (VTE) for the duration of pregnancy and, most notably, during the postpartum period.

The risk of VTE is estimated to be 4 to 6 times higher in pregnant and postpartum women compared to their non-pregnant peers, making thromboembolic events a leading cause of maternal morbidity and mortality, particularly in developed countries (Wendelboe et al., 2016). This risk is not uniform; it increases progressively throughout gestation, peaking significantly in the first six weeks following delivery (Kumar et al., 2010).

The heightened thrombotic risk associated with pregnancy stems from alterations affecting all three components of Virchow's Triad:

Hypercoagulability: Hormonal changes lead elevated production of coagulation factors (fibrinogen, Factors VII, VIII, X) and decreased activity of natural anticoagulant pathways (e.g., reduced Protein S levels) (Dimitriadis et al., 2020).

1. Venous Stasis: The enlarging uterus compresses pelvic veins (particularly the iliac veins), impairing venous return from the lower extremities. Reduced mobility in late pregnancy can also contribute (Phillips et al., 2014).

2. Vascular Changes/Injury: While less prominent than the other two factors throughout pregnancy, endothelial changes can occur, and potential vessel injury during labor and delivery can further contribute to risk at that specific time (Kumar et al., 2010)

While pregnancy itself is a potent risk factor, the likelihood of VTE is substantially magnified in women with additional underlying risk factors. A personal history of VTE is the strongest predictor. Furthermore, women with inherited thrombophilia, such as Factor V Leiden, the Prothrombin G20210A mutation, or deficiencies in Protein C, Protein S, or antithrombin, experience a synergistic increase in their VTE risk when combined with the prothrombotic state of pregnancy (Esmons, 1989; Wen et al., 2023).

The Role of Thrombosis in Recurrent Miscarriage (RM)

RM, also termed Recurrent Pregnancy Loss (RPL), is classically defined as the loss of two or three consecutive pregnancies before 20-24 weeks of gestation. Affecting 1–5% of couples attempting conception, RM has diverse causes, including genetic, anatomical, endocrine, and immunological factors (Lin et al., 2019). Thrombotic disorders, encompassing both inherited and acquired thrombophilia, are recognized contributors, potentially underlying a significant portion (estimated ~15–20%) of cases previously considered unexplained(Shaker et al., 2021).

The primary mechanism by which thrombophilia is thought to cause miscarriage involves impaired placental development and function due to microvascular thrombosis. The hypercoagulable state associated with thrombophilia (either baseline or exacerbated by pregnancy) can lead to the formation of microthrombi within the spiral arteries and intervillous spaces of the developing placenta (Hosseinzadegan et al., 2017). This micro thrombosis can result in:

- **Reduced Uteroplacental Blood Flow:** Obstructed vessels compromise the delivery of oxygen and nutrients to the fetus (Thornburg et al., 2013).
- **Defective Trophoblast Invasion:** Proper invasion of maternal spiral arteries by fetal trophoblast cells is crucial for establishing adequate blood flow; thrombosis can hinder this process (Whitley et al., 2010).
- **Placental Infarction:** Areas of placental tissue may die due to a lack of blood supply (Lei et al., 2022).
- **Inflammation and Oxidative Stress:** Thrombotic events can trigger local inflammatory responses and oxidative stress, further damaging placental cells and potentially contributing to pregnancy failure (Hosseinzadegan et al., 2017).

Specific Thrombophilia Implicated in RM:

- **Antiphospholipid Syndrome (APS):** This acquired autoimmune disorder is the most strongly established thrombotic cause of RM (Del Papa et al., 2010). Antiphospholipid antibodies (APL) are believed to cause pregnancy loss not only via direct prothrombotic effects (e.g., increasing tissue factor expression, potentially interfering with the natural anticoagulant annexin A5 shield on trophoblasts) but also through direct inflammatory and complement-mediated injury to placental cells (Esmons, 1989, Shaker et al., 2021). Diagnosis relies on clinical criteria (specific pregnancy morbidity like recurrent early miscarriages or later fetal death) combined with persistently positive laboratory tests for relevant antibodies (lupus anticoagulant, anticardiolipin, anti-β2 glycoprotein-I)(Devreese et al., 2021).
- **Inherited Thrombophilia:** The role of common inherited thrombophilia, such as Factor V Leiden (FVL) and the Prothrombin G20210A mutation, in causing early RM is more controversial, with conflicting study results. While strongly associated with later pregnancy complications and VTE, their impact on first-trimester loss is less certain (Ahangari et al., 2019, Lin et al., 2019). Rarer, more potent inherited deficiencies (Protein C, Protein S, Antithrombin) may carry a higher risk, particularly when combined or severe (Van Dijk et al., 2020).

Management of Thrombosis in RM:

The established link between APS and RM has led to effective treatment strategies. Standard therapy involving low-dose aspirin (LDA) combined with prophylactic doses of heparin (usually low-molecular-weight heparin, LMWH) significantly improves live birth rates in women with APS and RM, potentially from <20% untreated to around 70-80% with treatment (S. Ahmed et al., 2016). The benefit of anticoagulation for women with RM and *inherited* thrombophilia (but no personal history of VTE) is not well-established, and treatment decisions are often individualized (Van Dijk et al., 2020).

CYP2C19 Gene Polymorphisms and Functional Variability

Cytochrome P450 2C19 (CYP2C19) is an enzyme belonging to the cytochrome P450 superfamily, located primarily in the liver. Like other P450 enzymes, it plays a crucial role in the metabolism

of a wide range of endogenous substances and exogenous compounds, including numerous clinically used drugs (Schenkman et al., 2003).

The *CYP2C19* gene is highly polymorphic, meaning common variations (alleles) exist within the population. These genetic variations can significantly alter the enzyme's activity, leading to substantial inter-individual differences in metabolic capacity (Xu et al., 2018). Several key alleles have been well characterized based on their functional impact **(Table 2)**:

- **Normal Function (NF) Allele:** The *CYP2C19*1* allele is considered the wild-type or reference allele, associated with normal enzyme activity.
- **Loss-of-Function (LOF) Alleles:** Alleles like *CYP2C19*2* (c.681G>A) and *CYP2C19*3* (c.636G>A) result in non-functional or significantly reduced-function enzymes due to splicing defects or premature stop codons, respectively.
- **Gain-of-Function (GOF) Allele:** The *CYP2C19*17* allele (c.-806C>T), located in the promoter region, leads to increased enzyme transcription and thus higher-than-normal enzyme activity.

Based on the combination of these alleles inherited by an individual (their genotype), distinct metabolic phenotypes are predicted:

- **Poor Metabolizers (PM):** Individuals with two LOF alleles (e.g., *2/*2, *2/*3, *3/*3).
- **Intermediate Metabolizers (IM):** Individuals with one NF allele and one LOF allele (e.g., *1/*2, *1/*3) or potentially combinations involving decreased function alleles not discussed here.
- **Normal Metabolizers (NM):** Individuals with two NF alleles (*1/*1). *(Note: Often referred to as Extensive Metabolizers (EM) in older literature)*.
- **Ultrarapid Metabolizers (UM):** Individuals with one or two copies of the GOF allele (*1/*17, *17/*17).

The clinical significance of these polymorphisms is most profoundly demonstrated in pharmacogenetics, particularly concerning the antiplatelet drug clopidogrel. Clopidogrel is a

prodrug requiring metabolic activation by CYP2C19 to exert its therapeutic effect (Brown et al., 2018a). Consequently:

- PMs and IMs exhibit reduced conversion of clopidogrel to its active form, leading to diminished platelet inhibition and increased risk of thrombotic events (e.g., stent thrombosis) in cardiovascular patients treated with standard doses (Gower et al., 2020)
- UMs may metabolize clopidogrel more rapidly, which could potentially alter drug exposure profiles, although the clinical impact regarding bleeding risk is less consistently defined than the thrombotic risk in PMs (Zanger et al., 2013).

While the impact of *CYP2C19* variants on drug metabolism, exemplified by clopidogrel, is well-established and has led to pharmacogenetic testing recommendations in specific clinical contexts (e.g., post-PCI) (Perry et al., 2013).The potential role of this enzyme's variability in other physiological processes is less understood. Given that CYP enzymes metabolize endogenous substrates, it is conceivable that genetically determined alterations in CYP2C19 activity could influence pathways relevant to hemostasis or pregnancy physiology, independent of drug exposure. However, direct evidence linking *CYP2C19* polymorphisms to baseline thrombosis risk or miscarriage susceptibility (outside of drug response contexts) is currently limited and speculative (Brown et al., 2018b).

Therefore, investigating the frequency distribution of these functionally relevant *CYP2C19* polymorphisms (*2, *3, *17) in specific populations, such as women experiencing thrombosis-associated miscarriage in the TRNC, is a crucial first step. Such data can help determine if altered CYP2C19 function might be associated with the condition and provide a foundation for future mechanistic studies exploring potential non-pharmacogenetic roles of this enzyme (Alrajeh et al., 2022).

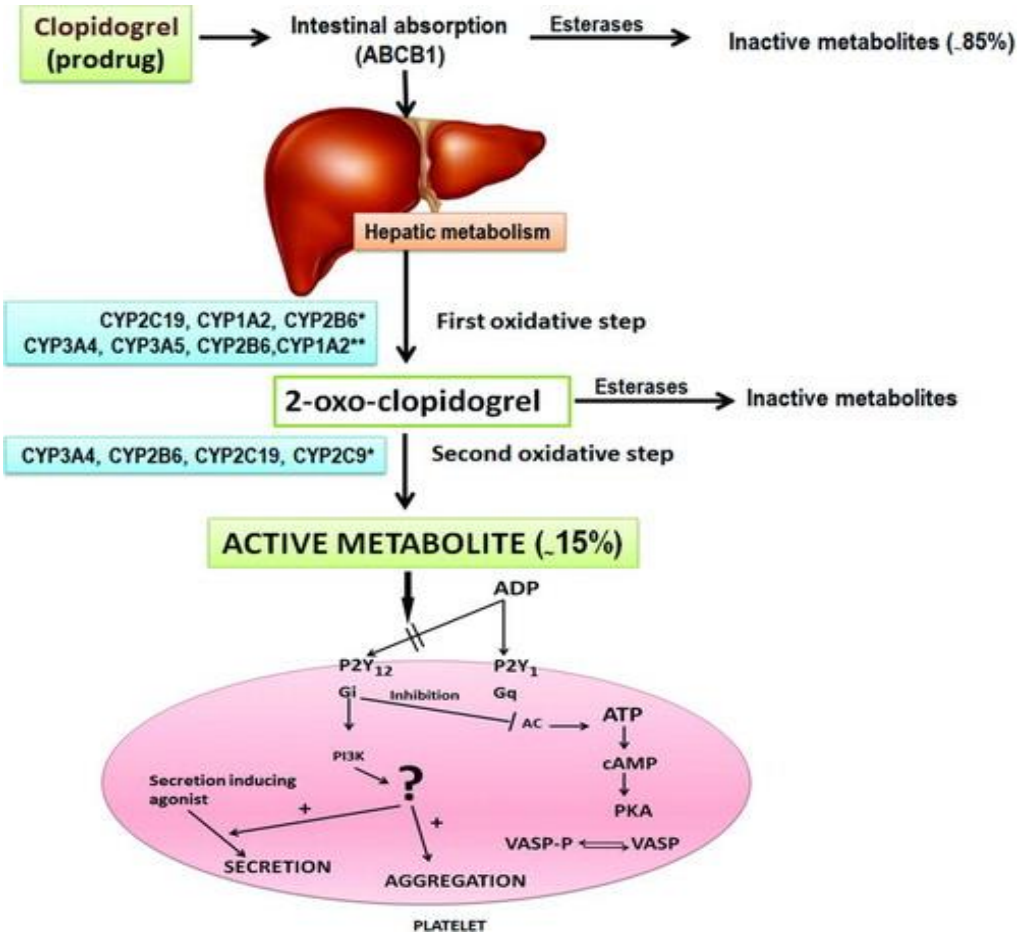
Table 2: Major functional alleles of the *CYP2C19* gene

Allele	**Common Nucleotide Change**	**Functional Consequence**	**Associated Phenotype Contribution**
*CYP2C19*1*	Wild-type	Normal enzyme activity	Normal Function (NF)
*CYP2C19*2*	c.681G>A	Splicing defect leading to non-functional enzyme	Loss-of-Function (LOF)
*CYP2C19*3*	c.636G>A	Premature stop codon leading to non-functional enzyme	Loss-of-Function (LOF)
*CYP2C19*17*	c.-806C>T	Increased transcription leading to increased activity	Gain-of-Function (GOF)

Note: Phenotypes like IM: Intermediate metabolizer; NM: Normal metabolizer; PM: Poor metabolizer; RM: Rapid metabolizer; UM: Ultra rapid metabolizer is determined by the combination of two alleles inherited.

Figure 1: Example of CYP2C19 metabolic activity

Activation of the prodrug Clopidogrel. Polymorphisms in *CYP2C19* alter this activation, illustrating the enzyme's pharmacogenetic importance (Adapted from,Hasan et al., 2013).



CHAPTER III

METHODOLOGY

Case Selection and Patient Information

We identified cases from the Medical Genetic Laboratory at the Near East University Hospital. Women experiencing RMs with a confirmed history of thrombosis were considered for inclusion in the study. The diagnosis of thrombosis-associated miscarriage was based on clinical history, laboratory findings, and prior medical records, including hypercoagulability markers, genetic predisposition, and pregnancy complications related to thrombophilia. The inclusion criteria were women between the ages of 16 to 45 with a history of recurrent spontaneous miscarriage, diagnosis or suspicion of thrombophilia disorders, availability of venous peripheral blood samples for genetic analysis, and Turkish Cypriot ethnicity. The exclusion criteria were insufficient quantity and quality of DNA sample, age, and other systemic diseases unrelated to thrombosis.

Sample Collection

Peripheral venous blood samples were collected using glass syringes into 2.5 mL EDTA tubes from 45 women with recent histories of recurrent spontaneous miscarriages, who presented to the laboratory for thrombosis gene panel testing between January and March 2025. DNA concentration and purity were subsequently evaluated.

DNA Extraction

Genomic DNA was extracted from whole blood using the Pure Link Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Genetic Analysis Using Clopidogrel Real-Time PCR Kits (3 Mutations)

CYP2C19 Genotyping

Genotyping for CYP2C19 loss-of-function alleles *2 (c.681G>A, rs4244285) and *3 (c.636G>A, rs4986893), and the gain-of-function allele *17 (c.-806C>T, rs12248560) was performed using a commercially available real-time PCR kit SNP Biotechnology R&D Ltd CLOPIDOGREL REAL TIME PCR KIT, Cat. No: 113R-20-03. Research has implicated genetic variations in the *CYP2C19* isozyme as at least partly responsible for the variable antiplatelet response seen with clopidogrel.

Product Specification

The assays utilize the 5' nuclease (TaqMan®) principle for allele discrimination. Reactions were prepared using the provided master mixes containing allele-specific primers and probes labeled with FAM, JOE/HEX, and Texas Red dyes, along with an internal control (CY5 dye). PCR amplification and real-time detection were carried out by the Real-Time PCR detection system. An increase in fluorescent signal (CT) is proportional to the amount of the specific PCR product. Each isolated DNA should be tested with wild-type and mutant real-time PCR master mixes **(Table 3)**.

Table 3: Contents of Clopidogrel real-time PCR kits for 3 mutations (636 G>A *3, 681 G>A *2, and 806 C>T *17)

TUBES/ Reagent (20 rxns)	MUTATIONS	DYE
Clopidogrel masters mix 1(400µL)	636 wild-type (G)	FAM
	806 wild-type (C)	JOE/HEX
	681 wild-type (G)	Texas red
Control DNA (45µL)	Internal Control	CY5
Clopidogrel masters mix 2(400µL)	636 Mutant (A)	FAM
	806 Mutant (T)	JOE/HEX
	681 Mutant (A)	Texas red
Control DNA (45µL)	Internal Control	CY5

rxns*:* Reaction, FAM: Green JOE/HEZ; Yellow, Texas Red; Red, CY5; Purple

PCR Program and Procedure

Commercial kits (Cat. No: 113R-20-03) offer pre-validated reagents **(Table 3)** and protocols for CYP2C19 genotyping, streamlining the process through careful primer and probe design, optimized cycling conditions **(Table 4),** and appropriate detection methods.

1. Different tubes were used to prepare each of the mix.

2. The master mixes were gently mixed by pipetting before beginning the experiment.

3. For each sample, 20 µL master mix was pipetted using micropipettes with sterile filter tips into each optical white strip or tube.

4.5µlbof DNA was added to each tube.

5. The programme was run with the details in the table below.

Note: Master mixes include HotstartTaq DNA polymerase

Table 4: PCR program for CYP2C19 genotyping cycling conditions

STAGE	**TEMPERATURE**	**TIME**	
Initial Denaturation	95 °C	3min	Holding
Denaturation	95 °C	15 sec	30 cycles
Annealing	60 °C	1min	30 cycles

Statistical Analysis

The Hardy-Weinberg Equilibrium (HWE) was assessed using the Pearson Chi-Square (χ^2) test to determine allele frequencies and genotype mutation distribution. A p-value greater than (> 0.05) signified the population is in HWE, indicating no significant evolutionary pressures. The exact calculations for HWE were performed using online tools available at https://wpcalc.com/en/equilibrium-hardy-weinberg/ and https://www.graphpad.com/quickcalcs/pValue2/, which provide user-friendly interfaces for determining both expected genotype frequencies and statistical significance. Additionally, Prism software (GraphPad Software, Inc., San Diego, CA, USA) was utilized to support the statistical analysis and visualization of the results.

CHAPTER IV

RESULTS

The Near East University Scientific Research Ethics Committee granted ethical approval for this study (Reference No: YDU/2024/124-1837). All participants in the research provided written consent with information prior to participation.

Case Selection and Patient Information

Initially, 100 women referred to the Near East University Medical Genetics laboratory with a history of thrombosis-associated miscarriage were identified as potential participants. From this group, 58 individuals (58%) were excluded primarily due to insufficient DNA quantity or quality obtained from available samples, or not meeting age criteria.

Blood samples were successfully collected and processed for DNA extraction from the remaining 42 Turkish Cypriot women who met the inclusion criteria. The final study cohort (N=42) consisted of female participants with a median age of 25 years (range: 16 to 42 years). All included participants had a documented history of at least one thrombosis-associated miscarriage and provided consent for genetic analysis.

CYP2C19 Allele and Genotype Frequencies

Genotyping for *CYP2C19* variants *3 (636 G>A), *2 (681 G>A), and *17 (-806 C>T) was performed on the cohort (N=42). Due to sample-specific amplification issues, the final number of successfully genotyped samples varied slightly for each variant. The observed genotype distributions, calculated allele frequencies, and assessment of HWE for each polymorphism are presented below.

*CYP2C19**3 (636 G>A) Variant

Genotyping for the *CYP2C19**3 variant was successful for 37 of the 42 participants (88.1%). The observed genotype counts were: GG n=14 (37.8%), GA n=17 (45.9%), and AA n=6 (16.2%). The calculated allele frequencies were G: 0.608 and A: 0.4. The genotype distribution for *CYP2C19**3 conformed to HWE ($\chi^2 = 0.079$, $p = 0.9$), The observed genotype frequencies are consistent with what would be expected under random mating, as indicated by the high p-value (0.9). **(Table 1)**.

Table 1: Genotype and Allele Frequencies for *CYP2C193 (636 G>A) in the Study Cohort (N=37)**

Genotypes	| Observed N (%)	Expected N	Allele	Frequency	HWE Test
GG	14 (37.8%)	14	G	0.6	χ^2=0.079
GA	17 (45.9%)	18	A	0.40	p=0.900
AA	6 (16.2%)	6			
Total	**37 (100%)**	**38**		**1.01**	

*(**Note**: Small difference in total Expected N due to rounding)* P 0.9 (bigger than P = 0.05). Not significant. The table presents statistics on the distribution of the CYP2C19 Gene Variations 636 G>A (*3) mutation in a sample population. It lists the expected and observed frequency of incidence, with predicted frequencies computed using a theoretical distribution under the null hypothesis. The observed frequencies represent the actual data obtained. The difference between the observed and expected values is quantified using the chi-square statistic (χ^2), which is computed as 0.079. The p-value (0,9) indicates that the observed difference from the predicted frequencies may not be statistically significant, indicating that the null hypothesis cannot be ruled out at conventional significance levels. The null hypothesis suggests that genetic drift, selection, or other processes controlling evolution through Hardy-Weinberg equilibrium may be controlling gene distribution.

*CYP2C19**2 (681 G>A) Variant

Genotyping for the *CYP2C19**2 variant was successful for all 42 participants (100%). The observed genotype counts were: GG n=20 (47.6%), GA n=12 (28.6%), and AA n=10 (23.8%). The calculated allele frequencies were G: 0.76 and A: 0.4. The result is statistically significant. A statistically significant deviation from HWE was observed in the genotype distribution of CYP2C19*2 (χ^2 = 9.63) p = 0.01). **(Table 2)**.

Table 2: Genotype and Allele Frequencies for CYP2C19*2 (681 G>A) in the Study Cohort (N=42)

Genotypes	Observed N (%)	Expected N	Allele	Frequency	HWE Test

GG	20 (47.6%)	24	G	0.76	χ^2=9.63
GA	12 (28.6%)	24	A	0.4	p=0.01*
AA	10 (23.8%)	6			
Total	**42 (100%)**	54		**1.16**	

**Indicates significant deviation from HWE.* P 0.01 (smaller than P = 0.05).

The table presents statistical analysis results for the CYP2C19 Gene 681 G>A (*2) mutation, revealing expected and observed frequencies of the normal genotype (GG) and the heterozygous mutant genotype (GA), with instances of the homozygous mutant genotype (AA) found. The expected frequencies are 16 for GG, 19 for GA, and 6 for AA. The observed frequencies of alleles G and A are 0.76 and 0.4, respectively. The difference between observed and expected frequencies is measured using the chi-square statistic (χ^2), with a calculated 9,63. p value is calculated as 0.01. The null hypothesis suggests that genetic drift, selection, or other processes controlling evolution through Hardy-Weinberg equilibrium may not be controlling gene distribution. The P-value is smaller than 0.05 (X2 is bigger than 3.84 for the degree of freedom is 1), therefore the null hypothesis is rejected, the population is not in Hardy–Weinberg equilibrium.

*CYP2C19**17 (-806 C>T) Variant

Genotyping for the *CYP2C19**17 variant was successful for all 41 participants (100%). The observed genotype counts were: CC n=12 (31.0%), CT n=21 (50.0%), and TT n=6 (19.0%). The calculated allele frequencies were C: 0.84 and T: 0.45. The result is statistically significant. (χ^2 = 7.774, p = 0.0082) **(Table 3)**. The P-value taken as 0.01 indicates significant correlation between the CYP2C19 Gene 806 C>T (*17) genetic variant and trait or outcome. The P-value is smaller than 0.05 (X2 is bigger than 3.84 for degree of freedom is 1), therefore the null hypothesis is rejected, the population is not in Hardy–Weinberg proportions (equilibrium). Deviation of expected from observed is significant. If a population's frequencies do not match those expected from Hardy-Weinberg, then the population is not in Hardy-Weinberg. Perhaps there is natural selection or non-random mating. Keeping in mind that the Hardy-Weinberg population is accepted as a scientific ideal, but it doesn't exist.

Table 3: Genotype and Allele Frequencies for CYP2C19*17 (-806 C>T) in the Study Cohort (N=42).

Genotypes	Observed N (%)	Expected N	Allele	Frequency	HWE Test
CC	12 (29.0%)	29	C	0.84	χ^2=7.774
CT	21 (51.0%)	15.5	T	0.45	p=0.0082
TT	6 (19.5%)	8.3			
Total	**41 (100%)**	**52.8**		**1.29**	

***Note**: Small difference in total Expected N due to rounding.* P = 0.0082 (smaller than P = 0.05). The table shows the distribution of the CYP2C19 Gene Variations 806 C>T (*17) mutation in a sample population, comparing expected and observed frequencies. The chi-square statistic quantifies the difference between the observed and expected values, with a p-value of 0.01, smaller than 0.05 and X2 is bigger than 3.84 for the degree of freedom is 1 suggesting that the observed difference statistically significant, The P-value is smaller than 0.05 (X2 is bigger than 3.84 for the degree of freedom is 1), indicating the null hypothesis can be rejected, and the population is not in Hardy–Weinberg equilibrium.

CHAPTER V

DISCUSSION

The cytochrome P450 superfamily comprises a diverse group of enzymes crucial for metabolizing endogenous and exogenous compounds. Within this superfamily, CYP2C19 is notable for its genetic polymorphism, first described in 1984 concerning the metabolism of the anticonvulsant mephenytoin (Mehta et al., 2023, Zhu et al., 2016. The *CYP2C19* gene, characterized in 1991, exhibits numerous variants that can alter enzyme function. The identification of loss-of-function alleles (like *2 and *3, first reported in 1994) and gain-of-function alleles (like *17) highlighted the significant inter-individual variability in CYP2C19 activity (Zhu et al., 2016). While much research has focused on the pharmacogenetic implications of these variants, particularly for drug metabolism, their potential roles in modulating endogenous pathways relevant to physiological processes like hemostasis or pregnancy maintenance remain less explored (Shah, 2005).

This study investigated the frequencies of key *CYP2C19* polymorphisms (*2, *3, *17) in a cohort of 42 women referred to the Near East University Medical Genetics laboratory due to a history of thrombosis-associated miscarriage in the TRNC. Following exclusions based on DNA quality, quantity, and age criteria, the final analysis included genotype data from 37 to 42 individuals for the different variants (specifically, N=37 for *3, N=42 for *2, and N=41 for *17). The median age of the included participants was 25 years, all with a history of at least one thrombosis-associated miscarriage.

The *CYP2C19**3 variant (636 G>A) results in early translation termination, leading to an incomplete and non-functional protein enzyme. Individuals homozygous for this allele lack CYP2C19 activity. The *CYP2C19**2 variant (681 G>A) creates a splicing defect, also resulting in a loss-of-function (LOF) enzyme with significantly impaired activity. Individuals carrying one (*1/*2) or two (*2/*2) copies of this allele exhibit reduced enzyme function. Conversely, the *CYP2C19**17 variant (-806 C>T) is located in the gene's promoter region and leads to increased transcription and, consequently, higher enzyme activity, representing a gain-of-function (GOF) phenotype. The altered enzyme activity associated with these common polymorphisms (*2, *3, *17) could potentially influence biological pathways beyond drug metabolism, potentially

impacting susceptibility to conditions like thrombosis or complications during pregnancy, although the specific endogenous substrates and mechanisms are not fully elucidated.

Recurrent pregnancy loss (RPL), which affects approximately 5% of women, is a significant and emotionally taxing condition. reproductive-aged women. Miscarriage, defined as pregnancy loss before 20 weeks of gestation, has numerous established causes, including chromosomal issues, uterine anomalies, endocrine disorders, and lifestyle factors (Cao et al., 2022). Importantly, thrombophilia, an inherited or acquired predisposition to thrombosis, is recognized as a significant contributor, potentially accounting for up to 40% of RPL cases, particularly early losses (D'Uva, 2010). Well-studied genetic risk factors for thrombophilia-associated RPL include variants in Factor V (Leiden G1691A) and Prothrombin (G20210A) genes (Padda et al., 2021). Emerging evidence also points towards potential roles for variants in genes like *PAI-1* (associated with hypofibrinolysis) and *MTHFR* (related to hyperhomocysteinemia), although consensus on their definitive contribution to RPL requires further large-scale investigation (Maghsudlu et al., 2024). Acquired thrombophilia, primarily antiphospholipid syndrome (APS), is another major factor where autoantibodies interfere with coagulation processes.

Against this background of known genetic and acquired thrombophilia, exploring other potential genetic contributors is essential. Genetic studies within the Cypriot population have highlighted significant variability. Research in Turkish Cypriots identified considerable heterozygosity for polymorphisms related to metabolic and thrombotic risk (*MTHFR C677T, A1298C*; Factor VII G353A), providing foundational data for this population (Ergoren et al., 2019). Similarly, studies in Greek Cypriots revealed notable frequencies of established thrombogenic mutations (Factor V Leiden, Prothrombin G20210A, *MTHFR C677T*), underscoring a potential genetic predisposition to thrombosis on the island (Papageorgiou et al., 2009). Our study adds to this by focusing specifically on *CYP2C19* variants within TRNC women experiencing thrombosis-associated miscarriage. The results indicated that the *CYP2C19**3 allele frequency was determined to be 0.40 (40%). The genotype frequencies that were found aligned with the concept of HWE.

The CYP2C19*3 variant was successfully genotyped in 37 out of 42 participants (88.1%). The genotype distribution was GG in 14 individuals (37.8%), GA in 17 (45.9%), and AA in 6 (16.2%). Allele frequencies were G: 0.608 and A: 0.4. The genotype distribution conformed to Hardy-Weinberg Equilibrium (HWE) ($\chi^2 = 0.079$, $p = 0.9$), indicating no significant deviation from

random mating patterns. This suggests that the CYP2C19*3 variant is distributed in the population as expected, without influence from selection, population structure, or genotyping errors.

In contrast, the CYP2C19*2 variant was successfully genotyped in all 42 participants (100%). Observed genotype counts were GG: 20 (47.6%), GA: 12 (28.6%), and AA: 10 (23.8%), with allele frequencies of G: 0.76 and A: 0.4. A significant deviation from HWE was observed (χ^2 = 9.63, p = 0.01), suggesting a possible selection pressure, population stratification, or other non-random mating influences acting on this allele in the studied population.

The CYP2C19*17 gain-of-function (GOF) variant was also successfully genotyped in all 41 participants (100%). Genotype counts were CC: 12 (31.0%), CT: 21 (50.0%), and TT: 6 (19.0%). The allele frequencies were C: 0.84 and T: 0.45. Like CYP2C19*2, the distribution deviated significantly from HWE (χ^2 = 7.774, p = 0.0082), which may suggest underlying non-random genetic structure or selection in this cohort. These LOF and GOF allele distributions point to a complex metabolic profile within this population, where both reduced and increased CYP2C19 enzyme activity may be present.

Notably, 27% (10/37) of individuals heterozygous for CYP2C19*3 were also heterozygous for both CYP2C19*2 and CYP2C19*17. Of the six individuals with the AA genotype (homozygous LOF) for CYP2C19*3, five also had the AA genotype for CYP2C19*2; this corresponds to 5/6 in 37 and 5/10 in 42 for CYP2C19*2. Additionally, 50% of the individuals with the AA genotype for CYP2C19*3 were also AA for CYP2C19*2 and TT (GOF) for CYP2C19*17. This combination may have contradictory functional outcomes but could ultimately lead to altered or imbalanced CYP2C19 enzyme activity.

This study, however, faces several limitations. The most significant is the small sample size (N=37-42), which limits statistical power and increases the risk of Type II errors, potentially failing to detect genuine associations. The cross-sectional design prevents the establishment of causality. Critically, the study lacks correlation with detailed clinical outcomes (e.g., specific type/timing of miscarriage, documented thrombotic events, placental pathology findings) and does not include functional assays to assess CYP2C19 activity directly or investigate its impact on potential endogenous substrates relevant to hemostasis or pregnancy. The lack of a control group such as healthy women from the TRNC with successful pregnancies or women who experienced

miscarriages unrelated to thrombosis limits the ability to assess whether the observed allele frequencies in women with thrombosis-associated miscarriage differ significantly from those in the general population or other comparison groups. The genetic scope was limited to three *CYP2C19* variants, and potential influences from other genetic, environmental, or acquired risk factors were not assessed. Finally, rigorous quality control reporting for genotyping, crucial in small cohorts, should be detailed.

Future research should address these limitations. Larger cohort studies, ideally multi-center and longitudinal, are necessary, including carefully matched control groups. Comprehensive assessment should include detailed clinical data, investigation of a wider panel of genetic variants related to thrombophilia and pregnancy outcomes, and analysis of potential gene-gene and gene-environment interactions. Incorporating functional assays exploring the impact of *CYP2C19* variants on relevant endogenous pathways (if candidates are identified) would be highly valuable. Such studies could clarify whether *CYP2C19* polymorphisms represent an independent risk factor for thrombosis-associated miscarriage in the TRNC population.

CHAPTER VI

CONCLUSION

This study is the inaugural examination of how often the major CYP2C19 polymorphisms (*2, *3, *17) occur in women living in the TRNC who have undergone thrombosis-related miscarriage. Our findings reveal the presence of significant genetic variability within the *CYP2C19* gene in this cohort, with notable frequencies observed for both loss-of-function (*2 and *3) and gain-of-function (*17) alleles. Specifically, the difference between observed and expected frequencies is measured using the chi-square statistic (χ^2). In *CYP2C19* variants *3 (636 G>A), the P-value is smaller than 0.05 (X2 is bigger than 3.84 for the degree of freedom is 1), therefore, the null hypothesis is rejected, and the population is not in Hardy–Weinberg proportions (equilibrium). The deviation of the expected from the observed is significant. 0.4 (38%) for the *2 LOF variant, and 0.45 (44%) for the *17 GOF variant. While these polymorphisms are well-known for altering the metabolism of certain drugs, this study explored their presence in the context of susceptibility to thrombosis-associated miscarriage itself. The prevalence of alleles conferring altered enzyme function in this specific patient group warrants further investigation to understand if *CYP2C19* is implicated in the pathogenesis of this condition, potentially through pathways not associated with drug metabolism.

This research represents a foundational step in characterizing the *CYP2C19* genetic landscape within this high-risk population in the TRNC. However, due to limitations, particularly the small sample size and lack of a control group and clinical correlation, no definitive conclusions can be drawn regarding an association between these polymorphisms and thrombosis-associated miscarriage risk. Future, larger studies incorporating control groups, detailed clinical phenotyping, and potentially functional analyses are required to elucidate the possible contribution of *CYP2C19* variants to the complex etiology of thrombosis-related pregnancy complications in this population.

Recommendation

Based on the findings of this study, which determined the frequencies of *CYP2C19* alleles (*2, *3, *17) in women with thrombosis-associated miscarriage in the TRNC, the following recommendations are made:

1. **Acknowledge Population-Specific Data:** This study provides initial, valuable data on the frequency of CYP2C19 polymorphisms with functional significance (*2, *3, *17) within a specific high-risk group (women with thrombosis-associated miscarriage) in the TRNC. The observed frequencies, particularly the notable presence of both loss-of-function (*2, *3) and gain-of-function (*17) alleles, contribute to understanding the genetic landscape of this population.

2. **Prioritize Established Thrombophilia Screening:** While this study explored *CYP2C19*, current clinical practice for investigating recurrent or thrombosis-associated miscarriage should continue to prioritize screening for established thrombophilia risk factors (e.g., Factor V Leiden, Prothrombin G20210A mutations, antiphospholipid antibodies) based on existing guidelines and individual patient history. Routine *CYP2C19* testing is *not* currently recommended for assessing baseline thrombosis or miscarriage risk based solely on these frequency findings.

3. **Further Research is Essential:** The primary recommendation arising from this study is the need for further, more extensive research:

    - **Larger Cohort Studies:** Conduct larger studies with adequate statistical power, including appropriately matched control groups (e.g., healthy women from the TRNC with successful pregnancies, women with miscarriage unrelated to thrombosis) to determine if the observed *CYP2C19* allele frequencies are significantly different in women with thrombosis-associated miscarriage compared to controls.

    - **Clinical Correlation:** Future studies must correlate *CYP2C19* genotypes with detailed clinical phenotypes, including specific miscarriage characteristics, documented thrombotic events (type, location), placental pathology, and response to any treatments received.

- **Investigate HWE Deviation:** The observed deviation from HWE for the *CYP2C19**2 variant warrants investigation in larger samples to determine if it represents a genuine association, sampling bias, population stratification, or a chance finding due to the small sample size.
- **Broader Genetic Context:** Integrate *CYP2C19* analysis with screening for other known genetic and acquired thrombophilia risk factors to understand potential interactions and build comprehensive risk profiles.
- **Functional Studies:** If feasible, explore potential mechanisms by which *CYP2C19* variants might influence endogenous pathways related to coagulation, vascular function, or pregnancy maintenance, independent of drug metabolism.

4. **Caution Regarding Direct Clinical Application:** Avoid extrapolating these frequency findings directly into immediate changes in clinical management regarding antiplatelet therapy choices *unless* a patient has a separate, established indication for such therapy and *CYP2C19* testing is performed for standard pharmacogenetic reasons (e.g., prior to Clopidogrel initiation for cardiovascular indications). This study did not assess drug response or establish *CYP2C19* as an independent risk factor for miscarriage.
5. **Future Guideline Consideration (Conditional):** Only if future, robust research definitively establishes a clinically significant association between specific *CYP2C19* genotypes and the risk of thrombosis-associated miscarriage, or demonstrates utility in guiding preventative strategies in this context, should incorporation into local clinical guidelines be considered.

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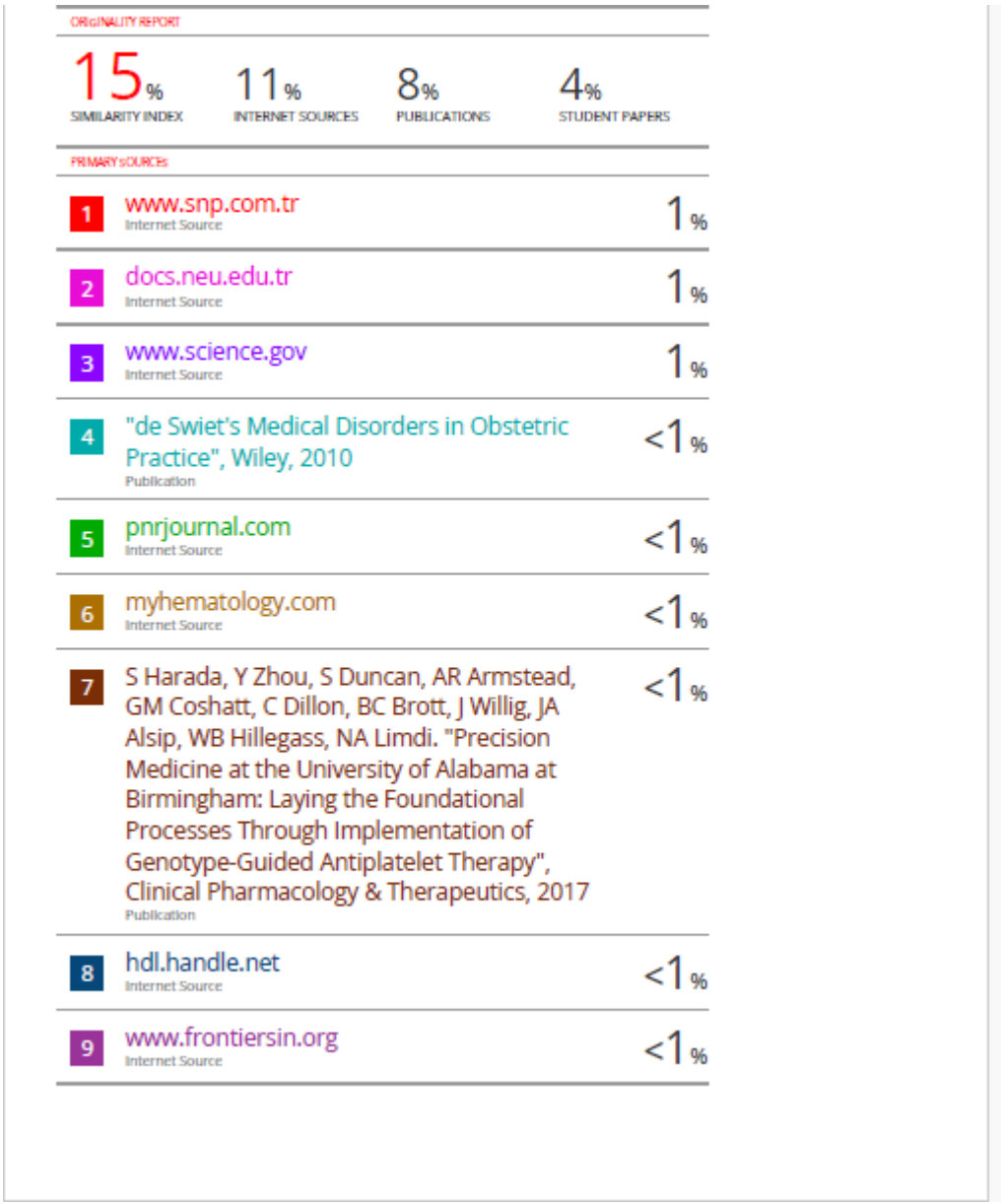

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