RISK ASSESSMENT OF INFLAMMATION RELATED CYP1A2, 2C9, 2D6, 3A4, AND 4F2 POLYMORPHISMS IN TURKISH PATIENTS WITH MULTIPLE SCLEROSIS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

BURAK BARUT

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOCHEMISTRY

JUNE 2023

Approval of the thesis:

RISK ASSESSMENT OF INFLAMMATION RELATED CYP1A2, 2C9, 2D6, 3A4, AND 4F2 POLYMORPHISMS IN TURKISH PATIENTS WITH MULTIPLE SCLEROSIS

submitted by **BURAK BARUT** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Biochemistry, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Assoc. Prof. Dr. Özgül Persil Çetinkol
Head of the Department, **Biochemistry** _____

Prof. Dr. Orhan Adalı
Supervisor, **Biology, METU** _____

Prof. Dr. Alaatin Şen
Co-Supervisor, **Molecular Biology and Genetics, Abdullah Gül University** _____

Examining Committee Members:

Prof. Dr. Şeref Demirkaya
Gülhane School of Medicine, University of Health Sciences _____

Prof. Dr. Orhan Adalı
Biology, METU _____

Assoc. Prof. Dr. Birsen Can Demirdöğen
Biomedical Engineering, TOBB ETU _____

Assoc. Prof. Dr. Özgül Persil Çetinkol
Chemistry, METU _____

Asst. Prof. Dr. Serkan Belkaya
Molecular Biology and Genetics, İhsan Doğramacı Bilkent University _____

Date: 13.06.2023

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Burak Barut

Signature :

ABSTRACT

RISK ASSESSMENT OF INFLAMMATION RELATED CYP1A2, 2C9, 2D6, 3A4, AND 4F2 POLYMORPHISMS IN TURKISH PATIENTS WITH MULTIPLE SCLEROSIS

Barut, Burak
Doctor of Philosophy, Biochemistry
Supervisor : Prof. Dr. Orhan Adalı
Co-Supervisor : Prof. Dr. Alattin Şen

June 2023, 123 pages

Multiple sclerosis (MS) is a complex, demyelinating, autoimmune disease that affects the central nervous system. Approximately, 2.8 million people in the world are suffering from MS. Cytochrome P450s (CYPs) are the superfamily of enzymes responsible for the metabolism of various endogenous, and exogenous compounds. There are a tremendous number of polymorphism studies investigating the role of CYPs in different diseases. However, polymorphism studies on inflammation related CYPs in MS are very limited. In this study, the relationship between SNPs in CYP1A2 (rs762551), CYP2C9 (rs1799853), CYP2D6 (rs16947), CYP3A4 (rs2740574), and CYP4F2 (rs2108622) and MS were investigated due to their significant roles in inflammation response via AA metabolism. 179 patients (125 female / 54 male), and 102 controls (58 female / 44 male) that have been recruited were genotyped by the PCR-RFLP method. Results were evaluated under additive, dominant, and recessive genetic models. Also, allele frequency differences between wild-type and polymorphic individuals were analyzed using SNPStats (Institut Català d'Oncologia). No significant difference between patients and the control group was observed in CYP2D6 (rs16947), CYP3A4 (rs2740574), and CYP4F2

(rs2108622). We found that the polymorphic CC allele of CYP1A2 (rs762551) might have disease causing effects for MS under additive (OR=3.02; 95%CI: 1.16-7.88; p=0.049), and recessive models (OR=2.84, 95%CI: 1.13-7.14, p=0.016). On the other hand, the polymorphic T allele in CYP2C9 (rs1799853) indicated protective effect (OR=0.14, 95%CI: 0.04-0.44, p<0.001) under the additive model.

Keywords: Multiple Sclerosis, Inflammation, Polymorphism, CYP

ÖZ

MULTİPL SKLEROZLU TÜRK HASTALARDA İNFLAMASYONLA İLİŞKİLİ CYP1A2, 2C9, 2D6 , 3A4 VE 4F2 POLİMORFİZMLERİNİN RİSK DEĞERLENDİRMESİ

Barut, Burak
Doktora, Biyokimya
Tez Yöneticisi: Prof. Dr. Orhan Adalı
Yardımcı Danışman: Prof. Dr. Alaattin Şen

Haziran 2023, 123 sayfa

Multipl skleroz (MS) myelin kılıf deformasyonu sonucu merkezi sinir sistemini etkileyen, kronik ve otoimmün bir hastalıktır. Tüm dünyada yaklaşık olarak 2.8 milyon kişi için MS tanısı konulmuştur. Sitokrom P450'ler (CYP'ler), çeşitli endojen (steroidler, safra asitleri, araşidonik asit dahil olmak üzere yağ asitleri, prostaglandinler, biyojenik aminler ve retinoidler) ve eksojen (ilaçlar, alkoller, antioksidanlar, organik çözücüler, anestezik ajanlar, boyalar, çevresel kirleticiler ve kimyasallar) bileşiklerin metabolizmasından sorumlu enzim süper ailesidir ve CYP'lerle ilgili literatürde oldukça fazla polimorfizm çalışması mevcuttur. Ancak, MS hastalığında inflamasyonla ilişkili CYP'ler üzerine yapılan polimorfizm çalışmaları çok sınırlıdır. Bu çalışmada araşidonik asit metabolizması yoluyla inflamasyon cevabındaki rolleri nedeniyle CYP1A2 (rs762551), CYP2C9 (rs1799853), CYP2D6 (rs16947), CYP3A4 (rs2740574) ve CYP4F2 (rs2108622) genlerindeki SNP'ler ile MS arasındaki bağlantı incelenmiştir. Mevcut çalışmaya 179 hasta (125 kadın / 54 erkek) ve 102 kontrol (58 kadın / 44 erkek) dahil edilmiş olup, bu kişilerin genotipleri PCR-RFLP yöntemiyle belirlenmiştir. Elde edilen sonuçların istatistiki analizi aditif, dominant ve resesif olmak üzere üç farklı genetik modele göre gerçekleştirilmiştir. Ayrıca, yabanıl tip ve polimorfik

bireylerin allel frekans farklılıkları da SNPStats (Institut Català d'Oncologia) kullanılarak belirlenmiştir. CYP2D6 (rs16947), CYP3A4 (rs2740574) ve CYP4F2 (rs2108622) genlerinde hasta ve kontrol grupları arasında anlamlı bir fark gözlenmemiştir. CYP1A2 (rs762551) kapsamında homozigot (CC) alelinin hem aditif (OR=3.02; 95%CI: 1.16-7.88; p=0.049) hem de resesif model (OR=2.84, 95%CI: 1.13-7.14, p=0.016) sonuçlarına göre, MS hastalığına neden olabileceği gösterilmiştir. Diğer taraftan, aditif model sonuçları (OR=0.10, %95CI: 0.03-0.37, p<0.001), CYP2C9 (rs1799853)'daki polimorfik T allelinin koruyucu etkisini işaret etmektedir.

Anahtar Kelimeler: Multiple Skleroz, İnflamasyon, Polimorfizm, CYP

This thesis is dedicated to my beloved wife and dearest daughter

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor, Professor Dr. Orhan Adalı and my co-supervisor Prof. Dr. Alaatin Şen for their guidance, advice, criticism and encouragement.

I would like to thank examining committee members, Prof. Dr. Şeref Demirkaya, Assoc. Prof. Dr. Birsen Can Demirdöğen, Assoc. Prof. Dr. Özgül Persil Çetinkol and Asst. Prof. Dr. Serkan Belkaya for serving as my examining committee members.

I would also like to thank Asst. Prof. Dr. Serkan Belkaya for his guidance, contribution and help throughout this study.

Additionally, I would like to thank lab mates Mr. Giray Bulut, Ms. Merve Akkulak, Mr. Deniz Çabuk and my friends Mr. Emre Evin and Mr. Bora Ergin for their support.

I would like to express my deepest appreciation to my wife, Ezgi. Without her absolute understanding and encouragement, it would be impossible to complete this study.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS

AA	Arachidonic acid
APC	Antigen-presenting cell
bp	Base pair
CNS	Central nervous system
COX	Cyclooxygenase
CYP	Cytochrome P450
DHET	Dihydroxyeicosatetraenoic acid
diHETE	Dihydroxyeicosatetraenoic acid
dNTP	Deoxynucleoside triphosphate
EAE	Experimental allergic encephalomyelitis
EDTA	Ethylenediaminetetraacetic acid
EET	Epoxyeicosatrienoic acid
FA	Fatty acid
gDNA	Genomic DNA
HETE	Hydroxyeicosatetraenoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
IL-17	Interleukin-17
IL-2	Interleukin-2
IL-4	Interleukin-4

IL-6	Interleukin-6
IL-10	Interleukin-10
LOX	Lipooxygenase
LTA_4	Leukotriene A_4
LTB_4	Leukotriene B_4
LTC_4	Leukotriene C_4
LTD_4	Leukotriene D_4
LTE_4	Leukotriene E_4
LX	Lipoxin
MAF	Minor allele frequency
MHC-II	Major histocompatibility complex class II
MS	Multiple sclerosis
NA	Not applicable
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NF-κB	Nuclear Factor κB
OD	Optical density
OG	Oligodendrocyte
OR	Odds Ratio
Oxo-ETE	Oxo-eicosatetraenoic acid
PCR	Polymerase chain reaction
PG	Prostaglandin

PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
PGHS	Prostaglandin H synthase
PGI_2	Prostacyclin
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PLD	Phospholipase D
PPARγ	Peroxisome proliferator-activated receptor gamma
RFLP	Restriction fragment length polymorphism
sEH	Soluble epoxide hydrolase
TBE	Tris-borate-EDTA
TNF-α	Tumor necrosis factor-α
Tris	2-amino-2(hydroxymethyl)-1,3-propanediol
TXA_2	Thromboxane A_2
TXB_2	Thromboxane B_2

LIST OF SYMBOLS

SYMBOLS

^{o} C	Centigrade degree
μ	Micro
p	Probability
g	Gravitational force

CHAPTER 1

INTRODUCTION

1.1 Multiple Sclerosis

Multiple sclerosis (MS) is a complex, demyelinating, autoimmune disease that affects central nervous system (CNS) and eventually results in lesions or plaques (Milo and Kahana, 2010; Tillery et al., 2017). Recent findings indicate that neurodegeneration and activation of astrocytes (gliosis) also take part in MS pathogenesis (Mañé-Martínez et al., 2016; Hauser and Cree, 2020). MS is mainly characterized by relapses and remissions and approximately 80% of individuals experience these relapses and remissions (Steinman, 2014). According to the classification of the National MS Society, relapsing-remitting, secondary progressive, primary progressive, and progressive relapsing are the four different subtypes of MS. However, there is no complete recovery from MS. The treatment strategy primarily focuses on diminishing the severe symptoms of MS, slowing the disease progression, and decreasing the frequency and duration of relapses (Tillery et al., 2017).

Roughly, 2.5 million people worldwide suffer from MS resulting in physical disabilities in young adults, according to a study published in 2015 (Dendrou et al., 2015). And in a more recent study, it is reported that 2.8 million people (35.9 per 100.000 population) are believed to suffer from MS (Walton et al.,2020). Women are more susceptible to this complex disease than men, and the average age of individuals with MS is 30. MS is typically seen between the ages of 20-40, peaking at 30 (Milo and Kahana, 2010; Dendrou et. al., 2015).

Early symptoms of MS are paresthesia and visual loss (Hauser and Cree, 2020; Kale, 2016). According to Kale, optic neuritis is believed to be one of the early

signs of MS (Kale, 2016). The major symptoms are limb, facial, and paroxysmal weaknesses, blurred vision, ataxia, vertigo, bladder, sexual, and cognitive dysfunctions, Lhermitte's symptom, heat sensitivity, and fatigue. Rare symptoms of MS include seizure, dementia and movement disorders (Hauser and Cree, 2020).

The underlying mechanisms of MS have not been fully identified yet, but strong evidence indicates that individuals' genetic susceptibility and environmental factors play a crucial role in disease development. These environmental factors include lack of sunlight exposure (Vitamin D deficiency), hygiene, smoking, and viral infections such as the Epstein-Barr virus (Correale, 2015). The prevalence of MS is estimated to increase with increasing latitude and decreasing average temperature. There are some exceptions to this estimation. Sardinia, which is relatively warmer, has a higher MS frequency, whereas Inuit communities in northern Canada have a lower MS frequency (Milo and Kahana, 2010). The highest prevalence is seen in the regions with high-income, including North America, Western Europe, and Australasia (more than 100 patients per 100.000). On the other hand, sub-Saharan Africa, and Oceania have the lowest MS prevalence (less than 30 patients per 100.000) (Hauser et al., 2020).

Since MS generally affects young adults and its prevalence tends to increase, the pathogenesis of the disease has been studied extensively. The ultimate consequence of the autoimmune response in MS is demyelination and axonal loss.

Pro-inflammatory T lymphocyte cells become active, when antigens on MHC-II (major histocompatibility complex class II) interact with these T-cells via T-cell receptors. Active T-cells then migrate to CNS with the help of various adhesion molecules and chemokines. In the CNS, T-cells are re-activated by CNS antigens on APCs (antigen-presenting cells), primarily microglial cells. Re-activated T-cells in CNS secrete different pro-inflammatory cytokines (i.e., interferon γ, interleukin 2, 6, and 17, tumor necrosis factor-α). These cytokines initiate CNS inflammation by activating the macrophages and other T and B cells. Macrophages and T cells then damage the myelin sheath of OGs (oligodendrocytes), mainly mediated by

TNF-α, NO, and O_2 radicals. On the other hand, plasma cells derived from activated B cells produce demyelinating antibodies that further activate macrophages. Activation of macrophages triggers the attack on the myelin membrane, which forms pores in membranes given in Figure 1.1 (Cosentino and Marino, 2013).

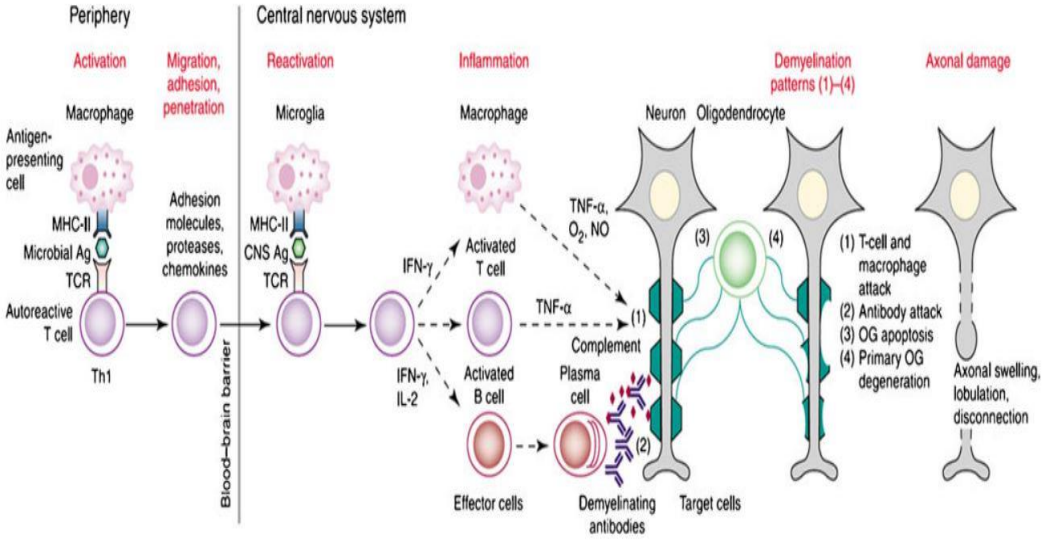

Figure 1. 1 MS pathogenesis and progression (Cosentino and Marino, 2013).

Four demyelination patterns that are believed to play a role in MS progression exist: T-cell and macrophage attack, antibody attack, apoptosis in oligodendrocytes, and primary oligodendrocyte degeneration (Cosentino and Marino, 2013).

1.2 Arachidonic Acid Metabolism

1.2.1 Arachidonic Acid

Arachidonic acid (AA) is an omega-6 (ω-6) polyunsaturated fatty acid with a twenty-carbon chain, and four cis-double bonds (Wang et al., 2019; Tallima and El Ridi, 2018). As seen in Figure 1.2, AA generally assumes a hairpin conformation.

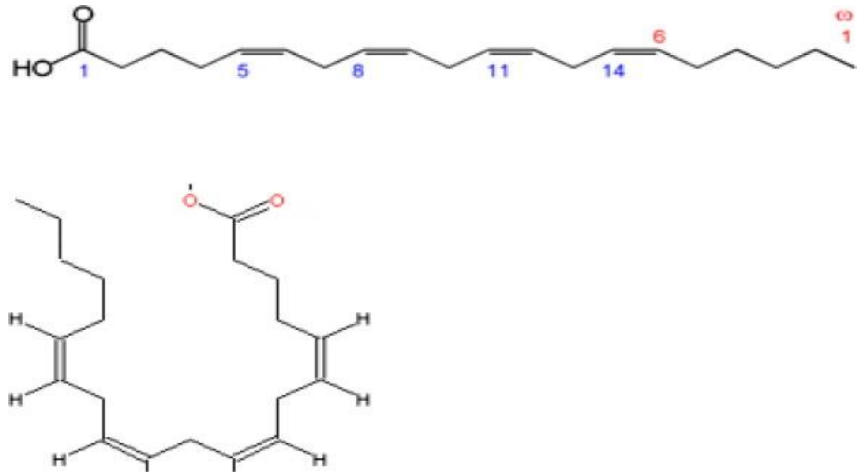

Figure 1. 2 Linear and hairpin conformation of Arachidonic acid (Tallima and El Ridi, 2018).

AA is either synthesized from an essential fatty acid, linoleic acid, or obtained from the diet. Since AA is derived only from an essential fatty acid (FA), it is also assumed to be essential for humans (Tallima and El Ridi, 2018). Table 1.1 summarizes the available food sources of AA (National Cancer Institute, Division of Cancer Control & Population Sciences).

Table 1. 1 Food sources of AA (National Cancer Institute, Division of Cancer Control & Population Sciences).

Rank	Food Item	Contribution to Intake (%)
1	Chicken	26.9
2	Egg	17.8
3	Beef	7.3
4	Sausage, franks, bacon, and ribs	6.7
5	Fish	5.8
6	Burger	4.6
7	Cold cuts	3.3
8	Pork	3.1
9	Pizza	2.8
10	Turkey	2.7
11	Pasta	2.3
12	Regular cheese	Less than 1

1.2.2 Arachidonic Acid Metabolism

AA and its metabolites are vital in many inflammatory processes (Wang et al., 2021). Figure 1.3 illustrates the AA metabolism. Once inflammatory stimulus reaches, phospholipases (PLA_2, PLC, PLD) release arachidonic acid from membrane-bound phospholipids. Free AA can undergo one of the three different enzymatic pathways.

In cyclooxygenase (COX) pathway, COX-1, COX-2, and COX-3 or so-called prostaglandin H synthases (PGHSs) act on AA, which yields prostanoids including prostaglandins, and thromboxane A_2 (TXA_2). After the action of cyclooxygenases prostaglandin G_2 (PGG_2) is produced, and converted to prostaglandin H_2 (PGH_2). All COX enzymes have two distinct enzymatic sites with different activities. Cyclooxygenase activity of COX enzymes helps to convert AA to PGG_2, while

peroxidase activity produces PGH_2 from PGG_2. PGH_2 is not a stable compound and quickly metabolized into prostaglandins (PGs) such as PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 (TXA_2) (Wang et al., 2019, Wang et al., 2021).

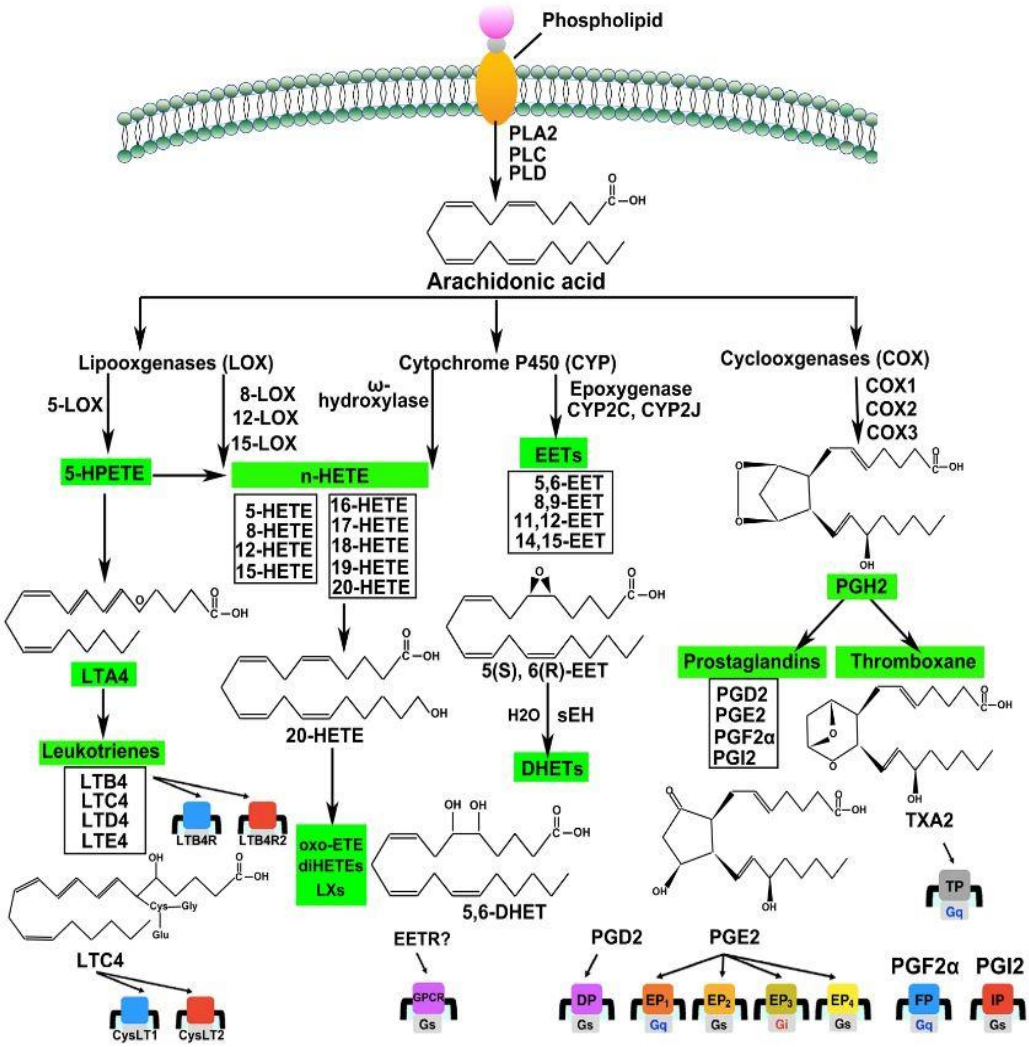

Figure 1. 3 Arachidonic acid metabolism (Wang et al., 2021).

In lipooxygenase (LOX) pathway, 5-LOX, 8-LOX, 12-LOX, and 15-LOX are responsible for the catalytic activities by inserting molecular oxygen. After the action of 5-LOX, 5-hydroperoxyeicosatetraenoic acid (5-HPETE) is produced, and 5-HPETE is converted into Leukotriene A_4 (LTA_4) in the first route of the LOX pathway. In this route, LTA_4 is further metabolized into different leukotrienes such as LTB_4, LTC_4, LTD_4, and LTE_4. In the second route of the LOX pathway, four

different hydroxyeicosatetraenoic acids (HETEs) are produced by the four different lipooxygenases (Wang et al., 2019, Wang et al., 2021).

Cytochrome P450 (CYP) pathway has two different routes, like in the LOX pathway. In the first route, AA is metabolized into HETEs by ω-hydroxylase. Oxo-ETE (oxo-eicosatetraenoic acid), diHETEs (dihydroxyeicosatetraenoic acid) and lipoxins (LXs) are produced from 20-HETE. In the second route, epoxygenase activity on AA yields EET regio-isomers (epoxyeicosatrienoic acids). These EETs are further metabolized into DHETs (dihydroxyeicosatrienoic acids) by soluble epoxide hydrolases (sEHs) (Wang et al., 2021).

In the CYP pathway, 5,6-EET and 20-HETE are further converted to prostaglandin analogs, 5,6-Epoxy PGE_1 and 20-Hydroxy PGE_2 respectively, after the actions of cyclooxygenases as seen in Figure 1.4 (Sacerdoti et. al., 2003).

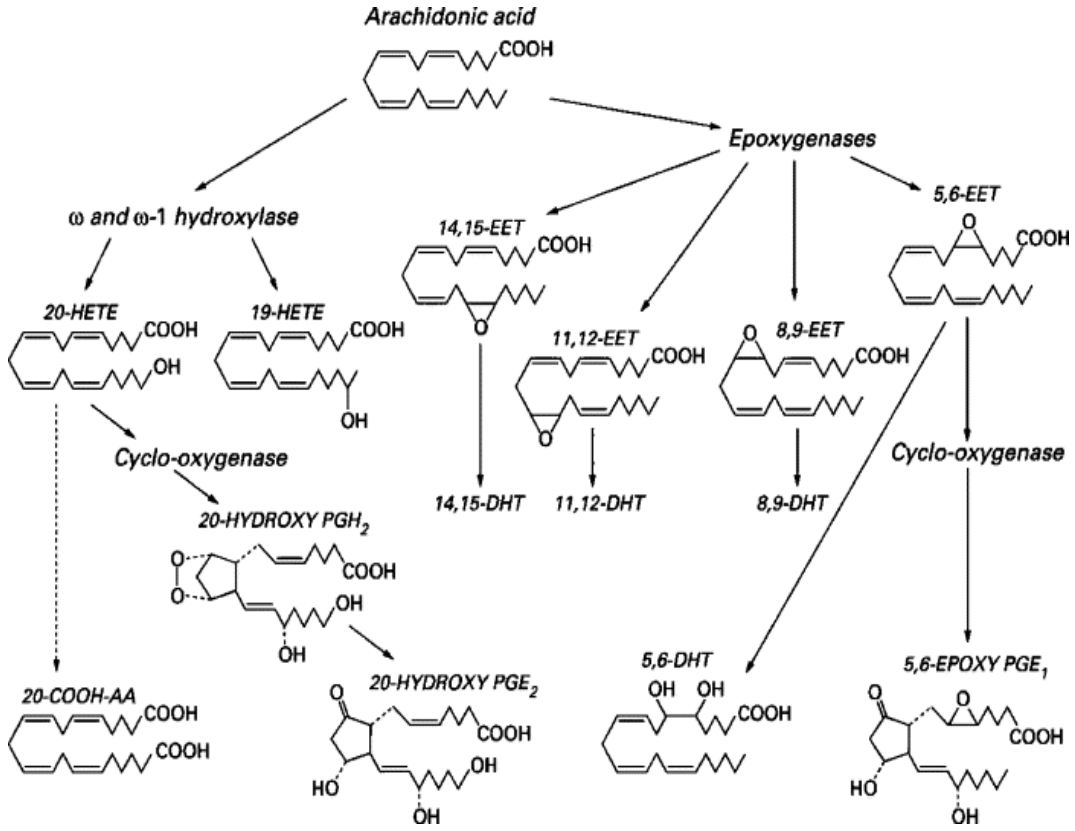

Figure 1. 4 CYP pathway of arachidonic acid metabolism (Sacerdoti et. al., 2003).

Studies have illustrated that the metabolism of AA is triggered in MS patients. This activated AA metabolism may facilitate neuroinflammation, and function in demyelination, motor dysfunctions, axonal, and oligodendrocyte loss. AA is metabolized via different pathways (COX, LOX, and CYP) producing various AA metabolites. Prostaglandins, and thromboxane are the ultimate end products of the COX pathway, and it has been shown that the COX pathway is activated in oligodendrocytes, and immune cells while demyelination is taking place. This activated COX pathway demonstrates its driving role in MS onset (Hoxha et al., 2022; Carlson et al., 2006). Animal models mimicking MS, and human studies including case-control groups pointed out that the level of prostaglandins (PGE, PGE_2, $PGF_{2\alpha}$, 8-epi-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$), and thromboxane (TXB_2) were significantly increased as well as activity of phospholipases (Hoxha et al., 2022).

Moreover, the LOX pathway of AA yields leukotrienes, and this pathway is activated in MS lesions, and cerebrospinal fluid of MS patients. Especially, LTB_4

concentration increases in MS according to experimental allergic encephalomyelitis (EAE) animal models, and case-control human studies (Hoxha et al., 2022).

1.3 Cytochrome P450s, Inflammation and MS

Cytochrome P450s (CYPs) are the super family of enzymes that contain a single molecule of heme at the active site (Gonzalez and Gelboin, 1992). They are responsible for the metabolism of a variety of endogenous (fatty acids including arachidonic acid and its metabolites, prostaglandins, steroids, and bile acids) and exogenous (drugs, alcohols, chemicals, organic solvents, environmental pollutants, antioxidants, and anaesthetic agents) compounds (Chang and Kam, 1999).

Evolutionarily, CYPs are well conserved and found in lower and higher organisms such as bacteria, archaea, fungi, plants, animals, and humans (Stavropoulou et al., 2018). CYPs function as terminal oxidase in the monooxygenase systems on the endoplasmic reticulum membrane. In simple, any endogenous or exogenous substance (RH) is hydroxylated via electrons from NADPH by CYPs, as shown below (Chang and Kam, 1999).

$$NADPH + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + ROH$$

Nevertheless the catalytic action of CYPs is far more complex (Figure 1.5). Once the substrates (RH) bind to CYPs, the first reduction reaction is catalyzed by P450 reductase yielding Fe^{2+}, followed by oxygen binding. After oxygen binding, a second reduction reaction occurs in which one electron is transferred from P450 reductase as in the first reduction reaction. Remaining steps cover the hydroxylation of substrates. Finally, the resulting product (ROH) is released from CYPs (Barnaba et al., 2017).

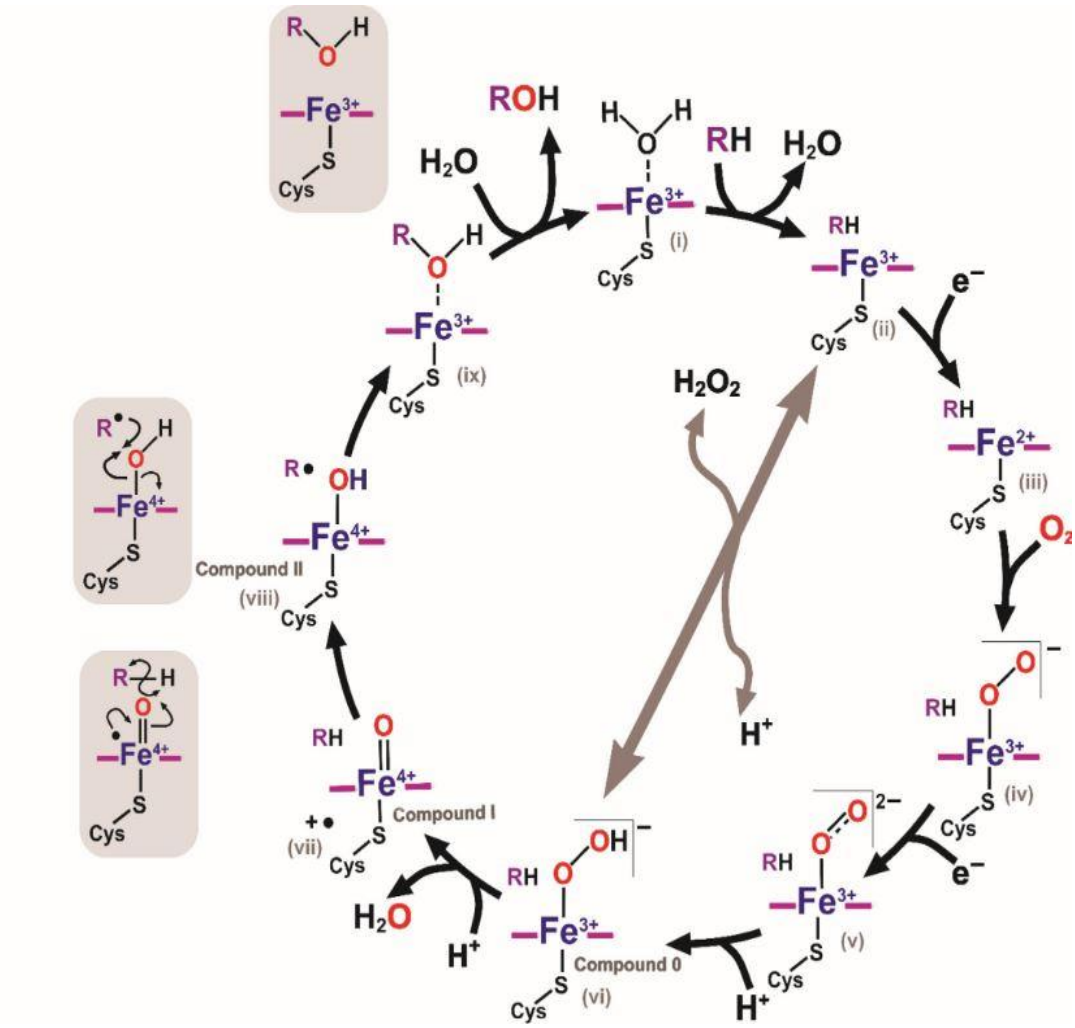

Figure 1. 5 General catalytic cycle of CYPs (Jeffreys et al., 2018).

There are 57 functional CYP genes and 58 pseudogenes encoded in humans. They are categorized into 18 families and 44 subfamilies according to sequence similarities (Zanger and Schwab, 2013). Human CYP families and their primary functions are given in Table 1.2.

Table 1. 2 Human CYP families, and their primary functions (Zhao et al., 2021).

CYP Family	Primary Functions	Subfamilies
1	Drug metabolism	3
2	Drug/steroid metabolism	13
3	Drug metabolism	1
4	Arachidonic acid/fatty acid metabolism	5
5	Thromboxane synthase	1
7	Steroid 7α-hydroxylase	2
8	Bile acid biosynthesis; prostacyclin synthase	2
11	Steroid biosynthesis	2
17	Steroid 7α-hydroxylase	1
19	Aromatase	1
20	Function not determined	1
21	Steroid biosynthesis	1
24	Vitamin D deactivation	1
26	Retinoic acid hydroxylase	3
27	Bile acid biosynthesis; vitamin D3 activation	3
39	Function not determined	1
46	Cholesterol 24-hydroxylase	1
51	Lanosterol 14α-demethylase	1

As indicated in Table 1.2, one of the essential roles of CYPs is the metabolism of drugs. More than 90 % of drug biotransformations are catalyzed by CYPs (Zanger and Schwab, 2013; Zhao et al., 2021). Due to their roles in drug metabolism, CYPs are generally expressed in the liver (Almazroo et al., 2017). However, CYP enzymes are also expressed in kidney, brain, vasculature, GI tract, adrenal gland, and placenta (Danielson, 2002; Sacerdoti et. al., 2003).

Inflammation is defined as the immune system's immediate response to infections or injuries and, is mainly identified by pain, heat, redness, and swelling (Calder, 2006; Chen et al., 2017). There are two types of inflammation, acute and chronic. Acute inflammation starts and gets severe rapidly. On the other hand chronic inflammation may take and last years as in the case of autoimmune responses, in which cytokines and eicosanoids take place.

It has been shown that AA and its metabolites have a central role in MS onset. Regarding AA, the regulation of nuclear factor κB (NF-κB) is strictly correlated with the balance between CYP ω-hydroxylase, and CYP epoxygenase activities (Hoxha and Zappacosta, 2020). NF-κB is a transcription factor that stimulates the expression of pro-inflammatory genes such as cytokines and chemokines. It also has crucial functions in controlling the survival, activation, and differentiation of T cells, and innate immune cells. Therefore, dysregulation of NF-κB may contribute to autoimmune disorders including MS and rheumatoid arthritis (Liu et al., 2017; Hoxha and Zappacosta, 2020).

Another critical aspect of MS onset, and progression is the increased levels of cytokines involving IL-2, 6, and 17, TNF-α, and interferon γ. These cytokines ignite the inflammation in CNS by activating the macrophages, T cells, and B cells. Macrophages and T cells then damage the myelin sheath of oligodendrocytes by NO and O_2 radicals. On the other hand, B cells produce demyelinating antibodies that further activate macrophages (Cosentino and Marino, 2013). Studies evidenced that these cytokines also inhibit the activities of CYPs (Abdel-Razzak, et al., 1993; Hoxha and Zappacosta, 2020).

Last but not least, CYPs generate reactive oxygen species (ROS) in their usual catalytic cycle from molecular oxygen. ROS then takes part in the pathogenesis of MS and contribute autoimmune inflammation by activating the inflammatory molecules. Genetic variations in CYPs may alter the ROS levels, thereby affecting the susceptibility of individuals to MS (Cosentino and Marino, 2013; Hoxha and Zappacosta, 2020).

On the other hand, Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) has protective effect on autoimmune response. PPARγ is a transcription factor, and has been known to take part in fatty acid metabolism and glucose homeostasis. Recent evidence indicates that PPARγ is also expressed in neurons, immune and glial cells in which it regulates anti-inflammatory mechanisms, neuronal death, neurogenesis, oxidative stress, and angiogenesis. PPARγ, alternatively, activates the immune cells by stimulating the expression of anti-inflammatory genes, and repressing the pro-inflammatory cytokines, interleukins and chemokines. Furthermore, it inhibits the action of NF-κB and NO release (Villapol, 2018).

Beside their role in drug metabolism, CYPs also function in inflammatory reactions via arachidonic acid metabolism, as mentioned previously. There are two distinct modes of action of CYPs. ω-hydroxylase activity of CYPs produces 7, 10, 12, 13, 15, 16, 17, 18, 19, and 20-HETE. 20-HETE is the main AA metabolite of ω-hydroxylation with pro-inflammatory properties. It is further metabolized into 20-hydroxy-prostaglandin analogs. 20-HETE is a potent vasoconstrictor, and it induces the generation of ROS in endothelial cells. However, more importantly, it stimulates the expression of cytokines and cellular adhesion molecules through NF-κB activation (Hoxha and Zappacosta, 2020).

Epoxygenase activity of CYPs on AA yields different EET regio-isomers (5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET). They are further converted to DHETs by soluble epoxide hydrolases (sEHs) (Wang et al., 2021). Although, CYPs are capable of producing four different EET regio-isomers, in many cases 11,12-EET and 14,15-EET are the predominant products of CYP action (Spector and Norris, 2007). EETs are produced in various cell types including astrocytes, leukocytes, cardiac, and endothelial cells. They are responsible for the regulation of MAPK activity, TXA_2 signalling, and ihbition of apoptosis (Hoxha and Zappacosta, 2020). EETs have also known to possess anti-inflammatory properties in addition to cardioprotective and vasodilatory properties (Wang et al., 2021). Cells treated with 5,6-EET, 8,9-EET and 11,12-EET regio-isomers showed protective effect on inflammatory cytokine upregulation of adhesion molecules and decreased pro-

inflammatory NF-κB signaling, and PGE_2 production (Iliff et al., 2010). 11,12-EET is the most potent inhibitor of NF-κB signaling (Spector and Norris, 2007). Moreover, predominant EET regio-isomers, 11,12-EET and 14,15-EET, are potent activators of PPARγ, and increases the PPARγ-mediated gene expression (Spector and Norris, 2007).

The concentration of AA and its metabolites greatly vary in MS patients, and mice with EAE. Cerebrospinal fluid, and serum samples of MS patients had distinct lipid signatures, including AA, with respect to healthy subjects. The lipid profile of EAE mice brains indicated a shift favoring AA production. (Pompura et al., 2022; Ferreira et al., 2021). It has been reported that AA metabolic pathways were overactivated in the CNS of MS patients. The concentrations of prostaglandins, and HETEs were increased in cerebrospinal fluid because of this overactivation, and neuroinflammation (Ferreira et al., 2021). Also, the overactivated metabolic pathway of AA resulted in a significant increase in EET regio-isomer levels in MS patients.

CYP-derived metabolites also play significant roles in cardiovascular diseases, inflammatory diseases, kidney inflammation, cancer, neurological and neurodegenerative disorders, and autoimmune diseases (Wang et al., 2021; Wang et al, 2019; Bosetti 2007). Nevertheless, 20-HETE and EETs exhibit partially opposite functions in vascular, renal, cardiac regulation and in angiogenesis (Hoxha and Zappacosta, 2020).

Since CYPs have diverse functions in autoimmune response in MS through AA catabolism, and their roles in metabolism of MS treatment drugs, we have selected CYP1A2, 2C9, 2D6, 3A4, and 4F2 and investigated their plausible roles within this study.

1.3.1 CYP1A2

The CYP1A2 gene is located on chromosome 15 as given in Figure 1.6. There are seven exons on the CYP1A2 gene, which encodes a protein with 515 amino acids (Tascioglu et al., 2021; Thorn et al., 2013).

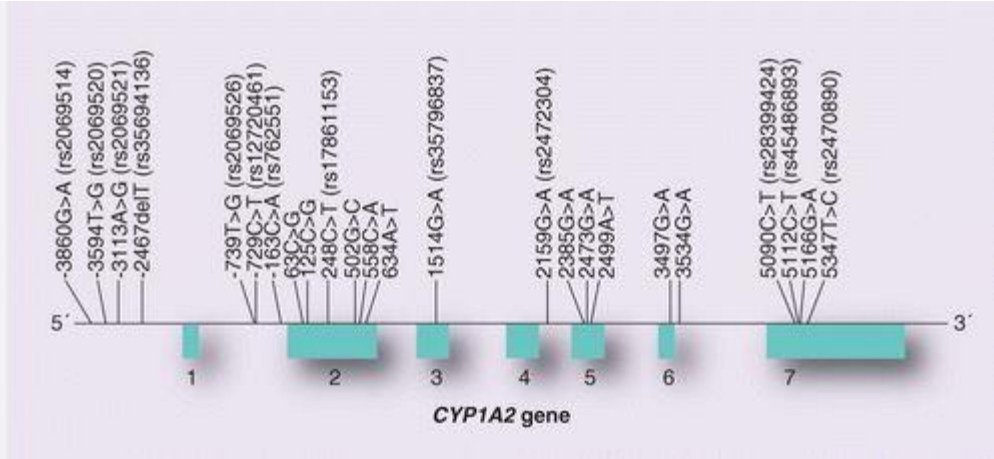

Figure 1. 6 Schematic representation of the CYP1A2 gene (Gunes and Dahl, 2008).

CYP1A2 is one of the main CYPs in the human liver (approximately 13-15 %). It shares a common 5'-flanking region with CYP1A1, unlike CYP1A2, which is mostly expressed in extrahepatic tissues such as intestine, lung, placenta, and lymphocytes. CYP1A2 and CYP1A1 have 80 % identical amino acid sequences and 74 % DNA sequences (Zhou et al., 2010; Koonrungsesomboon et al., 2018).

CYP1A2 is responsible for the metabolism of drugs such as clozapine (antipsychotics), tacrine (acetylcholinesterase inhibitor), tizanidine (adrenergic receptor agonist), and theophylline (antiasthmatics). It metabolizes procarcinogens including aromatic amines, and several endogenous compounds like melatonin, estrone, and estradiol (Zhou et al., 2010; Koonrungsesomboon et al., 2018).

CYP1A2 exhibits substantial inter-individual variability. As in the clozapine case, the plasma concentration of the drug differs as much as 40-fold between the patients who have taken the same dose of clozapine (Rasmussen et al., 2022). More than 30 allelic variants of CYP1A2 have been listed on PharmVar

(Koonrungsesomboon et al., 2018; https://www.pharmvar.org/gene/CYP1A2). Among these allelic variants, *CYP1A2*1F* (−163A>C) is one of the most common, and widely studied variant. *CYP1A2*1F* variant (rs762551) has decreased catalytic activity, protein expression, and mRNA level (Thorn et al., 2012). They are regarded as slow metabolizers. AA genotype was accepted as a fast metabolizer, conversely presence of the C allele indicated slow metabolizing individuals (Thorn et al., 2012). A meta-analysis concluded that the AA genotype showed fast metabolizer activity only in the presence of an inducer such as caffeine (Koonrungsesomboon et al., 2018). *CYP1A2*1F* variant has been associated with neurodegenerative diseases, kidney dysfunction, breast cancer, and lung cancer (Siokas et al., 2021; Kim et al., 2018; Siokas et al., 2022; Mahdavi et al., 2023; Tian et al., 2013; Khvostova et al., 2012). These studies pointed that the mutant C allele had increased the risk of disease development.

Besides drug metabolism, CYP1A2 also metabolizes AA. It displays high catalytic activity towards AA producing HETEs and EETs (El-Sherbeni and El-Kadi, 2014; Korbecki et al., 2023). CYP1A2 is responsible for the conversion of AA into 20-HETE via ω-hydroxylation. In addition to ω-hydroxylation activity, epoxidation of AA by CYP1A2 yields four different EETs (5,6-EET, 8,9-EET, 11,12-EET, or 14,15-EET) because AA has four cis-double bonds (Korbecki et al., 2023). The main EET product is 11,12-EET after CYP1A2 action. 11,12-EET is followed by 14,15-EET, 5,6-EET, and 8,9-EET in descending order (Lucas et al., 2010).

1.3.2 CYP2C9

The CYP2C9 gene is placed on chromosome 10 (Figure 1.7). There are nine exons on the CYP2C9 gene, encoding a protein consisting of 490 amino acids (Xie et al., 2002).

Figure 1. 7 Schematic representation of the CYP2C9 gene (Scott et al., 2009).

CYP2C9 is the most abundant enzyme belonging to the CYP2C subfamily in the human liver which is approximately 50% of the total CYP 2C subfamily. It metabolizes about 15-20 % of all clinically significant drugs such as Tolbutamide and Glimepiride (antidiabetics), Acenocoumarol and Warfarin (anticoagulants), Diclofenac, Ibuprofen, and Flurbiprofen (nonsteroidal anti-inflammatory drugs), Phenytoin and Valproate (antiepileptics), Losartan and Irbesartan (antihypertensives), Torsemide and Sulfinpyrazone (diuretics) (Isvoran et al., 2017; Fekete et al., 2021; Suzuki et al., 2006). Also CYP2C9, as along with CYP3A4, is responsible for the biotransformation of Siponimod which is used in MS treatment (Glaenzel et al., 2018).

More than 65 allelic variants of CYP 2C9 have been described in the human genome (Isvoran et al., 2017; Fekete et al., 2021). Like the other CYP enzymes, CYP2C9 displays an enormous genetic difference among individuals. *CYP2C9*2* (R144C) and CYP2C9*3 (I359L) are the most common and well-studied variants. However, these variants are accompanied by reduced CYP2C9 enzymatic activity (Fekete et al., 2021; Pratt et al., 2019; Sausville et al., 2018). Molecular dynamics simulations of the *CYP2C9*2* variant (rs1799853) predicted that the volume of the active site has expanded, the distance between metabolism site and heme center has increased, the size of substrate entry channel has decreased, hydrogen bonding with specific amino acids has changed. These alterations might contribute to the reduced enzymatic activity of CYP2C9 (Daly, et al., 2017).

CYP2C9 variants have been associated with warfarin dose prediction, and bleeding complications, Hodgkin lymphoma in young adults, drug-resistant epilepsy in

children, colorectal adenoma in adults, and breast cancer (Sanderson et al., 2005; King et al., 2004; Lindh et al., 2009; Oner Ozgon et al., 2009; Kocael et al., 2019; Chang et al., 2009; Makowska et al., 2021, Barry et al., 2009; Savas et al., 2006). Mutant T allele increases the risk of having these diseases.

Furthermore, CYP2C9 is also responsible for the metabolism of AA which catalyzes the epoxygenation reaction yielding the formation of EETs. CYP2C9 epoxidation activity mainly yields 14,15-EET. Also CYP2C9 produces 11,12-EET, and 8,9-EET, but not 5,6-EET. Due to the relatively short half-lives of EETs, they are converted to DHETs (dihydroxyeicosatrienoic) by sEH (soluble epoxide hydrolase) (Fan and Roman, 2017; Lucas et al., 2010).

1.3.3 CYP2D6

The CYP2D6 gene is located on chromosome 22. It has nine exons as seen in Figure 1.8 (Fuselli et al., 2004).

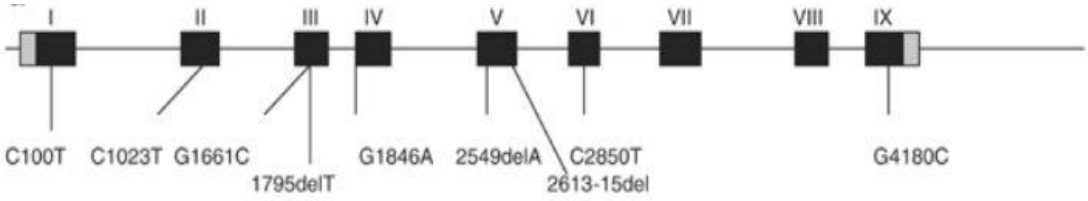

Figure 1. 8 Schematic representation of the CYP2C9 gene (Fuselli et al., 2004).

Despite CYP2D6's relatively low amount (2-4%) compared to other CYPs in the liver, it is responsible for the metabolism of approximately 20-25% of commercially available medications (Chan et al., 2019; Ramamoorthy et al., 2010). Atenolol and Bisoprolol (β blockers), Amiodarone and Disopyramide (antiarrhythmics), Amitriptyline, Citalopram and Clomipramine (antidepressants), Aripiprazole and Brexpiprazole (antipsychotics) and Tamoxifen (cancer treatment) and opioids are the substrates of CYP2D6 enzyme (Chan et al., 2019; Taylor et al., 2020).

The CYP2D6 is one of the most polymorphic CYP enzymes among all cytochrome P450 enzymes. Over 130 allelic variants have been identified and listed on PharmVar (Chan et al., 2019; Taylor et al., 2020). Variations of the CYP2D6 gene result in reduced or increased enzymatic activities, eventually altering the drug efficacy and causing adverse effects. According to *in vivo* studies, CYP2D6 enzyme activity ranges from ultra-rapid to poor or no activity. Individuals are classified into four groups, in which ultra-rapid, normal, intermediate and poor metabolizers take part, according to their CYP2D6 enzymatic activities. No enzymatic activity is observed in poor metabolizers. Intermediate metabolizers are observed to decrease CYP2D6 enzyme's activity than the normal metabolizers, and ultra-rapid metabolizers are observed to increase enzymatic activity (Kane, 2021).

The *CYP2D6*2* (R296C) allele has exhibited opposite results in the literature. In various *in vivo* and *in vitro* studies, the *CYP2D6*2* allele (rs16947) has been reported to have decreased, increased or unchanged activity. Moreover, *CYP2D6*2* haplotypes have harboured additional variants, such as the *CYP2D6*41* allele. However, according to Wang and his co-workers, *CYP2D6*2* (R296C) changes the splicing of exon six and results in a 2-fold decrease in CYP2D6 expression. The polymorphic A allele has been associated with decreased enzyme activity (Wang et al., 2014). X-ray crystallography and MD simulations suggest that the active site volume of the *CYP2D6*2* variant has diminished. *CYP2D6*2* variant exhibited 11 % shrinkage of active site volume. Also, this study reports that mutations have decreased the number of active sites due to the changes in protein structure. The *CYP2D6*2* variant has 13 active sites, whereas the wild-type enzyme has 14 (Dong et al., 2019). Furthermore, the *CYP2D6*2* variant has been an essential driving factor for the development of schizophrenia, epilepsy, and various cancer types (Ma et al., 2020; Elsaid et al., 2022; Zhou et al., 2012).

CYP2D6 has been also shown to participate in eicosanoid metabolism (Nebert et al., 2013). Eicosanoids are lipid-based signaling molecules critical in immune response (Sheppe and Edelman, 2021). Lucas et al. (2010) concluded that the epoxygenase activity of CYP2D6 yields 5,6-EET, 8,9-EET, 11,12-EET, or 14,15-

EET in varying levels. 14,15-EET has the highest concentration among other EET regio-isomers. 14,15-EET is followed by 8,9-EET, 11,12-EET, and 5,6-EET in descending order (Lucas et al., 2010).

1.3.4 CYP3A4

The CYP3A4 gene is located on chromosome 7. There are thirteen exons on the CYP3A4 gene as illustrated in Figure 1.9, and it encodes a protein with 503 amino acid residues (Zhou et al., 2011).

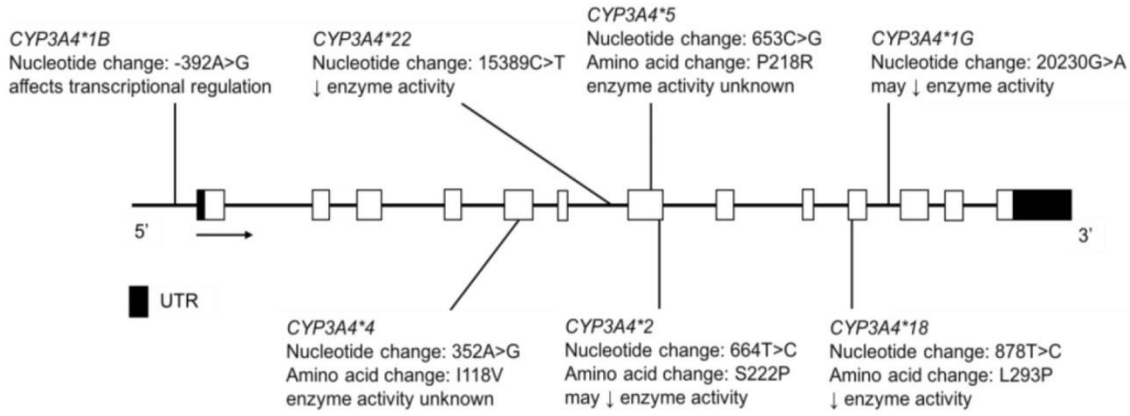

Figure 1. 9 Schematic representation of the CYP3A4 gene (Henderson et al., 2018).

CYP3A4 is the most abundant and vital CYP450 isoenzyme in the adult human liver (Werk and Cascorbi, 2014; Zhou et al., 2011). Due to its wide range of substrate specificity, it metabolizes half of the clinically used medications (Zhou et al., 2011; Zhou et al., 2019).

Clarithromycin and Troleandomycin (antibiotics), Voriconazole and Ketoconazole (antifungals), Nefazodone (antidepressants), Saquinavir, Darunavir and Lopinavir (antivirals), Nilotinib, Ribociclib and Midostaurin (anticancer agents) Loperamide (antidiarrheals), Terfenadine (antihistaminics) are the some of the substrates of CYP3A4 (Zhou et al., 2011; https://go.drugbank.com/categories/DBCAT002647). Most of the drugs used in MS treatment are also metabolized by CYP3A4. For instance biotransformations of Siponimod, Teriflunomide, Laquinimod, Ozanimod,

and Ponesimod are reported to be achieved by CYP3A4 (Glaenzel et al., 2018; Carazo et al., 2018; Thöne and Linker, 2016; Surapaneni et al., 2021; Alnaif et al., 2022).

CYP3A4 has relatively lower genetic differences between populations with respect to other CYPs, but the enzymatic activity of CPY3A4 exhibits extensive interindividual variability that causes drug toxicity, therapeutic failure, and adverse effects (Zhou et al., 2011). This interindividual variability can be as high as 43.2 fold, according to the study conducted by Zhou and co-workers (Zhou et al., 2019). In another study, it has been reported that interindividual variability of CYP3A4 enzymatic activity differs up to 60-fold (Zhou et al., 2011).

Although amino acid sequence changes in CYP3A4 variants are rare, more than 30 allelic variants of CYP3A4 have been identified and listed on PharmVar (Wang et al., 2011, Zhou et al., 2011; https://www.pharmvar.org/gene/CYP3A4). Among these allelic variants, *CYP3A4*1B* (A392G) is the most common allele located on the promoter region of the CYP3A4 gene and alters the CYP3A4 enzyme activity (Berges et al., 2011; Werk and Cascorbi, 2014). Generally, non-exonic variants have altered mRNA levels via common mechanisms affecting transcription, RNA elongation, and splicing (Wang et al., 2011). Despite the controversial reports related to the enzymatic activity of *CYP3A4*1B* variant (rs2740574), a more recent study exhibited a poor response to ifosfamide treatment in *CYP3A4*1B* carriers, which indicated the decreased enzymatic activity of this variant (Espindola et al., 2019). Furthermore, the *CYP3A4*1B* variant has been linked with the risk of development of different cancer types including ovarian cancer, breast cancer, small cell lung cancer, and prostate cancer (Zhou et al., 2013; Pearce et al., 2009; Kato et al., 2009; Dally et al., 2003; Zeigler-Johnson et al., 2004; Fernandez et al., 2012). The polymorphic C allele in CYP3A4 has been associated with decreased enzymatic activity, and risk of developing different cancer types.

Despite significant drug metabolism roles, CYP3A4 also bears ω-hydroxylation and epoxygenase activity on AA. (Zhou et al., 2011; Bishop-Bailey et al., 2014). It

has been reported that CYP3A4 can catalyze the formation of different EET regio-isomers and 20-HETE (Mitra et al., 2011; Lucas et al., 2010). Epoxygenase activity of CYP3A4 mainly yields 11,12-EET, and 14,15-EET. Also, 8,9-EET, and 5,6-EET are produced in lower amounts by CYP3A4 (Lucas et al., 2010).

1.3.5 CYP4F2

The CYP4F2 gene is located on chromosome 19. There are thirteen exons on the CYP4F2 gene as represented in Figure 1.10, and it encodes a protein with 520 amino acid residues (Meng et al., 2015).

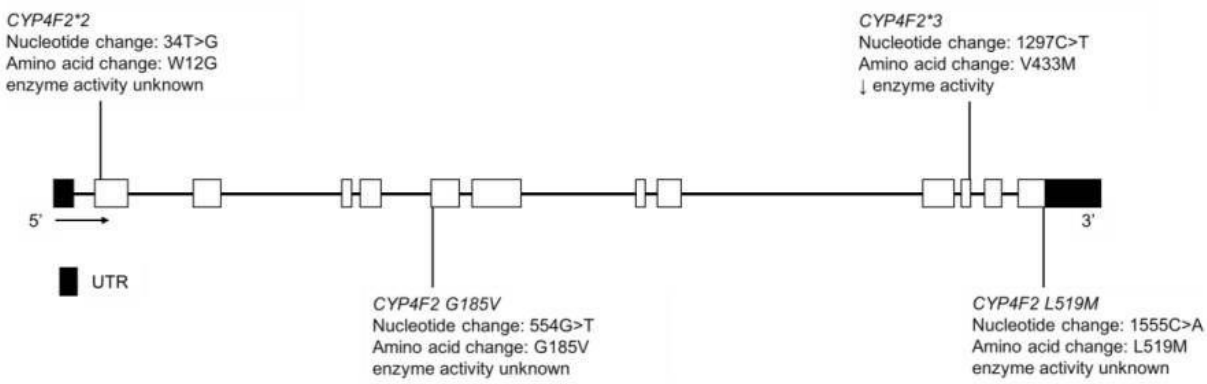

Figure 1. 10 Schematic representation of the CYP4F2 gene (Henderson et al., 2018).

CYP4F2 metabolizes drugs, such as Fingolimod, that is recently started to be used in MS treatment (David et al., 2012). However, its prominent role the is metabolism of endogenous compounds including fatty acids, arachidonic acid, prostaglandins, EETs, HETEs, and HPETEs (Nebert and Russell, 2002). ω-hydroxylating CYP4F2 primarily catalyzes 20-HETE production from AA (Kampschulte et al., 2020). 20-HETE is now regarded as a new therapeutic target for regulation of blood pressure. In general, 20-HETE is a potent vasoconstrictor that decreases blood flow. Also, it inhibits sodium ion transport in the kidneys causing hypertension (Williams et al., 2010). Moreover, CYP4F2 is found to ω-

hydroxylate leukotriene A4 (LTA4) as well, and it takes part in the catabolism of vitamin K and vitamin E (Jarrar and Lee, 2019; Henderson et al., 2018).

CYP4F2 is expressed in the human liver, kidney, heart, lung, and leukocytes, and there are three allelic variants of CYP4F2 (*CYP4F2*1*, *CYP4F2*2*, and *CYP4F2*3*) listed on PharmVar (Meng et al., 2015; https://www.pharmvar.org/gene/CYP4F2). *CYP4F2*3* (V433M) (rs2108622) has been the most studied allelic variant of CYP4F2. This variant is associated with decreased enzymatic activity and decreased 20-HETE metabolization in the kidneys (Stec et al., 2007). Computational analysis indicated that this decreased enzymatic activity of T allele carriers might have resulted in the size of mutant amino acid. Methionine is larger than the wild-type amino acid Valine, and possibly Methionine does not fit in the protein structure. Plus, the polymorphic enzymes have more open structures than the native ones. This variant affects the stability, and dynamics of CYP4F2 enzyme by diminishing the compactness, and stability of protein structure, which results in overall structural and conformational changes (Farajzadeh-Dehkordi et al., 2023).

Furthermore, the *CYP4F2*3* variant have been associated with metabolic syndrome, ischemic stroke, adrenoleukodystrophy, a neurodegenerative disease in which very long-chain fatty acids are accumulated, coronary heart disease, type II diabetes, and chronic obstructive pulmonary disease (Alvarellos et al., 2015; Fava et al., 2012; Liao et al., 2016; van Engen et al., 2016, Yu et al., 2014, Park et al., 2019; Ding et al., 2020).

Table 1.3 summarizes the genome locations of SNPs, arachidonic acid intermediates of selected CYPs, conformational, structural, and enzyme activity changes accompanied with each SNP, and minor allele frequencies according to dbSNP, and gnomAD.

Table 1. 3 Genome locations, arachidonic acid intermediates, conformational, structural, and enzyme activity changes, and minor allele frequencies of selected genes.

SNP	Genome Location	Enzyme Product(s)	Structural & Conformational Changes	Enzyme Activity	MAF (dbSNP; GnomAD)
CYP1A2 (rs762551) (A > C)	Intron	20-HETE (ω-hydroxylation) 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET (epoxidation)	-mRNA level and protein expression decreases	↓	0.32; 0.29
CYP2C9 (rs1799853) (C > T)	Exon (R144C)	8,9-EET, 11,12-EET, 14,15-EET (epoxidation)	-Active site volume decreases -Distance between the active site and heme center increases -Substrate entry channel size decreases	↓	0.11; 0.09
CYP2D6 (rs16947) (G > A)	Exon (R296C)	5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET (epoxidation)	-Active site volume decreases (11 % shrinkage) -Number of active sites decreases	↓	0.32; 0.34
CYP3A4 (rs2740574) (T > C)	Promoter	20-HETE (ω-hydroxylation) 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET (epoxidation)	-mRNA level and protein expression decreases -transcriptional regulation is affected	↓	0.11; 0.21
CYP4F2 (rs2108622) (C > T)	Exon (V433M)	20-HETE (ω-hydroxylation)	-Methionine does not fit in the protein structure -Polymorphic enzyme has a more open structure -Compactness of the enzyme decreases	↓	0.29; 0.27

1.4 Genetic Polymorphism

The human genome comprises two sets of 23 chromosomes, and the whole genome consists of more than 3 billion base pairs (Levy et al., 2007; Karki et al., 2015). Evolutionarily, the human genome has been very well conserved, and any two human genomes on earth are at least 99.5% identical. Genetic polymorphism is the variations in the DNA sequences among individuals in a population that occurs with a frequency of 1 % or higher. The high frequency of a polymorphism in a particular population indicates that polymorphism is naturally existing, with neutral or advantageous effects. (Karki et al., 2015). Several sources of genetic polymorphism include single nucleotide polymorphisms (SNPs), sequence repeats, insertions, deletions, inversions, duplications, and translocations (Bull, 2013; Karki et al., 2015). In general, polymorphisms can be grouped into two, synonymous and non-synonymous. In synonymous polymorphisms, encoded amino acid does not change and are called silent variations. So far, they have been thought to be neutral. However, recent evidence show that these silent variations might affect the protein expressions and functions, so they should not be considered as neutral or silent. They are associated with mRNA stability, splicing, translation efficiency, protein folding, and function (Edwards et al., 2012). On the other hand, non-synonymous variations change the amino acid sequence of the encoded proteins, leading to alterations in the protein structures and functions (Chu and Wei, 2019; Albert, 2011).

1.4.1 Single Nucleotide Polymorphism (SNP)

Over the last two decades, advancements in molecular biology techniques such as high-throughput sequencing, computational analysis methods, and human genome project have contributed to the understanding of human genome nature (Levy et al., 2007). These tools have reaccelerated the single nucleotide polymorphism (SNP)

studies. SNPs are single nucleotide substitutions with a population frequency of >1% (Edwards et al., 2012). They are the most common type of genetic variation among individuals, which exists in every 300-2000 base pairs and can be seen either in the coding, non-coding, or intergenic regions (Alwi, 2005).

Most SNPs do not directly affect an individual's health or development. However, some of these genetic variations have been determined to be important in human health. SNPs have been linked with susceptibility to environmental factors such as toxins, drug response (sensitivity or resistance to certain drugs), and risk of developing particular diseases (Alwi, 2005; Sripichai and Fucharoen, 2007).

1.5 Aim of the Study

MS is a complex, autoimmune, central nervous system disease affecting young adults. The average age of individuals suffering from MS is around 30. Since people at the most productive time of their life are affected by MS, and the physical capabilities of individuals with MS have gradually diminished, patients' participation in the general workforce has been interrupted. This brings a substantial socioeconomic burden to individuals and society.

On the other hand, it is very well known that polymorphisms are associated with susceptibilities to certain diseases. Besides their essential functions in drug metabolism, CYPs are considered immunologically significant for their roles in arachidonic acid metabolism. A balance between CYP ω-hydroxylase and CYP epoxygenase activities has been highly associated with NF-κB. This transcription factor induces the expression of pro-inflammatory genes including cytokines and chemokines. Disrupted regulation of NF-κB may contribute to the development of autoimmune responses, as in the case of MS, by stimulation of cytokines production such as IL-2, 6, and 17, TNF-α, interferon γ. These cytokines have been shown to inhibit the activity of CYPs. Furthermore, CYPs constantly generate ROS in their regular catalytic cycle. These ROS can activate inflammatory molecules in

MS or other autoimmune disorders. Therefore genetic variations in CYPs may alter the NF-κB, pro-inflammatory cytokine, and ROS levels, thereby affecting the individuals' susceptibility to MS.

Several *in vivo* studies including different animal models and case-control human subjects concluded that fatty acid metabolism is significantly altered in MS onset or during the progress of MS, especially in relapses. AA metabolism has been found to be overactivated in MS patients, and AA metabolites have been shown to take part in neuroinflammation, demyelination, motor dysfunctions, axonal damage, and oligodendrocyte loss. So, COX and LOX pathways of AA metabolism have been intriguing targets for new MS treatments. However, CYP-dependent AA metabolism and its potential role in MS have been undervalued. Although polymorphisms in vitamin D metabolizing CYPs such as CYP2R1, 24A1, 27A1, and 27B1 have been widely studied in different populations of MS patients, there is a limited number of polymorphism studies regarding the CYPs involving in AA metabolism. What is more important is that no polymorphism study related to these CYPs in MS patients in the Turkish population has been reported so far. Our main motivation in this study was to determine the possible effects of polymorphisms in CYP1A2, 2C9, 2D6, 3A4, and 4F2 in MS onset.

Therefore, we aimed to investigate the relationships between SNPs in AA-metabolizing CYP1A2, 2C9, 2D6, 3A4, and 4F2, and MS. By doing so, we also aimed to propose the possible protective or disease causing effects of SNPs in CYP1A2, 2C9, 2D6, 3A4, and 4F2.

This study also gives insight into the effectiveness of the specific MS treatment strategies since selected CYPs are responsible for the biotransformation of Siponimod, Teriflunomide, Fingolimod, Laquinimod, Ozanimod, and Ponesimod. This study will contribute to MS knowledge by adding of valuable and novel data.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Blood Sampling

Blood samples were collected from 179 MS patients and 102 healthy control subjects at Gülhane Education and Research Hospital, Ankara, Türkiye. All of the participants (patients and healthy controls) were Caucasians and they were from Central Anatolia of Türkiye. MS patients were diagnosed based on the 2010 McDonald Criteria, including neurological examinations, brain MRI scans, Lumbar puncture, and blood tests. In order to avoid any interferences, patients with other diseases such as cancer, neurological disorders, cerebrovascular diseases, cardiovascular diseases, rheumatological diseases, blood clotting defects, and hepatic and renal failure were eliminated. Healthy controls without any neurological disorders were selected randomly.

Blood samples were taken for the previously conducted "Association of the Vitamin D Metabolizing CYP24A1, CYP27A1, CYP27B1 and Vitamin D Receptor Genetic Polymorphisms with Multiple Sclerosis Risk in Turkish Population" titled study which was approved by the Ethical Committee of Gülhane Education and Research Hospital with the document number of 50687469-1491-31-16/1648.4-04. This thesis study was a continuation of the previous work and previously collected blood samples were used in this study. Current study was also approved by the Ethical Committee of the University of Health Sciences (Appendix A), and the principles of the Declaration of Helsinki were followed (World Medical Association, 2014).

2.1.2 Chemicals and Enzymes

Prona Agarose Biomax (HS-8000), 2-amino-2(hydroxymethyl)-1,3-propanediol (Tris) (T1503-1), and ethanol (24105) were purchased from Sigma-Aldrich Chemical Company, Saint Louis, Missouri, USA. Ethylenediaminetetraacetic acid (EDTA) (108421) was bought from E. Merck, Darmstadt, Germany. ClearBand SAFE DNA Gel Stain Solution (20000x) (SDGS1) was brought from Ecotech Biotechnology, Türkiye. WizPrep™ gDNA Mini Kit (Blood) (W71050) was purchased from Wizbiosolutions, Republic of Korea. Taq DNA polymerase (EP0402), dNTP set (R0181), and GeneRuler 1 kb DNA Ladder (SM0311) were the products of Thermo Fisher Scientific, Waltham, Massachusetts, USA. Restriction enzymes Apa I (R0114S), Ava II (R0153S), Fsp I (R0135S), Mbo II (R0148S), and Pvu II (R0151S) and their buffers were purchased from New England Biolabs, Ipswich, Massachusetts, USA. Only analytical grade, highest level purity chemicals were used in this work.

2.1.3 Primers

NCBI and Ensembl database were used to design suitable primers. Primers were purchased from Oligomer Biotechnology, Ankara, Türkiye, stored at -20^{o}C, and diluted to 10 μM before use. The sequences of forward and reverse primers for rs762551 (A → C) polymorphism of CYP1A2, rs1799853 (C → T) polymorphism of CYP2C9, rs16947 (G → A) polymorphism of CYP2D6, rs2740574 (T → C) polymorphism of CYP3A4, and rs2108622 (C → T) polymorphism of CYP4F2 are given in Table 2.1.

Table 2. 1 Sequence information of forward and reverse primers for selected gene regions

Region	Primers (5' → 3')	Product Size (bp)
CYP1A2 (rs762551)	Forward Primer CAAGGGTGCTGTCCTTGG Reverse Primer ATCCTTGACAGTGCCAGGTG	541
CYP2C9 (rs1799853)	Forward Primer GGAGGATGGAAAACAGACTAGCAGAGC Reverse Primer ACTCCAAATAAAAGATATGGCCACC	378
CYP2D6 (rs16947)	Forward Primer TGGACAGACATGCGTCC Reverse Primer GACAGGTGCAGAATTGGAG	420
CYP3A4 (rs2740574)	Forward Primer GAGGCTTCTCCACCTTGGAAGTTGGCAA Reverse Primer GGAATGAGGACAGCCATAGAGACAAGGGGA	219
CYP4F2 (rs2108622)	Forward Primer CCATCAACCCGTTCCCACCTCAG Reverse Primer TTAGGGACGACCTGGCCCATTTG	596

2.2 Methods

2.2.1 Genomic DNA isolation

Blood samples of the patients and control group were stored at -80°C until use. gDNA isolation from these blood samples was performed using WizPrep™ gDNA Mini Kit (Blood) according to in the manufacturer's instructions.

200 µl of whole blood sample was transferred to a 1.5 ml microcentrifuge tube. 200 µl of GB buffer and 20 µl of proteinase K were added to the blood sample and vortexed. This mixture was incubated at 56°C for 10 minutes. During incubation, the microcentrifuge tube was inverted at every 5 minutes. After incubation, 200 µl of absolute ethanol was added to the mixture and vortexed

briefly. Then, the mixture was transferred to the spin column supplied with the kit and centrifuged for 1 minute at 13.000 rpm by iFuge M08 Smart Personal Micro Centrifuge. The collection tube with flow-through was discarded, and the spin column was transferred to the new collection tube. 500 µl of W1 buffer was added to the spin column and centrifuged for 1 minute at 13.000 rpm. The flow-through solution was discarded, and the spin column was reconnected with the collection tube. 500 µl of W2 buffer was added to the spin column and centrifuged for 1 minute at 13.000 rpm. Again, the flow-through was discarded, and the spin column was reconnected with the collection tube and centrifuged for 2 minutes at 13.000 rpm. After centrifugation, the spin column was transferred to a new 1.5 ml tube. In order to elute gDNA from the column, 40 µl of elution buffer was added to the center of the spin column, incubated at room temperature for 1 minute, and centrifuged for 1 min at 13.000 rpm. Then, 30 µl of the elution buffer was added to the spin column. After 1 minute of incubation at room temperature, the tube was centrifuged for 1 min at 13.000 rpm. The spin column was discarded, and isolated DNA was taken to a new 1.5 ml Eppendorf tube. Isolated gDNA samples were stored at -20°C until further analysis.

The concentration and purity of gDNA were measured using Thermo Scientific™ NanoDrop™ Spectrophotometer.

2.2.2 Qualification of Genomic DNA Samples by Agarose Gel Electrophoresis

Biorad horizontal gel electrophoresis system (Wide Mini-Sub Cell GT Cell) was used to determine the intactness of isolated DNA samples. 10X TBE (Tris-Borate-EDTA) buffer is diluted to 0.5X. The final concentrations of Tris, Boric Acid, and EDTA were 45 mM, 45 mM, and 1mM, respectively. Then, 1 g agarose was added to 100 ml of 0.5X TBE buffer. The final mixture was microwaved until fully dissolved (no solid particle remained). While the solution was cooling down, it was stirred to provide homogenous cooling. After enough cooling down, 5 µl

SAFE DNA Gel Stain Solution (20000X) was added. Before pouring the solution into the tray, the equipment was cleaned with 70 % ethanol. The solution was poured into the tray by avoiding air bubbles, and let to solidify completely. Then, the solid gel was placed in an electrophoresis tank, and covered with 0.5X TBE buffer.

1 µl 10X orange dye was added to 5 µl of DNA samples, and 4 µl of MB grade water completed the total volume to 10 µl. Solution mixed well by pipetting, and loaded into the gel. DNA ladder was also loaded into the gel to determine the approximate sizes of DNA samples. After loading, the gel ran for 30 minutes at 120V. Then, the gel was visualized with Amersham™ Imager 680. Single DNA bands indicate that DNA samples are intact. If there had been any degradation, smear formation should have been seen.

2.2.3 Polymerase Chain Reactions (PCR)

PCR was performed to amplify the gene regions under investigation. The reaction mixture was prepared in a 1.5 mL Eppendorf tube, and each PCR tube contained 5 µl Thermo's Taq Buffer (10X) (with NH_4^+), 1 µl 10 mM dNTPs, 4 µl 25 nM $MgCl_2$, 2 µl forward primer, 2 µl reverse primer, 0.25 µl Taq Polymerase, and 35.75 µl MB grade water (Table 2.2) . After preparing the reaction mixture, 70 ng gDNA (~8 µl) was transferred into a PCR tube and 42 µl of the reaction mixture was added.

Table 2. 2 PCR mixture contents

Contents	**Volume**
Thermo's Taq Buffer (10X)	5 µl
dNTPs	1 µl
25 nM $MgCl_2$	4 µl
Forward primer	2 µl
Reverse primer	2 µl
Taq Polymerase	0.25 µl
MB grade water	35.75 µl
Total	**50 µl**

Since each primer has different GC content, a different reaction condition was employed. The final reaction conditions are given in Table 2.3. The product size of each reaction was confirmed by agarose gel electrophoresis, as explained in section 2.2.2.

Table 2. 3 PCR conditions of selected genes

	CYP1A2 (rs762551)		**CYP2C9 (rs1799853) CYP2D6 (rs16947)**		**CYP3A4 (rs2740574) CYP4F2 (rs2108622)**	
	Temperature	**Time**	**Temperature**	**Time**	**Temperature**	**Time**
Pre-denaturation	95 ºC	4 min	95 ºC	4 min	95 ºC	4 min
Denaturation (35 Cycles)	95 ºC	30 sec	95 ºC	30 sec	95 ºC	30 sec
Annealing (35 Cycles)	56 ºC	30 sec	60 ºC	30 sec	67 ºC	30 sec
Extension (35 Cycles)	72 ºC	40 sec	72 ºC	30 sec	72 ºC	40 sec
Final Extension	72 ºC	5 min	72 ºC	5 min	72 ºC	5 min

2.2.4 Sanger Sequencing

Amplified gene regions were verified with sanger sequencing. PCR products of each gene were shipped to Macrogen Europe, Amsterdam, Netherlands, and the DNA sequences of amplified gene regions were validated.

2.2.5 Restriction Endonuclease Reactions

The Restriction Fragment Length Polymorphism (RFLP) method can be defined as determining of differences in DNA sequences by detecting the lengths of DNA fragments after restriction enzyme digestion. In this study, five restriction enzyme reactions were performed for five different genes. Restriction enzyme digestions were performed according to the manufacturer's instructions.

For each PCR product, 5 µl buffer (supplied with restriction enzymes), 1 µl MB grade water, and 0.2 µl restriction enzyme were added into PCR tubes containing PCR product. Samples were mixed, spun down and incubated at 37°C (rs762551 PCR product was incubated at 30°C) for 4 hours in a water bath. After incubation, 20 µl from each sample were mixed with 2 µl orange dye (10X), and loaded into 2 % agarose gel. The gel ran for 1 hour at 100 V, and was visualized with Amersham™ Imager 680.

SNPs, restriction enzymes, their recognition sites, and expected DNA fragment numbers and sizes are given in Table 2.4.

Table 2. 4 SNPs, restriction enzymes, their recognition sites, wild-type (WT), and polymorphic (PM) alleles, DNA fragment numbers and sizes after restriction enzyme digestion.

SNP	Restriction Enzyme	Recognition Site	WT Allele / PM Allele	Number of DNA Fragments	DNA Fragment Size
CYP1A2 (rs762551)	ApaI	5′...GGGCC▼C...3′ 3′...C▲CCGGG...5′	A	1	541
			C	2	369/176
CYP2C9 (rs1799853)	AvaII	5′...G▼GWCC...3′ 3′...CCWG▲G...5′	C	2	221/154
			T	1	378
CYP2D6 (rs16947)	FspI	5′...TGC▼GCA...3′ 3′...ACG▲CGT...5′	G	2	305/115
			A	1	420
CYP3A4 (rs2740574)	MboII	5′...GAAGA(N)$_8$▼...3′ 3′...CTTCT(N)$_7$▲...5′	T	1	219
			C	2	179/40
CYP4F2 (rs2108622)	PvuII	5′...CAG▼CTG...3′ 3′...GTC▲GAC...5′	C	2	316/280
			T	1	596

2.2.5.1 Genotyping of rs762551 with ApaI

ApaI was used for identifying the nucleotide change of rs762551 on the CYP1A2 gene. In rs762551, Adenine (A) is replaced with Cytosine (C) in polymorphic individuals. After replacing C with A, the ApaI recognition site shows up. Therefore, polymorphic alleles can be cut by ApaI. Wild-type allele with the GGGCAC sequence does not carry ApaI recognition site, and remains uncut (Figure 2.1).

Figure 2. 1 Schematic representation of amplified gene region, forward and reverse primers, and ApaI recognition site of rs762551 (CYP1A2).

Wild-type genotype (AA) is expected to give one band, homozygous polymorphic genotype (CC) is expected to give two bands, and heterozygous genotype (AC) is expected to give three bands on the gel (Figure 2.2).

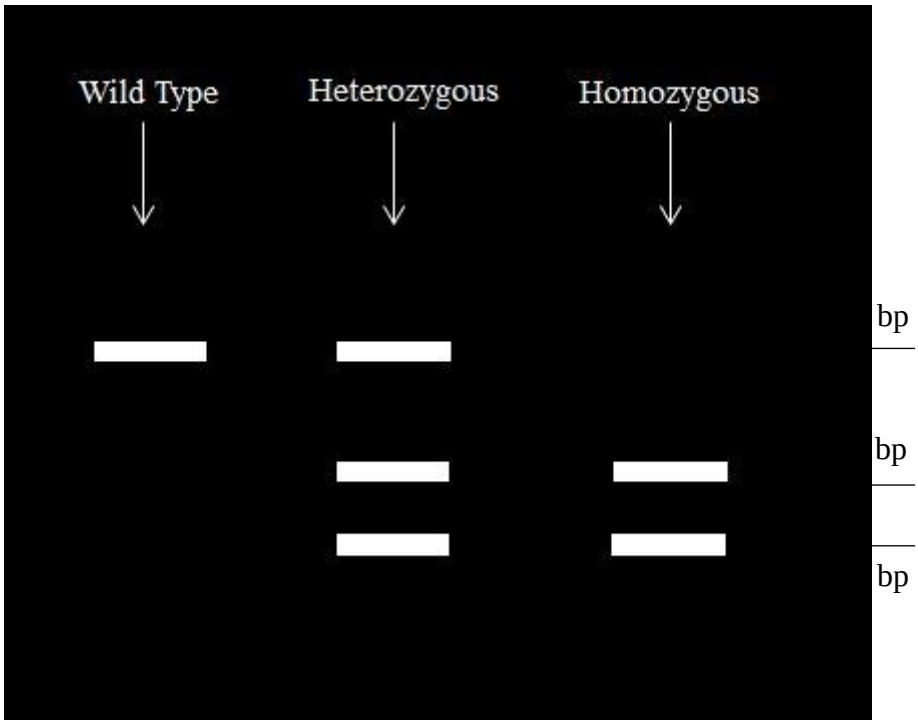

Figure 2. 2 Schematic representation of homozygous wild-type, heterozygous, and homozygous polymorphic genotypes for rs762551 (CYP1A2).

2.2.5.2 Genotyping of rs1799853 with AvaII

AvaII was used for determining the nucleotide change of rs1799853 on the CYP2C9 gene. In rs1799853, Cytosine (C) is replaced with Thymine (T) in polymorphic individuals. Wild-type individuals carry the AvaII recognition site (GGACC), whereas polymorphic individuals do not. The polymorphic allele becomes GGACT, after C replaces T (Figure 2.3).

Figure 2. 3 Schematic representation of amplified gene region, forward and reverse primers, AvaII recognition site of rs1799853 (CYP2C9).

Wild-type genotype (CC) is expected to have two bands, homozygous polymorphic genotype (TT) is expected to have a single band, and heterozygous genotype (CT) is expected to have three bands on the gel (Figure 2.4).

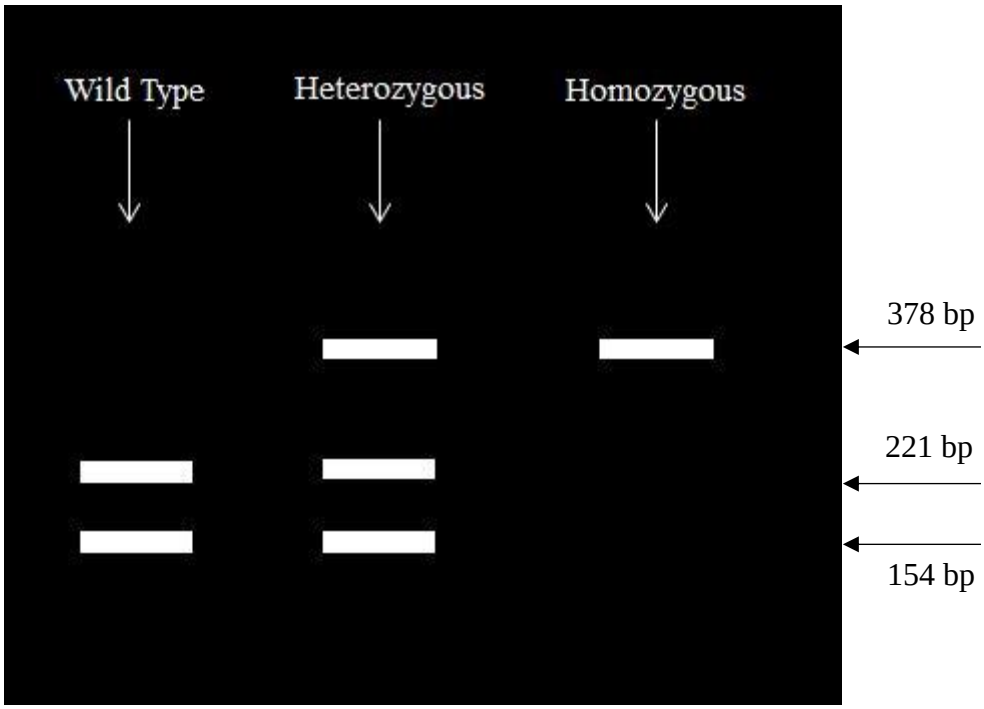

Figure 2. 4 Schematic representation of homozygous wild-type, heterozygous, and homozygous polymorphic genotypes for rs1799853 (CYP2C9).

2.2.5.3 Genotyping of rs16947 with FspI

FspI was used to identify the nucleotide change of rs16947 on CYP2D6. In rs16947, Guanine (G) replaces Adenine (A) in polymorphic individuals. After the replacement of G with A, the cut site of the FspI enzyme disappers. Wild-type alleles carry the FspI recognition site (TGCGAC). FspI cuts wild-type alleles, whereas polymorphic alleles remain uncut (Figure 2.5).

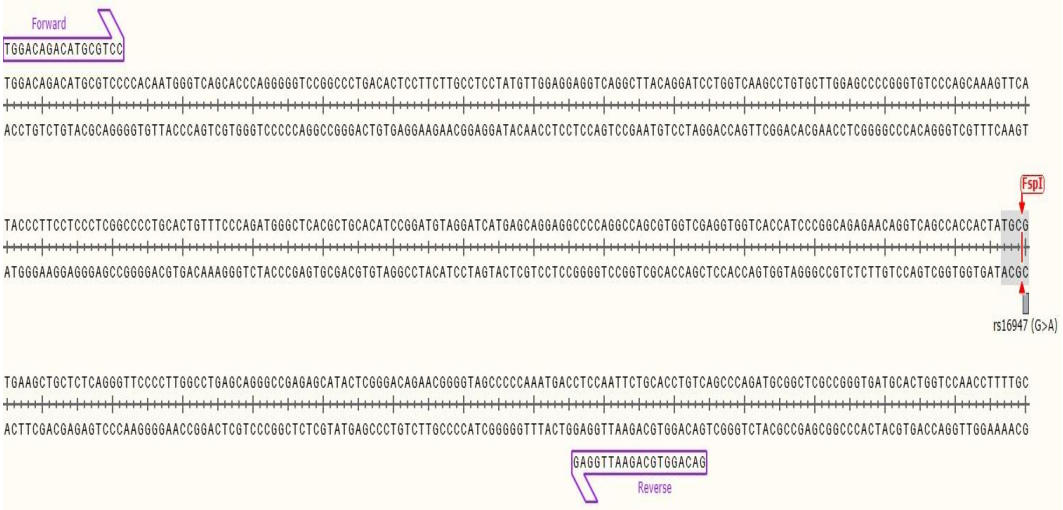

Figure 2. 5 Schematic representation of amplified gene region, forward and reverse primers, FspI recognition site of rs16947 (CYP2D6).

Wild-type genotype (GG) is expected to give two bands, homozygous polymorphic genotype (AA) is expected to give one band, and heterozygous genotype (GA) genotype is expected to give three bands as given in Figure 2.6.

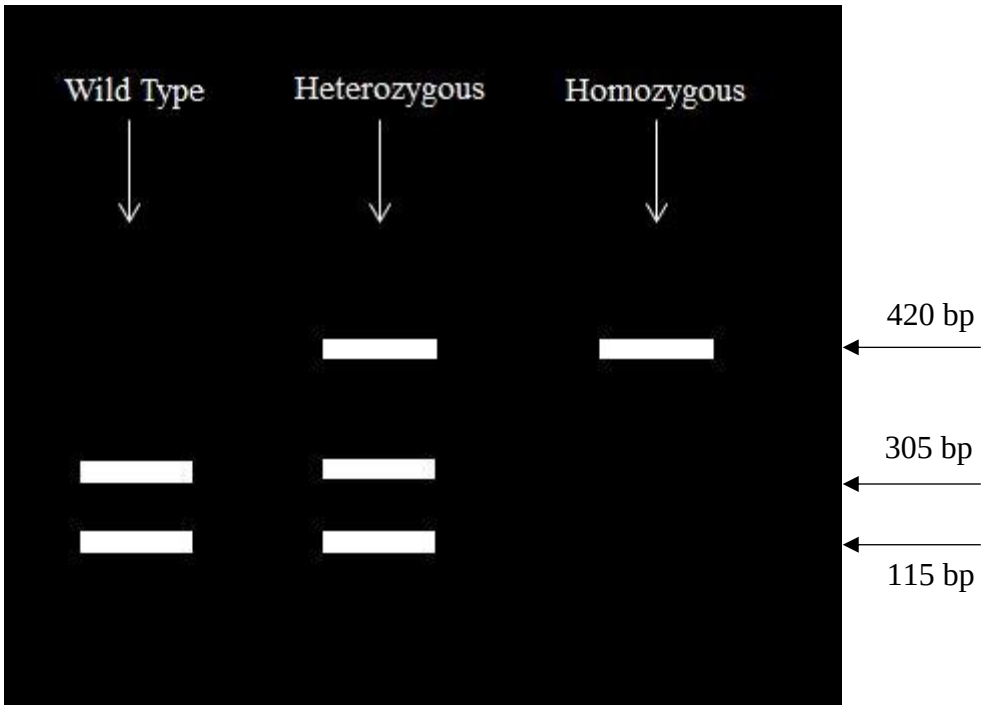

Figure 2. 6 Schematic representation of homozygous wild-type, heterozygous, and homozygous polymorphic genotypes for rs16947 (CYP2D6).

2.2.5.4 Genotyping of rs2740574 with MboII

MboII was used to determine the nucleotide change of rs2740574 on CYP3A4. In rs2740574, Thymine (T) replaces Cytosine (C) in polymorphic individuals. After T is replaced with C, the cut site of MboII appears. Polymorphic alleles carry the MboII recognition site ($GAAGA(N)_8$), and are cut by restriction enzyme, whereas wild-type alleles remain uncut (Figure 2.7).

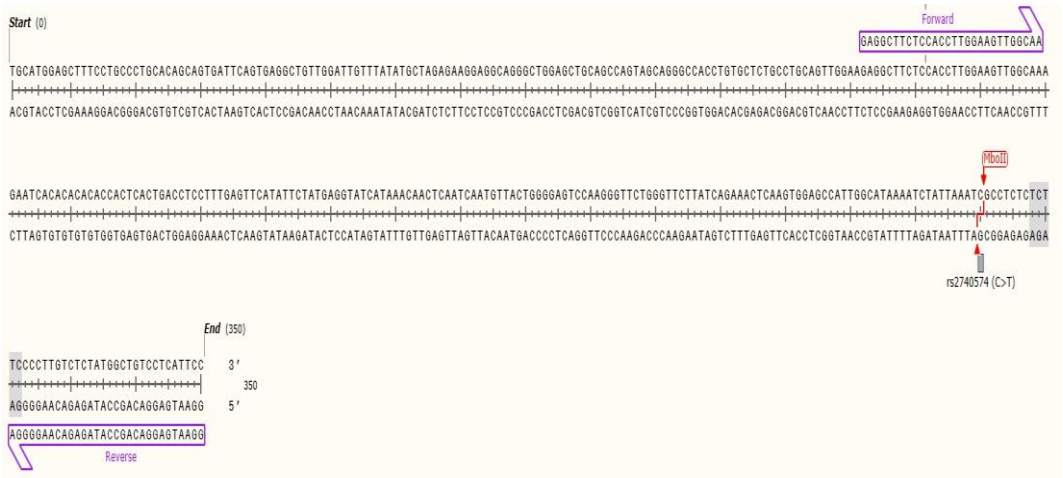

Figure 2. 7 Schematic representation of amplified gene region, forward and reverse primers, MboII recognition site of rs2740574 (CYP3A4).

Wild-type genotype (TT) is expected to be seen as a single band, homozygous polymorphic genotype (CC) is expected to be seen as two bands, and heterozygous genotype (TC) is expected to be seen as three bands as given in Figure 2.8.

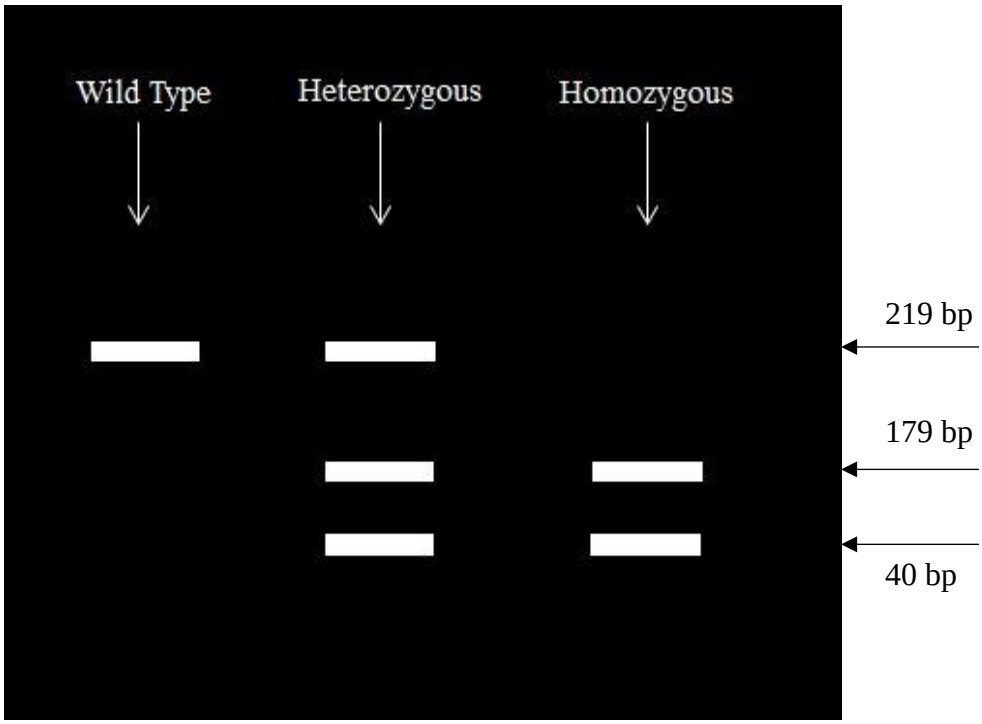

Figure 2. 8 Schematic representation of homozygous wild-type, heterozygous, and homozygous polymorphic genotypes for rs2740574 (CYP3A4).

2.2.5.5 Genotyping of rs2108622 with PvuII

PvuII was used to determine the nucleotide change of rs2108622 on the CYP4F2 gene (Figure 2.9). In rs2108622, Cytosine (C) is replaced with Thymine (T) in polymorphic individuals. Wild-type individuals have an AvaII recognition site (CAGCTG). Nevertheless, polymorphic individuals do not carry the recognition site of AvaII, since polymorphic allele becomes TAGCTG, after T replacement with C.

Figure 2. 9 Schematic representation of amplified gene region, forward and reverse primers, PvuII recognition site of rs2108622 (CYP4F2).

Wild-type genotype (CC) is expected to have two bands, homozygous polymorphic genotype (TT) is expected to have a single band, and heterozygous (CT) genotype is expected to have three bands on the gel (Figure 2.10).

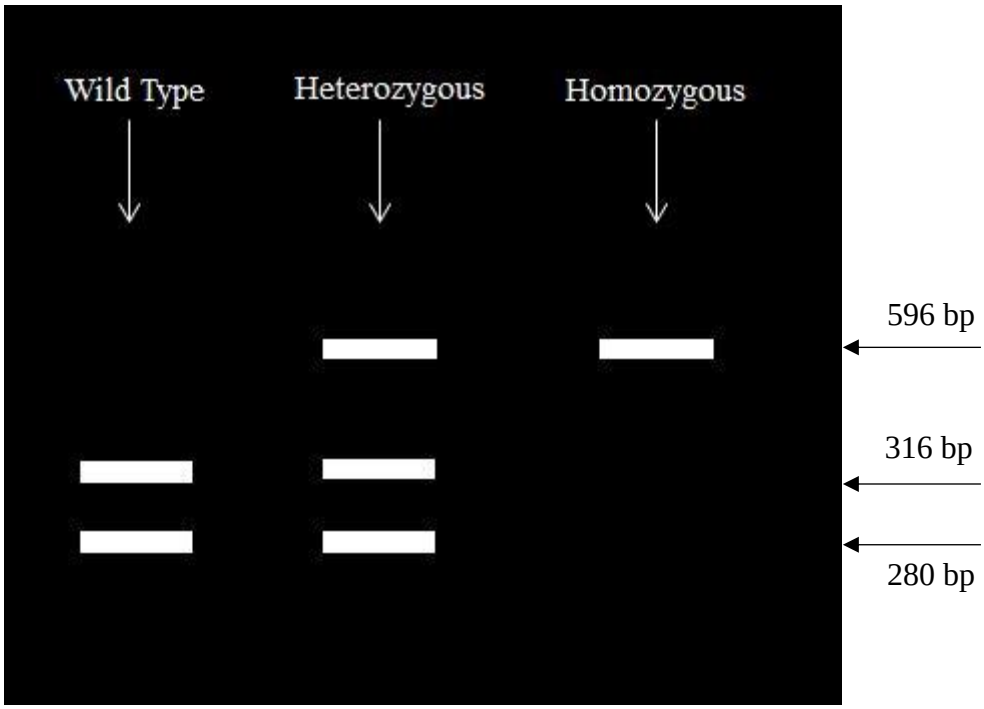

Figure 2. 10 Schematic representation of homozygous wild-type, heterozygous, and homozygous polymorphic genotypes for rs2108622 (CYP4F2).

2.2.6 Statistical Analysis

SNPStats (Institut Català d'Oncologia) was used for statistical analysis. SNPStats is a web-based, free, and useful software to analyze association studies based on SNPs. The software was designed to perform multiple analysis in only one session (Solé et al., 2006). All the statistical calculations and comparisons were provided by SNPStats software. Allele frequency indicates how an allele is common in a population, and is calculated by number of certain allele divided by the total number of alleles.

P-value was used to evaluate the significance of the results. If the *p*-value is less than 0.05, then it was accepted that the results are statistically significant. Higher *p*-values were regarded as statistically insignificant.

CHAPTER 3

RESULTS

3.1 Study Population

The population of this study comprised 179 MS patients and 102 healthy controls. The age of the patients and healthy control subjects differ from 24 to 74. Even though age is a significant factor for the onset of MS, it was focused on somatic cells in which the same genetic information from birth has been preserved. Therefore, the ages of the individuals in both groups were not compared, and the possible variations due to age were neglected. The gender distributions, the population means, and the median age are given in Table 3.1. Significant difference in gender distribution between patients and controls has been observed (p=0.028). As indicated previously, women are more prone to MS than men. Higher risk of having MS in women is directly related to the number of female participants recruited in patient group. On the other hand, the gender distribution of control group is more homogenous with respect to patient group.

Table 3. 1 Demographic data of the study population

	Patients (n:179)	**Controls (n:102)**	***p* value**
Age (years)	Mean ± SD: 43.8 ± 9.9 Median ± IQR: 44 ± 14.0	Mean ± SD: 41.1 ± 8.7 Median ± IQR: 39.5 ± 10.0	0.063
Gender, n (%)	Female: 125 (%69.8) Male: 54 (%30.2)	Female: 58 (%56.9) Male: 44 (%43.1)	0.028

3.2 Genotyping of SNPs

SNPs were genotyped according to the PCR-RFLP method. Gene regions having the polymorphic site were amplified by PCR, and these PCR products were subjected to restriction enzyme digestion. Digestion products were visualized with agarose gel electrophoresis.

After visualization, genotypes of patient and control groups were identified, and the results were analyzed according to Additive Model (AM), Dominant Model (DM), and Recessive Model (RM). The additive model is widely used for SNP studies. This model assumes that wild-type and mutant alleles have equal genetic contributions to heterozygous genotype, which means phenotype is linearly related to the number of minor alleles. In the dominant model, it is accepted that polymorphic alleles have dominant characteristics for wild-type alleles. So that, free from copy number (1 or 2), polymorphic allele produces an equal effect on phenotype. In the recessive model, it is assumed that polymorphic alleles have recessive characters concerning wild-type alleles. Therefore, the phenotype is directly related to the presence or absence of two copies of polymorphic alleles (Kitsche et al., 2016). Table 3.2 summarizes the numerical scores of additive, dominant, and recessive models.

Table 3. 2 Numerical scores of additive, dominant, and recessive models. A designates wild-type allele, and a designates polymorphic allele (Kitsche et al., 2016).

Genetic Model	AA	Aa	aa
Additive	0	0.5	1
Dominant	0	1	1
Recessive	0	0	1

As seen in Table 3.2., there are three different groups to be compared in the additive model. However, in the dominant and recessive models, there are two different groups.

The odds ratio (OR) was calculated to assess the risk factor between distinct groups in each model.

$$OR = \frac{\text{All patients with risky allele / All patients without risky allele}}{\text{All controls with risky allele / All controls without risky allele}}$$

If OR is 1.0 or close to 1.0, it means that SNP under investigation has no real effect on disease onset, while OR being higher than 1.0 suggests that related SNP might be a risk factor for the disease. On the other hand, OR less than 1.0 indicates that related SNP might have a protective effect.

In the additive model, homozygous and heterozygous polymorphic subjects were evaluated individually. Homozygous wild-type subjects were assumed to be non-risky. In the first round of additive model analysis, only homozygous polymorphic subjects were considered to have risky alleles. Subjects with heterozygous polymorphic and homozygous wild-type allele were accepted as non-risky groups. In the second round of analysis, heterozygous polymorphic subjects were considered to have risky alleles. Homozygous wild-type and homozygous polymorphic subjects were grouped as non-risky.

In the dominant model, subjects carrying polymorphic alleles were considered as risky groups. Homozygous wild-type individuals were included in the non-risky group.

In the recessive model, homozygous polymorphic subjects were evaluated in the risky group. Homozygous wild-type, and heterozygous individuals were grouped as non-risky.

In multiple comparisons, *p*-value correction methods can be applied. The corrected *p*-value is calculated as (α) / (number of polymorphisms), and can be used to determine the significance of the results (Lopes et al., 2015).

3.2.1 Genotyping and Analysis of rs762551 on CYP1A2 Gene

rs762551, also known as *CYP1A2*1F*, is one of the most studied SNPs on CYP1A2. It is an intron variant, and Adenine replaces with Cytosine in rs762551. Global minor allele frequency (MAF) of rs762551 is 0.32 according to dbSNP database, and 0.29 according to gnomAD, whereas MAF of Turkish population was slightly higher than global MAF, and calculated as 0.34 (Arici and Özhan, 2017; Yucesoy et al., 2017).

Initially, gDNA was amplified with the primers given in Table 2.1. PCR product of this amplified gene region is 541 nucleotides long (Figure 3.1).

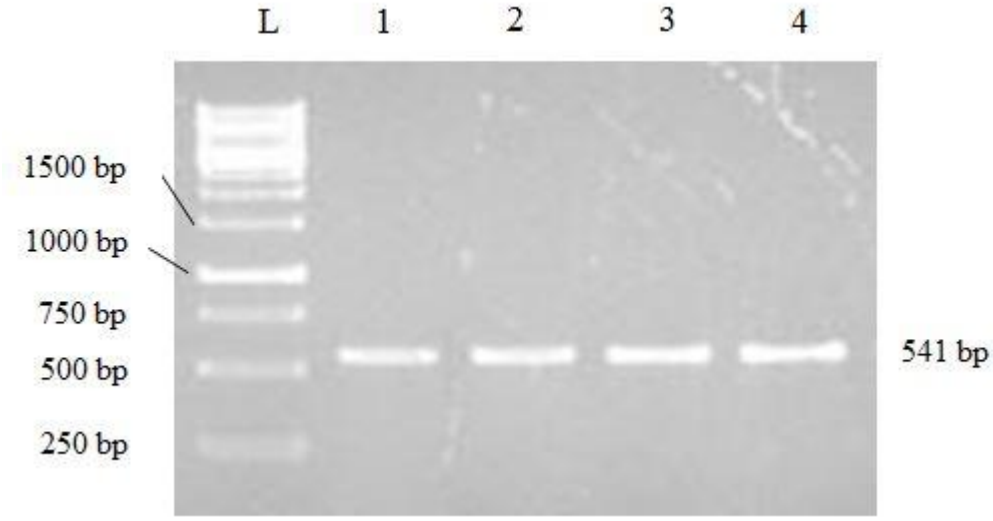

Figure 3. 1 Representative agarose gel (1%) electrophoresis image of rs762551 PCR products. L stands for GeneRuler 1 kb DNA Ladder. 1, 2, 3, and 4 are PCR products.

Then, PCR products were digested with ApaI enzyme. Wild-type allele does not carry the ApaI recognition site, whereas the polymorphic allele was cut by the enzyme which produced 369, and 176 nucleotide DNA fragments (Figure 3.2).

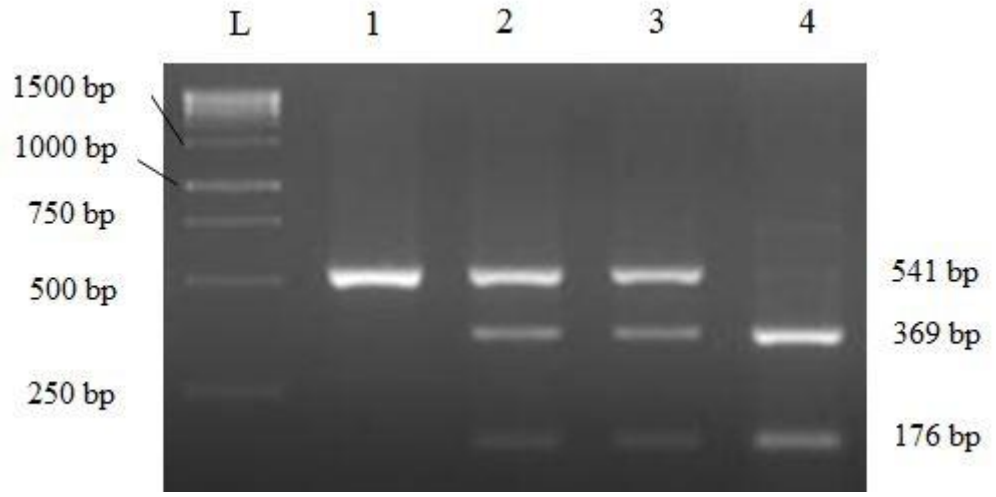

Figure 3. 2 Representative agarose gel (1 %) electrophoresis image of rs762551 restriction enzyme digestion. L stands for GeneRuler 1 kb DNA Ladder. 1 is wild-type (AA), 2 and 3 are heterozygous polymorphic (AC), and 4 is homozygous polymorphic (CC) genotypes.

Gel images after restriction enzyme digestion were used for genotyping of the 179 patients, and 102 control subjects. Statistical analysis regarding three different models, and the allele distribution of patients, and controls are shown in Table 3.3. There were 70 AA (39.1 %), 82 AC (45.8 %), 27 CC (15.1 %) individuals in the patient group, and 47 AA (46.1 %), 49 AC (48 %), 6 CC (5.9 %) individuals in the control group. Adenine and Cytosine frequencies were 0.65, 0.35 in patients, and 0.70, 0.30 in control subjects, respectively. Wild-type and polymorphic allele frequencies of both groups were found to be statistically insignificant (p=0.054).

In the additive model, the wild-type genotype (AA) was individually compared with the genotypes having at least one copy of polymorphic allele (AC or CC). Between compared groups (AA vs. AC and AA vs. CC), a statistically borderline significant difference (p=0.049) was observed. In the dominant model, at least one copy of polymorphic allele carriers (AC and CC) were grouped, and compared with wild-type individuals (AA). No statistically significant difference was observed (p=0.260). In the recessive model, at least one copy of wild-type allele carriers (AA and AC) were grouped, and compared with homozygous polymorphic individuals

(CC). As in the additive model, a statistically significant difference was obtained between compared groups (p=0.016).

Table 3. 3 Allele frequency distributions and allelic model results of CYP1A2 rs762551 for patients and controls.

Genotype/Allele	**Patients (n:179) (n, %)**	**Controls (n:102) (n, %)**	**Model**	**OR (95% CI) (n:281)**	***p*-values (n:281)**	**Age Adjusted OR (95% CI) (n:248)**	**Age Adjusted *p*-values (n:248)**
AA	70 (39.1 %)	47 (46.1 %)	Additive	Reference	Reference	Reference	Reference
AC	82 (45.8 %)	49 (48 %)		1.12 (0.67-1.87)	0.049	1.43 (0.82-2.50)	0.013
CC	27 (15.1 %)	6 (5.9 %)		3.02 (1.16-7.88)		4.18 (1.46-11.95)	
AA	70 (39.1 %)	47 (46.1 %)	Dominant	1.33 (0.81-2.18)	0.260	1.72 (1.01-2.93)	0.046
AC+CC	109 (60.9)	55 (53.9 %)					
AA+AC	152 (84.9 %)	96 (94.1 %)	Recessive	2.84 (1.13-7.14)	0.016	3.44 (1.26-9.41)	0.008
CC	27 (15.1 %)	6 (5.9 %)					
A	0.65	0.70	Allele Frequency	1.44 (0.99-2.08)	0.054	1.63 (1.10-2.41)	0.014
C	0.35	0.30					

3.2.1.1 Female Subgroup Analysis of rs762551 on CYP1A2 Gene

The female subgroup analysis of rs762551 covered 125 patients and 58 control subjects. Allele frequencies, and allelic model results are given in Table 3.4. In patients, 49 AA (39.2 %), 57 AC (45.6 %), 19 CC (15.2 %) subjects, and in controls, 22 AA (37.9 %), 32 AC (55.2 %), 4 CC (6.9 %) subjects were included. Adenine, and Cytosine frequencies were found to be 0.62, 0.38 in patients, and 0.66, 0.34 in control subjects, respectively. No statistically significant difference was seen between wild-type, and polymorphic allele frequencies (p= 0.517).

In the additive model, wild-type genotype (AA) was compared with the genotypes having at least one copy of polymorphic allele (AC or CC) individually. Between compared groups (AA vs. AC and AA vs. CC), no statistically significant difference was detected (p=0.200). In the dominant model, polymorphic allele carriers (AC and CC) were grouped, and compared with wild-type individuals (AA). Again, no statistically significant difference was observed under the dominant model (p=0.870). In the recessive model, wild-type allele carriers (AA and AC) were grouped, and compared with homozygous polymorphic individuals (CC). Like the other two models, recessive model gave a statistically insignificant difference (p=0.098).

Table 3. 4 Allele frequency distributions and allelic model results of the female subgroup of CYP1A2 rs762551 for patients and controls.

Genotype/Allele	**Patients (n:125) (n, %)**	**Controls (n:58) (n, %)**	**Model**	**OR (95% CI) (n:183)**	***p*-values (n:183)**
AA	49 (39.2 %)	22 (37.9 %)	Additive	Reference	Reference
AC	57 (45.6 %)	32 (55.2 %)		0.80 (0.41-1.55)	0.200
CC	19 (15.2 %)	4 (6.9 %)		2.13 (0.65-7.01)	
AA	49 (39.2 %)	22 (37.9 %)	Dominant	0.95 (0.50-1.80)	0.870
AC+CC	76 (60.8 %)	36 (62.1 %)			
AA+AC	106 (84.8 %)	54 (93.1 %)	Recessive	2.42 (0.78-7.47)	0.098
CC	19 (15.2 %)	4 (6.9 %)			
A	0.62	0.66	Allele Frequency	1.16 (0.74-1.85)	0.517
C	0.38	0.34			

3.2.1.2 Male Subgroup Analysis of rs762551 on CYP1A2 Gene

The male subgroup analysis of rs762551 covered 54 patients and 44 control subjects. Allele frequencies, and allelic model results are given in Table 3.5. There were 21 AA (38.9 %), 25 AC (46.3 %), 8 CC (14.8 %) genotypes in patients, and 25 AA (56.8 %), 17 AC (38.6 %), 2 CC (4.5 %) genotypes in the control group. The frequency of Adenine was 0.62, 0.76, and Cytosine was 0.38, 0.24 in patients and controls, respectively. A statistically significant difference was seen between allele frequencies of patients and the control group (p= 0.004).

In the additive model, wild-type genotype (AA) was compared with one copy of polymorphic allele (AC and CC) carriers, separately. No statistically significant difference was detected (p=0.095) between the compared groups (AA vs. AC and AA vs. CC). In the dominant model, individuals having at least one copy of polymorphic allele (AC and CC) were grouped, and compared with wild-type individuals. This comparison also produced statistically insignificant results (p=0.076). In the recessive model, individuals having at least one copy of wild-type allele (AA and AC) were compared with homozygous polymorphic individuals (CC), and the results under this model was statistically insignificant (p=0.082).

Table 3. 5 Allele frequency distributions and allelic model results of the male subgroup of CYP1A2 rs762551 for patients and controls.

Genotype/Allele	**Patients (n:54) (n, %)**	**Controls (n:44) (n, %)**	**Model**	**OR (95% CI) (n:98)**	***p*-values (n:98)**
AA	21 (38.9 %)	25 (56.8 %)	Additive	Reference	Reference
AC	25 (46.3 %)	17 (38.6 %)		1.75 (0.75-4.08)	0.095
CC	8 (14.8 %)	2 (4.5 %)		4.76 (0.91-24.91)	
AA	21 (38.9 %)	25 (56.8 %)	Dominant	2.07 (0.92-4.65)	0.076
AC+CC	33 (61.1 %)	19 (43.2 %)			
AA+AC	46 (85.2 %)	42 (95.5 %)	Recessive	3.65 (0.73-18.18)	0.082
CC	8 (14.8 %)	2 (4.5 %)			
A	0.62	0.76	Allele Frequency	1.95 (1.04-3.65)	0.004
C	0.38	0.24			

3.2.2 Genotyping and Analysis of rs1799853 on CYP2C9 Gene

In rs1799853, *CYP2C9*2*, Cytosine at the 430th position in exon 3 transforms into Thymine. The global MAF of rs1799853 is 0.11 according to the dbSNP database, and 0.09 according to gnomAD, and Turkish population MAF is around 0.11 (Aynacıoğlu et al., 1999; Arıcı and Özhan, 2017).

Using the primers given in Table 2.1., gDNA was amplified. This amplified gene region has 378 bp (Figure 3.3).

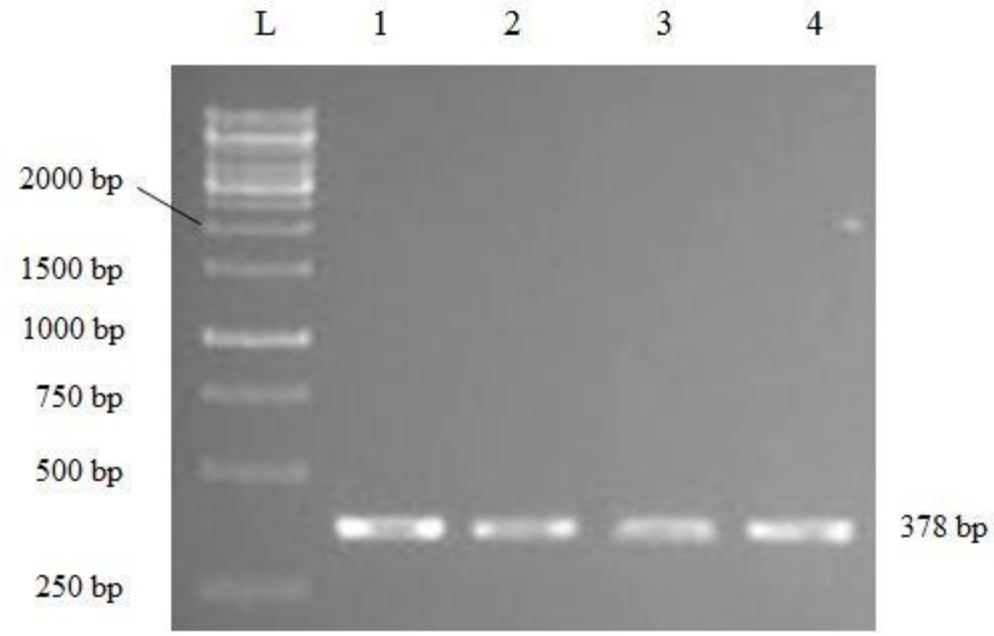

Figure 3. 3 Representative agarose gel (1%) electrophoresis image of rs1799853 PCR products. L stands for GeneRuler 1 kb DNA Ladder. 1, 2, 3, and 4 are PCR products.

Then, PCR products were treated with AvaII. Wild-type allele carries the AvaII recognition site. However polymorphic allele does not. In non-polymorphic subjects, 221 and 154 nucleotide DNA fragments were observed after restriction enzyme digestion (Figure 3.4).

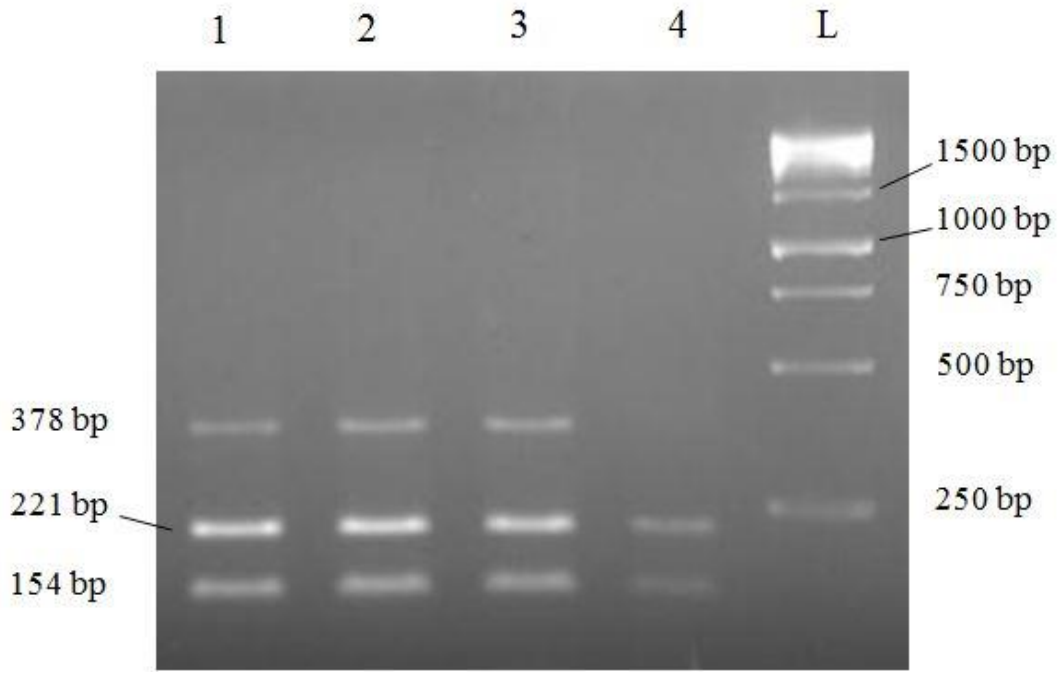

Figure 3. 4 Representative agarose gel (1 %) electrophoresis image of rs1799853 restriction enzyme digestion. L stands for GeneRuler 1 kb DNA Ladder. Lanes 1, 2, 3 are heterozygous polymorphic (CT), and 4 is wild-type (CC) genotypes.

After restriction enzyme digestion, genotyping of 179 patients, and 101 control subjects were performed. Statistical analysis, including three different models, and the distribution of alleles in both groups are given in Table 3.6. 176 CC (98.3 %) and 3 CT (1.7 %) genotypes in the patient group, 86 CC (85.2 %) and 15 CT (14.8 %) genotypes in the control group were identified. Cytosine and Thymine frequencies were calculated as 0.99, 0.01 in patients, and 0.93, 0.07 in control subjects, respectively. Wild-type and polymorphic allele frequencies between both groups were found to be statistically significant ($p<0.001$).

In the additive model, wild-type genotype (CC) was compared with CT and TT genotypes, separately. CC vs. CT comparison provided statistically significant ($p<0.001$) result. As mentioned earlier, in the dominant model, at least one copy of polymorphic allele carriers (CT and TT) were grouped, and compared with wild-type individuals (CC). In the recessive model, individuals with wild-type allele (CC and CT) would be compared with TT. However, there were no homozygous

polymorphic individuals in both groups. Therefore, calculations under dominant, and recessive models could not be performed.

Table 3. 6 Allele frequency distributions and allelic model results of CYP2C9 rs1799853 for patients and controls.

Genotype/Allele	**Patients (n:179) (n, %)**	**Controls (n:101) (n, %)**	**Model**	**OR (95% CI) (n:280)**	***p*-values (n:280)**	**Age Adjusted OR (95% CI) (n:247)**	**Age Adjusted *p*-values (n:247)**
CC	176 (98.3 %)	86 (85.2 %)	Additive	Reference	Reference	Reference	Reference
CT	3 (1.7 %)	15 (14.8 %)		0.10 (0.03-0.35)	<0.001	0.10 (0.03-0.35)	<0.001
TT	0 (0.0 %)	0 (0.0 %)		NA		NA	
CC	176 (98.3 %)	86 (85.2 %)	Dominant	NA	NA	NA	NA
CT+TT	3 (1.7 %)	15 (14.8 %)					
CC+CT	179 (100 %)	101 (100 %)	Recessive	NA	NA	NA	NA
TT	0 (0.0 %)	0 (0.0 %)					
C	0.99	0.93	Allele Frequency	0.11 (0.03-0.37)	<0.001	0.11 (0.03-0.38)	<0.001
T	0.01	0.07					

3.2.2.1 Female Subgroup Analysis of rs1799853 on CYP2C9 Gene

125 patients and 58 control subjects were included in the rs1799853 female subgroup analysis. Allele frequencies and allelic model results are given in Table 3.7. There were 122 CC (97.6 %) and 3 CT (2.4 %) subjects in the patient group, and 48 CC (82.8 %) and 10 CT (17.2 %) subjects in the control group. Cytosine and Thymine frequencies were found to be 0.99, 0.01 in patients, and 0.91, 0.09 in controls, respectively with a significant allele frequency difference ($p=0.001$).

In the additive model, CC vs. CT comparison was made, and a statistically significant difference was observed ($p<0.001$). However, dominant, and recessive models could not be performed due to the lack of TT genotype in the female subgroup of patients and controls.

Table 3. 7 Allele frequency distributions and allelic model results of the female subgroup of CYP2C9 rs1799853 for patients and controls.

Genotype/Allele	**Patients (n:125) (n, %)**	**Controls (n:58) (n, %)**	**Model**	**OR (95% CI) (n:183)**	***p*-values (n:183)**
CC	122 (97.6 %)	48 (82.8 %)	Additive	Reference	Reference
CT	3 (2.4 %)	10 (17.2 %)		0.12 (0.03-0.45)	<0.001
TT	0 (0.0 %)	0 (0.0 %)		NA	
CC	122 (97.6 %)	48 (82.8 %)	Dominant	NA	NA
CT+TT	3 (2.4 %)	10 (17.2 %)			
CC+CT	125 (100 %)	58 (100 %)	Recessive	NA	NA
TT	0 (0.0 %)	0 (0.0 %)			
C	0.99	0.91	Allele Frequency	0.12 (0.03-0.42)	0.001
T	0.01	0.09			

3.2.2.2 Male Subgroup Analysis of rs1799853 on CYP2C9 Gene

54 patients and 43 control subjects were included in the rs1799853 male subgroup analysis. Allele frequencies and allelic model results are given in Table 3.8. Only 54 CC (100 %) genotypes were present in the patient group, and 38 CC (88.4 %) and 5 CT (11.6 %) genotypes were present in the control group. The frequency of Cytosine was 1.00, 0.94 and the frequency of Thymine was 0, 0.06 in patients and controls, respectively. No significant allele frequency difference was observed between the two groups (p=0.071).

Since the male subgroup of patients was only comprised of wild-type individuals, allelic model results were “not applicable”.

Table 3. 8 Allele frequency distributions and allelic model results of the male subgroup of CYP2C9 rs1799853 for patients and controls.

Genotype/Allele	Patients (n:54) (n, %)	Controls (n:43) (n, %)	Model	OR (95% CI) (n:97)	*p*-values (n:97)
CC	54 (100 %)	38 (88.4 %)	Additive	Reference	Reference
CT	0 (0.0 %)	5 (11.6 %)		NA	NA
TT	0 (0.0 %)	0 (0.0 %)		NA	
CC	54 (100 %)	38 (88.4 %)	Dominant	NA	NA
CT+TT	0 (0.0 %)	5 (11.6 %)			
CC+CT	54 (100 %)	43 (100 %)	Recessive	NA	NA
TT	0 (0.0 %)	0 (0.0 %)			
C	1.00	0.94	Allele Frequency	0.07 (0.004-1.25)	0.071
T	0	0.06			

3.2.3 Genotyping and Analysis of rs16947 on CYP2D6 Gene

*CYP2D6*2*, an allele commonly known as rs16947 is an exon variant, where Guanine replaces Adenine in rs16947. The global MAF of rs16947 is 0.32 according to the dbSNP database and 0.34 according to gnomAD. Turkish population MAF is slightly higher than the global MAF, which is 0.35 (Aynacioglu et al., 1999; Taskin, et al., 2016).

gDNA was amplified with suitable primers, and the gene region has 420 bp (Figure 3.5).

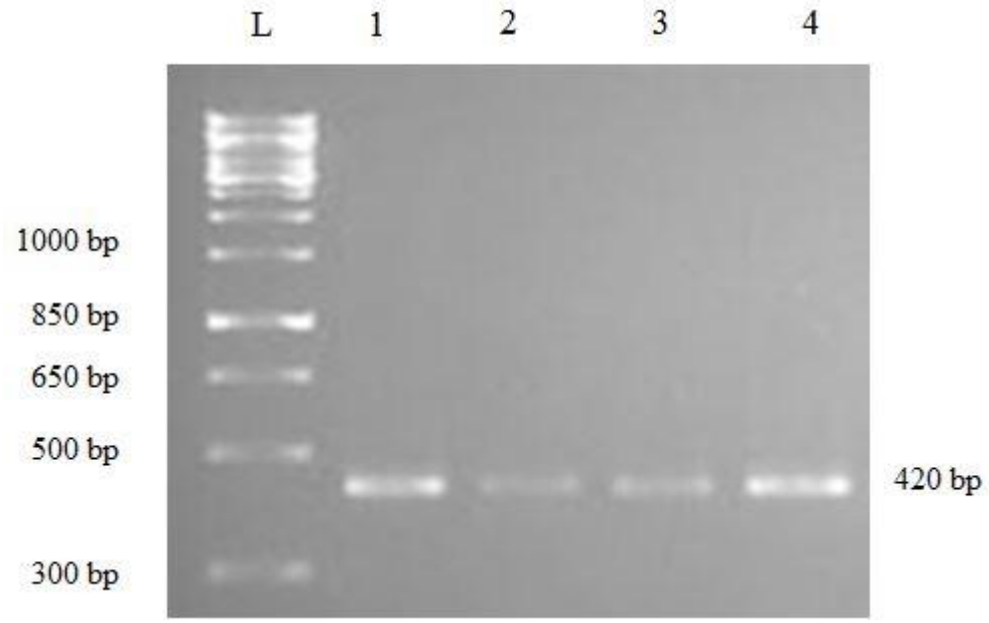

Figure 3. 5 Representative agarose gel (1%) electrophoresis image of rs16947 PCR products. L stands for GeneRuler 1 kb DNA Ladder. 1, 2, 3, and 4 are PCR products.

FspI was used to digest the PCR product. Only the wild-type allele carries the FspI recognition site. Therefore, polymorphic alleles remained uncut after FspI digestion. Restriction enzyme treatment produced 305 and 115 bp long DNA fragments in wild-type alleles (Figure 3.6).

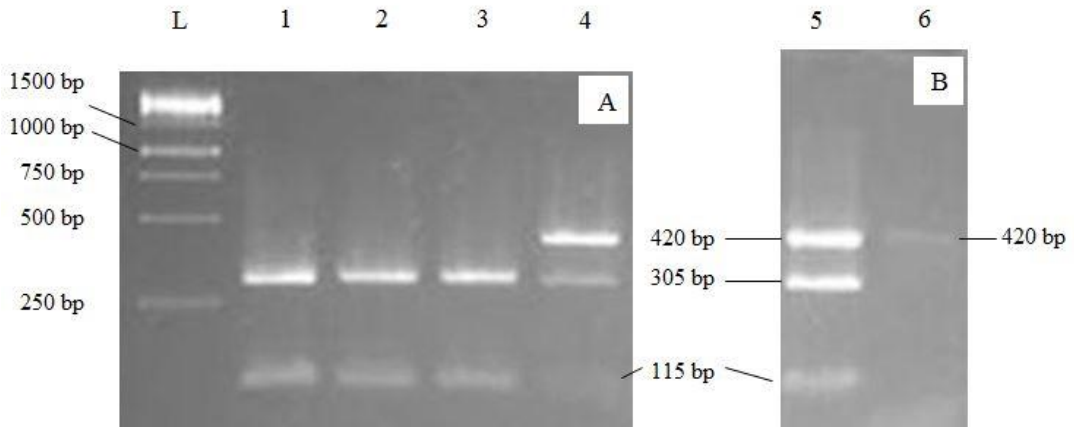

Figure 3. 6 Representative agarose gel (1 %) electrophoresis image of rs16947 restriction enzyme digestion. A) L stands for GeneRuler 1 kb DNA Ladder. Lanes 1-3 are wild-type (GG), 4 is heterozygous polymorphic (GA) genotypes. B) 5 is heterozygous polymorphic (GA), and 6 is homozygous polymorphic (AA) genotypes.

After restriction enzyme digestion, gel images were evaluated for genotyping of 179 patients and 100 controls. Three different models, and the allele distribution of patients and controls were analyzed statistically (Table 3.9). There were 69 GG (38.5 %), 109 GA (60.9 %) and 1 AA (0.6 %) individuals in the patient group, and 39 GG (39 %) and 61 GA (61 %) individuals in the control group. Guanine and Adenine frequencies were 0.69, 0.31 in both groups. Observed allele frequency difference was insignificant (p=0.901).

Since the control group did not have at least one homozygous polymorphic individual, only GG vs. GA comparison in additive model and GG vs. GA+AA comparison in the dominant model could be performed. No statistically significant results were obtained in any analysis.

Table 3. 9 Allele frequency distributions and allelic model results of CYP2D6 rs16947 for patients and controls.

Genotype/Allele	**Patients (n:179) (n, %)**	**Controls (n:100) (n, %)**	**Model**	**OR (95% CI) (n:279)**	***p*-values (n:279)**	**Age Adjusted OR (95% CI) (n:246)**	**Age Adjusted *p*-values (n:246)**
GG	69 (38.5 %)	39 (39 %)	Additive	Reference	Reference	Reference	Reference
GA	109 (60.9 %)	61 (61 %)		1.01 (0.61-1.67)	0.640	1.13 (0.66-1.95)	0.600
AA	1 (0.6 %)	0 (0.0 %)		NA		NA	
GG	69 (38.5 %)	39 (39 %)	Dominant	1.02 (0.62-1.68)	0.940	1.14 (0.67-1.96)	0.620
GA+AA	110 (61.5 %)	61 (61 %)					
GG+GA	178 (99.4 %)	100 (100 %)	Recessive	NA	NA	NA	NA
AA	1 (0.6 %)	0 (0.0 %)					
G	0.69	0.69	Allele Frequency	1.02 (0.70-1.49)	0.901	1.12 (0.75-1.66)	0.586
A	0.31	0.31					

3.2.3.1 Female Subgroup Analysis of rs16947 on CYP2D6 Gene

The female subgroup analysis of rs16947 covered 125 patients and 58 control subjects. Allele frequencies and allelic model results are illustrated in Table 3.10. In patients, 48 GG (38.4 %), 76 GA (60.8 %) and 1 AA (0.8 %) subjects, in controls, 23 GG (39.7 %) and 35 GA (60.3 %) subjects were included. The frequency of Guanine was 0.69, 0.70, and the frequency of Adenine was 0.31, 0.30 in patients, and controls respectively. The *p*-value for allele frequency was found to be 0.843.

GG vs. GA comparison was conducted in the additive model. Between compared groups, there was no statistically significant difference observed (p=0.680). GG vs. GA+AA comparison in the dominant model also did not generate a significant difference (p=0.870). Due to the lack of homozygous polymorphic individuals in the female subgroup of controls, the recessive model was “not applicable”.

Table 3. 10 Allele frequency distributions and allelic model results of the female subgroup of CYP2D6 rs16947 for patients and controls.

Genotype/Allele	**Patients (n:125) (n, %)**	**Controls (n:58) (n, %)**	**Model**	**OR (95% CI) (n:183)**	***p*-values (n:183)**
GG	48 (38.4 %)	23 (39.7 %)	Additive	Reference	Reference
GA	76 (60.8 %)	35 (60.3 %)		1.04 (0.55-1.97)	0.680
AA	1 (0.8 %)	0 (0.0 %)		NA	
GG	48 (38.4 %)	23 (39.7 %)	Dominant	1.05 (0.56-1.99)	0.870
GA+AA	77 (61.6 %)	35 (60.3 %)			
GG+GA	124 (99.2 %)	58 (100 %)	Recessive	NA	NA
AA	1 (0.8 %)	0 (0.0 %)			
G	0.69	0.70	Allele Frequency	1.05 (0.65-1.69)	0.843
A	0.31	0.30			

3.2.3.2 Male Subgroup Analysis of rs16947 on CYP2D6 Gene

The male subgroup analysis of rs16947 covered 54 patients and 42 control subjects. Allele frequencies and allelic model results are given in Table 3.11. There were 21 GG (38.9 %) and 33 GA (61.1 %) genotypes in patients, and 16 GG (38.1 %) and 26 GA (61.9 %) genotypes in the control group. Homozygous polymorphic individuals were not involved in both groups. The frequency of Guanine was 0.69, 0.68, and the frequency of Adenine was 0.31, 0.32 in patients and controls, respectively. The *p*-value for allele frequency was found to be 0.952.

Only GG vs. GA comparison could be calculated in the additive model, due to the lack of homozygous polymorphic (AA) individuals in both groups. We failed to observe statistically significant results (p=0.940)

Table 3. 11 Allele frequency distributions and allelic model results of the male subgroup of CYP2D6 rs16947 for patients and controls.

Genotype/Allele	**Patients (n:54) (n, %)**	**Controls (n:42) (n, %)**	**Model**	**OR (95% CI) (n:96)**	***p*-values (n:96)**
GG	21 (38.9 %)	16 (38.1 %)	Additive	Reference	Reference
GA	33 (61.1 %)	26 (61.9 %)		0.97 (0.42-2.22)	0.940
AA	0 (0.0 %)	0 (0.0 %)		NA	
GG	21 (38.9 %)	15 (35.7 %)	Dominant	NA	NA
GA+AA	33 (61.1 %)	27 (64.3 %)			
GG+GA	54 (100 %)	42 (100 %)	Recessive	NA	NA
AA	0 (0.0 %)	0 (0.0 %)			
G	0.69	0.68	Allele Frequency	0.98 (0.53-1.82)	0.952
A	0.31	0.32			

3.2.4 Genotyping and Analysis of rs2740574 on CYP3A4 Gene

*CYP3A4*1B* allele, also known as rs2740574, is located in the promoter region of CYP3A4 gene, and Thymine replaces with Cytosine. The global MAF of rs2740574 is 0.11 according to dbSNP and 0.21 according to gnomAD. On the other hand, the Turkish population MAF is much lower than the global MAF. The MAF of the Turkish population was calculated as 0.01 (Sayitoğlu et al., 2006; Arici and Özhan, 2016).

For the analysis of rs2740574, gDNA was amplified with the suitable primers given in Table 2.1. The amplified gene region comprises 219 bp (Figure 3.7).

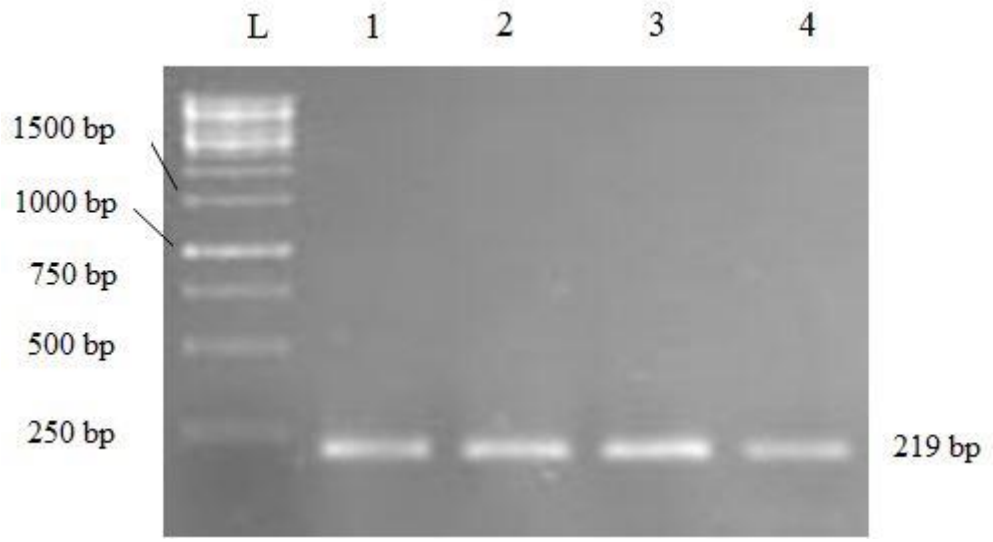

Figure 3. 7 Representative agarose gel (1%) electrophoresis image of rs2740574 PCR products. L stands for GeneRuler 1 kb DNA Ladder. 1, 2, 3, and 4 are PCR products.

MboII was used to digest PCR product having rs2740574. Wild-type allele does not carry a restriction enzyme recognition site. However, polymorphic allele does. After MboII digestion, 179 bp and 40 bp long DNA fragments were observed in polymorphic subjects (Figure 3.8).

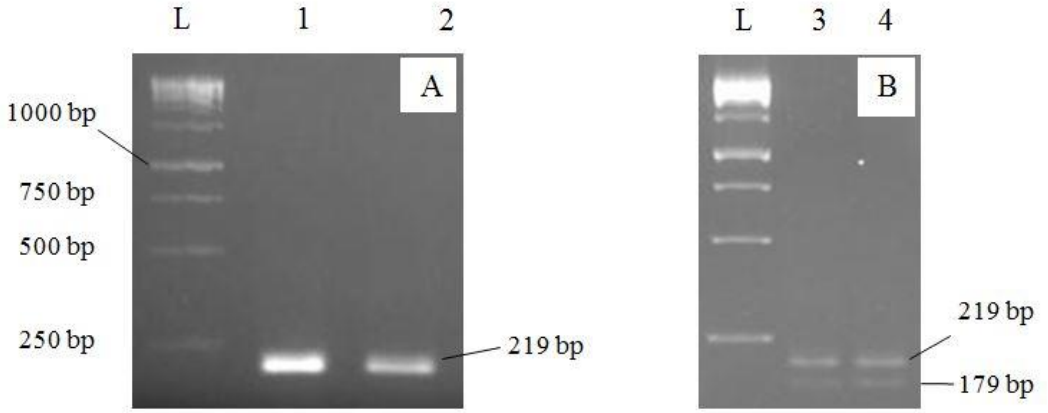

Figure 3. 8 Representative agarose gel (1 %) electrophoresis image of rs2740574 restriction enzyme digestion. A) L stands for GeneRuler 1 kb DNA Ladder. Lanes 1 and 2 are wild-type (TT) genotypes. B) L stands for GeneRuler 1 kb DNA Ladder. Lanes 3 and 4 are heterozygous polymorphic (TC) genotypes

After restriction enzyme digestion, 178 patients and 101 control subjects were genotyped. Statistical analysis, including three different models, and the distribution of alleles in both groups were illustrated in Table 3.12. In patient group, 166 TT (93.3 %), 12 TC (6.7 %) genotypes, and in control group 99 TT (98 %), 2 TC (2 %) genotypes were determined. Thymine and Cytosine frequencies were calculated as 0.97 and 0.03 in patients and 0.99 and 0.01 in the control group, respectively. Wild-type and polymorphic allele frequencies of both groups were found to be statistically insignificant (p=0.104).

There was no homozygous polymorphic individual in both groups. Therefore, only TT vs. TC comparison could be made. In the additive model, TT vs. TC testing did not give statistically significant results (p=0.061).

Table 3. 12 Allele frequency distributions and allelic model results of CYP3A4 rs2740574 for patients and controls.

Genotype/Allele	**Patients (n:178) (n, %)**	**Controls (n:101) (n, %)**	**Model**	**OR (95% CI) (n:279)**	***p*-values (n:279)**	**Age Adjusted OR (95% CI) (n:246)**	**Age Adjusted *p*-values (n:246)**
TT	166 (93.3 %)	99 (98 %)	Additive	Reference	Reference	Reference	Reference
TC	12 (6.7 %)	2 (2 %)		3.58 (0.78-16.32)	0.061	7.06 (0.89-55.85)	0.018
CC	0 (0.0 %)	0 (0.0 %)		NA		NA	
TT	166 (93.3 %)	99 (98 %)	Dominant	NA	NA	NA	NA
TC+CC	12 (6.7 %)	2 (2 %)					
TT+TC	178 (97.2 %)	101 (100 %)	Recessive	NA	NA	NA	NA
CC	0 (0.0 %)	0 (0.0 %)					
T	0.97	0.99	Allele Frequency	3.49 (0.77-15.75)	0.104	6.66 (0.85-52.01)	0.071
C	0.03	0.01					

3.2.4.1 Female Subgroup Analysis of rs2740574 on CYP3A4 Gene

125 patients and 58 control subjects were included in the rs2740574 female subgroup analysis. Allele frequencies and allelic model results are given in Table 3.13. There were 119 TT (95.2 %), 6 TC (4.8 %) genotypes in patients, and 56 TT (96.5 %), 2 TC (3.5 %) genotypes in controls. The frequency of Thymine and Cytosine was 0.98, 0.02 in both groups. The *p*-value for allele frequency was found to be 0.682.

TT vs. TC comparison in the additive model did not give a statistically significant difference (p=0.670). Since patients and controls in the female subgroup did not have at least one CC genotype, dominant and recessive model calculations could not be performed.

Table 3. 13 Allele frequency distributions and allelic model results of the female subgroup of CYP3A4 rs2740574 for patients and controls.

Genotype/Allele	**Patients (n:125) (n, %)**	**Controls (n:58) (n, %)**	**Model**	**OR (95% CI) (n:183)**	***p*-values (n:183)**
TT	119 (95.2 %)	56 (96.5 %)	Additive	Reference	Reference
TC	6 (4.8 %)	2 (3.5 %)		1.41 (0.28-7.22)	0.670
CC	0 (0.0 %)	0 (0.0 %)		NA	
TT	119 (95.2 %)	56 (96.5 %)	Dominant	NA	NA
TC+CC	6 (4.8 %)	2 (3.5 %)			
TT+TC	124 (99.2 %)	58 (100 %)	Recessive	NA	NA
CC	0 (0.0 %)	0 (0.0 %)			
T	0.98	0.98	Allele Frequency	1.40 (0.28-7.05)	0.682
C	0.02	0.02			

3.2.4.2 Male Subgroup Analysis of rs2740574 on CYP3A4 Gene

53 patients and 43 control subjects were included in the rs2740574 male subgroup analysis. Allele frequencies and allelic model results are given in Table 3.14. Only 43 TT (100 %) genotypes were present in the control group, and 47 TT (88.7 %) and 6 TC (11.3 %) genotypes were present in the patient group. The frequency of Thymine was 0.94, 1.00, and the frequency of Cytosine was 0.06 and 0 in patients and controls, respectively. No significant allele frequency difference was monitored between the two groups (p=0.102).

The control group was only comprised of wild-type individuals. Therefore allelic model results were “not applicable”.

Table 3. 14 Allele frequency distributions and allelic model results of the male subgroup of CYP3A4 rs2740574 for patients and controls.

Genotype/Allele	**Patients (n:53) (n, %)**	**Controls (n:43) (n, %)**	**Model**	**OR (95% CI) (n:96)**	***p*-values (n:96)**
TT	47 (88.7 %)	43 (100 %)	Additive	Reference	Reference
TC	6 (11.3 %)	0 (0.0 %)		NA	NA
CC	0 (0.0 %)	0 (0.0 %)		NA	
TT	47 (88.7 %)	43 (100 %)	Dominant	NA	NA
TC+CC	6 (11.3 %)	0 (0.0 %)			
TT+TC	53 (100 %)	43 (100 %)	Recessive	NA	NA
CC	0 (0.0 %)	0 (0.0 %)			
T	0.94	1.00	Allele Frequency	11.19 (0.62-201.49)	0.102
C	0.06	0			

3.2.5 Genotyping and Analysis of rs2108622 on CYP4F2 Gene

rs2108622, which is also known as *CYP4F2*3* allele, is an exon variant located in 11th exon. In rs2108622, Cytosine replaces Thymine, and the global MAF of rs2108622 is 0.29 according to dbSNP, and 0.27 according to gnomAD. The Turkish population MAF has been reported as 0.35 (Ongun et al., 2023).

Initially, gDNA was amplified with the suitable primers, and this amplified gene region is composed of 596 bp (Figure 3.9).

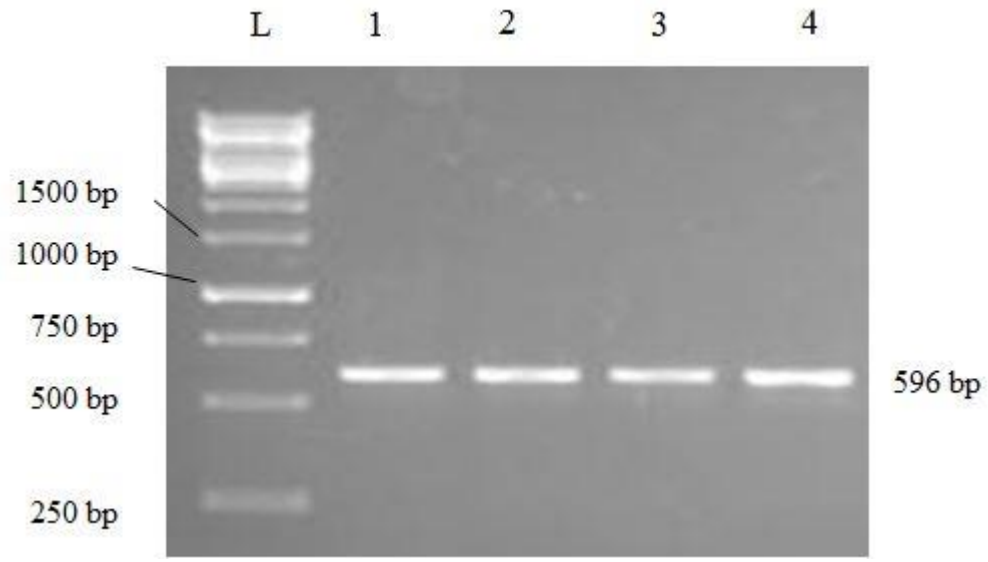

Figure 3. 9 Representative agarose gel (1%) electrophoresis image of rs2108622 PCR products. L stands for GeneRuler 1 kb DNA Ladder. 1, 2, 3, and 4 are PCR products.

Then, PvuII was used to digest PCR products expected to produce 316 bp and 280 bp DNA fragments. Wild-type allele carries the PvuII recognition site, but polymorphic alleles do not. After restriction enzyme digestion, 316 and 280 bp DNA fragments were obtained from wild-type subjects (Figure 3.10).

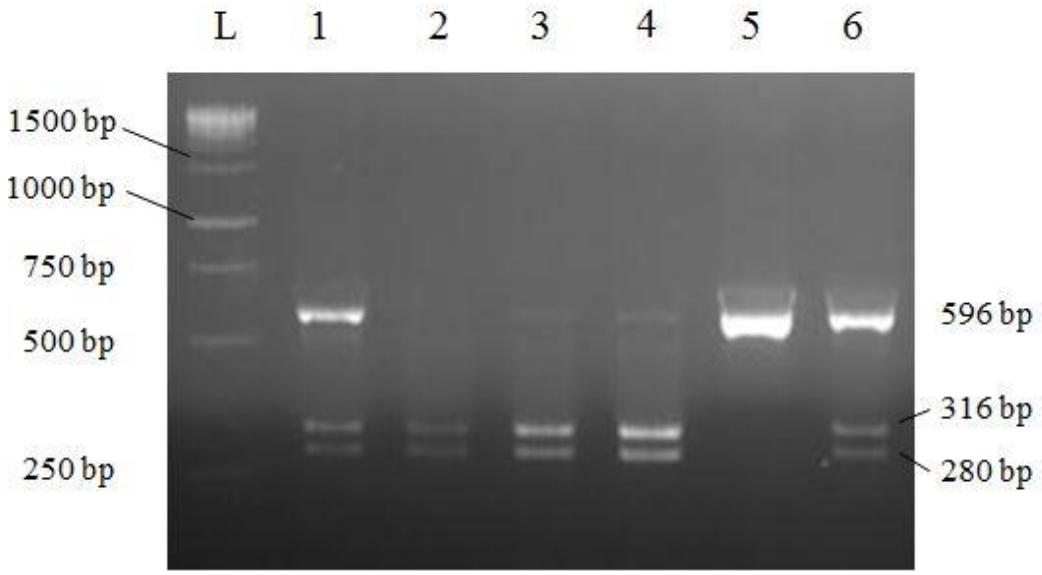

Figure 3. 10 Representative agarose gel (1 %) electrophoresis image of rs2108622 restriction enzyme digestion. L stands for GeneRuler 1 kb DNA Ladder. Lanes 1 and 6 are heterozygous polymorphic (CT), 2-4 are wild-type (CC), 5 is homozygous polymorphic (TT) genotypes.

After restriction enzyme digestion, 179 patients and 101 control group subjects were genotyped using gel images,. Three different models and the allele distribution of patients and controls were analyzed statistically (Table 3.15). In the patient group, 53 CC (29.6 %), 109 CT (60.9 %), 17 TT (9.5 %) genotypes, and in the control group 37 CC (36.6 %), 48 CT (47.5 %), 16 TT (15.8 %) genotypes were identified. Cytosine and Thymine frequencies were found to be 0.60, 0.40 in both groups. Wild-type and polymorphic allele frequencies were also statistically insignificant (p=0.937).

CC vs. CT and CC vs. TT comparisons (p=0.074) in the additive model, CC vs. CT+TT comparison (p=0.230) in the dominant model, and CC+CT vs. TT comparison (p=0.120) in the recessive model did not produce statistically significant results.

Table 3. 15 Allele frequency distributions and allelic model results of CYP4F2 rs2108622 for patients and controls.

Genotype/Allele	**Patients (n:179) (n, %)**	**Controls (n:101) (n, %)**	**Model**	**OR (95% CI) (n:280)**	***p*-values (n:280)**	**Age Adjusted OR (95% CI) (n:247)**	**Age Adjusted *p*-values (n:247)**
CC	53 (29.6 %)	37 (36.6 %)	Additive	Reference	Reference	Reference	Reference
CT	109 (60.9 %)	48 (47.5 %)		1.59 (0.92-2.72)	0.074	1.36 (0.76-2.43)	0.200
TT	17 (9.5 %)	16 (15.8 %)		0.74 (0.33-1.65)		0.68 (0.29-1.61)	
CC	53 (29.6 %)	37 (36.6 %)	Dominant	1.37 (0.82-2.30)	0.230	1.20 (0.69-2.09)	0.520
CT+TT	126 (70.4 %)	64 (63.4 %)					
CC+CT	162 (90.5 %)	85 (84.2 %)	Recessive	0.56 (0.27-1.16)	0.120	0.56 (0.25-1.23)	0.150
TT	17 (9.5 %)	16 (15.8 %)					
C	0.60	0.60	Allele Frequency	1.01 (0.71-1.44)	0.937	1.00 (0.69-1.45)	0.992
T	0.40	0.40					

3.2.5.1 Female Subgroup Analysis of rs2108622 on CYP4F2 Gene

The female subgroup analysis of rs2108622 covered 125 patients and 57 control subjects. Allele frequencies and allelic model results are illustrated in Table 3.16. In patients, 37 CC (29.6 %), 77 CT (61.6 %) and 11 TT (8.8 %) individuals, in controls, 21 CC (36.8 %), 27 CT (47.7 %) and 9 TT (15.8) subjects were included. The frequency of Cytosine was 0.60, 0.61, and the frequency of Thymine was 0.40, 0.39 in patients and controls, respectively. The *p*-value for allele frequency was found to be 0.982.

CC vs. CT and CC vs. TT comparison were conducted in the additive model. Between compared groups, no statistically significant difference was observed (p=0.160). Also, CC vs. CT+TT in the dominant model and CC+CT vs. TT in the recessive model did not produce significant differences with the *p*-values of p=0.330, and p=0.170, respectively.

Table 3. 16 Allele frequency distributions and allelic model results of the female subgroup of CYP4F2 rs2108622 for patients and controls.

Genotype/Allele	**Patients (n:125) (n, %)**	**Controls (n:57) (n, %)**	**Model**	**OR (95% CI) (n:182)**	***p*-values (n:182)**
CC	37 (29.6 %)	21 (36.8 %)	Additive	Reference	Reference
CT	77 (61.6 %)	27 (47.7 %)		1.62 (0.81-3.23)	0.160
TT	11 (8.8 %)	9 (15.8 %)		0.69 (0.25-1.94)	
CC	37 (29.6 %)	21 (36.8 %)	Dominant	1.39 (0.72-2.69)	0.330
CT+TT	88 (70.4 %)	36 (63.2 %)			
CC+CT	114 (91.2 %)	48 (84.2 %)	Recessive	0.51 (0.20-1.32)	0.170
TT	11 (8.8 %)	9 (15.8 %)			
C	0.60	0.61	Allele Frequency	1.01 (0.64-1.58)	0.982
T	0.40	0.39			

3.2.5.2 Male Subgroup Analysis of rs2108622 on CYP4F2 Gene

The male subgroup analysis of rs2108622 covered 54 patients and 44 control subjects. Allele frequencies and allelic model results are given in Table 3.17. In patients, 16 CC (31.5 %), 32 CT (57.4 %) and 6 TT (11.1 %) individuals, in controls, 16 CC (36.4 %), 21 CT (47.7 %) and 7 TT (15.9) subjects were included. The frequency of Cytosine was 0.59, 0.60 and the frequency of Thymine was 0.41, 0.40 in patients and controls, respectively. The *p*-value for allele frequency was found to be 0.891.

CC vs. CT and CC vs. TT comparisons in the additive model, CC vs. CT+TT comparison in the dominant model, and CC+CT vs. TT comparison in the recessive model were performed. Between compared groups in each model, no statistically significant difference was found with the *p*-values of p=0.510, p=0.480, and p=0.490, respectively.

Table 3. 17 Allele frequency distributions and allelic model results of the male subgroup of CYP4F2 rs2108622 for patients and controls.

Genotype/Allele	**Patients (n:54) (n, %)**	**Controls (n:44) (n, %)**	**Model**	**OR (95% CI) (n:98)**	***p*-values (n:98)**
CC	16 (31.5 %)	16 (36.4 %)	Additive	Reference	Reference
CT	32 (57.4 %)	21 (47.7 %)		1.52 (0.63-3.69)	0.510
TT	6 (11.1 %)	7 (15.9 %)		0.86 (0.24-3.12)	
CC	17 (31.5 %)	16 (36.4 %)	Dominant	1.36 (0.58-3.17)	0.480
CT+TT	37 (68.5 %)	28 (63.6 %)			
CC+CT	48 (88.9 %)	37 (84.1 %)	Recessive	0.66 (0.20-2.13)	0.490
TT	6 (11.1 %)	7 (15.9 %)			
C	0.59	0.60	Allele Frequency	1.04 (0.59-1.85)	0.891
T	0.41	0.40			

CHAPTER 4

DISCUSSION

MS is a chronic inflammatory and neurodegenerative disease displaying demyelination of neurons, which results in the deterioration of neuron impulses (Ahmadi et al., 2020; Bakr et al., 2021).

The global prevalence of MS has been increasing rapidly. Walton and her co-workers estimated that the prevalence of MS had risen 30 % from 2013 to 2020. 2.1 persons per 100.000 population increment had observed in a yearly manner. Walton's research group predicted that every 5 minutes, someone gets an MS diagnosis in the world. Also, the number of MS patients under 18 years of age has been increasing dramatically (Walton et al.,2020).

MS usually affects people at their highly productive stage of life. From this point of view, MS has a huge impact on young adults who are planning to start families and trying to build their careers. This brings an extraordinary burden to the patients' families and society. The advances in MS therapies are still offering only reduction in the disabilities and extending the survival of patients. Nonetheless, no known cure for MS exits, and the etiology still remains unsolved (Walton et al.,2020).

Since MS is an autoimmune disorder, inflammatory metabolites of AA metabolism including eicosanoids, leukotrines, prostaglandins, prostacyclin, and thromboxane play an important role in MS onset, or progression (Hoxha et al., 2022; Sonnweber et al., 2018). AA metabolites posses either pro-inflammatory or anti-inflammatory properties and produced via lipooxygenase, cyclooxygenase, and cytochrome P450 pathways. Lipooxygenase and cyclooxygenase pathways of AA have been paid much attention in MS. These pathways are shown to be overactivated in MS patients, which is resulted in increased concentrations of prostaglandins, thromboxanes, and leukotrienes. It is predicted that increased concentration of AA

metabolites have been linked with demyelination and autoimmunity of oligodendrocytes (Hoxha et al., 2022). On the other hand, CYP-dependent AA pathways and CYPs responsible for metabolism of AA have been mostly left behind in MS studies.

CYPs are responsible for the metabolism of various exogenous (drugs, alcohols, antioxidants, organic solvents, anaesthetic agents, dyes, environmental pollutants and chemicals) and endogenous (steroids, bile acids, fatty acids, including AA, prostaglandins, biogenic amines and retinoids) compounds (McMillan and Tyndale, 2018). This study investigated the polymorphisms in inflammation-related CYP1A2, CYP2C9, CYP2D6, CYP3A4, and CYP4F2 genes due to their roles in AA metabolism. In addition to their roles in the AA pathway, CYP2C9, CYP3A4 and CYP4F2 are the main enzymes that take part in biotransformation of MS treatment drugs such as siponimod, teriflunomide, fingolimod, laquinimod, ozanimod, and ponesimod (Glaenzel et al., 2018; Carazo et al., 2018; Thöne and Linker, 2016; Surapaneni et al., 2021; Alnaif et al., 2022; David et al., 2012). There is a limited number of MS polymorphism studies regarding the selected genes taking part in AA metabolism and biotransformation of MS treatment drugs, and so far, no polymorphism study in the Turkish MS patients has been reported.

179 MS patients and 102 healthy controls has been recruited for this study. There are 125 females and 54 males in the patient group, and 58 females and 44 males in the control group. The gender distribution difference between in case and control groups was found to be statistically significant (p=0.028). However, it is very well known that women are more susceptible to MS than men. Therefore, patient group with high number of women participant is somewhat expected. Conversely, control group exhibits more uniform gender distribution. Women's higher risk of having MS is directly related to the significant difference of gender distribution between case and control groups.

These recruited healthy control subjects' minor allele frequencies of selected variants are also compared with the results obtained from the Turkish population and other populations. Table 4.1 summarizes the MAFs of selected variants.

Table 4. 1 Minor allele frequencies of selected variants in different populations

	Global[1]	**European[1]**	**African[1]**	**Asian[1]**	**Caucasian**	**Turkish Population[2]**	**Present Study Control Group**
CYP1A2 (rs762551)	0.32	0.30	0.44	0.41	0.29 (Wang et al., 2012)	0.34 (Arici and Özhan, 2017; Yucesoy et al., 2017)	0.30
CYP2C9 (rs1799853)	0.11	0.12	0.03	< 0.01	0.14 (Cavallari and Momary, 2013)	0.11 (Aynacioglu et al., 1999; Arici and Özhan, 2017).	0.07
CYP2D6 (rs16947)	0.32	0.32	0.34	0.06	0.17 (Qiao et al., 2019)	0.35 (Aynacioglu et al., 1999; Taskin, et al., 2016)	0.31
CYP3A4 (rs2740574)	0.11	0.04	0.64	< 0.01	0.03 (Arici and Özhan, 2016)	0.01 (Sayitoğlu et al., 2006; Arici and Özhan, 2016)	0.01
CYP4F2 (rs2108622)	0.29	0.29	0.09	0.27	0.24 (Cavallari and Momary, 2013)	0.35 (Ongun et al., 2023)	0.40

1: dbSNP database minor allele frequencies
2: Meta-analysis of given studies for CYP1A2, 2C9, 2D6 and 3A4

Minor allele frequencies of selected variants in the control group are quite close to the previously conducted studies in Turkish population. MAFs of CYP1A2, 2C9, and 2D6 variants are slightly lower, MAF of CYP4F2 variant is slightly higher

than the previous Turkish population studies. This slight variation may arise from sampling location. Central anatolian residents were included in this study, but other Turkish population studies covered different regions of Türkiye. The racial and ethnic differences as well as sampling location may cause differences in allele frequencies and that is why our control group allele frequencies may differ from global, European, African, Asian and Caucasian populations.

SNPs on CYP1A2, 2C9, 2D6, 3A4 and 4F2 were statistically analyzed under the additive, dominant and recessive allelic models with female and male subgroup analysis.

The human liver expresses high levels of CYP1A2, comprising around 13-15 % of the total cytochrome P450 content. CYP1A2 metabolizes approximately 10 % of drugs used in clinical practices (Chen et al., 2017). The rs762551 variant has been studied extensively in neurodegenerative diseases such as Parkinson's, and Alzheimer's diseases and kidney dysfunction (Siokas et al., 2021; Kim et al., 2018; Siokas et al., 2022; Mahdavi et al., 2023). Researchers tried to correlate these diseases with caffeine intake since caffeine is capable of decreasing the inflammation in CNS (Madeira et al., 2017). Also, the rs762551 variant has been associated with certain types of cancer, such as breast and lung cancer. These studies concluded that the mutant C allele has increased the risk of cancer development (Tian et al., 2013; Khvostova et al., 2012). Zorlu et al., 2019 investigated 8 SNPs in the German population, which were assumed to be the potential risk factors for MS. Only rs762551 gave significant nominal results under the additive and recessive models (Zorlu et al., 2019). However, male and female subgroup analysis did not exhibit statistically significant differences under three allelic models. Only, allele frequency between case and control groups in male subgroup gave significant difference. Most likely, this difference is due to the relatively small sample size of male subgroup. Our results under the additive model ($p=0.049$) and recessive model ($p=0.016$) supported the results obtained from German population. CYP1A2 is responsible for the production of 20-HETE, and EET regio-isomers from AA. Most probably, distrupted balance between ω-

hydroxylase and epoxygenase activities of CYP1A2 may favor 20-HETE activity. Even though rs762551 variant has decreased enzyme activity, favored activity of pro-inflammatory 20-HETE with respect to anti-inflammatory EETs may upregulate NF-κB and induce ROS generation. This activation of NF-κB increases the expression of pro-inflammatory cytokines and chemokines. However, disease causing effect of this variant needs to be investigated deeper in order to uncover the underlying molecular mechanisms. Unlike our findings, polymorphic C allele in rs762551 variant has been shown to have protective effect in another autoimmune disease, rheumatoid arthritis in Korean population (Cornelis et al., 2010).

Around 20 % of the entire hepatic CYP content is constituted by CYP2C9, which is responsible for the metabolism of roughly 15-20 % of clinical drugs (Wang et al., 2009). CYP2C9 has diverse roles in the biotransformation of exogenous and endogenous compounds. Because of its diverse nature and reduced enzymatic activities of CYP2C9 variants, the Association for Molecular Pathology Clinical Practice Committee in the USA recommends genotype testing for CYP2C9-related medications (Pratt et al., 2019; Sausville et al., 2018). As in the case of Siponimod, which is the first oral therapy alternative for patients with secondary progressive MS, CYP2C9 genotyping is required before the medication starts in different countries (Díaz-Villamarín et al., 2022; Zhou et al., 2023). CYP2C9 variants have been associated with warfarin dose prediction and bleeding complications in different populations, including the Turkish population (Sanderson et al., 2005; King et al., 2004; Lindh et al., 2009; Oner Ozgon et al., 2009; Kocael et al., 2019). Warfarin is an anticoagulant agent used to prevent thrombosis in patients with thromboembolism, atrial fibrillation, and heart valve implants (Lindh et al., 2009; Takeuchi et al., 2009). The *CYP2C9*2* variant has also been linked with Hodgkin lymphoma in young adults, drug-resistant epilepsy in children, and colorectal adenoma in adults (Chang et al., 2009; Makowska et al., 2021, Barry et al., 2009). Also, the rs1799853 variant has been found possibly damaging in breast cancer development (Savas et al., 2006). To our knowledge, there is no polymorphism study conducted with rs1799853 in MS patients. Our results under additive and

dominant models were significant, and the OR values were 0.10 for both models. Also, female subgroup analysis gave a statistically significant difference under additive model with the OR value of 0.12. Relatively low OR values indicate that SNP under investigation might have protective effects on disease onset or decreased risk of having MS. Epoxygenase activities of different CYPs yield different amounts EET regio-isomers. CYP2C9's epoxygenase activity mainly produces 14,15-EET and 11,12-EET. These two metabolites are the most potent PPARγ activators, while 11,12-EET is the most potent inhibitor of NF-κB signaling. PPARγ stimulates anti-inflammatory genes, and represses the formation pro-inflammatory cytokines, interleukins and chemokines, and inhibits NF-κB and NO release. Simultaneously, activation of PPARγ and deactivation of NF-κB may accomplish this protective effect. Nevertheless, our findings pointing to the protective effects of rs1799853 for MS needs to be confirmed with further analysis, and underlying molecular mechanisms need to be enlightened.

The protective effect of rs1799853 has been demonstrated in mice with non-small cell lung cancer by Sausville et al., 2018. The rs1799853 variant exhibited diminished enzymatic activity, and individuals carrying rs1799853 allele metabolized drugs more slowly than wild-type individuals. Being a poor metabolizer might affect the drug response and change the risk of having certain diseases. Poor drug metabolizers (*CYP2C9*2*) also metabolized AA less efficiently, and leading to deficient EET biosynthesis. *CYP2C9*2* variants, in turn, produced lower levels of EET, and the tumors developed were fewer, smaller, and less vascularized with respect to wild-type. Therefore, mice carrying rs1799853 allele had increased survival rates (Sausville et al., 2018). In another study reported from Russia, a different CYP2C9 variant, rs4918758 was associated with the decreased risk of coronary heart disease (Polonikov et al., 2017). Unlike these protective effects of different CYP2C9 variants, including rs1799853 and rs4918758 in cancer and coronary heart disease, rs1799853 variant has been determined to be a risk factor for rheumatoid arthritis (Canet et al., 2019).

Furthermore, decreased enzyme activity of rs1799853 variant is highly associated with MS treatment strategy. CYP2C9 is the key enzyme responsible for the biotransformation of siponimod. Siponomid is an oral therapy alternative for secondary progressive MS, and dosage adjustments should be made for the individuals carrying polymorphic allele(s). Therefore, before siponimod treatment starts, CYP2C9 genotyping is recommended.

CYP2D6 is a key pharmacogene engaged with the metabolizing approximately 20% of clinical drugs and extensively expressed in the liver, brain, and intestine (Taylor et al., 2020). Mainly, CYP2D6 variants have been studied in neurodegenerative diseases. In Caucasians, CYP2D6 polymorphisms causing poor metabolizer phenotype significantly increased the genetic susceptibility to Parkison's disease (Lu et al., 2014; Anwarullah et al., 2017). Parkinson's disease patients had reduced CYP2D6 levels in different parts of their brains. Lower levels of CYP2D6 might diminish the ability to inactivate the disease causing neurotoxins, which eventually contributed disease onset (Mann et al., 2012). Also, the rs16947 variant has been associated with schizophrenia and epilepsy risk, and different cancer types development (Ma et al., 2020; Elsaid et al., 2022; Zhou et al., 2012). Moreover, different CYP2D6 (*CYP2D6*4* and *CYP2D6*3*) variants have been shown to possess disease causing effects in autoimmune diseases such as systemic lupus erythematosus and ankylosing spondylitis in Caucasians (Lee and Bae, 2017). Unlike these studies, our results pointed out that the rs16947 variant has no real effect on the genesis of MS. We failed to obtain significant differences between the case and control groups. Also, female and male subgroup analysis did not generate significant results. These findings are consistent with the results obtained in Sweden. Landtblom et al. suggested that CYP2D6 polymorphisms are not risk factors for MS development and have no pathogenic influence on Swedish population (Landtblom et al., 2003).

Depression is a quite common disorder among MS patients. It is estimated that the prevalance of depression is around 25% in MS patients, and 86% of these patients receive antidepressant drugs (Stamoula et al., 2021). The neuroprotective effects of

antidepressant drugs are intriguing. For instance, a phosphodiesterase inhibitor, Ibudilast is capable of boosting remyelanation in oligodendrocytes (Ainatzoglou et al., 2021). Clomipramine, an another antidepressant, has been shown to reduce crucial MS pathogenesis determinants including T-cell proliferation, oxidative stress and B-cell activity. (Faissner et al., 2017). Also, antidepressants suppress the pro-inflammatory cytokines such as IL-2, IL-6, IL-17, TNF-α and IFNγ, and reduce the anti-inflammatory cytokine IL-4 and IL-10 serum levels (Stamoula et al., 2021). Moreover, antidepressants are capable of ceasing the symptoms of MS. Escitalopram prevents stress-related relapses, and bupropion significantly reduces the fatique (Mitsonis et al., 2010; Siniscalchi et al., 2010). According to latest reports antidepressants can not only alter the course of MS, but also ease the MS symptoms. Therefore, study population with receiving antidepressant drugs may exhibit opposite results from our findings. One should keep in mind that CYP2D6 is an essential enzyme for the metabolism of antidepressants and antipsychotics, together with CYP2C19 (Stamoula et al., 2021; Austin-Zimmerman et al., 2021).

CYP3A4 is the most abundant cytochrome P450 enzyme in the liver and intestinal tract. It catalyzes a wide range of endogenous and exogenous compounds. It is responsible for the metabolism of half of the clinically used drugs. Therefore it is considered as one of the most essential CYPs (Chen et al., 2014; Zhou et al., 2011; Zhou et al., 2019). Generally, studies with CYP3A4 variants have focused on the risk of developing different cancer types. For instance, the rs2740574 variant (*CYP3A4*1B*) has been associated with ovarian cancer, breast cancer, small cell lung cancer, and prostate cancer (Zhou et al., 2013; Pearce et al., 2009; Kato et al., 2009; Dally et al., 2003; Zeigler-Johnson et al., 2004; Fernandez et al., 2012). This variant has also been linked with the occurrence of acute promyelocytic leukemia in MS patients treated with mitoxantrone (Hasan et al., 2011). To the best of our knowledge, there is no polymorphism study regarding with *CYP3A4*1B* variant in MS. Nevertheless our results did not show statistically significant differences between patient and control groups. The age-adjusted *p* value was found to be significant (p=0.018). However, this is most probably due to the decreased sample

size of the age-adjusted population. Also, female subgroup analysis did not produce significant result under additive model. On the other hand, our findings supported the results obtained from rheumatoid arthritis patients. It has been concluded that *CYP3A4*1B* variant is not a risk factor for rheumatoid arthritis, as well (Canet et al., 2019).

CYP3A4 is also one of the most prominent MS treatment drugs metabolizing enzyme. Teriflunomide, laquinimod, ozanimod and ponesimod are the substrates of CYP3A4. Considering the decreased enzyme activity of *CYP3A4*1B* variant, it may affect the biotransformation efficiency of these drugs. Therefore, dosage adjustments could be done for polymorphic allele(s) carriers with decreased enzymatic activity.

CYP4F2 functions in the metabolism of various endogenous and exogenous molecules. Its role in the metabolism of endogenous compounds is much more prominent (Nebert and Russell, 2002; Al-Eitan et al., 2021; Mockute et al., 2021). It is expressed highly in the liver and kidney and catalyzes some metabolically important subrates such as eicosanoids, AA, vitamin K and vitamin E (Hardwick, 2008; Nebert et al., 2013; Al-Eitan et al., 2021; Mockute et al., 2021). Mostly, CYP4F2 variants have been associated with metabolic syndrome-related disorders, including heart disease, stroke, and type II diabetes (Alvarellos et al., 2015; Hardwick, 2008). The *CYP4F2*3* phenotypes have decreased enzyme levels, and this variant has also been determined as a risk factor for metabolic syndrome (Alvarellos et al., 2015; Fava et al., 2012). In addition, the rs2108622 variant has been linked with ischemic stroke, adrenoleukodystrophy, a neurodegenerative disease in which very long-chain fatty acids are accumulated, coronary heart disease, type II diabetes, and chronic obstructive pulmonary disease (Liao et al., 2016; van Engen et al., 2016, Yu et al., 2014, Park et al., 2019; Ding et al., 2020). To our knowledge and understanding, no CYP4F2 polymorphism study in MS has been reported. And, our findings highlighted that rs2108622 has no real effect on MS development. We failed to obtain significant results between the case and control groups. Also, female and male subgroup analysis did not give significant

results. On the hand other, previously, rs2108622 variant has been significantly associated with Crohn's disease (chronic inflammatory bowel disease) which is also assumed to be an autoimmune disorder (Costea et al., 2010).

Fingolimod is an immune modulator used for MS treatment, and it is metabolized by CYP4F2. Due to the dimished activity of rs2108622 variant, dose adjustment may be required.

Autoimmune diseases have complex nature with the multiple factors involved. Selected SNPs showed different effects on different autoimmune diseases. For instance, CYP1A2 variant has been found to be a risk factor for MS, whereas it has protective effect against rheumatoid arthritis. On the contrary, our results showed that CYP2C9 variant has protective properties for MS, while it has been determined to be a risk factor for rheumatoid arthritis. CYP2D6 variants bear disease causing effects in systemic lupus erythematosus and ankylosing spondylitis in Caucasians, but no real effect on MS. In a similar manner, CYP4F2 variant has been linked with chronic inflammatory bowel disease, yet it has no real effect on MS like CYP2D6 variant. These opposing results indicate the complicated etiology of each autoimmune disorder. There are various metabolites, intermediates and inflammation-related pathways with opposite physiological roles involved in the pathogenesis of these disorders. In addition to genetic factors, enviromental factors such as diet, sunlight exposure, and smoking also contribute the onset of autoimmune diseases. Diets rich in lineloic acid or arachidonic acid, and high caffeine consumption may alter the susceptibility of individuals to the autoimmune diseases. Therefore, genetic and enviromental factors together may affect the risk of having autoimmune diseases negatively, or positively. Also induction and/or reduction mechanisms of CYP-independent pathways, LOX and COX pathways, may alter the susceptibility of specific autoimmune disorders.

In summary, we have obtained statistically significant results between case and control groups in CYP1A2 and CYP2C9 variants. CYP1A2 variant was found to possess disease causing effect, whereas CYP2C9 variant was found to have

protective effect on MS. Nevertheless, the remaining variants, CYP2D6, CYP3A4 and CYP4F2, did not give statistically significant differences between compared groups.

Despite our promising findings, this study also has limitations concerning the nature of polymorphism studies. Although a patient group with 179 individuals seems fair and enough for a rare disease, a larger population size would strengthen the results. Our study may give insights into future studies and can be regarded as the initial step. Studies with larger population sizes and individuals from different ethnicities should be encouraged. Moreover, findings pointing to the potential protective effect as in the case of rs1799853, and potential risk factors, such as rs762551 for MS, should be investigated further in more detail. Molecular mechanisms underlying these positive and negative effects should be enlightened.

Also, the PCR-RFLP method itself may bring limitations to our study. Especially, heterozygous and homozygous polymorphic alleles carrying restriction enzyme recognition sites might cause misleading interpretations regarding the genotyping of recruited subjects. Since restriction enzymes recognize specific sequences in the genes under investigation, the presence of possible rare alleles would be missed. An individual might be genotyped incorrectly due to this specifity of restriction enzymes. For instance, despite an individual carrying a heterozygous or homozygous rare allele, he/she might be marked as wild-type because these rare alleles do not have restriction enzyme recognition/cut sites. On the other hand, results obtained from wild-type alleles having recognition site for restriction enzymes would be more accurate.

CHAPTER 5

CONCLUSION

Multiple sclerosis (MS) is a common autoimmune disorder of the central nervous system characterized by chronic inflammation, demyelination, and axonal loss. The etiology of MS remains unsolved, and its prevalance has been increasing worldwide dramatically. In our study, we have examined the relationship between MS and specific single nucleotide polymorphisms (SNPs) on inflammation related cytochrome P450 enzymes (CYPs), including CYP1A2 (rs762551), CYP2C9 (rs1799853), CYP2D6 (rs16947), CYP3A4 (rs2740574), and CYP4F2 (rs2108622). These CYPs are all involved in arachidonic acid (AA) metabolism and take part in the production of AA metabolites. AA and metabolites possess pro-inflammatory and anti-inflammatory roles in inflammation response. Unlike cyclooxygenase (COX) and lipooxygenase (LOX) pathways of AA metabolism, the CYP pathway has been undervalued. Much of the interest in MS has been directed to LOX and COX pathways. We considered that impaired AA metabolism would also be a critical target in MS. Therefore, polymorphisms in selected CYPs functioning inflammatory processes through the AA pathway were thought to be worth investigating. Our results supported this initial consideration. SNPs on CYP1A2 (rs762551) and CYP2C9 (rs1799853) gave significant differences between patient and control groups. Primarily, SNP on CYP2C9 was found to be significant even after multiple comparisons correction. Findings highlighting the protective effect of CYP2C9 and the disease causing effect of CYP1A2 carry the potential to bring new insights to MS studies. In order to reveal the exact mechanisms of these possible protective and disease causing effects, studies with deeper mechanistic approaches and larger population size with inviduals from different etnicities should be encouraged and conducted.

REFERENCES

Abdel-Razzak, Z., Loyer, P., Fautrel, A., Gautier, J. C., Corcos, L., Turlin, B., Beaune, P., Guillouzo, A. 1993. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. Mol Pharmacol., 44(4):707-15.

Ahmadi, H., Yassaee, V. R., Mirfakhraie, R., Hashemi-Gorji, F. 2020. Association between single nucleotide polymorphisms rs12722489 and multiple sclerosis in Iranian patients with multiple sclerosis. Curr J Neurol., 5;19(1):26–31.

Ainatzoglou, A., Stamoula, E., Dardalas, I., Siafis, S., Papazisis, G. 2021. The effects of PDE inhibitors on Multiple Sclerosis: a Review of in vitro and in vivo Models. Curr Pharm Des., 27(20):2387-2397.

Albert, P. R. 2011. What is a functional genetic polymorphism? Defining classes of functionality. J Psychiatry Neurosci., 36(6):363-5.

Al-Eitan, L. N., Almasri, A. Y., Alnaamneh, A. H., Aman, H. A., Alrabadi, N. N., Khasawneh, R. H., Alghamdi, M. A. 2021. Influence of CYP4F2, ApoE, and CYP2A6 gene polymorphisms on the variability of Warfarin dosage requirements and susceptibility to cardiovascular disease in Jordan. Int J Med Sci., 18(3):826-834.

Almazroo, O. A., Miah, M. K., Venkataramanan, R. 2017. Drug metabolism in the liver. Clin. Liver Dis., 21:1-20.

Alnaif, A., Oiler, I., D'Souza, M. S. 2022. Ponesimod: An Oral Second-Generation Selective Sphingosine 1-Phosphate Receptor Modulator for the Treatment of Multiple Sclerosis. Ann Pharmacother, 10600280221140480.

Alvarellos, M. L., Sangkuhl, K., Daneshjoui R., Whirl-Carrillo, M., Altman, R. B., Klein, T. E. 2015. PharmGKB summary: very important pharmacogene information for CYP4F2. Pharmacogenet Genomics, 25(1):41-7.

Alwi, Z. B. 2005. The Use of SNPs in Pharmacogenomics Studies. Malays J Med Sci., 12(2):4-12.

Anwarullah, A. M., Badshah, M., Abbasi, R., Sultan, A., Khan, K., Ahmad, N., von Engelhardt, J. 2017. Further evidence for the association of CYP2D6*4 gene polymorphism with Parkinson's disease: a case control study. Genes Environ., 39:18.

Arici, M., Özhan, G. 2016. The evaluation of CYP3A4 and CYP3A5 genetic profiles in Turkish population. J. Fac. Pharm. Istanbul, 46(1): 15-22.

Arici, M., Özhan, G. 2017. CYP2C9, CYPC19 and CYP2D6 gene profiles and gene susceptibility to drug response and toxicity in Turkish population. Saudi Pharm J., 25(3):376-380.

Arici, M., Özhan, G. 2017. The genetic profiles of CYP1A1, CYP1A2 and CYP2E1 enzymes as susceptibility factor in xenobiotic toxicity in Turkish population. Saudi Pharm J., 25(2):294-297.

Austin-Zimmerman, I., Wronska, M., Wang, B., Irizar, H., Thygesen, J. H., Bhat, A., Denaxas, S., Fatemifar, G., Finan, C., Harju-Seppänen, J., Giannakopoulou, O., Kuchenbaecker, K., Zartaloudi, E., McQuillin, A., Bramon, E. 2021. The Influence of CYP2D6 and CYP2C19 Genetic Variation on Diabetes Mellitus Risk in People Taking Antidepressants and Antipsychotics. Genes (Basel), 12(11):1758.

Aynacioglu, A. S., Brockmöller, J., Bauer, S., Sachse, C., Güzelbey, P., Ongen, Z., Nacak, M., Roots, I. 1999. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Br J Clin Pharmacol., 48(3):409-15.

Aynacioglu, A. S., Brockmöller, J., Bauer, S., Sachse, C., Güzelbey, P., Ongen, Z., Nacak, M., Roots, I. 1999. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Br J Clin Pharmacol., 48(3):409-15.

Aynacioglu, A. S., Sachse, C., Bozkurt, A., Kortunay, S., Nacak, M., Schröder, T., Kayaalp, S. O., Roots, I., Brockmöller, J. 1999. Low frequency of defective alleles of cytochrome P450 enzymes 2C19 and 2D6 in the Turkish population. Clin Pharmacol Ther., 66(2):185-92. doi: 10.1053/cp.1999.v66.100072001. PMID: 10460072.

Bakr, N. M., Hashim, N. A., El-Baz, H. A. E., Khalaf, E. M., Elharoun, A. S. 2021. Polymorphisms in proinflammatory cytokines genes and susceptibility to Multiple Sclerosis. Mult Scler Relat Disord., 47:102654.

Barnaba, C., Gentry, K., Sumangala, N., Ramamoorthy, A. 2017. The catalytic function of cytochrome P450 is entwined with its membrane-bound nature. F1000Res., 6:662.

Barry, E. L., Poole, E. M., Baron, J. A., Makar, K. W., Mott, L. A., Sandler, R. S., Ahnen, D. J., Bresalier, R. S., McKeown-Eyssen, G. E., Ulrich, C. M. 2012. CYP2C9 variants increase risk of colorectal adenoma recurrence and modify associations with smoking but not aspirin treatment. Cancer Causes Control, 24(1):47-54.

Berges, R., Gsur, A., Feik, E., Höfner, K., Senge, T., Pientka, L., Baierl, A., Michel, M. C., Ponholzer, A., Madersbacher, S. 2011. Association of polymorphisms in CYP19A1 and CYP3A4 genes with lower urinary tract symptoms, prostate volume, uroflow and PSA in a population-based sample. World J Urol., 29(2):143-8.

Bishop-Bailey, D., Thomson, S., Askari, A., Faulkner, A., Wheeler-Jones, C. 2014. Lipid-metabolizing CYPs in the regulation and dysregulation of metabolism. Annu Rev Nutr., 34:261-79.

Bosetti, F. 2007. Arachidonic acid metabolism in brain physiology and pathology: lessons from genetically altered mouse models. J Neurochem., 102(3):577-86.

Bull, L. 2000-2013. Genetics, Mutations, and Polymorphisms. In: Madame Curie Bioscience Database. Landes Bioscience.

Calder, P. C. 2006. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr., 83(6):1505S-1519S.

Canet, L. M., Sánchez-Maldonado, J. M., Cáliz, R., Rodríguez-Ramos, A., Lupiañez, C. B., Canhão, H., Martínez-Bueno, M., Escudero, A., Segura-Catena, J., Sorensen, S. B., Hetland, M. L., Soto-Pino, M. J., Ferrer, M. A., García, A., Glintborg, B., Filipescu, I., Pérez-Pampin, E., González-Utrilla, A., Nevot, M. Á. L, Conesa-Zamora, P., Broeder, A. D., De Vita, S., Jacobsen, S. E. H., Collantes-Estevez, E., Quartuccio, L., Canzian, F., Fonseca, J. E., Coenen, M. J. H., Andersen, V., Sainz, J. 2019. Polymorphisms at phase I-metabolizing enzyme and hormone receptor loci influence the response to anti-TNF therapy in rheumatoid arthritis patients. Pharmacogenomics J., 19(1):83-96.

Carazo, A., Dusek, J., Holas, O., Skoda, J., Hyrsova, L., Smutny, T., Soukup, T., Dosedel, M., Pávek, P. 2018. Teriflunomide Is an Indirect Human Constitutive Androstane Receptor (CAR) Activator Interacting With Epidermal Growth Factor (EGF) Signaling. Front Pharmacol., 9:993.

Carlson, N. G., Hill, K. E., Tsunoda, I., Fujinami, R. S., Rose, J. W. 2006. The pathologic role for COX-2 in apoptotic oligodendrocytes in virus in-duced demyelinating disease: implications for multiple sclerosis. J. Neuroimmunol., 174(1-2): 21-31.

Cavallari, L., Momary, K. 2013. Pharmacogenetics in Cardiovascular Diseases. Pharmacogenomics, 133-182.

Chan, W., Li, M. S., Sundaram, S. K., Tomlinson, B., Cheung, P. Y., Tzang, C.H. 2019. CYP2D6 allele frequencies, copy number variants, and tandems in the population of Hong Kong. J Clin Lab Anal., 33(1):e22634.

Chang, E. T., Birmann, B. M., Kasperzyk, J. L., Conti, D. V., Kraft, P., Ambinder, R. F., Zheng, T., Mueller, N. E. 2009. Polymorphic variation in NFKB1 and other aspirin-related genes and risk of Hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev., 18(3):976-86.

Chang, G. W., Kam, P. C. 1999. The physiological and pharmacological roles of cytochrome P450 isoenzymes. Anaesthesia, 54(1):42-50.

Chen, J., Zhao, K. N., Chen, C. The role of CYP3A4 in the biotransformation of bile acids and therapeutic implication for cholestasis. 2014. Ann Transl Med., 2(1):7.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L. 2017. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 14;9(6):7204-7218.

Chen, Y., Zeng, L., Wang, Y., Tolleson, W. H., Knox, B., Chen, S., Ren, Z., Guo, L., Mei, N., Qian, F., Huang, K., Liu, D., Tong, W., Yu, D., Ning, B. 2017. The expression, induction and pharmacological activity of CYP1A2 are post-transcriptionally regulated by microRNA hsa-miR-132-5p. Biochem Pharmacol., 145:178-191.

Chu, D., Wei, L. 2019. Nonsynonymous, synonymous and nonsense mutations in human cancer-related genes undergo stronger purifying selections than expectation. BMC Cancer, 19:359

Cornelis, M. C., Bae, S. C., Kim, I., El-Sohemy, A. 2010. CYP1A2 genotype and rheumatoid arthritis in Koreans. Rheumatol Int., 30(10):1349-54.

Correale, J., Gaitán, M. I. 2015. Multiple sclerosis and environmental factors: the role of vitamin D, parasites, and Epstein-Barr virus infection. Acta Neurol Scand., 132(199):46-55.

Cosentino, M., Marino, F. 2013. Adrenergic and dopaminergic modulation of immunity in multiple sclerosis: teaching old drugs new tricks? J Neuroimmune Pharmacol., 8(1):163-79.

Costea, I., Mack, D. R., Israel, D., Morgan, K., Krupoves, A., Seidman, E., Deslandres, C., Lambrette, P., Grimard, G., Levy, E., Amre, D. K. 2010. Genes involved in the metabolism of poly-unsaturated fatty-acids (PUFA) and risk for Crohn's disease in children & young adults. PLoS One, 5(12):e15672.

Dally, H., Edler, L., Jäger, B., Schmezer, P., Spiegelhalder, B., Dienemann, H., Drings, P., Schulz, V., Kayser, K., Bartsch, H., Risch, A. 2003. The CYP3A4*1B allele increases risk for small cell lung cancer: effect of gender and smoking dose. Pharmacogenetics, 13(10):607-18.

Daly, A. K., Rettie, A. E., Fowler, D. M., Miners, J. O. 2017. Pharmacogenomics of CYP2C9: Functional and Clinical Considerations. J Pers Med., 8(1):1.

Danielson, P. B. 2002. The cytochrome P450 superfamily: Biochemistry, evolution and drug metabolism in humans. Curr. Drug Metab., 3:561-597.

David, O. J., Kovarik, J. M., Schmouder, R. L. 2012. Clinical pharmacokinetics of fingolimod. Clin Pharmacokinet., 51(1):15-28.

Dendrou, C. A., Fugger, L., Friese, M. A. 2015. Immunopathology of multiple sclerosis. Nat Rev Immunol., 15(9):545-58.

Díaz-Villamarín, X., Piñar-Morales, R., Barrero-Hernández, F. J., Antúnez-Rodríguez, A., Cabeza-Barrera, J., Morón-Romero, R. 2022. Pharmacogenetics of siponimod: A systematic review. Biomed Pharmacother., 153:113536.

Ding, Y., Yang, Y., Li, Q., Feng, Q., Xu, D., Wu, C., Zhao, J., Zhou, X., Niu, H., He, P., Liu, J., Yao, H. 2020. The correlation between CYP4F2 variants and chronic obstructive pulmonary disease risk in Hainan Han population. Respir Res., 21(1):86.

Dong, A. N., Ahemad, N., Pan, Y., Palanisamy, U. D., Yiap, B. C., Ong, C. E. 2019. Functional and structural characterisation of common cytochrome P450 2D6 allelic variants-roles of Pro34 and Thr107 in catalysis and inhibition. Naunyn Schmiedebergs Arch Pharmacol., 392(8):1015-1029.

Drugbank Online.
https://go.drugbank.com/categories/DBCAT002647
Last visited: 29/05/2023

Edwards, N. C., Hing, Z. A., Perry, A., Blaisdell, A., Kopelman, D. B., Fathke, R., Plum, W., Newell, J., Allen, C. E., Shapiro, A., Okunji, C., Kosti, I., Shomron, N., Grigoryan, V., Przytycka, T. M., Sauna, Z. E., Salari, R., Mandel-Gutfreund, Y., Komar, A. A., Kimchi-Sarfaty, C. 2012. Characterization of coding synonymous and non-synonymous variants in ADAMTS13 using ex vivo and in silico approaches. PLoS One, 7(6):e38864.

Elsaid, A. M., Zahran, R. F., Elmetwaly, S. M., Wahba, Y., Megahed, H., Elshazli, R. M. 2022. The potential impact of CYP2D6 (*2/*4/*10) gene variants among Egyptian epileptic children: A preliminary study. Gene, 832:146585.

El-Sherbeni, A. A., El-Kadi, A. O. 2014. Characterization of arachidonic acid metabolism by rat cytochrome P450 enzymes: the involvement of CYP1As. Drug Metab Dispos., 42(9):1498-507.

Espindola, L. M. T., López, M. J. C., Flores, A. U., Espinosa, L. R., Granados, J., Pacheco, J. L. C., García, M. P., Cervantes, M. T. R., Méndez, V. C. B., Doño, S. H., Ruíz Gómez, D. 2019. Genetic polymorphism of CYP3A4 is associated with poor response to ifosfamide treatment in children with solid embryonic tumors. Arch Med Sci., 17(6):1766-1771.

Faissner, S., Mishra, M., Kaushik, D. K., Wang, J., Fan, Y., Silva, C., Rauw, G., Metz, L., Koch, M., Yong, V. W. 2017. Systematic screening of generic drugs for progressive multiple sclerosis identifies clomipramine as a promising therapeutic. Nat Commun., 8(1):1990.

Fan, F. and Roman, R. J. 2017. Effect of Cytochrome P450 Metabolites of Arachidonic Acid in Nephrology. J Am Soc Nephrol., 28(10):2845-2855.

Farajzadeh-Dehkordi, M., Mafakher, L., Samiee-Rad, F., Rahmani, B. 2023. Computational analysis of missense variant CYP4F2*3 (V433M) in association with human CYP4F2 dysfunction: a functional and structural impact. BMC Mol Cell Biol., 24(1):17.

Fava, C., Montagnana, M., Danese, E., Sjögren, M., Almgren, P., Guidi, G. C., Hedblad, B., Engström, G., Minuz, P., Melander, O. 2012. The functional variant V433M of the CYP4F2 and the metabolic syndrome in Swedes. Prostaglandins Other Lipid Mediat., 98(1-2):31-6.

Fekete, F., Mangó, K., Déri, M., Incze, E., Minus, A., Monostory, K. 2021. Impact of genetic and non-genetic factors on hepatic CYP2C9 expression and activity in Hungarian subjects. Sci Rep., 11(1):17081.

Fernandez, P., Zeigler-Johnson, C. M., Spangler, E., van der Merwe, A., Jalloh, M., Gueye, S. M., Rebbeck, T. R. 2012. Androgen Metabolism Gene Polymorphisms, Associations with Prostate Cancer Risk and Pathological Characteristics: A Comparative Analysis between South African and Senegalese Men. Prostate Cancer, 2012:798634.

Ferreira, H. B., Melo, T., Monteiro, A., Paiva, A., Domingues, P., Domingues, M. R. 20201. Serum phospholipidomics reveals altered lipid profile and promising biomarkers in multiple sclerosis. Arch Biochem Biophys., 697:108672.

Fuselli, S., Dupanloup, I., Frigato, E., Cruciani, F., Scozzari, R., Moral, P., Sistonen, J., Sajantila, A., Barbujani, G. 2004. Molecular diversity at the CYP2D6 locus in the Mediterranean region. Eur J Hum Genet., 12(11):916-24.

General Assembly of the World Medical Association. (2014). World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. The Journal of the American College of Dentists, 81(3), 14-18.

Glaenzel, U., Jin, Y., Nufer, R., Li, W., Schroer, K., Adam-Stitah, S., Peter van Marle, S., Legangneux, E., Borell, H., James, A. D., Meissner, A., Camenisch, G., Gardin, A. 2018. Metabolism and Disposition of Siponimod, a Novel Selective S1P1/S1P5 Agonist, in Healthy Volunteers and In Vitro Identification of Human Cytochrome P450 Enzymes Involved in Its Oxidative Metabolism. Drug Metab Dispos., 46(7):1001-1013.

Gonzalez, F. J. and Gelboin, H. V. 1992. Human cytochromes P450: evolution and cDNA-directed expression. Environ Health Perspect., 98:81-85.

Gunes, A., and Dahl, M. L. 2008. Variation in CYP1A2 activity and its clinical implications: Influence of environmental factors and genetic polymorphisms. Pharmacogenomics, 9(5):625-37.

Hardwick, J. P. 2008. Cytochrome P450 omega hydroxylase (CYP4) function in fatty acid metabolism and metabolic diseases. Biochem Pharmacol., 75(12):2263-75.

Hasan, S. K., Buttari, F., Ottone, T., Voso, M. T., Hohaus, S., Marasco, E., Mantovani, V., Garagnani, P., Sanz, M. A., Cicconi, L., Bernardi, G., Centonze, D., Lo-Coco, 2011. F. Risk of acute promyelocytic leukemia in multiple sclerosis: Coding variants of DNA repair genes. Neurology, 76(12):1059-65.

Hauser, S. L. and Cree, B. A. C. 2020. Treatment of Multiple Sclerosis: A review. Am J Med., 133(12):1380-1390.

Henderson, L. M., Claw, K. G., Woodahl, E. L., Robinson, R. F., Boyer, B. B., Burke, W., Thummel, K. E. 2018. P450 Pharmacogenetics in Indigenous North American populations. J Pers Med., 8(1):9.

Hoxha, M., Spahiu, E., Prendi, E., Zappacosta, B. 2022. A Systematic Review on the Role of Arachidonic Acid Pathway in Multiple Sclerosis. CNS Neurol Disord Drug Targets, 21(2):160-187.

Hoxha, M., Zappacosta, B. 2020. CYP-derived eicosanoids: Implications for rheumatoid arthritis. Prostaglandins Other Lipid Mediat., 146:106405.

Iliff, J. J., Jia, J., Nelson, J., Goyagi, T., Klaus, J., Alkayed, N. J. 2010. Epoxyeicosanoid signaling in CNS function and disease. Prostaglandins Other Lipid Mediat., 91(3-4):68-84.

Ingelman-Sundberg, M., Sim, S. C., Gomez, A., Rodriguez-Antona, C. 2007. Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol. Ther., 116:496–526.

Isin, E. M. and Guengerich, F. P. 2007. Complex reactions catalyzed by cytochrome P450 enzymes. Biochim Biophys Acta., 1770(3):314-29.

Isvoran, A., Louet, M., Vladoiu, D. L., Craciun, D., Loriot, M. A., Villoutreix, B. O., Miteva, M. A. 2017. Pharmacogenomics of the cytochrome P450 2C family: Impacts of amino acid variations on drug metabolism. Drug Discov Today, 22(2):366-376.

Jarrar, Y. B. and Lee, S. J. 2019. Molecular Functionality of Cytochrome P450 4 (CYP4) Genetic Polymorphisms and Their Clinical Implications. Int J Mol Sci., 20(17):4274.

Jeffreys, L. N., Girvan, H. M., McLean, K. J., Munro, A. W. 2018. Characterization of Cytochrome P450 Enzymes and Their Applications in Synthetic Biology. Methods Enzymol., 608:189-261.

Kale, N. 2016. Optic neuritis as an early sign of multiple sclerosis. Eye Brain, 8:195-202.

Kampschulte, N., Alasmer, A., Empl, M. T., Krohn, M., Steinberg, P., Schebb, N. H. 2020. Dietary Polyphenols Inhibit the Cytochrome P450 Monooxygenase Branch of the Arachidonic Acid Cascade with Remarkable Structure-Dependent Selectivity and Potency. J Agric Food Chem., 68(34):9235-9244.

Kane, M. 2021. CYP2D6 Overview: Allele and Phenotype Frequencies. Medical Genetics Summaries.

Karki, R., Pandya, D., Elston, R. C., Ferlini, C. 2015. Defining "mutation" and "polymorphism" in the era of personal genomics. BMC Med Genomics, 8:37.

Kato, I., Cichon, M., Yee, C. L., Land, S., Korczak, J. F. African American-preponderant single nucleotide polymorphisms (SNPs) and risk of breast cancer. 2009. Cancer Epidemiol., 33(1):24-30.

Khvostova, E. P., Pustylnyak, V. O., Gulyaeva, L. F. 2012. Genetic polymorphism of estrogen metabolizing enzymes in Siberian women with breast cancer. Genet Test Mol Biomarkers., 16(3):167-73.

Kim, I. Y., O'Reilly, É. J., Hughes, K. C., Gao, X., Schwarzschild, M. A., McCullough, M. L., Hannan, M. T., Betensky, R. A., Ascherio, A. 2018. Interaction between caffeine and polymorphisms of glutamate ionotropic receptor NMDA type subunit 2A (GRIN2A) and cytochrome P450 1A2 (CYP1A2) on Parkinson's disease risk. Mov Disord., 33(3):414-420.

King, B. P., Khan, T. I., Aithal, G. P., Kamali, F., Daly, A. K. 2004. Upstream and coding region CYP2C9 polymorphisms: correlation with warfarin dose and metabolism. Pharmacogenetics, 14(12):813-22.

Kitsche A, Ritz C, Hothorn LA. A General Framework for the Evaluation of Genetic Association Studies Using Multiple Marginal Models. 2016. Hum Hered., 81(3):150-172.

Kocael, A., Eronat, A. P., Tüzüner, M. B., Ekmekçi, A., Orhan, A. L., İkizceli, İ., Yılmaz-Aydoğan, H., Öztürk, O. 2019. Interpretation of the effect of CYP2C9, VKORC1 and CYP4F2 variants on warfarin dosing adjustment in Turkey. Mol Biol Rep., 46(2):1825-1833.

Koonrungsesomboon, N., Khatsri, R., Wongchompoo, P., Teekachunhatean, S. 2018. The impact of genetic polymorphisms on CYP1A2 activity in humans: a systematic review and meta-analysis. Pharmacogenomics J., 18(6):760-768.

Korbecki, J., Rębacz-Maron, E., Kupnicka, P., Chlubek, D., Baranowska-Bosiacka, I. 2023. Synthesis and Significance of Arachidonic Acid, a Substrate for Cyclooxygenases, Lipoxygenases, and Cytochrome P450 Pathways in the Tumorigenesis of Glioblastoma Multiforme, Including a Pan-Cancer Comparative Analysis. Cancers (Basel), 15(3):946.

Kwon, Y. J., Shin, S., Chun, Y. J. 2021. Biological roles of cytochrome P450 1A1, 1A2, and 1B1 enzymes. Arch Pharm Res., 44(1):63-83.

Landtblom, A. M., Wastenson, M., Ahmadi, A., Söderkvist, P.2003. Multiple sclerosis and exposure to organic solvents, investigated by genetic polymorphisms of the GSTM1 and CYP2D6 enzyme systems. Neurol Sci., 24(4):248-51.

Lee, Y. H., Bae, S. C. 2017. Association between Functional CYP2D6 Polymorphisms and Susceptibility to Autoimmune Diseases: A Meta-Analysis. Immunol Invest., 46(2):109-122.

Levy, S., Sutton, G., Ng, P. C., Feuk, L., Halpern, A. L., Walenz, B. P., Axelrod, N., Huang, J., Kirkness, E. F., Denisov, G., Lin, Y., MacDonald, J. R., Pang, A. W., Shago, M., Stockwell, T. B., Tsiamouri, A., Bafna, V., Bansal, V., Kravitz, S. A., Busam, D. A., Beeson, K. Y., McIntosh, T. C., Remington, K. A., Abril, J. F., Gill, J., Borman, J., Rogers, Y. H., Frazier, M. E., Scherer, S. W., Strausberg, R. L., Venter, J. C. 2007. The diploid genome sequence of an individual human. PLoS Biol., (10):e254.

Liao, D., Yi, X., Zhang, B., Zhou, Q., Lin, J. 2016. Interaction Between CYP4F2 rs2108622 and CPY4A11 rs9333025 Variants Is Significantly Correlated with Susceptibility to Ischemic Stroke and 20-Hydroxyeicosatetraenoic Acid Level. Genet Test Mol Biomarkers, 20(5):223-228.

Lindh, J. D., Holm, L., Andersson, M. L., Rane, A. 2009. Influence of CYP2C9 genotype on warfarin dose requirements--a systematic review and meta-analysis. Eur J Clin Pharmacol., 65(4):365-75.

Liu, J., Chen, H., Wang, S. H., Zhou, Q., Geng, P. W., Zhou, Y. F., Wu, H. L., Shi, H. F., Wang, F., Yang, J. F., Cai, J. P., Dai, D. P. 2021. Functional characterization of the defective CYP2C9 variant CYP2C9*18. Pharmacol Res Perspect., 9(1):e00718.

Liu, T., Zhang, L., Joo, D., Sun, S. C. 2017. NF-κB signaling in inflammation. Signal Transduct Target Ther., 2:17023.

Lopes, B. A., Emerenciano, M., Gonçalves, B. A., Vieira, T. M., Rossini, A., Pombo-de-Oliveira, M. S. 2015. Polymorphisms in CYP1B1, CYP3A5, GSTT1, and SULT1A1 Are Associated with Early Age Acute Leukemia. PLoS One, 10(5):e0127308.

Lu, Y., Peng, Q., Zeng, Z., Wang, J., Deng, Y., Xie, L., Mo, C., Zeng, J., Qin, X., Li, S. 2014. CYP2D6 phenotypes and Parkinson's disease risk: a meta-analysis. J Neurol Sci., 336(1-2):161-8.

Lucas, D., Goulitquer, S., Marienhagen, J., Fer, M., Dreano, Y., Schwaneberg, U., Amet, Y., Corcos, L. 2010. Stereoselective epoxidation of the last double bond of polyunsaturated fatty acids by human cytochromes P450. J Lipid Res., 51(5):1125-33.

Ma, L., Shcherbina, A., Chetty, S. 2020. Variations and expression features of CYP2D6 contribute to schizophrenia risk. Mol Psychiatry., 26(6):2605-2615.

Madeira, M. H., Boia, R., Ambrósio, A. F., Santiago, A. R. 2017. Having a Coffee Break: The Impact of Caffeine Consumption on Microglia-Mediated Inflammation in Neurodegenerative Diseases. Mediators Inflamm., 2017:4761081.

Mahdavi, S., Palatini, P., El-Sohemy, A. 2023. CYP1A2 Genetic Variation, Coffee Intake, and Kidney Dysfunction. JAMA Netw Open., 6(1):e2247868.

Makowska, M., Smolarz, B., Bryś, M., Forma, E., Romanowicz, H. 2021. An association between the rs1799853 and rs1057910 polymorphisms of CYP2C9, the rs4244285 polymorphism of CYP2C19 and the prevalence rates of drug-resistant epilepsy in children. Int J Neurosci., 131(12):1147-1154.

Mañé-Martínez, M. A., Olsson, B., Bau, L., Matas, E., Cobo-Calvo, Á., Andreasson, U., Blennow, K., Romero-Pinel, L., Martínez-Yélamos, S., Zetterberg, H. 2016. Glial and neuronal markers in cerebrospinal fluid in different types of multiple sclerosis. J Neuroimmunol., 15(299):112-117.

Mann, A., Miksys, S. L., Gaedigk, A., Kish, S. J., Mash, D. C., Tyndale, R. F. 2012. The neuroprotective enzyme CYP2D6 increases in the brain with age and is lower in Parkinson's disease patients. Neurobiol Aging., 33(9):2160-71.

McMillan, D. M., Tyndale, R. F. 2018. CYP-mediated drug metabolism in the brain impacts drug response. Pharmacol Ther., 184:189-200.

Meng, C., Wang, J., Ge, W. N., Tang, S. C., Xu, G. M. 2015. Correlation between CYP4F2 gene rs2108622 polymorphism and susceptibility to ischemic stroke. Int J Clin Exp Med., 8(9):16122-6.

Milo, R. and Kahana, E. 2010. Multiple sclerosis: Geoepidemiology, genetics and the environment. Autoimmunity Reviews, Volume 9, Issue 5, Pages A387-A394.

Mitra, R., Guo, Z., Milani, M., Mesaros, C., Rodriguez, M., Nguyen, J., Luo, X., Clarke, D., Lamba, J., Schuetz, E., Donner, D. B., Puli, N., Falck, J. R., Capdevila, J., Gupta, K., Blair, I. A., Potter, D. A. 2011. CYP3A4 mediates growth of estrogen receptor-positive breast cancer cells in part by inducing nuclear translocation of phospho-Stat3 through biosynthesis of (±)-14,15-epoxyeicosatrienoic acid (EET). J Biol Chem., 286(20):17543-59.

Mitsonis, C. I., Zervas, I. M., Potagas, C. M., Mitropoulos, P. A., Dimopoulos, N. P., Sfagos, C. A., Papadimitriou, G. N., Vassilopoulos, D. C. 2010. Effects of escitalopram on stress-related relapses in women with multiple sclerosis: an open-label, randomized, controlled, one-year follow-up study. Eur Neuropsychopharmacol., 20(2):123-31.

Mockute, R., Vilkeviciute, A., Balciuniene, V. J., Zemaitiene, R., Liutkeviciene, R. 2021. ABCA1 rs1883025 and CYP4F2 rs2108622 Gene Polymorphism Association with Age-Related Macular Degeneration and Anti-VEGF Treatment. Medicina (Kaunas), 57(9):974.

Nebert, D. W. and Russell, D. W. 2002. Clinical importance of the cytochromes P450. Lancet, 360(9340):1155-62.

Nebert, D. W., Wikvall, K., Miller, W. L. 2013. Human cytochromes P450 in health and disease. Philos Trans R Soc Lond B Biol Sci., 368(1612): 20120431.

NIH, National Cancer Institute. Identification of Top Food Sources Of Various Dietary Components.
https://epi.grants.cancer.gov/diet/foodsources/top-food-sources-report-02212020.pdf
Last visited: 29/05/2023

Oner Ozgon, G., Langaee, T. Y., Feng, H., Buyru, N., Ulutin, T., Hatemi, A. C., Siva, A., Saip, S., Johnson, J. A. 2008. VKORC1 and CYP2C9 polymorphisms are associated with warfarin dose requirements in Turkish patients. Eur J Clin Pharmacol. 64(9):889-94.

Ongun, M. C., Tonyali, N. V., Kaplan, O., Deger, I., Celebier, M., Basci Akduman, N. E., Sahin, D., Yucel, A., Babaoglu, M. O. 2023. Effects of genetic polymorphisms of CYP2J2, CYP2C9, CYP2C19, CYP4F2, CYP4F3 and CYP4A11 enzymes in preeclampsia and gestational hypertension. Placenta, 137:88-95.

Ongun, M. C., Tonyali, N. V., Kaplan, O., Deger, I., Celebier, M., Basci Akduman, N. E., Sahin, D., Yucel, A., Babaoglu, M. O. 2023. Effects of genetic polymorphisms of CYP2J2, CYP2C9, CYP2C19, CYP4F2, CYP4F3 and CYP4A11 enzymes in preeclampsia and gestational hypertension. Placenta, 137:88-95.

Park, T. J., Lee, H. S., Kim, Y. J., Kim, B. J. 2019. Identification of novel non-synonymous variants associated with type 2 diabetes-related metabolites in Korean population. Biosci Rep., 39(10):BSR20190078.

Pearce, C. L., Near, A. M., Van Den Berg, D. J., Ramus, S. J., Gentry-Maharaj, A., Menon, U., Gayther, S. A., Anderson, A. R., Edlund, C. K., Wu, A. H., Chen, X., Beesley, J., Webb, P. M., Holt, S. K., Chen, C., Doherty, J. A., Rossing, M. A., Whittemore, A. S., McGuire, V., DiCioccio, R. A., Goodman, M. T., Lurie, G., Carney, M. E., Wilkens, L. R., Ness, R. B., Moysich, K. B., Edwards, R., Jennison, E., Kjaer, S. K., Hogdall, E., Hogdall, C. K., Goode, E. L., Sellers, T. A., Vierkant, R. A., Cunningham, J. M., Schildkraut, J. M., Berchuck, A., Moorman, P. G., Iversen, E. S., Cramer, D. W., Terry, K. L., Vitonis, A. F., Titus-Ernstoff, L., Song, H., Pharoah, P. D., Spurdle, A. B., Anton-Culver, H., Ziogas, A., Brewster, W., Galitovskiy, V., Chenevix-Trench, G.; Australian Cancer Study; Australian Ovarian Cancer Study Group. 2009. Validating genetic risk associations for ovarian cancer through the international Ovarian Cancer Association Consortium. Br J Cancer, 100(2):412-20. Erratum in: Br J Cancer. 2009 Nov 17;101(10):1805. Cunningham, J C [corrected to Cunningham, J M].

Pharmacogene Variation Consortium.
https://www.pharmvar.org/gene/CYP1A2
last visited: 19/05/2023

Pharmacogene Variation Consortium.
https://www.pharmvar.org/gene/CYP3A4
last visited: 19/05/2023

Pharmacogene Variation Consortium.
https://www.pharmvar.org/gene/CYP4F2
last visited: 19/05/2023

Polonikov, A., Kharchenko, A., Bykanova, M., Sirotina, S., Ponomarenko, I., Bocharova, A., Vagaytseva, K., Stepanov, V., Bushueva, O., Churnosov, M., Solodilova, M.. 2017. Polymorphisms of CYP2C8, CYP2C9 and CYP2C19 and risk of coronary heart disease in Russian population. Gene, 627:451-459.

Pompura, S. L., Hafler, D. A., Dominguez-Villar, M. 2022. Fatty Acid Metabolism and T Cells in Multiple Sclerosis. Front Immunol., 13:869197.

Pratt, V. M., Cavallari, L. H., Del Tredici, A. L., Hachad, H., Ji, Y., Moyer, A. M., Scott, S. A., Whirl-Carrillo, M., Weck, K. E. 2019. Recommendations for Clinical CYP2C9 Genotyping Allele Selection: A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn., 21(5):746-755.

Qiao, W., Martis, S., Mendiratta, G., Shi, L., Botton, M. R., Yang, Y., Gaedigk, A., Vijzelaar, R., Edelmann, L., Kornreich, R., Desnick, R. J., Scott, S. A. 2019. Integrated CYP2D6 interrogation for multiethnic copy number and tandem allele detection. Pharmacogenomics, 20(1):9-20.

Ramamoorthy, A., Flockhart, D. A., Hosono, N., Kubo, M., Nakamura, Y., Skaar, T. C. 2010. Differential quantification of CYP2D6 gene copy number by four different quantitative real-time PCR assays. Pharmacogenet Genomics, 20(7):451-4.

Rasmussen, B. B., Brix, T. H., Kyvik, K. O., Brøsen, K. 2002. The interindividual differences in the 3-demthylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. Pharmacogenetics, 12(6):473-8.

Ray, B., Ozcagli, E., Sadee, W., Wang, D. 2019. CYP2D6 haplotypes with enhancer single-nucleotide polymorphism rs5758550 and rs16947 (*2 allele): implications for CYP2D6 genotyping panels. Pharmacogenet Genomics, 29(2):39-47.

Sacerdoti, D., Gatta, A., McGiff, J. C. 2003. Role of cytochrome P450-dependent arachidonic acid metabolites in liver physiology and pathophysiology. Prostaglandins Other Lipid Mediat., 72(1-2):51-71.

Sanderson, S., Emery, J., Higgins, J. 2005. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. Genet Med., 7(2):97-104.

Sausville, L. N., Gangadhariah, M. H., Chiusa, M., Mei, S., Wei, S., Zent, R., Luther, J. M., Shuey, M. M., Capdevila, J. H., Falck, J. R., Guengerich, F. P., Williams, S. M., Pozzi, A. 2018. The Cytochrome P450 Slow Metabolizers CYP2C9*2 and CYP2C9*3 Directly Regulate Tumorigenesis via Reduced Epoxyeicosatrienoic Acid Production. Cancer Res., 78(17):4865-4877.

Sausville, L. N., Gangadhariah, M. H., Chiusa, M., Mei, S., Wei, S., Zent, R., Luther, J. M., Shuey, M. M., Capdevila, J. H., Falck, J. R., Guengerich, F. P., Williams, S. M., Pozzi, A. 2018. The Cytochrome P450 Slow Metabolizers CYP2C9*2 and CYP2C9*3 Directly Regulate Tumorigenesis via Reduced Epoxyeicosatrienoic Acid Production. Cancer Res., 78(17):4865-4877.

Savas, S., Schmidt, S., Jarjanazi, H., Ozcelik, H. 2006. Functional nsSNPs from carcinogenesis-related genes expressed in breast tissue: potential breast cancer risk alleles and their distribution across human populations. Hum Genomics, 2(5):287-96.

Sayitoğlu, M. A., Yıldız, İ., Hatırnaz, Ö., Özbek, U. 2006. Common Cytochrome p4503A (CYP3A4 and CYP3A5) and Thiopurine S-Methyl Transferase (TPMT) Polymorphisms In Turkish Population. Turkish Journal of Medical Sciences, 36 (1):11-15.

Scott, S. A., Edelmann, L., Kornreich, R., Desnick, R. J. 2008. Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. Am J Hum Genet., 82(2):495-500.

Shen, Q., Otoki, Y., Sobel, R. A., Nagra, R. M., Taha, A. Y. 2022. Evidence of increased sequestration of pro-resolving lipid mediators within brain esterified lipid pools of multiple sclerosis patients. Mult Scler Relat Disord., 68:104236.

Sheppe, A. E. F., Edelmann, M. J. 2021. Roles of Eicosanoids in Regulating Inflammation and Neutrophil Migration as an Innate Host Response to Bacterial Infections. Infect Immun., 15;89(8):e0009521.

Siniscalchi, A., Gallelli, L., Tolotta, G. A., Loiacono, D., De Sarro, G. 2010. Open, uncontrolled, nonrandomized, 9-month, off-label use of bupropion to treat fatigue in a single patient with multiple sclerosis. Clin Ther., 32(12):2030-4.

Siokas, V., Aloizou, A. M., Tsouris, Z., Liampas, I., Liakos, P., Calina, D., Docea, A. O., Tsatsakis, A., Bogdanos, D. P., Hadjigeorgiou, G. M., Dardiotis, E. 2021. ADORA2A rs5760423 and CYP1A2 rs762551 Polymorphisms as Risk Factors for Parkinson's Disease. J Clin Med., 10(3):381.

Siokas, V., Mouliou, D. S., Liampas, I., Aloizou, A. M., Folia, V., Zoupa, E., Papadimitriou, A., Lavdas, E., Bogdanos, D. P., Dardiotis, E. 2022. Analysis of ADORA2A rs5760423 and CYP1A2 rs762551 Genetic Variants in Patients with Alzheimer's Disease. Int J Mol Sci., 23(22):14400.

Solé, X., Guinó, E., Vall,s J., Iniesta, R., Moreno, V. 2006. SNPStats: a web tool for the analysis of association studies. Bioinformatics, 22(15):1928-9.

Sonnweber, T., Pizzini, A., Nairz, M., Weiss, G., Tancevski, I. 2018. Arachidonic Acid Metabolites in Cardiovascular and Metabolic Diseases. Int J Mol Sci., 19(11):3285.

Spector, A. A., Norris, A. W. 2007. Action of epoxyeicosatrienoic acids on cellular function. Am J Physiol Cell Physiol., 292(3):C996-1012.

Sripichai, O. and Fucharoen, S. 2007. Genetic polymorphisms and implications for human diseases. J Med Assoc Thai., 90(2):394-8.

Stamoula, E., Siafis, S., Dardalas, I., Ainatzoglou, A., Matsas, A., Athanasiadis, T., Sardeli, C., Stamoulas, K., Papazisis, G. 2021. Antidepressants on Multiple Sclerosis: A Review of In Vitro and In Vivo Models. Front Immunol. 12:677879.

Stavropoulou, E., Pircalabioru, G. G., Bezirtzoglou, E. 2018. The Role of Cytochromes P450 in Infection. Front Immunol., 9:89.

Stec, D. E., Roman, R. J., Flasch, A., Rieder, M. J. 2007. Functional polymorphism in human CYP4F2 decreases 20-HETE production. Physiol Genomics, 30(1):74-81.

Steinman, L. 2014. Immunology of relapse and remission in multiple sclerosis. Annu Rev Immunol., 32:257-81.

Surapaneni, S., Yerramilli, U., Bai, A., Dalvie, D., Brooks, J., Wang, X., Selkirk, J. V., Yan, Y. G., Zhang, P., Hargreaves, R., Kumar, G., Palmisano, M., Tran, J. Q. 2021. Absorption, Metabolism, and Excretion, In Vitro Pharmacology, and Clinical Pharmacokinetics of Ozanimod, a Novel Sphingosine 1-Phosphate Receptor Modulator. Drug Metab Dispos., 49(5):405-419.

Suzuki, K., Yanagawa, T., Shibasaki, T., Kaniwa, N., Hasegawa, R., Tohkin, M. 2006. Effect of CYP2C9 genetic polymorphisms on the efficacy and pharmacokinetics of glimepiride in subjects with type 2 diabetes. Diabetes Res Clin Pract., 72(2):148-54.

Takeuchi, F., McGinnis, R., Bourgeois, S., Barnes, C., Eriksson, N., Soranzo, N., Whittaker, P., Ranganath, V., Kumanduri, V., McLaren, W., Holm, L., Lindh, J., Rane, A., Wadelius, M., Deloukas, P. 2009. A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet., 5(3):e1000433.

Tallima, H. and El Ridi, R. 2018. Arachidonic acid: Physiological roles and potential health benefits – A review. Journal of Advanced Research, 11:33-41.

Taskin, B., Percin, F. E., Ergun, M. A. 2016. Investigation of CYP2D6 Gene Polymorphisms in Turkish Population. Psychopharmacol Bull., 46(1):67-72.

Taşçıoğlu, N., Saatçi, Ç., Emekli, R., Tuncel, G., Eşel, E., Dundar, M. 2021. Investigation of cytochrome p450 CYP1A2, CYP2D6, CYP2E1 and CYP3A4 gene expressions and polymorphisms in alcohol withdrawal. Turkish J Clinical Psychiatry, 24:298-306.

Taylor, C., Crosby, I., Yip, V., Maguire, P., Pirmohamed, M., Turner, R. M. 2020. A Review of the Important Role of CYP2D6 in Pharmacogenomics. Genes (Basel)., 11(11):1295.

Thorn CF, Aklillu E, Klein TE, Altman RB. 2012. PharmGKB summary: very important pharmacogene information for CYP1A2. Pharmacogenet Genomics, 22(1):73-7.

Thöne, J., Linker, R. A. 2016. Laquinimod in the treatment of multiple sclerosis: a review of the data so far. Drug Des Devel Ther., 10:1111-8.

Tian, Z., Li, Y. L., Zhao, L., Zhang, C. L. 2013. Role of CYP1A2 1F polymorphism in cancer risk: evidence from a meta-analysis of 46 case-control studies. Gene, 524(2):168-74.

Tillery, E. E., Clements, J. N., Howard, Z. 2018. What's new in multiple sclerosis? Ment Health Clin., 23;7(5):213-220.

van Engen, C. E., Ofman, R., Dijkstra, I. M., van Goethem, T. J., Verheij, E., Varin, J., Vidaud, M., Wanders, R. J., Aubourg, P., Kemp, S., Barbier, M. 2016. CYP4F2 affects phenotypic outcome in adrenoleukodystrophy by modulating the clearance of very long-chain fatty acids. Biochim Biophys Acta, 1862(10):1861-70.

Villapol S. 2018. Roles of Peroxisome Proliferator-Activated Receptor Gamma on Brain and Peripheral Inflammation. Cell Mol Neurobiol., 38(1):121-132.

Walton, C., King, R., Rechtman, L., Kaye, W., Leray, E., Marrie, R. A., Robertson, N., La Rocca, N., Uitdehaag, B., van der Mei, I., Wallin, M., Helme, A., Angood Napier, C., Rijke, N., Baneke, P. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. 2020. Mult Scler., (14):1816-1821.

Wang, B., Wang, J., Huang, S. Q., Su, H. H., Zhou, S. F. 2009. Genetic polymorphism of the human cytochrome P450 2C9 gene and its clinical significance. Curr Drug Metab., 10(7):781-834.

Wang, B., Wu, L., Chen, J., Dong, L., Chen, C., Wen, Z., Hu, J., Fleming, I., Wang, D. W. 2021. Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. Signal Transduct Target Ther., 6(1):94.

Wang, D., Guo, Y., Wrighton, S. A., Cooke, G. E., Sadee, W. 2011. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J., 11(4):274-86.

Wang, D., Poi, M. J,, Sun, X., Gaedigk, A., Leeder, J. S,, Sadee, W. 2014. Common CYP2D6 polymorphisms affecting alternative splicing and transcription: long-range haplotypes with two regulatory variants modulate CYP2D6 activity. Hum Mol Genet., 23(1):268-78.

Wang, H., Zhang, Z., Han, S., Lu, Y., Feng, F., Yuan, J. 2012. CYP1A2 rs762551 polymorphism contributes to cancer susceptibility: a meta-analysis from 19 case-control studies. BMC Cancer, 12:528.

Wang, T., Fu, X., Chen, Q., Patra, J. K., Wang, D., Wang, Z., Gai, Z. 2019. Arachidonic Acid Metabolism and Kidney Inflammation. Int J Mol Sci., 20(15):3683.

Werk, A. N. and Cascorbi, I. 2014. Functional gene variants of CYP3A4. Clin Pharmacol Ther., 96(3):340-8.

Williams, J. M., Murphy, S., Burke, M., Roman, R. J. 2010. 20-hydroxyeicosatetraeonic acid: a new target for the treatment of hypertension. J Cardiovasc Pharmacol., 56(4):336-44.

Xie, H. G., Prasad, H. C., Kim, R. B., Stein, C. M. 2002. CYP2C9 allelic variants: ethnic distribution and functional significance. Adv Drug Deliv Rev., 54(10):1257-70.

Yu, C., Yan, Q., Fu, C., Shi, W., Wang, H., Zeng, C., Wang, X. 2014. CYP4F2 genetic polymorphisms are associated with coronary heart disease in a Chinese population. Lipids Health Dis., 13:83.

Zanger, U. M. and Schwab M. 2013. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther., 138(1):103-41.

Zeigler-Johnson, C., Friebel, T., Walker, A. H., Wang, Y., Spangler, E., Panossian, S., Patacsil, M., Aplenc, R., Wein, A. J., Malkowicz, S. B., Rebbeck, T. R. 2004. CYP3A4, CYP3A5, and CYP3A43 genotypes and haplotypes in the etiology and severity of prostate cancer. Cancer Res., 64(22):8461-7.

Zhao, M., Ma, J., Li, M., Zhang, Y., Jiang, B., Zhao, X., Huai, C., Shen, L., Zhang, N., He, L., Qin, S. 2021. Cytochrome P450 Enzymes and Drug Metabolism in Humans. Int J Mol Sci., 22(23):12808.

Zhou, L. P., Luan, H., Dong, X. H., Jin, G. J., Man, D. L., Shang, H. 2012. Genetic variants of CYP2D6 gene and cancer risk: a HuGE systematic review and meta-analysis. Asian Pac J Cancer Prev., 13(7):3165-72.

Zhou, L. P., Yao, F., Luan, H., Wang, Y. L., Dong, X. H., Zhou, W. W., Wang, Q. H. 2013. CYP3A4*1B polymorphism and cancer risk: a HuGE review and meta-analysis. Tumour Biol., 34(2):649-60.

Zhou, Q., Yu, X., Shu, C., Cai, Y., Gong, W., Wang, X., Wang, D. M., Hu, S. 2011. Analysis of CYP3A4 genetic polymorphisms in Han Chinese. J Hum Genet., 56(6):415-22.

Zhou, S. F., Wang, B., Yang, L. P., Liu, J. P. 2010. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. Drug Metab Rev., 42(2):268-354.

Zhou, X. Y., Hu, X. X., Wang, C. C., Lu, X. R., Chen, Z., Liu, Q., Hu, G. X., Cai, J. P. 2019. Enzymatic Activities of CYP3A4 Allelic Variants on Quinine 3-Hydroxylation In Vitro. Front Pharmacol., 10:591.

Zhou, Y., Nevosadová, L., Eliasson, E., Lauschke, V. M. 2023. Global distribution of functionally important CYP2C9 alleles and their inferred metabolic consequences. Hum Genomics, 17(1):15.

Zorlu, N., Hoffjan, S., Haghikia, A., Deyneko, I. V., Epplen, J. T. 2019. Evaluation of variation in genes of the arylhydrocarbon receptor pathway for an association with multiple sclerosis. J Neuroimmunol., 334:576979.

APPENDICES

A. ETHICAL COMMITTEE APPROVAL

T.C.
SAĞLIK BİLİMLERİ ÜNİVERSİTESİ
Gülhane Bilimsel Araştırmalar Etik Kurulu

Sayı : 46418926
Konu : Gülhane Bilimsel Araştırmalar Etik Kurul Kararları

14.02.2023

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

TOPLANTI TARİHİ : 14.02.2023
TOPLANTI SAATİ : 13:30 (Toplantı Online Yapılmıştır.)
TOPLANTI NO : 2023/02
PROJE/ KARAR NO : 2023-71(Değerlendirilme Tarihi: 14.02.2023)

Üniversitemiz Gülhane Sağlık Bilimleri Enstitüsü'nde, görevli Prof. Dr. Şeref DEMİRKAYA'nın sorumlu araştırmacı olduğu, Prof. Dr. Orhan ADALI ve Burak BARUT'un yardımcı araştırmacı oldukları, 2023/71 kayıt numaralı, **"Multiple Sklerozlu Türk hastalarda İnflamasyonla İlişkili CYP1A2, 2C9, 2D6, 3A4 ve 4F2 Polimorfizmlerin Risk Değerlendirmesi"** başlıklı doktora tezi proje önerisi, araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş olup, etik açıdan uygun bulunmuştur. Rica ederim.

Sıra No	Unvanı ve Adı/Soyadı	Kuruldaki Görevi	İmza
1	Prof. Dr. Ahmet COŞAR	Başkan	
2	Prof. Dr. Alper GÖZÜBÜYÜK	Başkan Yardımcısı	
3	Prof. Dr. Selahattin BEDİR	Üye	
4	Prof. Dr. Levent KENAR	Üye	
5	Prof. Dr. Yusuf İZCİ	Üye	
6	Prof. Dr. Ali Kağan COŞKUN	Üye	
7	Prof. Dr. Cantürk TAŞÇI	Üye	
8	Prof. Dr. Necmiye Ün YILDIRIM	Üye	
9	Prof. Dr. Fulya TOKSOY	Üye	
10	Prof. Dr. Ayten TÜRKKANI	Sekreter	
11	Prof. Dr. Gülten GÜVENÇ	Üye	
12	Prof. Dr. Dilek YILDIZ	Üye	
13	Prof. Dr. Türkan YILDIRIM	Üye	
14	Prof. Dr. Eda PURUTÇUOĞLU	Üye	
15	Doç. Dr. Mehmet Erhan YUMUŞAK	Üye	
16	Doç. Dr. Umut BEYLİK	Üye	
17	Doç. Dr. Serkan PEKÇETİN	Üye	
18	Dr. Öğr. Üyesi Mustafa GÜNEY	Üye	
19	Dr. Öğr. Üyesi Eray Serdar YURDAKUL	Üye	

Sağlık Bilimleri Üniversitesi Gülhane Bilimsel Araştırmalar Etik Kurulu

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Barut, Burak

EDUCATION

Degree	Institution	Year of Graduation
MS	METU Biochemistry	2011
BS	METU Molecular Biology and Genetics	2007
High School	Antalya Anatolian High School, Antalya	2003

WORK EXPERIENCE

Year	Place	Enrollment
2009-Present	TÜBİTAK	Scientific Programmes Chief Expert
2006-2006	New York University	Internship

FOREIGN LANGUAGES

Advanced English