Joint Biotechnology Master Program

Palestine Polytechnic University
Deanship of Higher Studies and Scientific Research

Bethlehem University
Faculty of Science

Allele frequencies of CYP2C9, CYP2C19, and CYP2D6 in the Palestinian population

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In Partial Fulfillment of the Requirements for the Degree Master of Science

May 2022

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ABSTRACT:

The polymorphism of CYP2C9, CYP2C19 and CYP2D6 differs among different individuals and shows ethnic variability. This will affect the metabolism of several drugs and may cause differences in the clinical response. In this study, we analyzed the genotype profile of a random sample of the Palestinian population to compare the allele frequencies of CYP2C9, CYP2C19 and CYP2D6. Data have been taken from 67 unrelated Palestinian volunteers (35 males and 32 females, aged from 16 to 60 years). Next generation sequencing (NGS) was conducted to sequence the most frequent variants of the CYP2C9, CYP2C19, and CYP2D6. To compare the frequency of different variants with the global data, we used two libraries of python, i.e. Matplotlib, and Pandas. The frequency of CYP2C9 and CYP2D6 between the Palestinian population and the global populations shows a high correlation (0.95, and 0.96 respectively). However, we found a low correlation for CYP2C19 (0.34). Only rs9332239 and rs28371685 in CYP2C9 variants' frequencies showed a significant difference between the Palestinian population and the global population, (p-value=0.00311776) for the reference variant and (p-value=0.003118) for the alternative variant, respectively. For CYP2C19 variants: rs28399504 and rs41291556 showed a significant difference between the Palestinian population and the global population (p-value=0.014664745) for the reference variant and (p-value=0.014664745) for the alternative variant. There were no statistically significant differences for all the other variants examined for CYP2C9 (P-value = 0.847232), CYP2C19 (P-value= 0.479754), and CYP2D6 (P-value= 0.923857). Up to our knowledge this is the first study done to compare the Palestinian pharmacogenetics profile in those specific genes. Further studies on a larger population sample are necessary to confirm our findings.

الترددات الاليلية ل CYP2C9 و CYP2C19 و CYP2D6 في السكان الفلسطينيين

لميس وليد وزوز

ملخص:

يختلف تعدد البلورية في CYP2C9 و CYP2C19 و CYP2D6 من شخص الى اخر و يبين التباين العرقي. و يؤثر هذا الاختلاف على استقلاب عدد من الأدوية و قد يسبب اختلافات في الاستجابة الاكلينيكية. في هذه الدراسة قمنا بتحليل النمط الجيني لعينة عشوائية من الشعب الفلسطيني و مقارنة ترددات الاليل ل CYP2C9 و CYP2C19 و CYP2D6. تم اخذ العينات من 67 متطوع/ة فلسطيني غير مترابطون (35 ذكر و 32 انثى). تم اجراء تسلسل الجيل القادم لسلسلة للمتغيرات الأكثر شيوعا في CYP2C9 و CYP2C19 و CYP2D6. قمنا باستخدام مكتبتان من لغة البرمجة بايثون (Matplotlib و Pandas) لمقارنة ترددات المتغيرات بين الشعب الفلسطيني و باقي العالم. اظهرت النتائج وجود ارتباط كبير بين الشعب الفلسطيني و العالم في ترددات CYP2C9 و CYP2D6 (0.95 و 0.96 على التوالي). و مع ذلك وجدنا ارتباط ضعيف بين الشعب الفلسطيني و العالم في تردد CYP2C19 حيث كان المعامل 0.34. فقط المتغيرات rs9332239 وrs28371685 في CYP2C9 اظهرت اختلافات مهمة بين الشعب الفلسطيني و العالم حيث كانت القيمة الاحتمالية للمتغير المرجعي 0.00311776 و القيمة الاحتمالية للمتغير البديل 0.003118. أما بالنسبة ل CYP2C19 فقط المتغيرات rs28399504 CYP2C9 and rs41291556 اظهرت اختلافات مهمة بين الشعب الفلسطيني و العالم حيث كانت القيمة الاحتمالية للمتغير المرجعي 0.00.14664745 و القيمة الاحتمالية للمتغير البديل 0.004664745. لم يكن هناك اي اختلافات هامة بين الشعب الفلسطيني و العالم في باقي المتغيرات ل CYP2C9 (القيمة الاحتمالية= 0.847232) و CYP2C19 (القيمة الاحتمالية= 0.479754) و CYP2D6 (القيمة الاحتمالية = 0.923857). على حسب معرفتنا فان هذه هي الدراسة الاولى التي قارنت ملف علم الوراثة الدوائي الفلسطيني لهذه الجينات المحددة. من الضروري اجراء المزيد من الدراسات المشابهة على عينة اكبر لتأكيد نتائجنا.

DECLARATION

I declare that the Master Thesis entitled "dissertation title" is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

Name and signature: "Lamees Waleed Wazwaz"

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Dedication

I dedicate this thesis to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared. I have a special feeling of gratitude to my loving parents, Waleed and Maysoon who has encouraged me attentively with their fullest and truest attention to accomplish my work with truthful self-confidence.

Thanks to my homeland Palestine, the warmest womb

Thanks to my academic adviser Dr.Areej Khatib who guided me in this process and the committee Dr.Omar Dar-Issa and Prof.Yousef Najajreh who kept me on track.

This dissertation is dedicated to Mr.Ibrahim Elias who encouraged me to pursue my dreams and finish my dissertation.

Acknowledgment

First and foremost, I have to thank my research supervisors Dr.Areej Khatib for her continued guidance and an endless supply of fascinating projects. His unassuming approach to research and science is a source of inspiration. This approach is reflected by his simple but clear writing style, which is something I hope to carry forward throughout my career. I would like to thank you very much for your support and understanding over these past months. Dr.Areej Khatib was my second-year pathology professor at Bethlehem University. Her teaching style and enthusiasm for the topic made a strong impression on me and I have always carried positive memories of her classes with me. She raised many precious points in our discussion and I hope that I have managed to address several of them here.

I would also like to show gratitude to my committee, including Dr.Omar Dar-Issa and Prof.Yousef Najajreh for their comments on the manuscript. I was delighted when Dr.Omar Dar-Issa and Prof.Yousef Najajreh agreed to be on my thesis committee.

I would like to thank the faculty at Bethlehem University and Palestine Polytechnic University for their support during the study period.

I would like to thank Mr.Ghaith Khanfer for his invaluable assistance. Much of the analysis presented in the results section is owed to Mr.Ghaith who kindly assisted me with the statistical analysis in this dissertation and was very patient with my knowledge gaps.

Abbreviations

CYP450	cytochrome P450
CYP2C9	Cytochrome P450 2C9
CYP2C19	Cytochrome P450 2C19
CYP2D6	Cytochrome P450 2D6
Ref allele	Reference allele
Alt allele	Alternative allele
NGS	Next generation sequencing
RM	rapid metabolizer
IM	intermediate metabolizer
PM	poor metabolizer
DDI	drug-drug interaction
DDGI	drug–drug–gene interaction
CPIC	Clinical Pharmacogenetics Implementation Consortium
DPWG	Dutch Pharmacogenetics Working Group
CPNDS	Canadian Pharmacogenomics Network for Drug Safety
PharmGKB	Pharmacogenomics Knowledgebase
AA	arachidonic acid
DHA	docosahexaenoic acid

EPA	eicosapentaenoic acid
PXR	pregnane X receptor
CAR	constitutive androstane receptor
PXR	pregnane X receptor
UGTs	UDP-glucuronosyltransferases
VDR	vitamin D receptors
ADRs	adverse drug reactions
NSAIDs	non-steroidal anti-inflammatory drugs
COAG	Optimal Anticoagulation through Genetics
FDA	Food and Drug Administration
(S)-MP	S-mephenytoin
HBD	heme-binding domain
PPI	proton pump inhibitors
OP	omeprazole
AUC	area under the curve
HGVS	Human Genome Variation Society
GMAF	Global minor allele frequency
SNP	single nucleotide polymorphism
NGS	Next generation sequencing

MPS	Massively parallel sequencing
cDNA	complementary DNA
GU	gastric ulcers
SNV	single nucleotide polymorphism
WT	Wild type
QC	quality control
CLIA	Clinical Laboratory Improvement Amendments
CAP	College of American Pathologists
dbGaP	database of Genotypes and Phenotypes
ALFA	Allele Frequency Aggregator
HWE	Hardy-Weinberg equilibrium
OCs	Oral contraceptives

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1 Introduction:

Pharmacogenetics is an emerging area of clinical medicine focused on understanding how genes might affect the individual responses to treatment (Spear, 2001). When drugs enter our body, they are metabolized and structurally modified by enzymes that either activate or deactivate these medications (Ingelman-Sundberg, 2004). The body cannot clear the original forms of drugs, so it must convert the drug into a more familiar form that can be execrated (Totah, 2005). The body's conversion process of the drug is a two-phase process; Phase one metabolism is characterized as a functionalization reaction, where they add or reveal a functional group by oxidation, reduction, or hydrolysis. Hence leading to an increase in overall polarity of the drug which facilitates its excretion in the urine (Ingelman-Sundberg, 2004). Phase two reactions are commonly called conjugation reactions (Daly, 2018). Owing to the fact that they add a functional group on the drug for the purpose of increase in its polarity (Ingelman-Sundberg, 2000). The conjugation process requires an enzyme generally termed as transferees, that transfers the large polar molecule called a cofactor on to the drug (Spear, 2001).

The first step in pharmacokinetics is absorption (Bahar, 2017). Once the drug gets absorbed either through skin or through stomach, it gets into your bloodstream, and then it gets distributed into the fluids outside and inside the cells (Bahar, 2017). So once the drug gets distributed all over the body, the body starts metabolizing it, basically modifying the drug so that it's easy to excrete (Bahar, 2017). This is done primarily by a liver but it can also be done by other tissues, then it gets bio transformed, and finally it gets eliminated (Bahar, 2017). Elimination is the last step in which drug and its metabolites get excreted primarily in bile urine and feces (Bahar, 2017).

Metabolism is the protective biochemical process by which our bodies alter xenobiotic either enzymatically or none enzymatically (Malki, 2020). Generally drug metabolism begins with a hydrophobic drug, and converts it to a more hydrophilic metabolite, to facilitate its elimination (Bahar, 2017). An understanding of the drug metabolism process and the potential outcomes is critical for developing safe and useful pharmaceuticals (Malki, 2020). Drug metabolism can result in one of two products: an active metabolite or an inactive metabolite (Malki, 2020). In active metabolites of the drugs basically have no pharmacological activity of the original drug

(Malki, 2020). On the other hand an active metabolite can mean that the metabolite can retain the same activity as the parent drug (Malki, 2020). That's a parent when codeine is demethylated to a more active drug which is morphine (Malki, 2020).

The most well-known family of drug-metabolizing enzymes is the cytochrome P450 (CYP450) enzymes. This large family of enzymes is essential for the metabolism of drugs, these enzymes catalyze vast majority of phase 1 reactions (Bahar, 2017). Many drug interactions arise from drug's ability to induce or inhibit these enzymes (Storelli, 2018). Some of the important inducers include Phenytoin, Carbamazepine, Rifampin, alcohol (with chronic use), barbiturates and St. John's Wort (Storelli, 2018). On the other hand some of the important inhibitors are grapefruit, protease inhibitors, azole antifungals, Cimetidine macrolides (with exception of Azithromycin) Amiodarone, nondihydropyridine calcium channel blockers such as Diltiazem and Verapamil (Storelli, 2018). The major role of the CYP450 is to transform drugs into aqueous molecules so that they can be excreted out properly (Ingelman-Sundberg, 2004; Meyer, 2013). The CYP450 gene encodes 57 genes in the human genome (Ingelman-Sundberg, 2005). CYP2 is the most diverse family, and is responsible for phase one metabolism of a large number of foreign compounds (Bahar, 2017). The main enzymes that affect drug metabolism are those related to drug oxidation by CYP2C9, CYP2C19, and CYP2D6 (Malki, 2020). The aforementioned enzymes have the most significant clinical consequences as they are involved in metabolizing over 35% of medications as shown in figure 1 (Meyer, 2013).

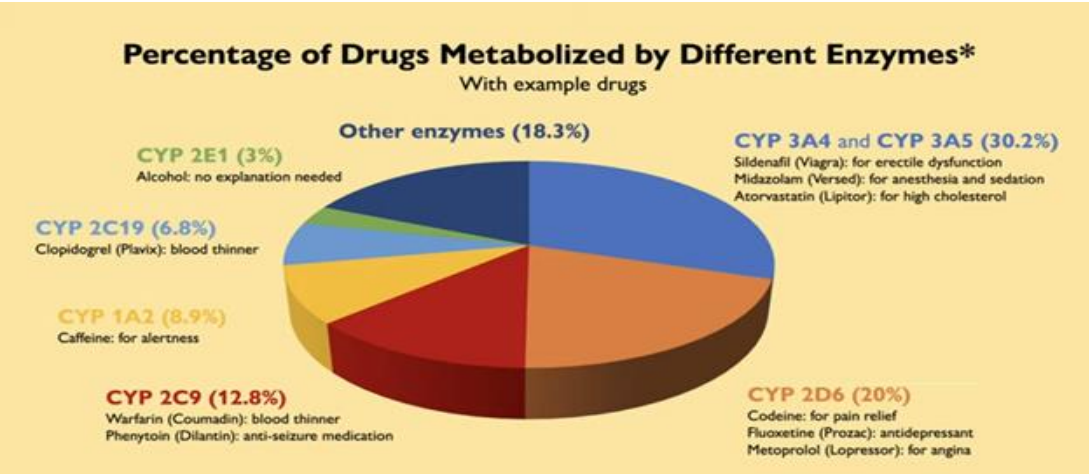


Figure 1: percentage of drugs metabolized by different enzymes (Meyer, 2013)

The CYP450 gene sequence varies among people. The genetic variants result in slight structural changes in CYP450 which alter how effective the enzyme is at metabolizing drugs (Ingelman-Sundberg, 2004). Broadly, the variants can be grouped as a normal function, decreased function and non-functional. Accordingly, individuals can be classified as rapid, intermediate or poor metabolizers. A rapid metabolizer (RM) should have at least one functional allele (Zhou, 2017). However, an intermediate metabolizer (IM) should have a decreased function and non-functional allele (Zhou, 2017). And poor metabolizers (PM) should have two non-functional alleles (Zhou, 2017). Impaired metabolism can alter drug's effectiveness and toxicity (Ingelman-Sundberg, 2005). In rare cases, a person can be classified as an ultra-rapid metabolizer (Koren, 2006). These people have three normal alleles of CYP2D6 rather than two due to gene duplication, and so they metabolize certain drugs extremely rapidly and efficiently because they have more enzymes (Koren, 2006). If all metabolizer types were given the same codeine dose, an ultra-rapid metabolizer would convert way too much codeine into morphine, and theoretically should be receiving a lower dose to prevent overdose (Koren, 2006).

When a drug is rapidly metabolized, its efficacy is decreased (Zanger, 2013). On the other hand, when a drug slowly metabolize, it may result in toxicity (Zanger, 2013). Hence the drug dosage must be considered depending on the speed at which it is metabolized by CYP450 (Spear, 2001). Other drugs may function as inhibitors or inducers of CYP450 enzymes activity of CYP450 enzymes expression that will lead to decreased or increased CYP450 activity respectively (Storelli, 2018). If an inhibitor and an inducer drug are taken at the same time, as a drug is metabolized by CYP450 enzyme, the first drug may affect the metabolism rate of the second through what is known as a drug-drug interaction (DDI) (Zanger, 2013).

Most human CYP450 variants are biallelic which means that two allelic variants are segregated in the population (Arici, 2017). On the other hand, there are a tri-allelic variant, which means that there are three different alleles at the same locus (Zhao, 2003). Natural selection on the variations' region is a clear candidate (Hodgkinson, 2010). It has been discovered that there are too many locations in the human genome with three alleles that segregate in the population (Hodgkinson, 2010). Natural selection or a higher mutation rate at specific locations cannot

account for the excess (Zhao, 2003). Rather, there are signs that a part of triallelic sites are result of a single mutation mechanism in which two new alleles are generated at an even comparable times, with the same or related genetic background; the minor alleles at a triallelic variants are fairly close together on the evolutionary tree than one might expect by chance (Hodgkinson, 2010). There is no relationship between recombination rates and genes with triallelic variants, indicating that the clustering is unlikely to be driven by a mutational process associated to recombination (Hodgkinson, 2010).

Ethnicity is a factor that influences drug metabolic capability and pharmacokinetics in different populations (Shah, 2005). Even though CYP450 genotypes and metabolic phenotypes have been extensively investigated throughout the world, there are still populations that are understudied; i.e. Palestinians. Studies on population genetic variation of relevant molecular biomarkers among understudied ethnic groups such as Palestinians are both needed as well as essential for personalized medicine and rational therapies that guarantee the health and well-being people.

Drug metabolizing enzymes like CYP2D6, CYP2C9, and CYP2C19 are also crucial for personalized medicine because they confer significant interpersonal differences in the clinical effects of commonly used drugs (Pratt, 2019).

1.1 Drug metabolism role in drug pharmacokinetics

Drug metabolism is most commonly seen in the liver; where large number of CYP450 is expressed (Zhou, 2009). Drug metabolism can also occur in the kidney, intestine, lung, adrenals, blood, stomach, and skin (Ingelman-Sundberg, 2004). The fact that most medications are processed by the liver is critical for pharmacokinetics, because pharmaceuticals administered orally may be metabolized at the liver prior reaching the systemic circulation (Zhou, 2009). A phenomenon referred to as “first pass effect” (Ingelman-Sundberg, 2007). First pass effect can be dramatic that delivering a medicine intravenously is necessary since providing it orally would result in much less active drug in the systemic circulation (Ingelman-Sundberg, 2007). Morphine is one example of this; therefore it is frequently administered intravenously rather than orally. The liver has a large number of CYP450 (Zhou, 2009).

Conjugated glucuronic acid to drug metabolite is done via the action of UDP-glucuronosyltransferase, which catalyzes the attachment of a glucuronic acid moiety to various drugs and other xenobiotics, as well as endogenous compounds as shown in figure 2 (Allain, 2020) (Ritter, 2000). This is the same process that occurs in bilirubin metabolism, which is a catabolic product of heme metabolism (Ingelman-Sundberg, 2007). In any case, the glucuronic acid contains many polar groups linked to it, including three hydroxyl groups and a carboxylic acid group, which makes the molecule hydrophilic (Ritter, 2000). As a result, medications are solubilized and easily eliminated by the kidneys (Ritter, 2000). The initial stage of the process is known as phase 1 metabolism, while the second stage is known as phase 2 metabolism (Ritter, 2000).

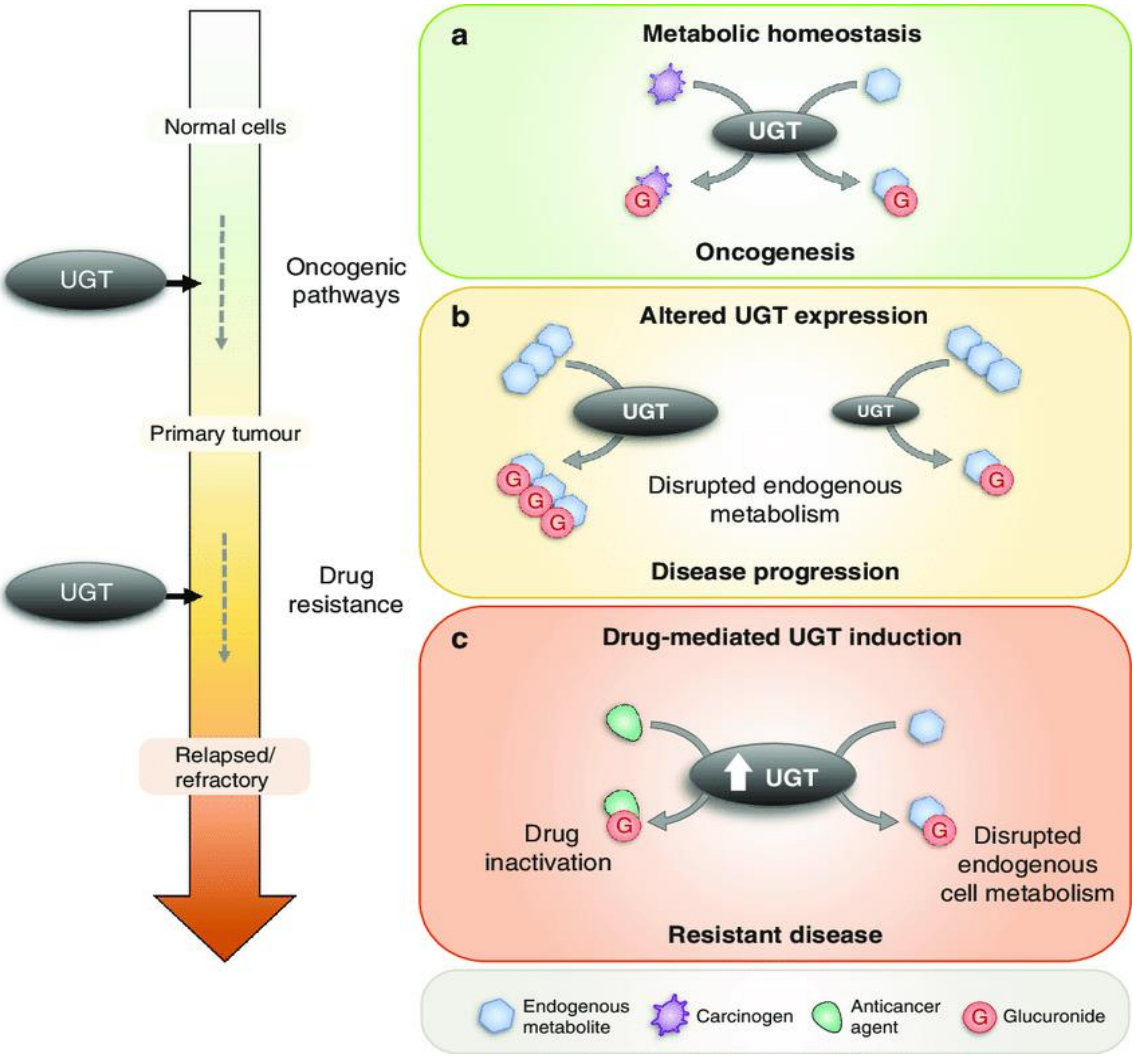


Figure 2: UGTs as mediators of oncogenic pathways and drug response. a UDP-glucuronosyltransferases (UGTs) regulate metabolic homoeostasis through the inactivation of endogenous metabolites (such as steroid hormones) and xenobiotics (such as carcinogens) by their conjugation with glucuronic acid (GlcA, G). b The modulation of UGT expression and activity observed in normal and cancer cells influences the bioactivity of metabolites, including some with oncogenic potential, thereby promoting tumour development and progression. Endogenous metabolites such as steroid hormones induce or repress the expression of specific UGTs. c Therapeutic drugs generally induce UGT expression, promote drug inactivation and resistance to treatment and further perturb endogenous metabolites (Allabi, 2004).

Another example is aspirin (or, more correctly, acetylsalicylic acid), whose phase 1 metabolism includes a CYP450 hydrolyzing the aspirin molecule and producing salicylic acid as a byproduct (Testa, 2003). Aspirin must be digested before it has any action, which is why it is referred to as a prodrug (Ritter, 2000). UDP-glucuronosyltransferase can then operate on salicylic acid to attach the glucuronic acid group in place of the hydroxyl group. The product may now be readily eliminated by the kidneys (Testa, 2003).

1.2 Drug-drug-gene interaction:

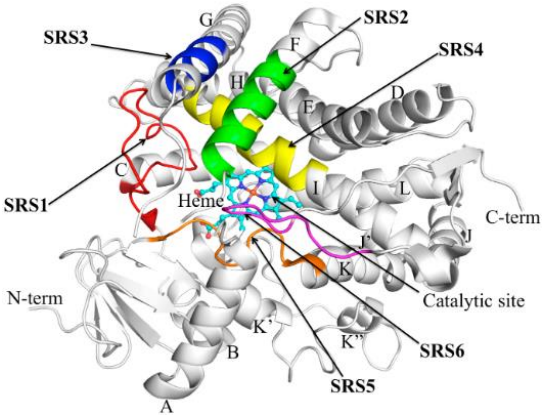
Drug-drug interaction occurs due to the effect of the patient's genetic profile of CYP450 type affecting the ability to eliminate a drug (Verbeurgt, 2014). A drug–drug–gene interaction (DDGI) occurs when the genotype of CYP450 and another drug in the individual's treatment (e.g., a CYP450 inhibitor) affect that individual's ability to eliminate a drug (Verbeurgt, 2014). More importantly, since the CYP450 genes have many variants and the function of CYP450 enzymes is important to the impact of DDIs, variations can affect their magnitude (Bahar, 2017) (Storelli, 2018). Drug-gene interaction has been combined in some guidelines, but these guidelines do not take into account the difference in CYP450 genotypes after taking these drugs (Verbeurgt, 2014). Therefore, this interaction is rarely taken into consideration in clinical practice and tests, and the individual's genetic profile of CYP450 enzymes' effects on DDIs remains lacking (Verbeurgt, 2014) (Bahar, 2017).

The genotype of patients has a very important impact on DDIs related to metabolizing pathways involving CYP2C9, CYP2C19 and CYP2D6 (Bahar, 2017). When the gene goes a long way to the drug metabolism, the disruption of the drug produced becomes stronger (Bahar, 2017). Generally, the disruption of DDIs is largest in NMs, less in IMs and least in PMs (Storelli, 2018). PMs do not get influenced by an effector drug, as they have no metabolic activities (Zanger, 2013). Genotype also plays an important role in DDGIs because it affects substrates metabolized by multiple pathways. This increases the importance of the secondary pathway (Verbeurgt, 2014).

Pharmacogenetics testing is essential to prevent the condition of DDI and DDGI. Pharmacogenetics testing should be conducted to detect genetic variations to define DDI and DDGI (Storelli, 2018). This pharmacogenetics test detects the variations in three major drug-metabolizing enzymes; CYP2C9, CYP2C19, and CYP2D6, as they have a considerable impact on DDIs and DDGIs (Bahar, 2017).

1.3 Cytochrome P450 2C (CYP2C) subfamily: CYP2C9

The most prevalent human CYP2C isoform in the liver is Cytochrome P450 2C9 (CYP2C9) (Daly, 2018). There is more than one allele that occupies the CYP2C9 gene's locus within a population, and its expression impacts tolbutamide metabolism but is unrelated to CYP2D6 (Veronese, 1991). CYP2C9 was discovered to be the enzyme responsible for tolbutamide hydroxylation in humans by isolating one or a few proteins from a complex mixture and using complementary DNA (cDNA) cloning methods (Meehan, 1988). This enzyme was also shown to have a function in phenytoin hydroxylation (Veronese, 1991). The CYP2C9 gene product was originally considered to be responsible for mephenytoin metabolism, however this was disproved in 1991 with the discovery of CYP2C19 (Romkes, 1991). CYP2C9 was shown to be the primary enzyme involved in the hydroxylation of S-warfarin and diclofenac in subsequent investigations (Rettie, 1992). Following that, numerous CYP2C9 substrates were discovered, encompassing a wide range of pharmacological classes (Sangkuhl, 2021).



When comparing cDNA sequences, it was discovered that there were two common variants (c.430C>T, also known as CYP2C9*2 and c.1075A>C, also known as CYP2C9*3) (Stubbins, 1996). Early research on these variations found that the presence of CYP2C9*2 and *3 alleles were associated with reduced metabolism of tolbutamide and S-warfarin in vivo and in vitro (Veronese, 1991). By sequencing, more variations were discovered, including the low function CYP2C9*5 allele and the nonfunctional CYP2C9*6 allele, as well as many others by sequencing (Blaisdell, 2004) .

Figure 3: CYP2C9 structure. The figure shows the structure of CYP2C9 (Anon., 2022)

Guidelines for several CYP2C9 gene–drug combinations, including warfarin, phenytoin, and nonsteroidal anti-inflammatory medications (NSAIDs), have been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC). The Royal Dutch Pharmacogenetics Working Group (DPWG) and the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) have also produced recommendations; information on these guidelines may be found on the Pharmacogenomics Knowledgebase (PharmGKB) (Sangkuhl, 2021).

1.3.1 CYP2C9 allele function

CYP2C9 is a critical enzyme that is important in oxidative metabolism of both xenobiotics (foreign substances), and endogenous compounds that are already present in the body (Blaisdell, 2004). CYP2C9 accounts for approximately 18% of the CYP450 proteins, found mostly in the liver, duodenum, and small intestine (Daly, 2018). CYP2C9 metabolizes around 100 medications, including some with a limited therapeutic index and others that are commonly prescribed (Daly, 2018). On the other hand, none liver CYP450s often metabolize key endogenous chemicals because of their epoxygenase activity (Spector, 2015). It also metabolizes different polyunsaturated fatty acids, by converting them into a diverse spectrum of biologically active metabolites (Spector, 2015).

CYP2C9 metabolizes polyunsaturated fatty acids such as arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) to its epoxy isomerism form' which are epoxyeicosatrienoic acids, Epoxydocosapentaenoic acid and epoxyeicosatetraenoic acids respectively. Human epoxides are different than animal ones. Thus, studying those products is challenging. These products have been implicated in many pathways in human pathology such as protecting against heart attacks and some cancers, and have a range of effects on brain tissues (Spector, 2015).

Other fatty acids such as linoleic acid and coronary acid are metabolized by CYP2C9 to leukotoxin and isoleukotoxin respectively. Those products are toxic in animal models, as they may lead to multiple organ dysfunction syndrome and they may lead to similar disorders in humans (Spector, 2015).

1.3.2 Additional factors influencing CYP2C9 activity:

A wide spectrum of medications can both inhibit and induce CYP2C9 (Sangkuhl, 2021). CYP2C9 inhibitors include sulfamethoxazole, fluconazole, voriconazole, metronidazole, and amiodarone (Flockhart, 2021). Because amiodarone is frequently administered alongside warfarin, it poses a danger to anticoagulated individuals (Sangkuhl, 2021). Amiodarone and warfarin have a well-documented drug–drug interaction that results in a 6–65% reduction in warfarin dosage requirements (O'Reilly, 1987). When an inhibitor like one of these is added to a treatment regimen that contains medicines with a low therapeutic index like S-warfarin, tolbutamine, and phenytoin, a dangerous drug-drug interaction can occur (Bahar, 2017). Numerous studies, for example, have shown that warfarin's anticoagulant action is potentiated in individuals who are also taking amiodarone (Lu, 2008).

Rifampicin, clotrimazole, nifedipine, hyperforin (an active component of the herbal medicine St John's wort), phenobarbital, phenytoin, carbamazepine, dicloxacillin, flucloxacillin, and tamoxifen, are reported inducers of CYP2C9 (Flockhart, 2021). Rifampicin treatment has repeatedly been demonstrated to enhance the clearance of medicines transformed by CYP2C9 (Miners, 1998). In healthy volunteers or patients treated with rifampicin, the clearance of losartan, phenytoin, tolbutamide, and S-warfarin is about quadrupled (Vormfelde, 2009). Although numerous pathways are reported to be implicated in CYP2C9 activation, nuclear receptors such as the pregnane X receptor (PXR), constitutive androstane receptor (CAR), and glucocorticoid, estrogen, and vitamin D receptors (VDR) have the max eminent (Daly, 2018). Finally, cytochrome b5 reductase (are ubiquitous electron transport hemoproteins) has been demonstrated to affect the catalytic activity of CYP2C9, and variations in its gene (CYB5A) may lead to varied CYP2C9-mediated drug metabolism (Sangkuhl, 2021).

1.3.3 Frequencies of CYP2C9 in different populations

Frequency data is restricted for CYP2C9 alleles in different groups, particularly for CYP2C9*12 and above, and is restricted to CYP2C9*2 and *3 in Oceanians (Daly, 2018). The lack of stated data does not mean the absence of an allele in that group, nor does it rule out the possibility that an individual in that community possesses the allele (Daly, 2018). It's crucial to remember these frequencies for each group are averages of collected data from various articles, each

one reports on smaller, more specialized research groups (Daly, 2018). CYP2C9 frequencies within each category might vary greatly (Sangkuhl, 2021). For example CYP2CP ranged from 0.5 % to 19.6%, in Ecuadorian Mestizos and Brazilian admixed populations respectively, and both those frequencies are within the frequency of the Latino group, which is 8% (Sangkuhl, 2021). Similarly, CYP2C9*3 frequencies ranged from 1% to 15% in Malay and Vietnamese respectively, and both of are within the frequency of East Asian group, which is 4% (Sangkuhl, 2021). Since CYP2C9 frequencies are rating values, they mustn't be applicable to individual patients' pharmacogenetics tests (Sangkuhl, 2021). The PharmGKB/CPIC provides detailed allele frequency statistics for particular studies as well as across populations (Theken, 2020).

Individual genomes may contain several genotypes of CYP2C9 variants (Daly, 2018). Thus, hundreds of variant combinations are conceivable in a population or a group of patients (Daly, 2018). However, in a particular population, the number of those variants is substantially fewer, depending on the number of genotypes and their frequencies (Sangkuhl, 2021).

Table 1: Frequencies of CYP2C9 Ref alleles in biogeographical groups (Sangkuhl, 2021)

Variant	South Asian	Other Asian	Latin American 2	Latin American 1	European	East Asian	Asian	African Others	African American	african
rs1799853	0.9538	0.9976	0.9179	0.871	0.87906	0.9995	0.9988	1	0.9639	0.9652
rs1057910	0.8853	0.9515	0.9607	0.94	0.933308	0.9564	0.955	0.997	0.9869	0.9873
rs9332131	1	1	1	1	1	1	1	0.994	0.9927	0.9928
rs28371686	1	1	1	0.999	0.99997	1	1	1	0.9949	0.9951
rs1934954	0.977	0.98	0.9393	0.909	0.92158	1	0.995	1	0.9871	0.9876
rs2185570	0.9512	0.999	0.9229	0.881	0.869273	0.9997	0.9995	1	0.9769	0.9778
rs2256871	0.9996	1	0.9981	0.979	0.999196	1	1	0.92	0.9309	0.93054
rs28371685	0.9971	1	0.9989	0.9961	0.997817	1	1	0.984	0.9837	0.9837
rs7900194	1	1	1	0.974	0.9997	0.994	0.995	0.982	0.994	0.9936
rs9332239	1	1	0.9992	0.994	0.997446	1	1	1	0.9994	0.9994

1.3.4 CYP2C9 variants

Unexpected variations in CYP2C9 enzyme activity caused by genetic polymorphisms are a common cause of adverse drug reactions (ADRs) (Bahar, 2017). Reduced metabolic capacity due to genetic polymorphisms or drug-drug interactions can induce toxicity at normal therapeutic dosages, especially for CYP2C9 substrates such as warfarin and phenytoin (Xie, 2002). CYP2C9 enzyme plays a significant role in metabolizing more than 12% of clinical drugs, including antidiabetics, antiepileptic, antihypertensive drugs & anticoagulants (Meyer, 2013). There are about thirty CYP2C9 variations and sub-variants known.

There are differences in the frequency of CYP450 genetic variants all over the world, leading to population-specific drug-metabolizing enzymes activity patterns, such as warfarin, phenytoin, and antidiabetic drugs (tolbutamide and glipizide), as well as non-steroidal anti-inflammatory drugs (NSAIDs) (Daly, 2018).

rs1799853 (CYP2C9*2)

The presence of a missense mutation in exon 3 (rs1799853) in the CYP2C9*2 allele causes a reduction in CYP2C9 enzymatic activity toward most of its substrates, resulting in reduced function (Crespi, 1997). The allele frequency of CYP2C9*2 varies between 11 and 13 percent in European, Middle Eastern, and South/Central Asian groups, but only about 2 percent in African-American communities and 1% in East Asian populations (Niinuma, 2014) (Crespi, 1997).

rs1057910 (CYP2C9*3)

A missense mutation in exon 7 (rs1057910) causes a substantial drop in enzymatic activity in the CYP2C9*3 allele with reduced function. For most major pharmacological substrates, the amount of enzyme impairment induced by CYP2C9*3 is greater than that caused by CYP2C9*2 (Niinuma, 2014). In populations of European, Middle Eastern, and South/Central Asian heritage, CYP2C9*3 frequency varies from 7% to 10%, but is significantly lower in populations of African (about 1%) and East Asian (roughly 3%) ancestry (Hiratsuka, 2016).

The Pharmacogene Variation Consortium (PharmVar) has given the most frequent human gene variation the designation CYP2C9*1 (Sangkuhl, 2021). PharmVar catalogs other relevant variations as a series of integers that are written following a star character to construct an allele label. The two most well-known variant alleles are rs1799853 (also known as CYP2C9*2) and rs1057910 (also known as CYP2C9*3), which cause 30% and 80% enzyme activity decreases, respectively (Pratt, 2019). The most prevalent variations, CYP2C9*2 and CYP2C9*3, have a negative effect on enzyme activity (Sangkuhl, 2021).

EM or NM are those who have the homozygous CYP2C9*1 variation, i.e. the *1/*1 genotype. Intermediate metabolizers IM are carriers of the CYP2C9*2 or CYP2C9*3 alleles in a heterozygous form, i.e. just one of these alleles (*1/*2, *1/*3), while PM are individuals carrying

two of these alleles, i.e. homozygous (*2/*3, *2/*2 or *3/*3). As a result, PMs have a greater metabolic ratio (the ratio of unmodified drug to metabolite).

rs28371686 (CYP2C9*5)

A missense mutation in exon 7 (rs28371686) characterizes the CYP2C9*5 allele, which has been identified almost exclusively in people of African origin and is linked to decreased enzymatic activity (Niinuma, 2014).

rs9332131 (CYP2C9*6)

The CYP2C9*6 allele is characterized by a frameshift caused by a single nucleotide loss in exon 5. (rs9332131). Although this allele is less common among African Americans and Africans than the other decreased activity alleles, its null activity and association with central nervous system phenytoin toxicity and reduced warfarin dose requirements make it an important allele to investigate when clinically genotyping CYP2C9 (Kidd, 2001).

rs7900194 (CYP2C9*8)

A missense mutation in exon 3 (rs7900194) defines the CYP2C9*8 allele, which is the most common reduced function allele among African Americans and Africans (Cavallari, 2010). In most other groups, the CYP2C9*8 variation is quite uncommon. Even though this genotype causes reduced enzymatic activity when it comes to warfarin and phenytoin, it has been shown to have substrate specificity (Liu, 2012). It may, for example, give enhanced enzymatic activity toward tolbutamide, according to an in vitro research (Patel, 2014). However, two promoter variations (rs9332094) and (rs4918758), are insignificant linkage disequilibrium with the defining CYP2C9*8 variant (rs7900194) and have been linked to reduced CYP2C9 production, may also contribute to the *8 allele's effects (Patel, 2014). When used to guide medicines other than phenytoin or warfarin, substrate specificity may restrict the categorization of this gene as a reduced function allele when assigning a probable phenotype (eg, sulfonylureas) (Cavallari, 2010). Reduced warfarin clearance has been linked to CYP2C9*8 in both in vivo and in vitro investigations. Although its functional impact is less well understood than that of the *2 and *3 alleles, the CYP2CP*8 allele was chosen as a tier 1 gene due to its predominance in African-

American communities, where warfarin is often utilized (Pratt, 2019). Clinicians should be careful when interpreting genotypic data and taking therapeutic action for any substrate other than those that have been thoroughly described due to concerns about substrate specificity for the CYP2C9*8 allele (Liu, 2012).

rs28371685 (CYP2C9*11)

A missense mutation (rs28371685) in exon 7 defines the CYP2C9*11 allele. This allele has been found in people of many races, although it is most common among African Americans and Africans. CYP2C9*11 has been linked to decreased warfarin metabolism and lower dosage needs, like other tier 1 recommended CYP2C9 alleles (Tai, 2005).

1.3.5 Clinical relevance of CYP2C9

Polymorphisms in the CYP2C9 gene have important implications for medicines with a limited therapeutic index, such as warfarin and phenytoin (Sangkuhl, 2021). While CYP2C9 variation impacts the oral clearance of losartan, it is unclear if this has clinically relevant implications (Yasar, 2002).

The most prevalent clinical application of CYP2C9 genotype information documented so far is its use to guide warfarin dosage in conjunction with VKORC1 and potentially CYP4F2 (Johnson, 2017). Patients with one or two reduced or no function alleles have impaired metabolism of the more powerful S-enantiomer of warfarin and a higher risk of bleeding with standard warfarin dosages, necessitating a smaller warfarin dose to maintain effective results (Johnson, 2017). Three clinical studies have looked at the efficacy of CYP2C9 plus VKORC1 genotype-guided warfarin, with two of them showing that a genotype-guided approach reduced the risk of diseases symptoms (Gage, 2017). On the other hand, the third one; Clarification of Optimal Anticoagulation through Genetics (COAG) study found it did not affect anticoagulation management (Kimmel, 2013). Unlike the previous studies, which were mostly performed in European ancestry populations, almost 30% of patients in the COAG study were of African ancestry, and genotype-guided dosage resulted in worse anticoagulation management than a nongenotype-guided strategy (Kimmel, 2013). All three studies concluded that CYP2C9*2 and *3 alleles, which are the most frequent reduced or no function alleles in European ancestry

patients but are less common in African ancestry patients (Gage, 2017) (Kimmel, 2013). The CYP2C9*5, *6, *8 and *11 variants are primarily present in individuals of African ancestry, and studies suggest that not accounting for them leads to a substantial overestimation of warfarin dosage needs in African Americans (Drozda, 2015). The COAG trial's failure to account for these alleles may have contributed to the trial's poor results (Kimmel, 2013). In 2017, the CPIC guidelines were updated to indicate that in African ancestral patients, genotyping should only be used to advise warfarin dose if the CYP2C9*5, *6, *8, and *11 alleles are examined (Sangkuhl, 2021). These variations, along with the CYP2C9*2 and *3 alleles, are included in the Association for Molecular Pathology's Tier 1 allele recommendations (Pratt, 2019).

Reduced and no function variants of CYP2C9 lead to higher susceptibility to other CYP2C9 substrates (Sangkuhl, 2021). This may raise the risk of severe side effects such as phenytoin neurotoxicity and gastrointestinal hemorrhage, and severe cardiovascular consequences from NSAIDs (Macías, 2020) as well as bradycardia from siponimod (Selmaj, 2013). PMs with the CYP2C9*3/*3 genotype, who are predicted to have little to no enzyme activity, should avoid taking siponimod without a genotype test (Selmaj, 2013). Patients having genotypes linked to substantial decreases in enzyme activity should take lower phenytoin maintenance dosage (Veronese, 1991). Reduced CYP2C9-mediated metabolism is predicted to have the largest impact on NSAIDs with long elimination half-lives and be less important for NSAIDs with shorter half-lives (Macías, 2020). Piroxicam, tenoxicam, and melocoxicam should be avoided in PMs (e.g., CYP2C9*2/*3 or *3/*3 genotypes), but celecoxib and ibuprofen can be used but should be begun at lower dosages than normal, according to CPIC guidelines (Theken, 2020).

1.4 Cytochrome P450 2C (CYP2C) subfamily: CYP2C19

Many medications are hydrolyzed by the enzyme protein CYP2C19 (Botton, 2021). It was initially characterized in 1984, following the discovery of impaired metabolism of the anticonvulsant medication mephenytoin in healthy volunteers and family studies (Küpfer, 1984). Mephenytoin was then employed as a probe drug to differentiate between NM and PM, which was eventually revealed to be attributable to CYP2C19 expression variability (Romkes, 1991). In 1991, researchers discovered the CYP2C19 gene (Botton, 2021). With 1994, the first CYP2C19 variant allele associated with PM (CYP2C19*2) was discovered. Following then,

several variations were reported, and new allelic variants are still being discovered (Goldstein, 1994) (Botton, 2021). Even though many CYP2C19 variations have been found to have no function, the finding of the CYP2C19*17 allele, which is associated with enhanced enzyme activity and faster drug clearance, was a significant discovery in CYP2C19 pharmacogenetics (Sim, 2006). In 2000, a systematic naming scheme for CYP2C19 alleles was created, and it is still being updated as new variants are reported (Ingelman-Sundberg, 2000). The Food and Drug Administration (FDA) amendment of medication labeling based on CYP2C19 pharmacogenetics, as well as the evidence-based CPIC guidelines published for various CYP2C19 gene-drug combinations, are two more landmarks in CYP2C19 history (Botton, 2021).

Figure 4: CYP2C19 structure. The figure shows the structure of CYP2C19 (Anon., 2022)

1.4.1 CYP2C19 allele function

The CYP2C19 gene is found mostly in liver cells, and in the endoplasmic reticulum (Anon., n.d.). At least 10% of routinely prescribed medications, including clopidogrel citalopram, clomipramine, diazepam, omeprazole, S-mephenytoin, and biguanides, are processed or metabolized by the CYP2C19 enzyme (Botton, 2021). Clopidogrel is an antiplatelet medication that stops platelets from adhering to each other and creating blood clots (Sim, 2006). This drug is also converted to its active form by CYP2C19 enzyme, which is required for the medicine to work (Lasker, 1998). The active drug then blocks a receptor protein on the surface of platelets called P2RY12. This receptor protein assists platelets in clumping together to create a clot that seals off damaged blood arteries and prevents blood loss during clot formation (Spector, 2015).

Studies have found a link between CYP2C19 protein levels and microsomal S-mephenytoin 4-prime-hydroxylase activity (Lasker, 1998). The gene expression of important human CYP450 genes was also studied in various locations of explanted hearts from six individuals with dilated

cardiomyopathy, one with arterial trunk transposition, and two samples of the normal heart (Lasker, 1998). The right ventricle was the most abundant source of CYP2C19 mRNA (Lasker, 1998). There was a significant link discovered between tissue-specific gene expression and enzyme activity (Thum, 2000). According to the findings, the expression of genes for CYP450 monooxygenases and verapamil metabolism is mostly located on the right side of the heart, which might explain why some cardioselective medications are ineffective (Thum, 2000).

1.4.2 Additional factors influencing CYP2C19 activity:

One or more drugs can have a significant influence on an individual's metabolic profile (Bahar, 2017). The degree of a DDI and DDGI is determined by the particular enzyme's phenotype and the drug(s) (Bahar, 2017). Fluconazole, fluoxetine, and fluvoxamine, for instance, are known to be CYP2C19 inhibitors that may cause misdirection between the patient's genotype ability to metabolize a drug and its genuine capacity due to no genetic variables in NM or IM (Malki, 2020). On the other hand Rifampin, efavirenz, and St. John's wort are all recognized inducers (Malki, 2020). DDI is unimportant for CYP2C19 PM which lacks enzyme activity (Bahar, 2017). DDI is especially concerning among the elderly, who are subject to multi-drugs (Bahar, 2017).

Moreover, it has been proposed that inflammation may have an impact on effectiveness of the drug (Gravel, 2019). According to recent research, chronic inflammation is related with a 46% reduction in CYP2C19 activity in type 2 diabetes patients (Gravel, 2019). Angiolillo et al showed that individuals with type 2 diabetes were poor responders even when maintenance dosages of clopidogrel were increased and enzyme activity was decreased by 60% (Gravel, 2019). (Angiolillo, 2007).

1.4.3 Frequencies of CYP2C19 in different populations

The predicted frequencies for specific alleles vary significantly between biogeographical regions (Zhou, 2017). In the CYP2C19*2 allele, no function has been reported in high frequency, for example, the frequency in the Oceanian population is 60% and in South and East Asia is 28% (Roco, 2012). This allele is less prevalent than in Sub-Saharan African populations where the frequency is 16–18, although it is still widespread in Europeans (15%) (Zhou, 2017). CYP2C19*3, which is likewise a no-function allele, is more common among Oceanians (15%)

than East Asians (7%), and Near Eastern groups (2%), and is uncommon in other communities throughout the world as 0.2% in Europeans (Zhou, 2017). In African people, other alleles such as CYP2C19*9 which causes decreased enzyme function, CYP2C19*15 which causes normal enzyme function, and CYP2C19*35 which causes loss of function are more common (Zhang, 2019). The CYP2C19*17 enhanced function allele, on the other hand, is commonly found in Europeans with a frequency of 22%, 19% for Near Eastern groups , and 17% for Sub-Saharan Africans, but very infrequently (2%) in East Asians (Roco, 2012). As a result, Oceanic and East Asian populations have the highest prevalence of CYP2C19 PMs (Roco, 2012).

Hundreds of allele combinations are conceivable between CYP2C19*1 and CYP2C19*35, eliminating deletion alleles like CYP2C19*36 and CYP2C19*37, resulting in a significant variety of variants in a population or patient cohort (Zhou, 2017). However, depending on the alleles and their frequency, the possible variations that occur in any population may be much lower (Céspedes-Garro, 2016).

Table 2: Frequencies of CYP2C19 Ref alleles in biogeographical groups (Botton, 2021)

variant	South Asian	Other Asian	Latin American 2	Latin American 1	European	East Asian	Asian	African Others	African American	african
rs4244285	0.633	0.66	0.8913	0.8384	0.852732	0.7199	0.7081	0.816	0.8253	0.825
rs4986893	0.9967	0.9136	1	0.9993	0.994193	0.9214	0.9192	0.997	0.9996	0.9995
rs12248560	0.93	0.96	0.897	0.836	0.76865	0.99	0.982	0.789	0.773	0.7736
rs28399504	0.9996	0.997	0.9974	0.9965	0.996702	0.9994	0.999	1	0.9993	0.9993
rs3814637	1	0.88	0.9617	0.979	0.96169	1	0.97	0.99	0.9943	0.9941
rs41291556	1	1	0.999	0.999	0.997328	1	1	1	0.9987	0.9988

1.4.4 CYP2C19 variants

Gastroenterology, cardiology, psychiatry, mycology, and cancer are among the medical domains where the CYP2C19 polymorphism has a significant impact on clinical outcome (Wang, 2011). Since CYP2C19 is involved in the metabolism of proton pump inhibitors, it can have an impact on reflux treatment, ulcer prevention, and Helicobacter pylori eradication (Sim, 2006). The CYP2C19 enzyme is also involved in both of clopidogrel's bioactivation phases, resulting in a decreased (rs12248560 carriers) or increased (rs4244285 carriers) risk of significant adverse cardiovascular events (Scott, 2011). It has an impact on antidepressant therapy, methadone replacement therapy, and voriconazole prophylaxis (Xu, 2018). In breast cancer patients treated with tamoxifen, the presence of an rs4244285 allele is related to a longer relapse-free time or higher survival, while the presence of an rs12248560 allele is associated with better results (Scott, 2011; Zordoky, 2010).

In many circumstances, knowing about the CYP2C19 polymorphism might have a significant impact on an individual's treatment and drive better patient outcomes (de Leon, 2006). Pharmacogenetics testing in medical practice would be a smart method to avoid needless medical expenses while also minimizing poor therapeutic results (de Leon, 2006; Zordoky, 2010). Polymorphisms in CYP2C19 generally cause dissimilar effect (Wang, 2011). Polymorphisms such as rs56337013 (CYP2C19*5) or rs4986893 (CYP2C19*3) cause poor metabolism while rs12248560 (CYP2C19*17) cause ultra-rapid metabolism (Wang, 2011). On the other hand, Polymorphisms such as rs4244285 (CYP2C19*2) and rs4986893 (CYP2C19*3) causes a non-functional protein (Zi, 2010; Zordoky, 2010). CYP2C19 Polymorphisms and their effect are summarized in table 3.

Table 3: CYP2C19 Polymorphisms and their effect, and Global minor allele frequency (Anon., 2022) (Anon., 2022)

Allele Name	#Rs	Protein function	nomenclature recommendations of the Human Genome Variation Society (HGVS)	Amino acid change	Global minor allele frequency (GMAF)
CYP2C19*1	n/a	Wild-type (WT)			
CYP2C19*2	rs4244285	nonfunctional	NC_000010.10:g.96541616G>A	G to A	0.1983
CYP2C19*2B	rs17878459	nonfunctional	NC_000010.11:g.94775165G>A	G to A	0.01653
CYP2C19*3	rs4986893	Poor metabolism	NC_000010.10:g.96540410G>A	G to A	0.01423
CYP2C19*4	rs28399504	nonfunctional	NC_000010.10:g.96522463A>G	A to G	0.001377
CYP2C19*5	rs56337013	poor metabolizer	NC_000010.10:g.96612495C>T	C to T	
CYP2C19*6	rs72552267	nonfunctional	NC_000010.11:g.94775453G>A	G to A	
CYP2C19*8	rs41291556	nonfunctional	NC_000010.10:g.96535173T>C	T to C	0.001837
CYP2C19*9	rs17884712			G to A	0.003214
CYP2C19*11	rs58973490			G to A	0.001377
CYP2C19*13	rs17879685			T to C	0.005051
CYP2C19*17	rs12248560	ultra-rapid metabolizer	NC_000010.10:g.96521657C>T	C to T	0.1524

rs4244285 (CYP2C19*2)

CYP2C19*2 is another name for this variant (Ingelman-Sundberg, 2000). It was discovered that the main flaw in CYP2C19 that causes the S-mephenytoin ((S)-MP) PM phenotype is a G-to-A mutation at nucleotide 681 in exon 5 that causes an erroneous splicing site (Kosaki, 2004; Fung, 2009). From the AA number 215, the reading frame (RF) of the mRNA was changed, resulting in a premature stop codon which causes in a shortened, nonfunctional protein (Kosaki, 2004; Fung, 2009). The defective allele's inheritance pattern matched that of the PM characteristic (Wang, 2011). Researchers experimentally constructed primers for the intron 4/exon 5 junctions to assess the nature of the problem (Kosaki, 2004). This required the use of numerous primers, a closely related gene that shares 95% of the upstream region and many exons with CYP2C19, as well as a specialized reverse primer for exon 5 of CYP2C19 (de Leon, 2006). A 40-base pair loss occurred at the start of exon 5 as a result of the abnormal splicing site, leading to the deletion of AA 215 to 227 (de Leon, 2006; Fung, 2009). Because it lacked the heme-binding domain (HBD), the shortened protein contained 234 AA and would be catalytically inactive (Wang, 2011). A simple PCR-based test to detect the faulty CYP2C19 allele was created (de Leon, 2006).

Studies demonstrated a link between carriers of the rs4244285 polymorphism and greater residual platelet reactivity in patients who were taking dual antiplatelet therapy with clopidogrel and aspirin (Scott, 2011). Clopidogrel's active metabolite is the result of a series of metabolic processes involving many P450 isoforms, including CYP2C19 (Scott, 2011; Zordoky, 2010).

rs4986893 (CYP2C19*3)

CYP2C19*3 is another name for this variant (Ingelman-Sundberg, 2000). It's a G-to-A mutation that leads to a premature termination codon locate at the nucleotide 636 in the exon 4 of the CYP2C19 gene (Wang, 2011). This variant has been related to the poor metabolism of some compounds (Wang, 2011; Fung, 2009).

Further research revealed that homozygosity or compound heterozygosity for rs4244285 and rs4986893 was linked to poor proguanil metabolism, which is used in malaria chemoprophylaxis

(Zi, 2010). According to the findings, 348 of the 493 people investigated (70.6%) exhibited PM phenotype, which has substantial implications for proguanil effectiveness in this population (Hoskins, 1998).The rs4986893 allele was found in 7.2 % of Han, 8.0 of Kazakh, and 9.4% of Uygur people, and these frequencies were higher than that found in Caucasians (0%) (Hoskins, 1998).

rs28399504 (CYP2C19*4)

CYP2C19*4 is another name for this allelic variation (Ingelman-Sundberg, 2000). It's an A-to-G mutation located at the initiation codon (methionine) of the CYP2C19 gene (Fung, 2009). This variant causes a substitution in methionine for valine has been related to the poor metabolism of some compounds (Fung, 2009).

In Caucasians, the allele was found in 0.6% of the population (Salazar-Flores, 2012). rs28399504 is a faulty allele, according to expression studies and in vitro experiments (Salazar-Flores, 2012).

rs56337013 (CYP2C19*5)

CYP2C19*5 is another name for this allelic variation (Ingelman-Sundberg, 2000). It's a C-to-T mutation that is located at nucleotide 1297 in exon 9 of the CYP2C19 gene (Wang, 2011). This variant has been related to the poor metabolism of some compounds (Wang, 2011).

The rs4244285 allele resulting in substitution in the HBD causes a poor mephenytoin metabolizer phenotype (Fung, 2009) (Zordoky, 2010). The rs56337013 variation was also discovered, with the allele's frequency expected to be low in Chinese and Caucasians (Salazar-Flores, 2012). rs56337013 rendered the recombinant enzyme inactive against drugs, such as (S)-MP and tolbutamide (Wang, 2011).

rs12248560 (CYP2C19*17)

CYP2C19*17 is another name for this allelic variation (Ingelman-Sundberg, 2000). It's a C-to-T mutation that is related to the rapid metabolism of some compounds like antidepressants and proton pump inhibitors (PPI) such as omeprazole (OP) (Sim, 2006). In addition, rs12248560 carrier Cancer patients will be more able to metabolize tamoxifen

treatment, thus being more benefited; it is probably because they metabolite the therapy faster into endoxifen (4-OH-N-desmethyl-tamoxifen) (Wang, 2011).

Carriers of the rs12248560 allele had a 0.77x lower risk of having breast cancer (Schroth, 2007; Justenhoven, 2009). Moreover, a larger effect was seen in number of carriers who were exposed to hormone treatment for ten years or more (Schroth, 2007). The assumption is that estrogen hormone metabolism is sped up, resulting in reduced levels leading to a lower risk of breast cancer (Justenhoven, 2009).

1.4.5 Clinical relevance of CYP2C19

As mentioned before, CYP2C19 has been demonstrated to metabolize a variety of medicinal drugs, including omeprazole and other major PPIs, some tricyclic antidepressants like imipramine, barbiturates, and β-adrenoceptor blockers (Bertilsson, 1995; Ahmad, 2017). OP is a proton pump inhibitor (PPI) that is used to treat gastric ulcers (GU) and other hyper acidic disorders (Furuta, 1998). *Helicobacter pylori* (H.pylori) infections, which are intimately linked to this condition, are frequently treated with OP in conjunction with antimicrobial therapies (Lin, 2017). CYP2C19 is responsible for the 5-hydroxylation of OP, and OP metabolism is distinct from that of S-MP (Lin, 2017). The introduction of a hydroxyl group (-OH) to the OP is done by CYP2C19, causing OP metabolism to be segregated from the metabolism of (S)-MP (Ahmad, 2017). Because (S)-MP is a CYP2C19 substrate and possesses anticonvulsant properties (S) MP can be used to screen for such mutations by examining its metabolites in urine (Ahmad, 2017).

Due to decreased 5-hydroxylation, the actual body exposure to OP following administration of a dosage of the medication is substantially larger in PMs of MP than in IMs (the area under the curve (AUC) plasma concentration vs time curves is much higher in PMs), and the oral clearance of OP is roughly 10 times greater in PMs of MP (Ahmad, 2017) (Lin, 2017). PMs of MP also exhibit greater OP/5-hydroxyomeprazole ratios in their blood than IMs (Bergmeijer, 2018). The majority of OP PMs were found to be homozygous for CYP2C19 PM alleles (Patel, 2020) (Pratt, 2018). Recent research has revealed that the CYP2C19 genotype may influence H. pylori infection cure rates in GU patients (Pratt, 2018). Patients with GU who have CYP2C19 PMs have a cure rate of 100%, with 60% of heterozygotes carrying one mutant allele and 29% of

patients who have two wild-type alleles (Patel, 2020). The remarkable cure rate in CYP2C19 PMs has been attributed to the PM phenotype's significantly higher plasma concentration vs time curves of OP (Ahmad, 2017). Gastric pH was highest in CYP2C19 PMs, lowest in homozygous IMs, and intermediate in heterozygous IMs after OP treatment (Pratt, 2018).

CYP2C19 genotyping is an important application to predict the patient's response to clopidogrel and guide antiplatelet medication selection following cardiac surgery used to open clogged coronary arteries (Bousman, 2017). Data on the efficacy of this genotype-guided strategy to antiplatelet medication are starting to appear. Furthermore, research in multi-ethnic, more diversified communities is being conducted, including those in minority and under-represented groups (Bousman, 2017). The outcomes of these investigations are likely to considerably contribute to addressing the field's present gap regarding the detection of new ethnic-specific variations of therapeutic relevance, as well as verifying prior findings in different populations at large (Bousman, 2017) (Ahmad, 2017).

In addition, CYP2C19 testing can also be used to prescribe selective serotonin reuptake inhibitors in patients with depression, dose information of PPIs for digestive disorders, and dose information of voriconazole drugs for invasive aspergillosis (Goldstein, 1994) (Ahmad, 2017). A new analysis of CYP2C19-guided voriconazole dosing found that the genotype-guided method reduced the percentage of patients with low dose concentrations and achieve high treatment success rates contrasted to comparative controls, the expected cost reductions per patient are $4,700. As a result, CYP2C19 genotyping is critical that test results be correct and that practitioners understand the limits of a genotyping test (Lin, 2017) (Pratt, 2018).

Table 4: Medications affected by CYP2C19 (Kaneko, 1999; Goldstein, 1994)

Medication	**Usual Use**	**Action to be taken in Poor Metabolizer**	**Action to be taken in Normal Metabolizer**	**Action to be taken in Rapid Metabolizer**
Clopidogrel (Plavix ®)	Blood thinner	Select alternative drug	Use standard dose	Use standard dose
Voriconazole (Vfend®)	Fungal infection	Decrease starting dose – check drug levels	Use standard dose	Use standard dose – check drug levels
Amitriptyline	Antidepressant	Select alternative drug or decrease starting dose and check drug levels	Use standard dose	Select alternative drug or standard dose and check drug levels
Imipramine	Antidepressant	Select alternative drug or decrease starting dose and check drug levels	Use standard dose	Select alternative drug or standard dose and check drug levels
Clomipramine	Antidepressant	Select alternative drug or decrease starting dose and check drug levels	Use standard dose	Select alternative drug or standard dose and check drug levels
Trimipramine	Antidepressant	Select alternative drug or decrease starting dose and check drug levels	Use standard dose	Select alternative drug or standard dose and check drug levels
Doxepin	Antidepressant	Select alternative drug or decrease starting dose and check drug levels	Use standard dose	Select alternative drug or standard dose and check drug levels

Citalopram (Celexa®)	Antidepressant	Decrease starting dose or select alternative drug	Use standard dose	Use standard starting dose
Escitalopram (Lexapro®)	Antidepressant	Decrease starting dose or select alternative drug	Use standard dose	Use standard starting dose
Sertraline (Zoloft ®)	Antidepressant	Decrease starting dose or select alternative drug	Use standard dose	Use standard starting dose

1.5 Cytochrome P450 2D (CYP2D) subfamily: CYP2D6

CYP2D6 is an enzyme with a long history in pharmacogenetics, and it is currently considered to metabolize up to 25% of all medications used in clinical practice (Bahar, 2017). Researchers noticed that some Caucasian responded to different drugs in a bimodal pattern (Gaedigk, 2018). This discovery prompted researchers to believe that frequent mutations and their pharmacokinetics are still not identified (Gaedigk, 2018). CYP2D6 protein was isolated from human liver microsomes, then the protein was identified on chromosome 22, then cloning the cDNA was done using an antibody against the rat orthologue (Bousman, 2017). The inferred human protein has a 73 % sequence similarity to the rat protein, and the gene was located on human chromosome 22 using human–rodent somatic cell hybrids (Spear, 2001). This gene was given the name CYP2D6 and is a member of the CYP450 gene family, which is responsible for phase I metabolism and elimination of a wide range of endogenous substrates and medications (Arici, 2017) (Bahar, 2017). CYP2D6 is the sole no inducible CYP450 enzyme among the drug-metabolizing CYP450 enzymes, resulting in a considerable proportion of genetic variation to inter individual variance in enzyme activity (Bahar, 2017). With over 90 documented allelic variations, CYP2D6 is a highly polymorphic gene (Gaedigk, 2018).

Figure 5: CYP2D6 structure. The figure shows the structure of CYP2D6 (Anon., 2022)

1.5.1 CYP2D6 allele function

The liver is the primary site of CYP2D6 expression (Haertter, 2013). It is also found to be expressed in parts of the central nervous system, such as the midbrain's basal ganglia structure (Taylor, 2020). CYP2D6 belongs to the CYP450 family of mixed-function enzymes that catalyze oxidations and are involved in the body's xenobiotic metabolism (Nofziger, 2020). CYP2D6 is responsible for the metabolism and clearance of approximately 25% of clinically used drugs by introducing or removing functional groups – specifically, hydroxylation, which introduces a hydroxyl group, demethylation which removes a methyl group (CH3), and dealkylation which removes an alkyl group from a molecule (Nofziger, 2020) (Wang, 2015). Some prodrugs are also activated by CYP2D6 (Wang, 2015) (Haertter, 2013). This enzyme also breaks down endogenous compounds including serotonin, neuroactive steroids, and both m- tyramine and p-tyramine, which CYP2D6 converts to dopamine in the brain and liver (Haertter, 2013).

Individuals with various types of CYP2D6 function may react differently to different amounts of medicines that CYP2D6 metabolizes (Black, 2012). The pattern of the influence on pharmacogenetics is determined not only by the kind of CYP2D6 function, but also by the amount to which CYP2D6 has to process of the drug to its active effect comparable to, stronger than, weaker than, or no effect at all than the original drug (Nofziger, 2020) (Black, 2012).

1.5.2 Additional factors influencing CYP2C9 activity

CYP2D6 expression has been linked to the gender difference in research, and its activity has been linked to pregnancy (Tamargo, 2017). Although CYP2D6 activity is not detectable throughout fetal development, it is detectable two weeks after birth and reaches adult-like levels during the first year (Ryu, 2016).

1.5.3 Frequencies of CYP2D6 in different populations

Many testing systems employ the allele default assignment technique. Most platforms screen for a subset of the most often seen single nucleotide variants (SNVs) (Nofziger, 2020). As a result, allele assignments are made by "default," as demonstrated by those assigned to CYP2D6*10 (Gaedigk, 1999; Black, 2012). It is critical to understand which SNVs are examined in order to completely comprehend how phenotypic is formed as well as the limits of a test (Shen, 2007).

The Hardy-Weinberg equation may be used to compute genotype frequencies, which are the outcome of allele frequencies in a population (Friedrich, 2014). Thousands of allele permutations exist for CYP2D6*1 to CYP2D6*139 (Haertter, 2013). If individuals of a community have recent ancestry from two or more independent sources, the number of genotypes in that group might be relatively enormous (Haertter, 2013). However, based on the number of variants and their distributions, the total permutations that occur in a population might be substantially lower (Black, 2012).

There is a lot of diversity amongst populations when it comes to the estimated frequencies of individual alleles (Nofziger, 2020). CYP2D6*17 and CYP2D6*29 cause a decrease in allele function and they are more prevalent in the Sub-Saharan African and African American/Afro-Caribbean groups than in others, but other alleles as CYP2D6*10 which also cause a decreased function allele, is the most common variant allele in the East Asian group (Dorji, 2019). The European group has the most nonfunctional CYP2D6*4 alleles, whereas the Oceanian group has the most functional CYP2D6*1 duplication/multiplication alleles (Haertter, 2013). Some alleles, such as CYP2D6*2, CYP2D6*4, and CYP2D6*5, have been discovered in nearly every community tested, whilst others, such as CYP2D6*44 and CYP2D6*49 among Asians, have only been found in a few other population groups (Zhang, 2019; Haertter, 2013).

Table 5: Frequencies of CYP2CD6 Ref alleles in biogeographical groups (Botton, 2021)

variant	South Asian	Other Asian	Latin American 2	Latin American 1	European	East Asian	Asian	African Others	African American	african
rs3892097	0.79	1	0.889	0.86	0.80875	0.994	0.995	0.975	0.9046	0.907
rs28371706	1	1	1	0.969	0.99779	1	1	0.851	0.9125	0.9107
rs1065852	0.8	0.41	0.876	0.832	0.78161	0.427	0.421	0.948	0.8608	0.8637
rs1135840	0.4	0.28	0.577	0.425	0.43263	0.282	0.28	0.45	0.3772	0.3795
rs28371725	0.886	0.97	0.9623	0.881	0.89579	0.954	0.957	0.975	0.9606	0.9611
rs5030655	1	1	1	1	0.99833	1	1	1	0.9978	0.9979
rs16947	0.97	0.9	0.979	0.677	0.68182	0.97	0.944	0.58	0.6627	0.6602

1.5.4 CYP2D6 variants

The CYP2D6 genotype, located on chromosome 22, provides the genetic foundation for CYP2D6-mediated metabolic variability (Bertilsson, 2002; Gaedigk, 2007). Depending on the allele, subjects with particular allelic variations will have normal, reduced, or no CYP2D6 function (Wang, 2015). Pharmacogenomic assays are now available to detect individuals with CYP2D6 allele variants, and they've been proven to be useful in clinical practice (Kramer, 2009). Databases such as PharmVar include data regarding the currently recognized variants of CYP2D6 and their clinical function (Gaedigk, 1999).

Table 6: CYP2D6 Polymorphisms and their effect, and Global minor allele frequency (Anon., 2022) (Anon., 2022)

Allele Name	#Rs	Protein function	HGVS	Amino acid change	GMAF
CYP2D6*1		WT normal			
CYP2D6*2	rs1135840	normal (except *2XN subvariant)	NC_000022.10:g.42522613G	G to C	0.4008
CYP2D6*3A	rs35742686	Non-functional	NC_000022.10:g.42524244delT	A to -	0.009183
CYP2D6*4	rs3892097	splicing defect; has several subvariants	NC_000022.10:g.42524947C>T	G to A	0.1061
CYP2D6*5		whole-gene deletion			
CYP2D6*6	rs5030655	Non-functional	NC_000022.10:g.42525086delA	T to -	0.0101
CYP2D6*9	rs5030656	decreased		AAG to ---	0.01148
CYP2D6*10	rs1065852	decreased	NC_000022.10:g.42526694G>A	C to T	0.2557
CYP2D6*14	rs5030865	nonfunctional	NC_000022.10:g.42525035C>A	G to A	0.0009183

CYP2D6*17	rs28371706	decreased		T to C	0.04867
CYP2D6*29	rs1135840	decreased	NC_000022.10:g.42522613G\x3d	G to C	0.4008
CYP2D6*39	rs1135840	normal	NC_000022.10:g.42522613G\x3d	G to C	0.4008

rs16947 (CYP2D6*1)

CYP2D6*1 is another name for this variant. When a normal number of copies of this allele are inherited, CYP2D6 function is identical to WT (normal) activity (Wang, 2015). It can't be utilized to detect the existence of any given variation since it exists in so many distinct versions (Black, 2012). However, this mutation has been reported in larger copy numbers, which can result in RM in some situations (Taylor, 2020).

rs3892097 (CYP2D6*4)

CYP2D6*4 is another name for the related variant. The most prevalent nonfunctioning version of CYP2D6 is the rs3892097 allele (Chan, 2020).

PM of debrisoquine is found when two copies of rs3892097 are inherited (Kagimoto, 1999). Many additional medicines, such as dextromethorphan, sparteine, metoprolol, nortriptyline, and many other antidepressants and codeine, are often processed first by CYP2D6 (Gough, 1990). However, the active form of medicine is not always the one created after CYP2D6 metabolism; for example, tamoxifen's active form (endoxifen) is formed predominantly through CYP2D6 metabolism; less functional CYP2D6 might imply less benefit from taking the drug (Taylor, 2020) (Bertilsson, 2002).

Researchers have proposed that the rs3892097 SNP has a variety of possible implications, both favorable and negative, over the years (Bijl, 2009). On the positive side, this genotype may lower

the incidence of some malignancies, such as bladder and lung tumors, and it may be linked to milder Alzheimer's neurodegeneration (Journa, 1995; Chen, 1995).

however, many studies have found that carrying the rs3892097 SNP increases the chance of getting Parkinson's disease when exposed to pesticides by 3 to 8 times (Deng, 2004). The risk to rs3892097 carriers appears to be proportional to the degree of pesticide exposure, with no additional risk of Parkinson's disease seen in rs3892097 carriers who have never been exposed to pesticides and the highest increased risk of Parkinson's disease seen in rs3892097 carriers who have been exposed to pesticides frequently (Deng, 2004).

rs5030655 (CYP2D6*6)

CYP2D6*6 is another name for this SNP. The rs5030655 version of this SNP, which has a one-nucleotide deletion, induces a frameshift, rendering the CYP2D6 protein nonfunctional (Kagimoto, 1999). rs5030655 has numerous subtypes, all of which are nonfunctional (Wang, 2015). CYP2D6 PM is detected when two copies of this (or comparable) alterations are inherited (Bertilsson, 2002).

Drugs metabolized by CYP2D6, such as dextromorphan, sparteine, nortriptyline, venlafaxine, and codeine, may have less effectiveness or have more adverse effects if rs5030655 is inherited (Van Nieuwerburgh, 2009).

rs1065852 (CYP2D6*10)

CYP2D6*10 is another name for this SNP. The rs1065852 mutation is a C to T mutation (Taylor, 2020). It occurs in CYP2D6 as one of the most prevalent variations (Taylor, 2020). While it is impossible to identify this mutation to a specific variation, all of the variants in which it is found have little or no CYP2D6 activity. PM of debrisoquine is detected if two copies of rs1065852 are inherited (Nofziger, 2020).

Because the rs1065852 single nucleotide polymorphism (SNP) is more common in Asians, research imply that thermal instability and decreased intrinsic clearance by the enzyme encoded by this SNP are the major reasons Asians have lower metabolic activity than Caucasians for medicines metabolized primarily by CYP2D6 (Nakamura, 2002).

In addition, having rs1065852 gives a therapeutic advantage in specific patient subgroups (Wagner, 1987). Toremifene is the alternative drug for tamoxifen in breast cancer patients who have a different metabolic route than tamoxifen (Chan, 2020). Toremifene may have a therapeutic advantage in specific patient subgroups, such as individuals with the rs1065852 SNP who would benefit less from adjuvant tamoxifen therapy (Shen, 2007; Chan, 2020). The rs1065852 SNP was not linked to disease-free survival in Toremifene patients (Nakamura, 2002). Toremifene patients showed a greater 5-year disease-free survival rate than tamoxifen patients (Wagner, 1987).

rs5030865 (CYP2D6*14)

CYP2D6*14 is another name for this SNP. The SNP rs5030865 in the CYP2D6 gene results in two inactive variants, rs5030865 and rs5030865 (Taylor, 2020). Because this SNP can have more than two allele alterations and has altered direction in different reference genome builds, references to it can be quite misleading (Nofziger, 2020) (Kramer, 2009).

1.5.5 Clinical relevance of CYP2D6

CYP2D6 is a drug metabolizing enzyme that has been widely investigated and is undoubtedly one of the most significant (Nofziger, 2020). CYP2D6 activity have wide range of activity, which can have serious clinical implications (Taylor, 2020). Many medicines, including antidepressants, atypical and typical antipsychotics, antineoplastic therapies, adrenergic antagonists, and analgesics, are metabolized by CYP2D6 (Wagner, 1987) (Van Nieuwerburgh, 2009).

One or more co-medications may have a significant influence on a patient's metabolic profile (Shen, 2007). In psychiatry, for example, antipsychotics and antidepressants are commonly used in combination (Van Nieuwerburgh, 2009). Each medication may be transformed at a slower pace if both (or numerous) are CYP2D6-dependent (Nofziger, 2020). CYP2D6-inhibiting medications may also impair CYP2D6 metabolic capability (Zhang, 2019) (Wagner, 1987). DDI and polymorphic enzyme inhibitors have the least impact on PMs since they have no function to block (Wagner, 1987). UMs, NMs, and IMs, on the other hand, frequently pheno-convert to IM or PM status (Zhang, 2019). Fluoxetine, for example, not only is metabolized by CYP2D6, but it also functions as a nonreversible inhibitor, causing most people to become PM (Zhang, 2019).

Additionally, some popular dietary supplements, such as herbal medicines, might have clinical implications by decreasing CYP2D6 activity, sesamin, turmeric, and lotus herbals in cosmetics and teas (Wang, 2015) (Wagner, 1987). There are no known clinical CYP2D6 inducers, despite the fact that several drugs have been found to induce different CYP450 enzymes (Taylor, 2020) (Nofziger, 2020).

2 Goal of study and aims:

In this research we aimed to determine the genotype profile for the most frequent variants of CYP2C9, CYP2C19 and CYP2D6 of a random sample from the Palestinian population. In addition, we tried to find inter-ethnic variation by comparing the allele frequencies of CYP2C9, CYP2C19 and CYP2D6 among different populations and the Palestinian population. We aimed to find the meaningful differences of the CYP2C9, CYP2C19 and CYP2D6 frequencies in the Palestinian population comparing to the global population.

3 Methodology and Research design:

3.1 Study group

Our data have been taken from 67 healthy unrelated Palestinian volunteers (35 males and 32 females, aged from 16 to 60 years). Volunteers came to have a commercial genetic test to test their genetic profile for cancers, vitamin deficiency, mineral deficiency, diabetes, metabolic disorder, hormonal disorder, and other conditions. They were interviewed for family history of risk of familial related disorders.

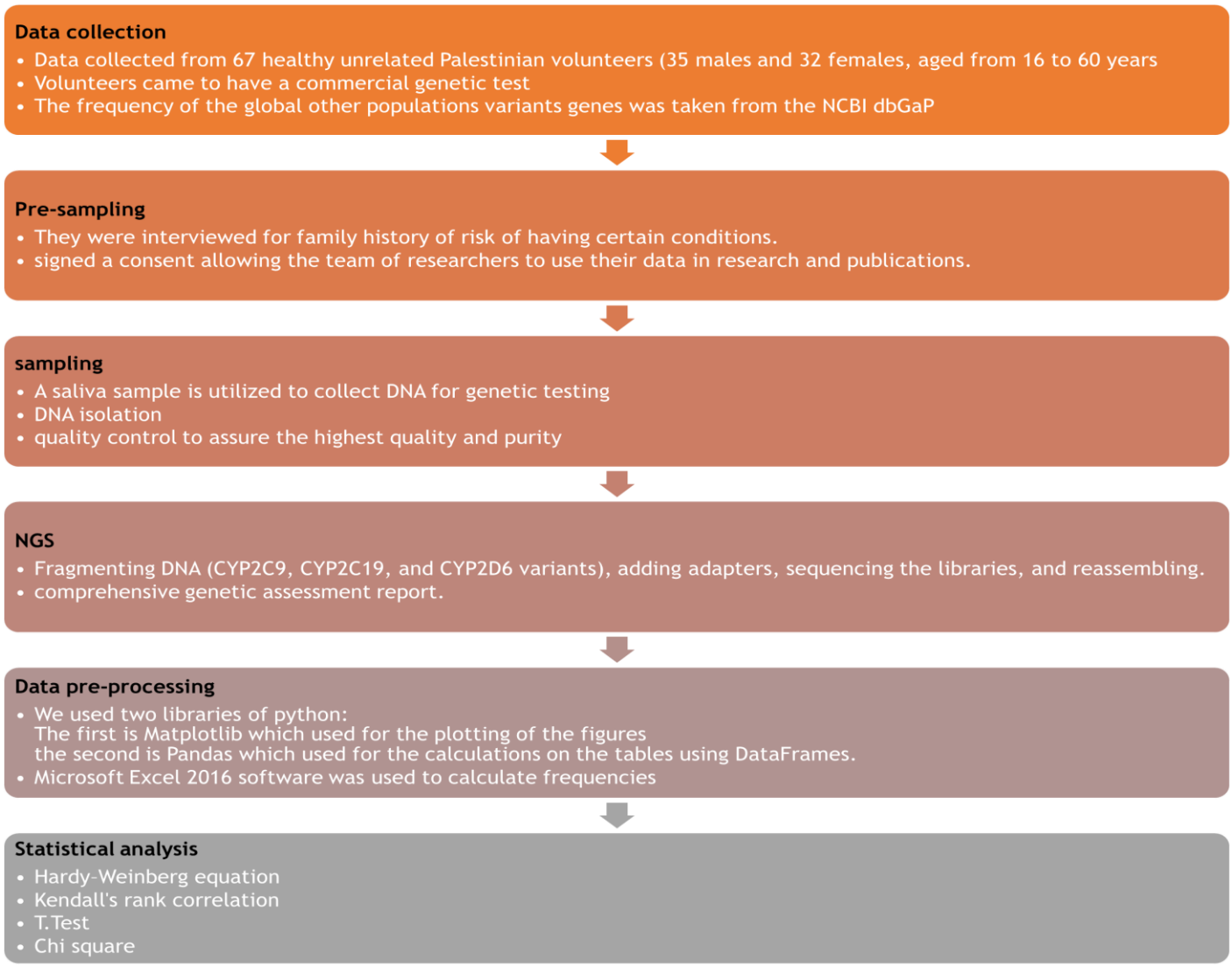


Figure 6: Methodology workflow

3.2 Sample

The testing procedure is straightforward. Each individual provided 2mL of saliva sample by spitting in a test tube and mix it with the preservation liquid provided in the kit. The test tube was sealed, gently hacked for 10 seconds, and placed it in the bag. Test kits were delivered to Anantlife laboratories, Canada (https://www.anantlife.com/), where they examined, and analyzed in cutting-edge facilities. The procedure takes from two weeks to a month. Isolation of DNA is done by breaking cells open to release the DNA, separating DNA from proteins and other cellular debris, precipitating the DNA with an alcohol, cleaning the DNA, and confirming the presence and quality of the DNA. DNA isolation is followed by a quality control check (QC) to assure the highest quality and purity, as shown in figure 6.

The NGS was run at the Anantlife laboratories. The test is entirely non-invasive and relies on DNA. Isolation of DNA and QC testing to assure the highest quality and purity are the following stages. It is necessary to achieve a minimum coverage of 50x, which means that each fragment of DNA is read at least 50 times in order to achieve higher than 99% accuracy. A saliva sample is utilized to collect DNA for genetic testing, making this the most complete health and wellness test available. These tests are suited for children and adults of all ages due to the simplicity of the test and collecting procedure (Anon, 2022).

Figure 7: the steps for the genetic test in Anantlife laboratory.

Anantlife lab meets the Clinical Laboratory Improvement Amendments (CLIA) criteria and is accredited by the College of American Pathologists (CAP), a team of experts uses cutting-edge genomic technologies to run the samples in a high-throughput sequencing laboratory dedicated to the highest standards of process and quality control. To undertake risk stratification analysis, our patented algorithms take into account an individual's genetic data as well as their medical history (Anon, 2022).

3.3 Next generation sequencing (NGS)

Prior to starting the extraction, a 37 °C water bath, and an ice bucket should be prepared. Three 15 ml conical centrifuge tubes will be needed for each extracted sample. The three tubes will be used to hold the cell and protein pellet, the final extracted gDNA, and the isopropanol and ethanol supernatants. The sample Retrieved from storage, and inverted several times then vortex step takes place at medium speed for 15 sec. the sample then Dispensed into a clean 15 ml

centrifuge tube, and 5 ml of Cell Lysis Solution is added. the sample should be mixed 50 times by inversion, and incubated at room tempteture for 30 min. The next step is the RNA Removal. In this step, 40 µl of RNase A Solution at 100 mg/ml is added, and incubated at 37 °C for 15 min. the smple removed from the 37 °C water bath and cooled on ice for 3 min. After the RNase A incubation, the temperature of the water bath should be increased to 65 °C for the DNA rehydration step. Then protein and Lipid Removal takes place by Adding 50 µl of Proteinase K Solution at 20 mg/ml. the mix is mixed several times by inversion, and incubated at room temperature for a minimum of 30 min. After the addition of the Proteinase K Solution, the sample can be stored at 4 °C until the extraction can be completed. 1.7 ml of Protein Precipitation Solution is added, and then vortex vigorously took place for 20 sec at high speed, and the mix placed on ice for 10 min. Once the samples have cooled on ice for 10 min, the sample centrifuged for 10 min at 3,000 x g and 4 °C. The precipitated proteins must form a tight pellet to continue. If the pellet is not tight or the solution is still cloudy, the samples can be cooled on ice for 5 min more and centrifugation repeated. The samples must be kept on ice to ensure a tight pellet. The next step is isolation and Purification of gDNA. In this step, 5 ml of Isopropanol and 8 µl of pure Glycogen Solution at 20 mg/ml should be pipetted into a clean 15 ml centrifuge tube. The supernatant containing the gDNA Poured into the tube containing the Isopropanol and Glycogen Solution, the precipitated protein pellet should be left. Once the supernatant has been added, the sample mixed gently 50 times by inversion and centrifuged for 30 min at 3,000 x g and 4 °C. The supernatant Poured slowly into a clean 15 ml tube. After removal of the supernatant, 1 ml of 70% ethanol is added to wash the pellet. the ethanol Retained in the tube. After the initial wash, the sample centrifuged for 1 min at 2,000 x g and 20 °C. This centrifugation step can be done at either 4 °C or 20 °C. Following the initial wash and centrifugation of the pellet, second wash is done by similar steps. After the removal of the supernatant from the second wash, the pellet allowed to air dry for 15 min. the last step is Rehydration of gDNA. Once the sample has dried, 300 µl of Tris-EDTA is added to rehydrate the dried gDNA pellet. the sample Vortexed for 5 sec at medium speed and placed in a 65 °C hot water bath for 1 hr. the samples removed from the water bath and incubated at room temperature (Goode, 2014).

Next generation sequencing (NGS) were done to sequence variants of CYP2C9 (rs1799853, rs1057910, (rs9332131, rs28371686, rs1934954, rs2185570, rs2256871, rs28371685, rs7900194, rs9332239), variants of CYP2C19 (rs4244285, rs4986893, rs12248560, rs28399504, rs3814637, rs41291556), and variants of CYP2D6 (rs3892097, rs28371706, rs1065852, rs1135840, rs28371725, rs5030655, rs16947).

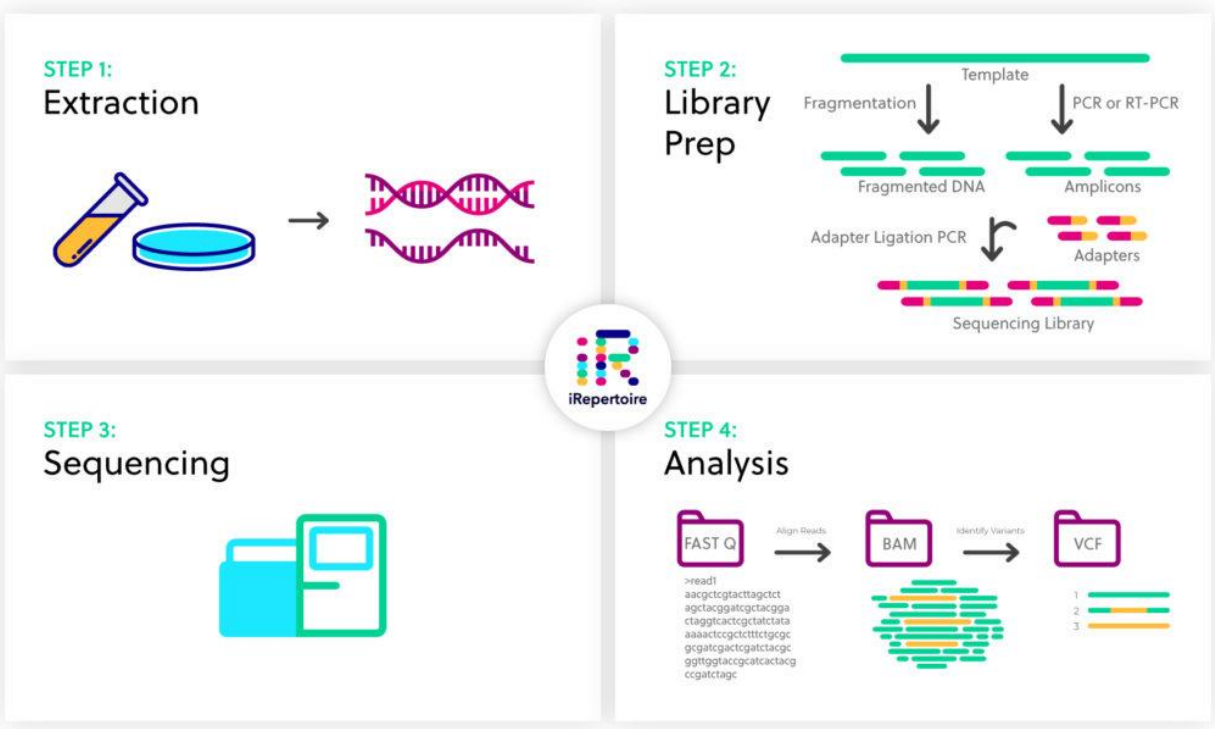


Figure 8: NGS steps

A high-throughput approach is used to determine a section of an individual's genome's nucleotide sequence. The amplicon DNA was purified and prepared as much as 500 ng for sequencing using Illumina's Next Generation Sequencing method. For whole genome and exome sequencing, libraries were prepared as per manufacturer's protocol using Illumina Nextera DNA Flex Library Prep kit and Illumina TrueSeq DNA LP for enrichment kit, respectively. Sequencing of above library was performed on Illumina NovaSeq 6000 system. The NovaSeq 6000 is a sequencing platform from Illumina that enables the sequencing of short reads with an output up to 6 Tb. The NovaSeq 6000 uses the typical Illumina sequencing workflow based on library preparation,

cluster generation by in situ amplification, and sequencing by synthesis. Flexibility is one of the major features of the NovaSeq 6000. Several types of sequencing kits coupled with dual flow cell mode enable high scalability of sequencing outputs to match a wide range of applications from complete genome sequencing to metagenomics analysis (Nizamuddin, 2021).

This method makes use of DNA sequencing tools that can process numerous DNA sequences at the same time. Massively parallel sequencing (MPS) and next-generation sequencing (NGS) are other terms for the same method. The three fundamental phases of next-generation sequencing are library preparation, sequencing, and data processing. Fragmenting DNA/RNA into many pieces, adding adapters, sequencing the libraries, and reassembling them to generate a genomic sequence are all part of the fundamental next-generation sequencing process as shown if figure 8. The approach is comparable to capillary electrophoresis in theory. NGS, on the other hand, reads millions of fragments in a massively parallel method, enhancing speed and accuracy while lowering sequencing costs. NGS technology is known for being extremely scalable, allowing the complete genome to be sequenced at the same time. This is usually performed by slicing the genome into little bits, randomly sampling for a segment, then sequencing it using one of many methods. A full genome may be sequenced because many sections are sequenced at the same time in an automated process (thus the phrase "massively parallel" sequencing). A full human genome may be sequenced in a single day using NGS technology. NGS technology has greatly aided researchers in their quest for health insights and is fueling the "Personalized Medicine" movement (Behjati, 2013).

3.4 Consent

Each individual signed a consent allowing the team of researchers to use their data in research and publications. The consent stated that the data will be used anonymously for purposes of research and publications by the mother company (Anantlife) and the research team. They confirm that they have seen and been given the opportunity to read and discuss the consent.

3.5 Statistics

The frequency of the global other populations variants genes was taken from the NCBI database of Genotypes and Phenotypes (dbGaP). The populations include South Asian, Other Asian (Asian Individuals excluding South or East Asian), Latin American 2 (Latin American individuals with mostly European and Native American Ancestry), Latin American 1 (Latin American individuals with Afro-Caribbean ancestry), European, East Asian, Asian (Asian Individuals excluding South Asian), African Others (Individuals with African ancestry), African American, and African (All Africans, AFO and AFA Individuals).

NCBI developed the Allele Frequency Aggregator (ALFA) pipeline to compute allele frequency for variants in dbGaP across approved unrestricted studies and provide the data as open-access to the public through dbSNP in order to comply with this updated Genomic Summary Results policy and to promote research toward identifying genetic variants that contribute to health and disease. The ALFA project's purpose is to make frequency data from over 1 million dbGaP individuals open-access in future releases, making it easier to find and analyze common and uncommon variations that have biological consequences or cause disorders. To that purpose, GRAF-pop was used to assess over 925K dbGaP participants having genotyping data as ALFA project candidates, requiring study permission and processing.

Chi square value and P-value for allele and genotype frequencies for CYP2C9, CYP2C19, and CYP2D6 were calculated with the Hardy–Weinberg equation.

A Jupyter notebooks was used write the code (script). To draw the figures and the tables we used two libraries of python 3.8.10. The first is seaborn0.11.2-Matplotlib3.5.1, which used for the plotting of the figures, and the second is Pandas 1.3.5 which used for the calculations on the tables using DataFrames. Microsoft Excel 2016 software was used to calculate frequencies of the Palestinian population and the P value for the T test. Kendall's rank correlation was used to measure the strength and direction of between populations' frequencies as it's a small data. The genotype distributions were evaluated for Hardy-Weinberg equilibrium (HWE). by using Hardy-Weinberg equation. A value of $P<0.05$ was considered statistically significant.

4 Results:

Ref variant alleles for the CYP2C9, CYP2C19 and CYP2D6 and their frequencies in the Palestinian population (n=67) and the global population is shown in table 7A. There were no statistically significant differences for the CYP2C9 (P-value = 0.847232), CYP2C19 (P-value= 0.479754), and CYP2D6 (P-value= 0.923857) Ref variants between the Palestinian population (n=67) and the global population.

Alt variant alleles for the CYP2C9, CYP2C19 and CYP2D6 and their frequencies in the Palestinian population (n=67) and the global population shown in table 7B. There were no statistically significant differences for the CYP2C9 (P-value = 0.4768), CYP2C19 (P-value= 0.450072), and CYP2D6 (P-value= 0.675789) Alt variants between the Palestinian population (n=67) and the global population.

Table 7A: Frequencies of Ref variant alleles for the CYP2C9, CYP2C19 and CYP2D6 in the Palestinian population (n=67) and the global population and their P-value.

Gene	variant	Ref allele	Palestine Ref Allele frequency (n=67)	Global Ref Allele frequency	Ref Allele P-value
CYP2C9	rs1799853	C	0.8880597	0.890019	0.847232
	rs1057910	A	0.910447761	0.9353	
	rs9332131	A	1	0.99962	
	rs28371686	C	1	0.999764	
	rs1934954	T	0.962686567	0.93141	
	rs2185570	T	0.902985075	0.877132	
	rs2256871	A	1	0.994902	
	rs28371685	C	1	0.997368	

	rs7900194	G	1	0.99884	
	rs9332239	C	1	0.997647	
CYP2C19	rs4244285	G	0.9	0.850252	0.479754
	rs4986893	G	1	0.992434	
	rs12248560	C	0.975806452	0.77771	
	rs28399504	A	1	0.99681	
	rs3814637	C	0.895522388	0.96545	
	rs41291556	T	1	0.997506	
CYP2D6	rs3892097	C	0.892307692	0.81761	0.923857
	rs28371706	G	0.985074627	0.98694	
	rs1065852	G	0.76119403	0.78846	
	rs1135840	G	0.425373134	0.42619	
	rs28371725	C	0.798387097	0.90893	
	rs5030655	A	1	0.99823	
	rs16947	A	0.669230769	0.67841	

Table 7B: frequencies of Alt variant alleles for the CYP2C9, CYP2C19 and CYP2D6 in the Palestinian population (n=67) and the global population and their P-value.

Gene	variant	Alt Allele	Palestine Alt Allele frequency (n=67)	Global Alt Allele frequency	Alt Allele P-value
CYP2C9	rs1799853	T	0.082089552	0.109981	0.617751
	rs1057910	C	0.089552239	0.0647	
	rs9332131	Deletion	0	0.00038	
	rs28371686	G	0	0.000236	
	rs1934954	C	0.02238806	0.06859	
	rs2185570	C	0.082089552	0.122868	
	rs2256871	G	0	0.005098	
	rs28371685	T	0	0.002632	
	rs7900194	A	0	0.00114	
	rs9332239	T	0	0.002353	
CYP2C19	rs4244285	A	0.1	0.149748	0.432077
	rs4986893	A	0	0.007566	
	rs12248560	T	0	0.22229	
	rs28399504	G	0	0.00319	
	rs3814637	T	0.104477612	0.03455	
	rs41291556	C	0	0.002494	

CYP2D6	rs3892097	T	0.107692308	0.18239	0.938957
	rs28371706	A	0.014925373	0.01306	
	rs1065852	A	0.194029851	0.21154	
	rs1135840	G	0.574626866	0.57381	
	rs28371725	T	0.161290323	0.09107	
	rs5030655	G,A,T	0	0.00177	
	rs16947	A	0.284615385	0.32159	

In our study group, the observed genotype frequency distributions did not show a significant deviation from the HWE, except for the variants rs3892097 and rs2185570 (p-value= 0.013, 0.003741 respectively). Chi square value and P-value for the CYP2C9, CYP2C19, and CYP2D6 genotype variants are shown in table 8. In our study population, none of the individuals were heterozygous for any of the rs9332131, rs28371686, rs2256871, rs28371685, rs7900194, rs9332239, rs4986893, rs28399504, rs41291556, and rs5030655 variants, as shown in figure 9A, 6B, 6C. Thus there are no Chi square and P-values for these variants. Among the Palestinian population, rs1799853, rs1057910, rs9332131, rs28371686, rs1934954, rs2185570, rs2256871, rs28371685, rs7900194, rs9332239, rs4244285, rs4986893, rs12248560, rs28399504, rs3814637, rs41291556, rs3892097, rs28371706, rs1065852, rs1135840, rs28371725, rs5030655, and rs16947 are biallelic variants, while rs1799853, rs1934954, rs2185570, rs1065852, rs28371725, and rs16947 are tri-allelic variants.

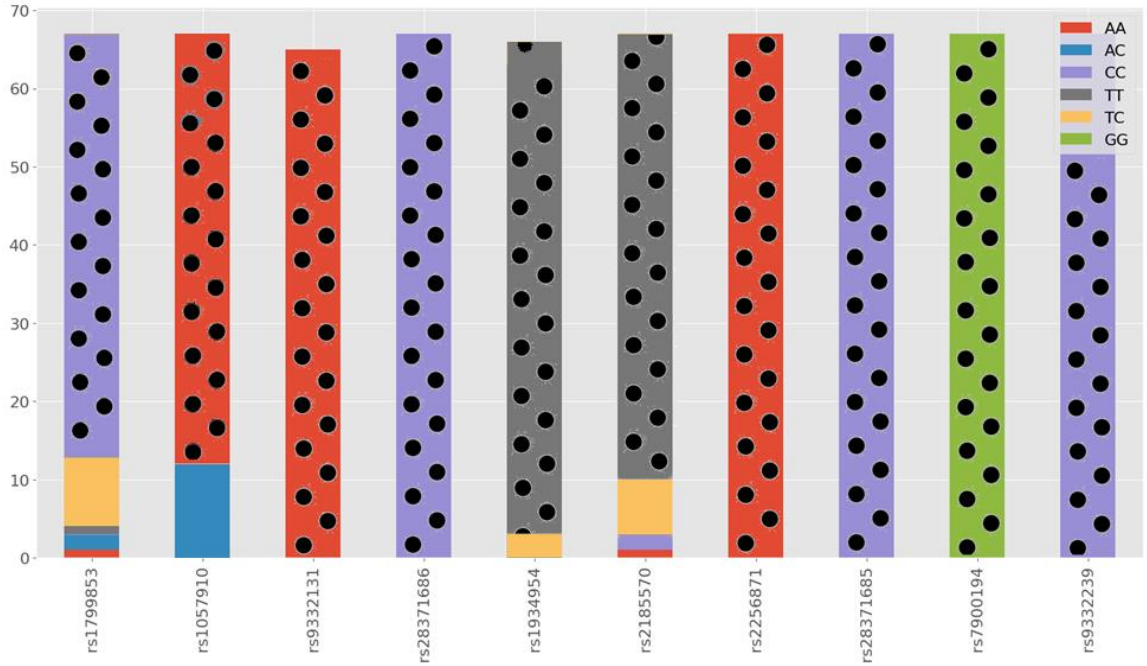


Figure 9A: Histogram of the frequency of individuals per genotype in the Palestinian population for CYP2C9 variants. The dotted bar refers to the reference homogenous genotype.

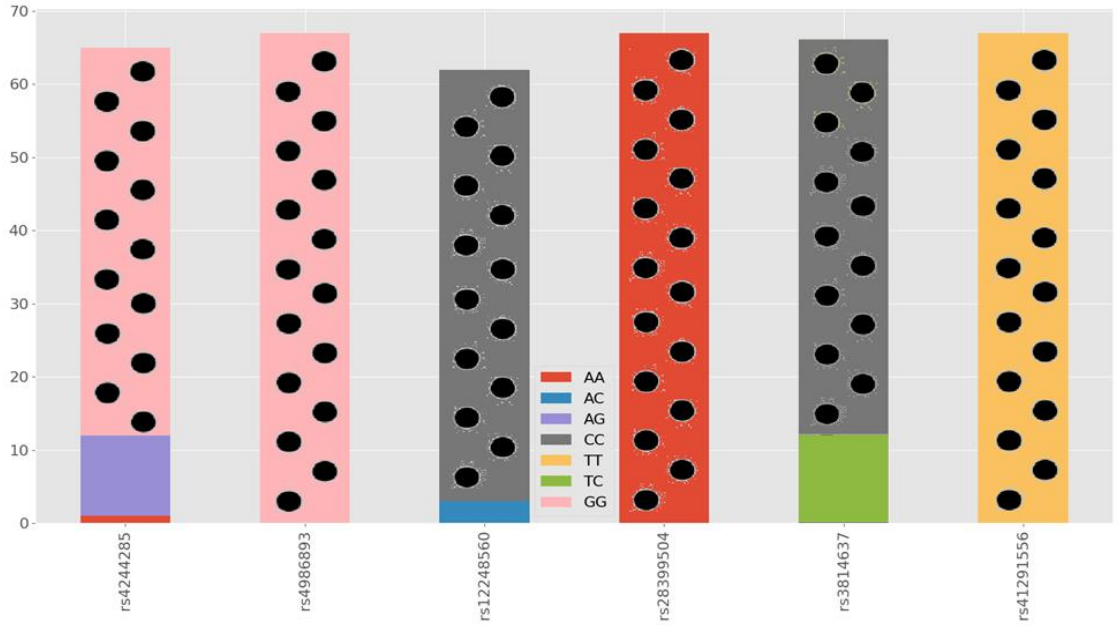


Figure 9B: Histogram of the frequency of individuals per genotype in the Palestinian population for CYP2C19 variants. The dotted bar refers to the reference homogenous genotype.

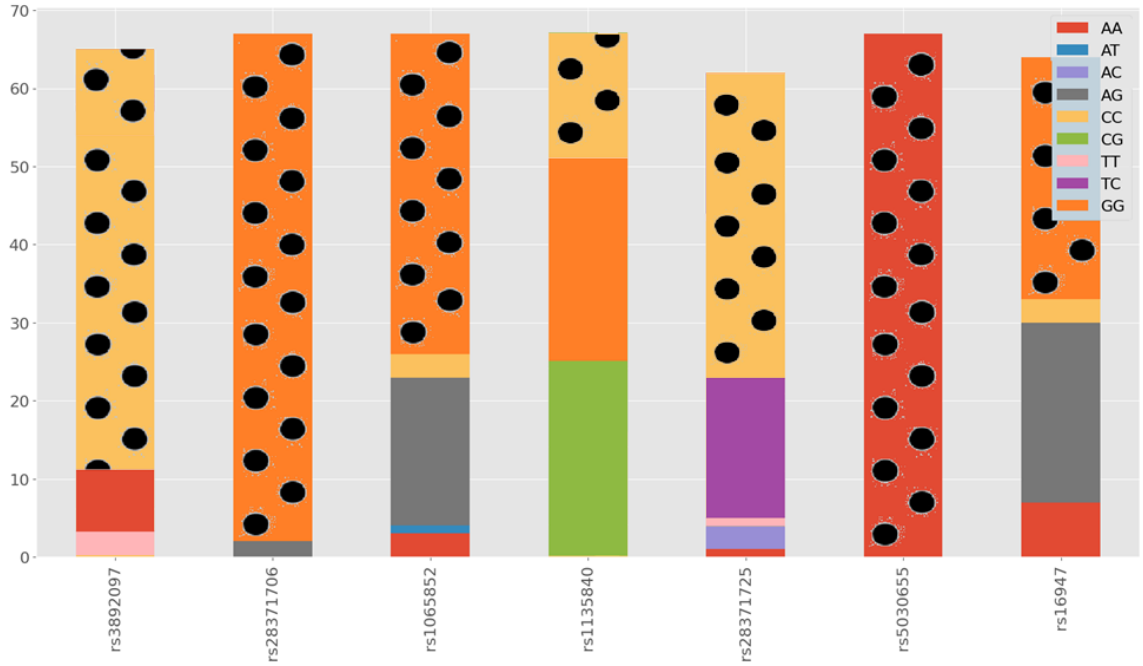


Figure 9C: Histogram of the frequency of individuals per genotype in the Palestinian population for CYP2D6 variants. The dotted bar refers to the reference homogenous genotype.

Table 8: Chi square value and P-value for allele and genotype frequencies for the CYP2C9, CYP2C19, and CYP2D6.

	Homozygote reference	Heterozygote	Homozygote alternative	chi2	P Value
rs1799853	CC	CT	TT	0.7042	0.4013
rs1057910	AA	AC	CC	0.6482128	0.420752
rs9332131	AA	A(Deletion)	(Deletion) (Deletion)	NA	NA
rs28371686	CC	CG	GG	NA	NA
rs1934954	TT	TC	CC	0.0357	0.850147

rs2185570	TT	TC	CC	6.1713	0.013
rs2256871	AA	AG	GG	NA	NA
rs28371685	CC	CT	TT	NA	NA
rs7900194	GG	GA	AA	NA	NA
rs9332239	CC	CT	TT	NA	NA
rs4244285	GG	GA	AA	0.23267	0.629552
rs4986893	GG	GA	AA	NA	NA
rs12248560	CC	CT	TT	0.0381	0.845218
rs28399504	AA	AG	GG	NA	NA
rs3814637	CC	CT	TT	0.66	0.41656
rs41291556	TT	TC	CC	NA	NA
rs3892097	CC	CT	TT	8.405567	0.003741
rs28371706	GG	GA	AA	0.0154	0.9013
rs1065852	GG	GA	AA	0.17	0.68
rs1135840	CC	CG	GG	3.7547	0.052659
rs28371725	CC	CT	TT	0.444	0.505
rs5030655	AA	A(C or G or T)	(C or G or T) (C or G or T)	NA	NA
rs16947	GG	GA	AA	0.78	0.3998

4.1 CYP2C9 variants frequencies

The Palestinian population and the global population frequency of the Ref and Alt CYP2C9 allelic variants were compared in table 9. Only rs9332239 and rs28371685 frequencies showed a significant difference between the Palestinian population and the global population for Ref variants (p-value=0.00311776) and Alt variants (p-value=0.003118). rs9332239 and rs28371685 frequencies between the Palestinian population and the global population were tested with the other CYP2C9 variables for Ref frequencies and Alt frequencies. For Ref allele frequencies, there were statistically significant differences in rs9332239 and rs28371685 with rs7900194 (p-value= 0.010516) and rs2256871 (p-value= 0.01825). The p-value of the four variants together (rs9332239, rs28371685, rs7900194 and rs2256871) were 0.002377 which shows a statistically significance differences. T.Test was also done for these for variables between the Palestinian population and all other populations (P-value = 0.003817). On the other hand, For the Alt allele frequencies, there were statistically significant differences in rs9332239 and rs28371685 with rs7900194 (p-value= 0.011177) and rs2256871 (p-value= 0.01825). The p-value of the four variants together (rs9332239, rs28371685, rs7900194 and rs2256871) were 0.014841 which shows a statistically significance differences. T.Test was also done for these for variables between the Palestinian population and all other populations (P-value = 0.005033).

Table 9: The Palestinian and the global population frequency of the CYP2C9 allelic variants p-values using T-Test. (the Palestinian and global population frequencies were taken from table 7A and 7B)

Allele_a	Allele_b	T_value (Ref allele)	P_value (Ref allele)	T_value (Alt allele)	P_value (Alt allele)
rs1799853	rs1057910	-0.5307817	0.64861448	-0.06623	0.953223
rs1799853	rs9332131	-0.0100809	0.99287189	-0.20646	0.855542
rs1799853	rs28371686	-0.010993	0.99222699	-0.20524	0.856381
rs1799853	rs1934954	0.34354744	0.76394066	-0.77842	0.517793
rs1799853	rs2185570	1.21170937	0.34935526	-1.85222	0.205189
rs1799853	rs2256871	0.02046111	0.98553333	-0.24769	0.827483
rs1799853	rs28371685	0.00433735	0.99693304	-0.22587	0.842286
rs1799853	rs7900194	-0.0051199	0.99637974	-0.21295	0.851097
rs1799853	rs9332239	0.0025353	0.99820728	-0.22344	0.843942
rs1057910	rs9332131	-0.2219558	0.84495154	0.221956	0.844952
rs1057910	rs28371686	-0.2230917	0.84417724	0.223092	0.844177
rs1057910	rs1934954	0.12264044	0.91360437	0.542366	0.64192
rs1057910	rs2185570	0.01706607	0.98793334	-0.90925	0.459197
rs1057910	rs2256871	-0.1836353	0.87123131	0.183635	0.871231
rs1057910	rs28371685	-0.2039323	0.85727438	0.203932	0.857274
rs1057910	rs7900194	-0.2157683	0.84917413	0.215928	0.849065

rs1057910	rs9332239	-0.2061917	0.85572584	0.206192	0.855726
rs9332131	rs28371686	4.27777778	0.05053971	-4.27778	0.05054
rs9332131	rs1934954	0.40716401	0.72333007	-0.09947	0.929836
rs9332131	rs2185570	0.16788775	0.88211323	-0.9747	0.432512
rs9332131	rs2256871	1.16108521	0.3654532	-1.16109	0.365453
rs9332131	rs28371685	1.3374778	0.31287926	-1.33748	0.312879
rs9332131	rs7900194	1.97435897	0.1870379	-2	0.183503
rs9332131	rs9332239	1.3852002	0.30025931	-1.3852	0.300259
rs28371686	rs1934954	0.40465422	0.72490605	-0.09777	0.931031
rs28371686	rs2185570	0.16684561	0.88283492	-0.97238	0.433427
rs28371686	rs2256871	1.09707939	0.38705705	-1.09708	0.387057
rs28371686	rs28371685	1.19699499	0.35394682	-1.19699	0.353947
rs28371686	rs7900194	1.51082251	0.26993678	-1.52212	0.267402
rs28371686	rs9332239	1.22295701	0.34589293	-1.22296	0.345893
rs1934954	rs2185570	0.70804145	0.55231368	-1.63562	0.24355
rs1934954	rs2256871	0.49392039	0.67027668	-0.15748	0.889328
rs1934954	rs28371685	0.44745512	0.69834063	-0.12661	0.910827
rs1934954	rs7900194	0.42089588	0.71474704	-0.10852	0.923488
rs1934954	rs9332239	0.44235523	0.70147079	-0.1232	0.913214

rs2185570	rs2256871	0.20284704	0.85801857	-1.05375	0.402511
rs2185570	rs28371685	0.18437582	0.87072067	-1.01166	0.418189
rs2185570	rs7900194	0.17355795	0.87818987	-0.98702	0.427678
rs2185570	rs9332239	0.18231358	0.87214289	-1.007	0.419963
rs2256871	rs28371685	3.13463098	0.08847389	-3.13463	0.088474
rs2256871	rs7900194	1.58913154	0.25297646	-1.57605	0.255713
rs2256871	rs9332239	2.7143898	0.11314869	-2.71439	0.113149
rs28371685	rs7900194	2.57608696	0.12340585	-2.52815	0.127266
rs28371685	**rs9332239**	**17.8673835**	**0.00311776**	**-17.8674**	**0.003118**
rs7900194	rs9332239	2.94467728	0.09856874	-2.87964	0.102403

The difference of the CYP2C9 Ref and Alt allele frequencies between the Palestinian population and the global population is shown in figure 10A and 10B. And figure 11 shows the correlation between the CYP2C9 frequencies between different populations. According to the Kendall rank correlation coefficient, there are a high correlation (Clint= 0.95) between the Palestinian population and the global population. The European population are the closest population to the Palestinian population for the CYP2C9 variants according to the data that reported in the 1000 Genomes database (Clint= 0.94). The farthest population to the Palestinian population for the CYP2C9 variants according to the data that reported in the 1000 Genomes database is the African others population (Clint= -0.37) as shown in figure 11.

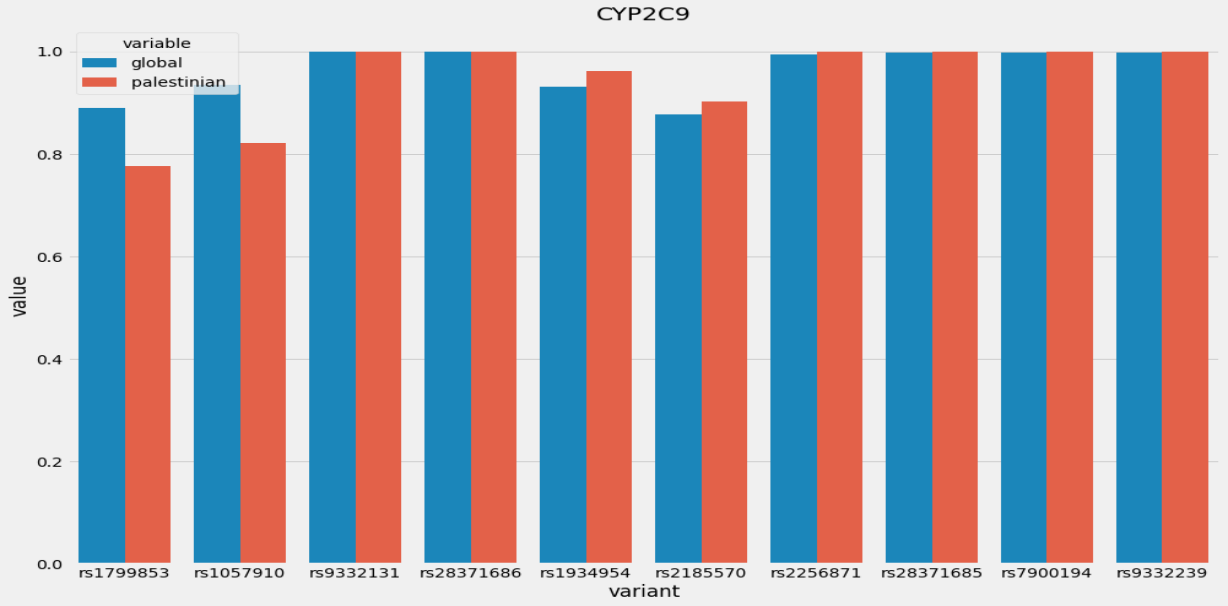


Figure 10A: The difference of CYP2C9 Ref allele frequencies between Palestinian population and the global population.

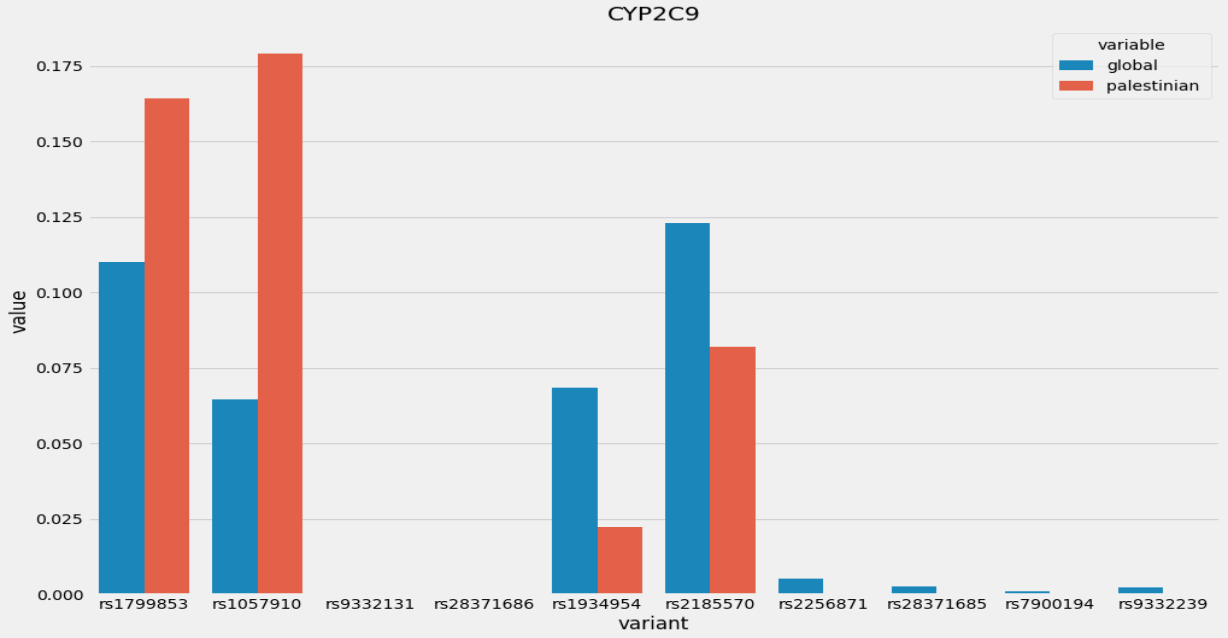


Figure 10B: The difference of the CYP2C9 Alt allele frequencies between the Palestinian population and the global population.

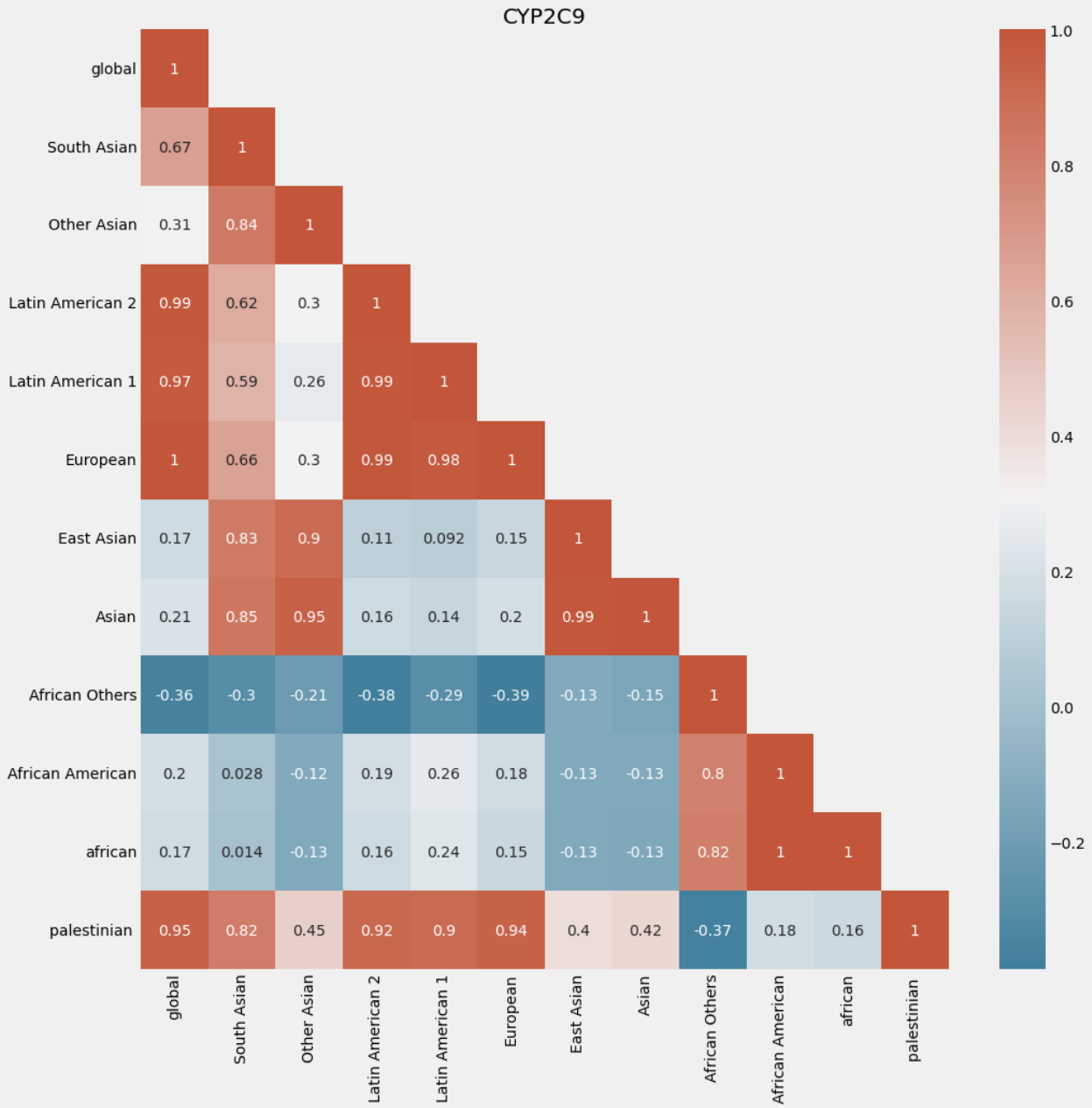


Figure 11: The correlation between the CYP2C9 enzyme frequencies between the different populations.

4.2 CYP2C19 variants frequencies

The Palestinian and the global population frequency of the Ref and Alt CYP2C19 allelic variants were compared in table 10. Only rs28399504 and rs41291556 frequencies showed a significant difference between the Palestinian population and the global population for Ref variants (p-value=0.014664745) and Alt variants (p-value=0.014664745). rs28399504 and rs41291556 frequencies between the Palestinian population and the global population were tested with other CYP2C19 variables for Ref frequencies and Alt frequencies. For Ref allele frequencies, there were statistically significant differences in rs28399504 and rs41291556 with rs4986893 (p-value= 0.049703).

n the other hand, For Alt allele frequencies, there were statistically significant differences in rs28399504 and rs41291556 with rs4986893 (p-value= 0.049703)

Table 10: The Palestinian and the global population frequency of the CYP2C19 allelic variants p-values using T-Test. (the Palestinian and global population frequencies were taken from table 7A and 7B)

allele_a	allele_b	t_value (Ref allele)	p_value (Ref allele)	t_value (Alt allele)	p_value (Alt allele)
rs4244285	rs4986893	0.3297192	0.772942778	0.453948528	0.694369255
rs4244285	rs12248560	2.3621423	0.142015222	-0.10809403	0.923788275
rs4244285	rs28399504	0.29837014	0.793564888	0.464189751	0.688138041
rs4244285	rs3814637	-0.1750411	0.877164569	2.438275786	0.134970906
rs4244285	rs41291556	0.29349558	0.79679711	0.46580299	0.687160109
rs4986893	rs12248560	0.95177667	0.441663135	-0.94055795	0.446217724
rs4986893	rs28399504	2.45795247	0.133229488	-2.45795247	0.133229488
rs4986893	rs3814637	-0.5779253	0.621712944	0.926563054	0.451969877

rs4986893	rs41291556	1.98343849	0.185775939	-1.98343849	0.185775939
rs12248560	rs28399504	0.91314654	0.457558291	-0.9022142	0.462165885
rs12248560	rs3814637	0.62770694	0.594310839	0.598952966	0.610011092
rs12248560	rs41291556	0.9071421	0.460082934	-0.89625388	0.464698444
rs28399504	rs3814637	-0.6118079	0.602948865	0.933729872	0.449014379
rs28399504	**rs41291556**	**8.16666667**	**0.014664745**	**-8.16666667**	**0.014664745**
rs3814637	rs41291556	-0.617045	0.60009169	0.934864398	0.448548405

The difference of the CYP2C19 Ref and Alt allele frequencies between the Palestinian population and the global population is shown in figure 12A and 12B. And figure 13 shows the correlation between the CYP2C19 frequencies between the different populations. According to the Kendall rank correlation coefficient, there are no high correlation (Clint= 0.34) between the Palestinian population and the global population. Other Asian population is the closest population to the Palestinian population for the CYP2C19 variants according to the data that reported in the 1000 Genomes database (Clint= 0.78). The farthest population to the Palestinian population for the CYP2C19 variants according to the data that reported in the 1000 Genomes database is the African and African American populations (Clint= 0.3) as shown in figure 13.

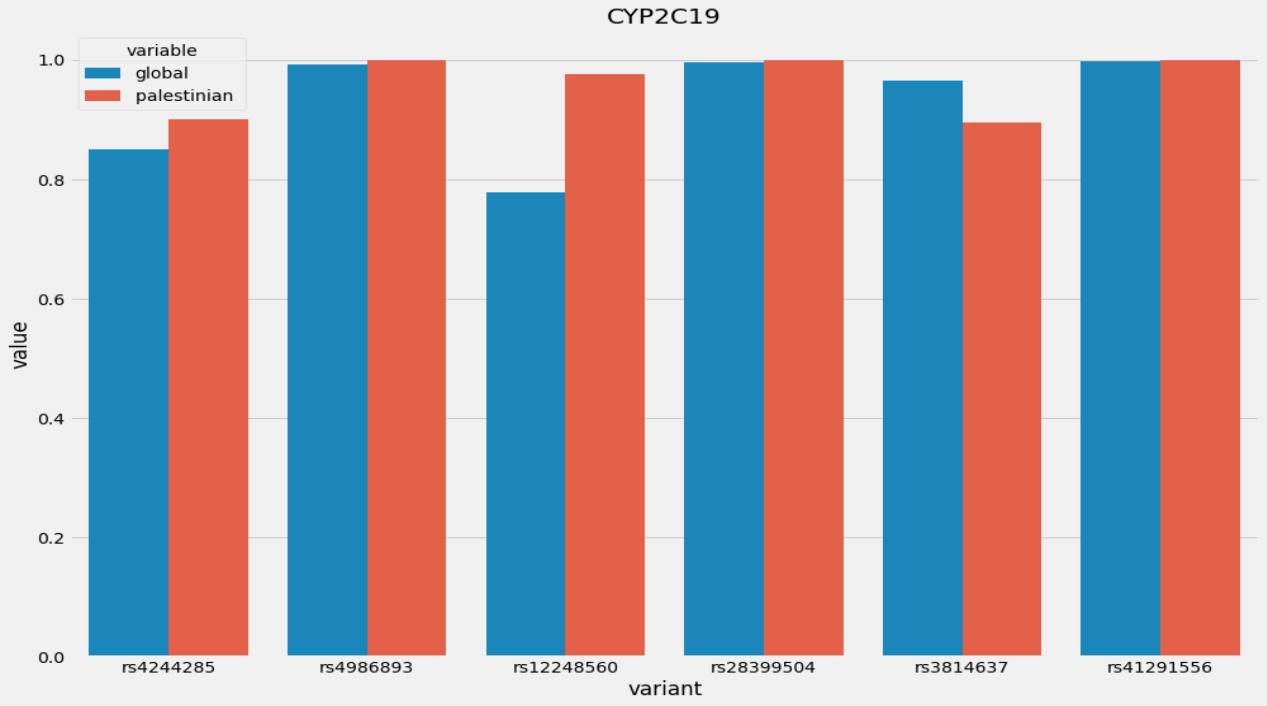


Figure 12A: The difference of the CYP2C19 Ref allele frequencies between Palestinian population and the global population.

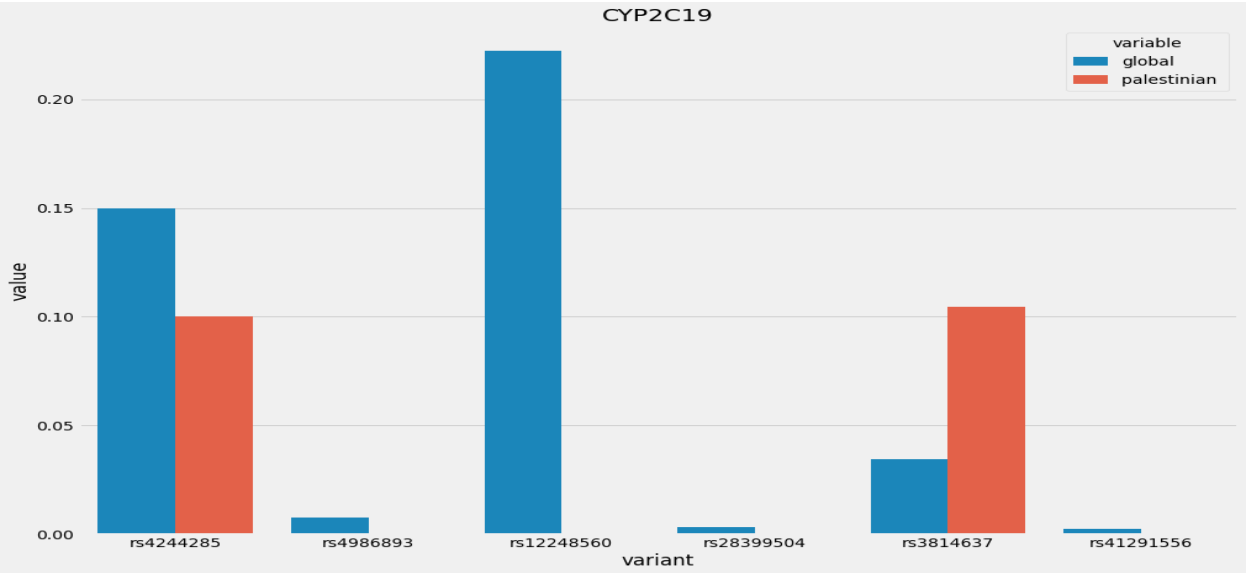


Figure 12B: The difference of the CYP2C19 Alt allele frequencies between the Palestinian population and the global population.

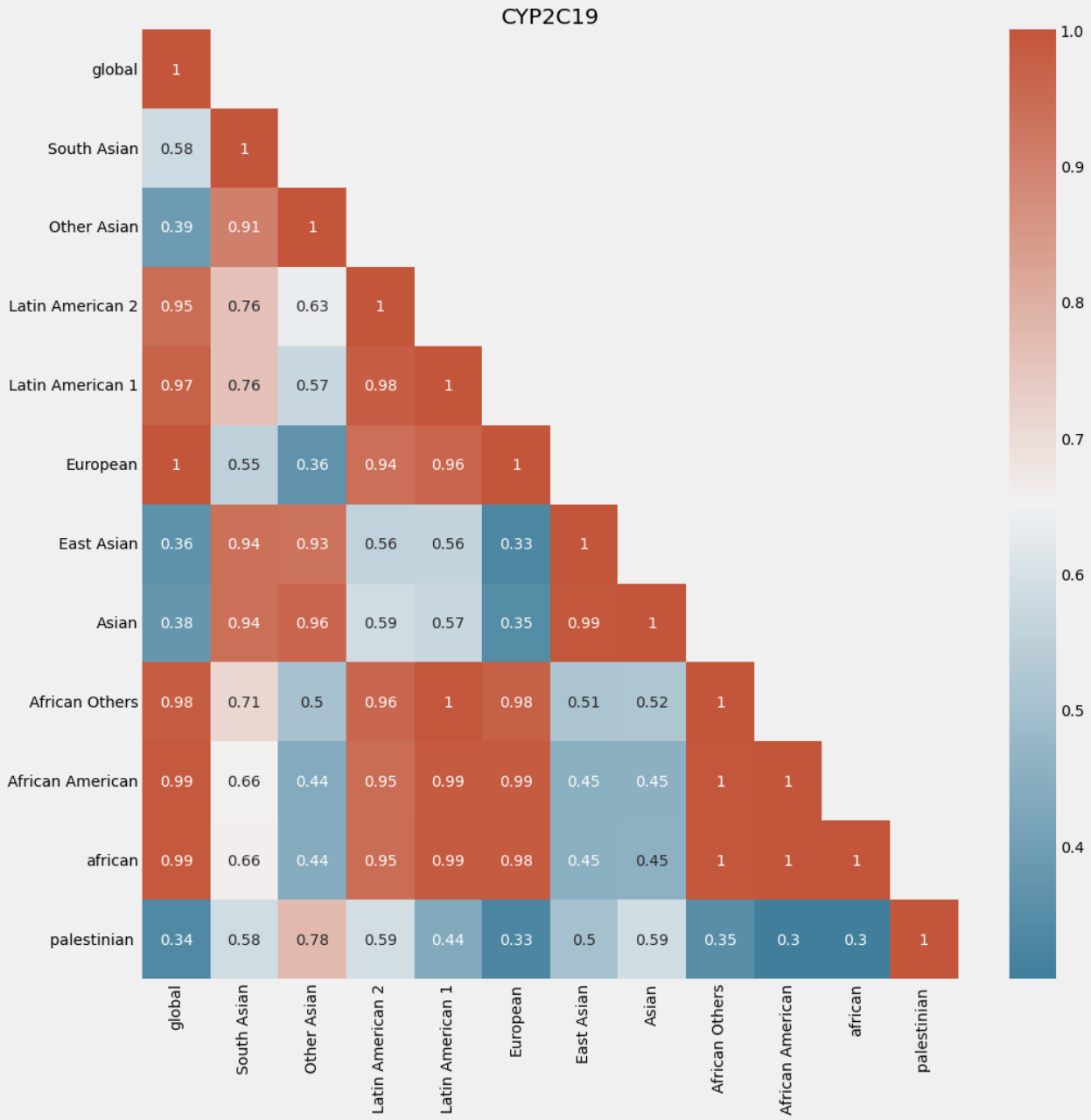


Figure 13: The correlation between the CYP2C19 enzyme frequency between the different populations.

4.3 CYP2D6 variants frequencies

The Palestinian population and the global population frequency of the Ref and Alt CYP2D6 allelic variants were compared in table 11. None of the allelic variants frequencies showed a significant difference between the Palestinian population and the global population.

Table 11: The Palestinian population and the global population frequencies of the CYP2D6 allelic variants p-values using T-Test. (the Palestinian and global population frequencies were taken from table 7A and 7B).

allele_a	allele_b	t_value (Ref allele)	p_value (Ref allele)	t_value (Alt allele)	p_value (Alt allele)
rs3892097	rs28371706	0.377220929	0.742275278	-1.00986	0.418871
rs3892097	rs1065852	0.35313798	0.757732609	0.146684	0.896832
rs3892097	rs1135840	0.121256723	0.91457199	-0.97114	0.43392
rs3892097	rs28371725	-0.273632183	0.810036024	0.151578	0.893429
rs3892097	rs5030655	0.363632453	0.750973389	-0.88576	0.469192
rs3892097	rs16947	0.249171949	0.826481537	-2.84335	0.104635
rs28371706	rs1065852	-0.097366186	0.931314305	0.435056	0.705968
rs28371706	rs1135840	-0.003385472	0.997606117	-0.49149	0.671722
rs28371706	rs28371725	-0.555566526	0.634357533	0.624107	0.596257
rs28371706	rs5030655	-0.005096228	0.996396446	-1.31355	0.319451
rs28371706	rs16947	-0.025014432	0.982314892	-0.84996	0.484865
rs1065852	rs1135840	-0.056850265	0.959833233	-0.19156	0.865772

rs1065852	rs28371725	-1.093021087	0.38847554	2.313813	0.146754
rs1065852	rs5030655	-0.080212604	0.943372137	0.454222	0.694202
rs1065852	rs16947	-0.254121695	0.823141429	-0.09363	0.933937
rs1135840	rs28371725	-0.182538033	0.871988058	-0.19035	0.866605
rs1135840	rs5030655	0.001175522	0.999168781	-0.46593	0.687081
rs1135840	rs16947	-0.028492732	0.979856684	-1.66714	0.237416
rs28371725	rs5030655	-0.493290989	0.67065125	0.654582	0.579954
rs28371725	rs16947	-0.453087368	0.694895031	-0.16159	0.886475
rs5030655	rs16947	-0.016103481	0.988613858	-0.78666	0.513894

The difference of the CYP2D6 Ref and Alt allele frequencies between the Palestinian population and the global population is shown in figure 14A and 14B. And figure 15 shows the correlation between the CYP2D6 frequencies between the different populations. According to the Kendall rank correlation coefficient, there are a high correlation (Clint= 0.96) between the Palestinian population and the global population. Latin American 1 population is the closest population to the Palestinian population for the CYP2D6 variants according to the data that reported in the 1000 Genomes database (Clint= 0.98). The farthest population to the Palestinian population for CYP2D6 variants according to the data that reported in the 1000 Genomes database is the east Asian population (Clint= 0.75) as shown in figure 15.

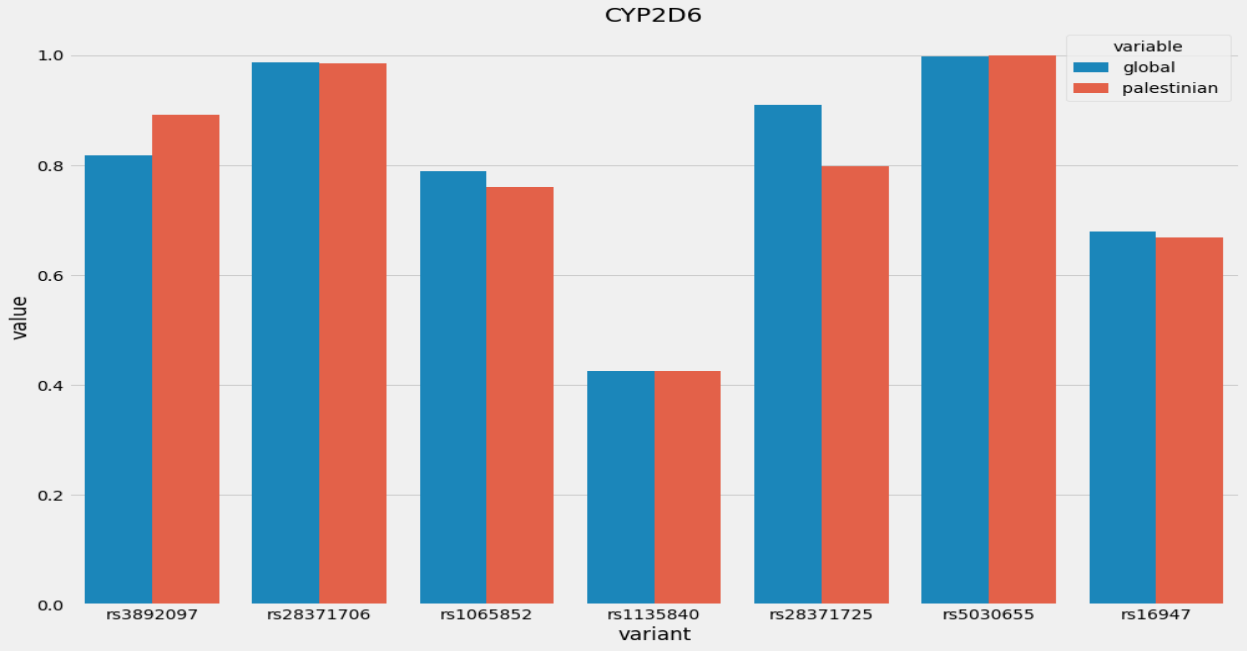


Figure 14A: The difference of the CYP2D6 Ref allele frequencies between the Palestinian population and the global population.

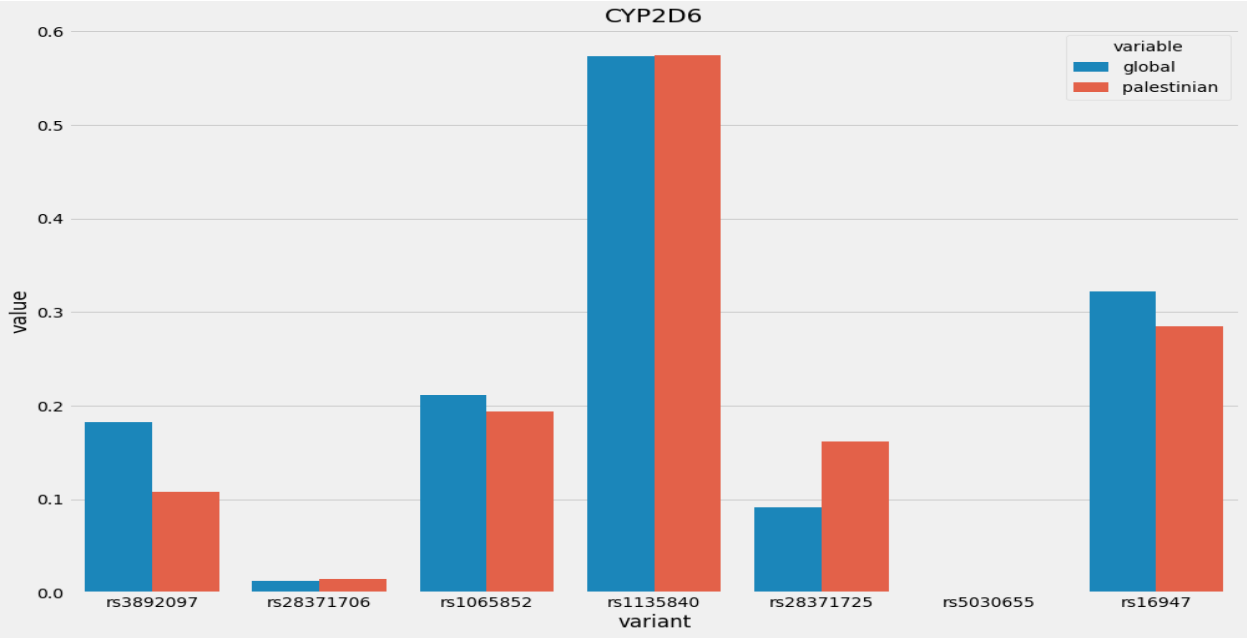


Figure 14B: The difference of the CYP2D6 Alt allele frequencies between the Palestinian population and the global population.

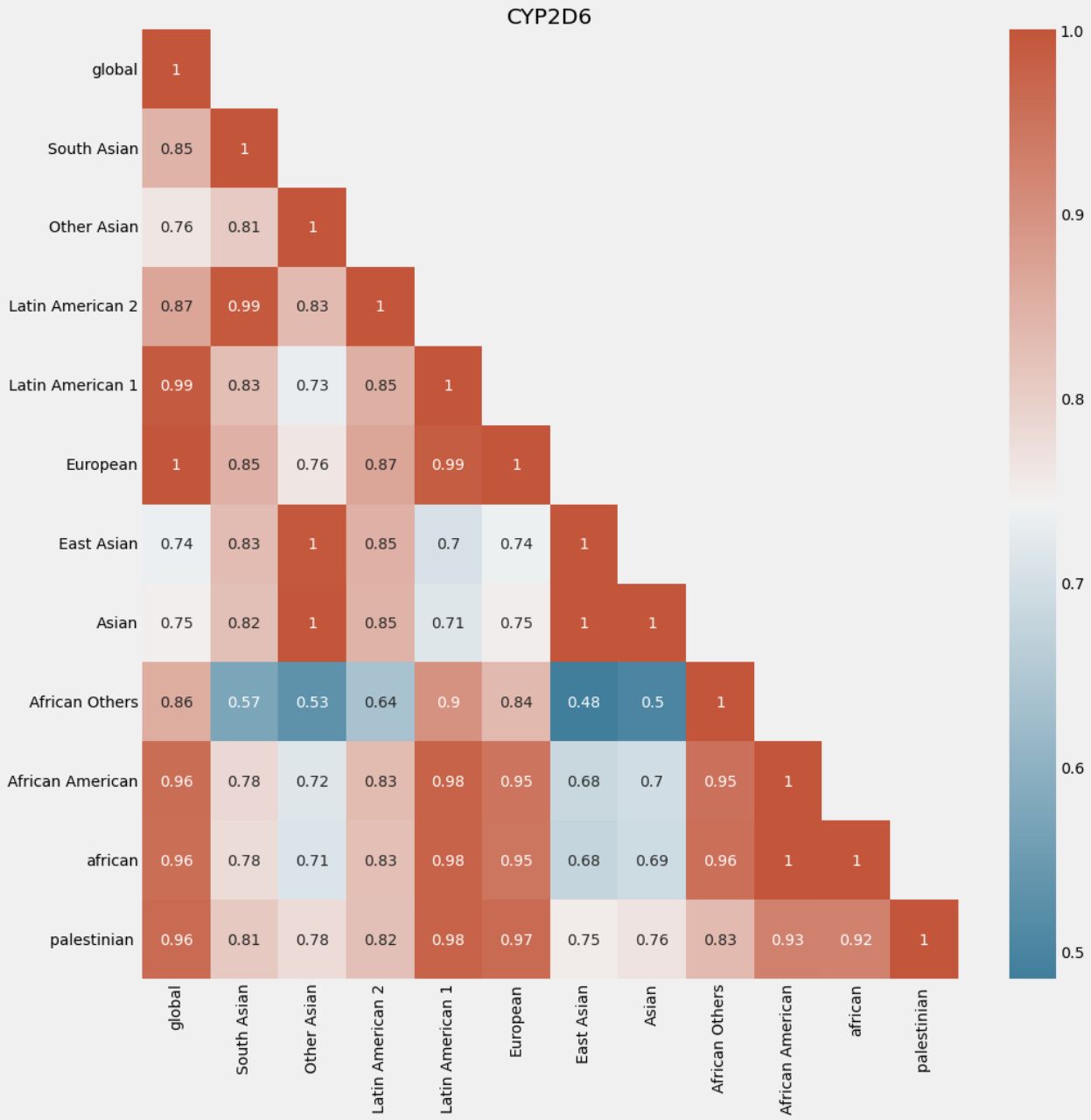


Figure 15: The correlation between the CYP2D6 enzyme frequencies between the different populations.

5 Discussion:

The genetic variables that determine CYP450 levels and activities are crucial to understand because inter-individual variance in CYP450 expression can lead to significant diversity in drug responsiveness, drug activity, or detoxification (Ganoci, 2017).

The observed interethnic difference in exposure could be due to differences in enzyme activity for subjects in each ethnic group with the same activity classification, or it could be due to interethnic differences in other determinants of drug pharmacokinetics, such as net absorption, body weight, or parallel elimination pathways (Xie, 2002). Furthermore, some medicines, such as amitriptyline, are metabolized by many polymorphic enzymes, each of which has documented interethnic activity variations (Zhang, 2019). As a result, changes in these additional pharmacokinetic factors between ethnic groups will contribute to the relative exposures reported in persons with the same CYP2C9, CYP2C19, or CYP2D6 activity (Xie, 2002).

The impact of DDIs on metabolizing pathways including CYP2C9, CYP2C19, and CYP2D6 is greatly influenced by the patients' genotype (Bahar, 2017). The greater the gene's involvement to drug metabolism, the greater the disruption caused by perpetrator medicines (Bahar, 2017). In general, the interaction distortion is greatest in NMs, least in IMs, and least in PMs (Storelli, 2018). PMs don't have any metabolic activities, therefore an effector medicine won't have much of an effect on them (Storelli, 2018). The gene is the most important determinant of the relationship in this category (Storelli, 2018). As a result, applying DDI principles to these people as a rule may lead to medication management errors. Furthermore, DDIs can only affect a single genotype (Storelli, 2018). CYP2C9*3 is the only enzyme that mediates the interaction between simvastatin and warfarin. As a result, only patients with this genotype require particular care (Storelli, 2018).

In addition, the NSAID and coumarin interaction demonstrates the combined influence of polymorphism and effector medication on the effects of a DDI (Malki, 2020). In coumarin-treated individuals, NSAIDs or reduced CYP2C9 activity do not enhance the risk of bleeding (Storelli, 2018). Co-administration of an NSAID with a coumarin, on the other hand, greatly

increases the risk of hemorrhage in individuals with a less active CYP2C9. As a result, genotyping is critical for managing the DDI (Malki, 2020).

The clinical impact of metabolites is another factor to consider in DDI (Malki, 2020). The addition of an inhibitor will not significantly modify the pharmacodynamic effect of the medication in NMs and PMs if the influence of the parent drug and its metabolite is equivalent, despite pharmacokinetic changes (Malki, 2020). The active moiety concentration accounts for pharmacokinetic values that vary due to the influence of interfering drugs and polymorphisms (Bahar, 2017).

In substrates metabolized by numerous routes, polymorphism is also important, resulting in DDGIs (Malki, 2020). The relevance of the secondary metabolic route grows as the primary metabolic pathway of substrates is impaired due to polymorphism (Storelli, 2018). As a result of the subsequent obstruction of the secondary metabolic pathway, the plasma concentration of the substrates rises dramatically (Storelli, 2018). Nonetheless, depriving the secondary route in a sequential manner without affecting the main pathway results in a less severe metabolic deficit. A drug transporter might also be implicated in the drug interaction (Storelli, 2018). The interaction between clarithromycin and PPI has been observed to exist in all genotypes (Storelli, 2018).

However, because the baseline concentration of PPIs is genotype dependent, the substrate's net drug exposure following the addition of the inhibitor is similarly genotype dependent (Bahar, 2017). The number of functioning CYP alleles determines the amount of DDGI. PMs are more likely than IMs and NMs to create DDGI (Bahar, 2017).

In this research, we genotyped variants that related in drug absorption, distribution, metabolism, and excretion (Ganoci, 2017). Thus, the three enzymes found in the Palestinian population, CYP2C9, CY92C19, and CYP2D6, were linked to medicines used to treat cardiovascular conditions (statins, beta-blockers, anticoagulants, and antiplatelet agents). CYP2D6 overlaps with four distinct therapeutic areas; which are cancers, psychiatric, primary care, and cardiovascular (Taylor, 2020). However, CYP2C19 overlaps with four distinct therapeutic areas;

which are antimicrobial, psychiatric, primary care, and cardiovascular (Taylor, 2020). CYP2C9 overlaps with only one distinct therapeutic area which is the cardiovascular (Taylor, 2020) as shown in figure 16.

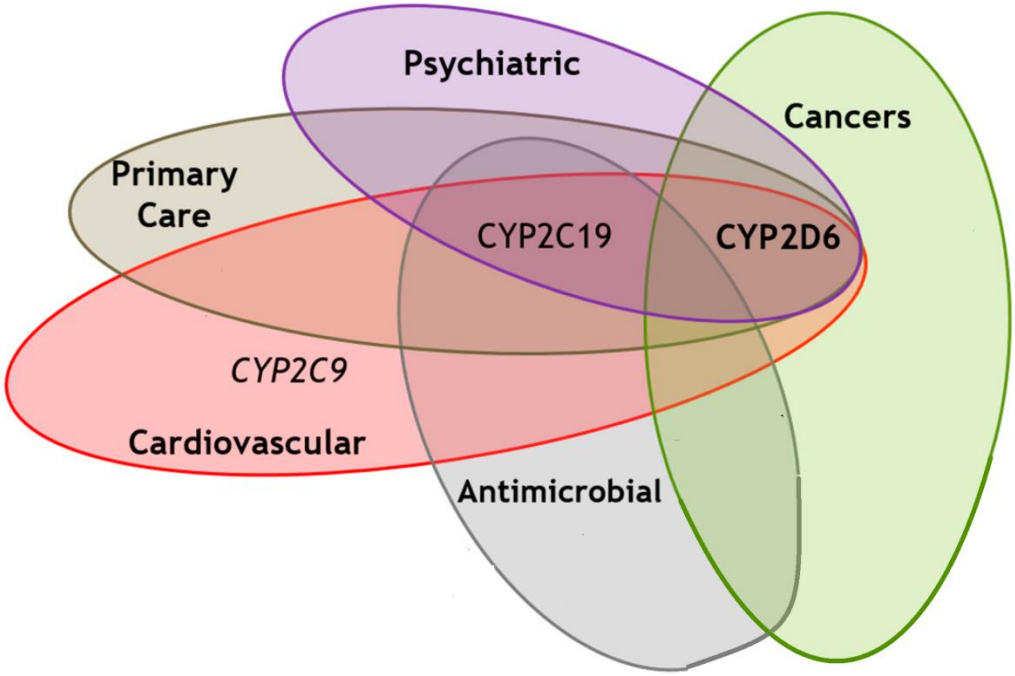


Figure 16: Venn Diagram showing recognized (non-HLA) pharmacogenes according to their association with drugs used in different therapeutic areas.

Genotyping of 23 variants from 67 DNA samples showed that 10 variants didn't have the Alt allele (Table 8) meaning that the entire population sample has the same genotype at that locus. The frequencies of many of these variants were very high in other populations as well. However, we could not conclude that the Alt allele for these variants did not occur (or were only present in a very low frequency) in the Palestinian population. In addition, 21 of 23 variants identified in the Palestinian population were not in HWE. We expect that this result because at least one of the five basic Hardy-Weinberg assumptions: no mutation, random mating, no gene flow, infinite population size, and no selection didn't happen (Zhang, 2019). The highest probability is that there is a natural selection against these alt allele variables. The rs3892097 (CYP2D6*4) allele is

in HWE in the Palestinian population. Researchers have proposed that the CYP2D6*4 allele has a variety of potential implications, both positive and negative (Chen, 1995).

On the plus side, this genotype may lower the incidence of certain cancers, such as bladder and lung tumors, and it may be linked to milder Alzheimer's neurodegeneration (Chen, 1995). On the other hand, at least two studies have found that carrying the CYP2D6*4 allele increases the chance of acquiring Parkinson's disease when exposed to pesticides by 3 to 8 times (Deng, 2004). The risk to CYP2D6*4 carriers appears to be proportional to the degree of pesticide exposure, with no additional risk of Parkinson's disease seen in CYP2D6*4 carriers who have never been exposed to pesticides and the highest increased risk of Parkinson's disease seen in CYP2D6*4 carriers who have been exposed to pesticides frequently (Iskakova, 2016).

The rs2185570 polymorphism in the CYP2C9 gene region is linked to serum Dehydroepiandrosterone sulphate levels (Johnson, 2008). This variation has significant linkage disequilibrium with rs4086116 and rs4917639, which have been linked to acenocoumarol and warfarin maintenance dose in recent genome-wide association studies, respectively (Zhai, 2011). These results need more exploration as they might be new results and need more studies, or it might be that we didn’t search enough in the literature to explain them.

5.1 CYP2C9

There are clinically significant interactions caused by DDI mediated by CYP2C9 (Bahar, 2017). First, as with NSAIDs and coumarin interactions, co-administration of CYP2C9 substrates induced a competitive inhibition in individuals with CYP2C9 variant alleles (Bahar, 2017). Because they are both CYP2C9 substrates, NSAIDs tended to reduce coumarin metabolism much more in individuals with low CYP2C9 activity (Bahar, 2017). As a result, NSAIDs increased the incidence of overanticoagulation in CYP2C9*1/*2 and CYP2C9*1/*3 patients treated with coumarin (Bahar, 2017).

Second, as seen in the warfarin and simvastatin interaction, a CYP2C9*3 selective inhibition exacerbated the metabolic interference caused by genetic polymorphism (Bahar, 2017). Warfarin was metabolized less efficiently by CYP2C9*3, and simvastatin, which inhibited CYP2C9*3 specifically, produced an even greater impairment in warfarin metabolism (Bahar, 2017). As a

result, individuals with CYP2C9*3 needed a larger warfarin dosage decrease than noncarriers (Bahar, 2017). CYP2C9*2 was likewise said to have had no part in this interaction (Bahar, 2017).

Third, as with fluconazole and flurbiprofen interactions, the extent of the interaction was determined by CYP2C9 activity (Bahar, 2017). Fluconazole reduced flurbiprofen metabolism in NMs and IMs of CYP2C9 more effectively than in PMs. Fluconazole's inhibitory power was boosted when the dosage was increased from 200 to 400 mg, as evidenced by a twofold rise in the AUC (area under curve) value in NMs and IMs but not in PMs (Bahar, 2017). In PMs, the diminished function of CYP2C9*3 resulted in resistance to inhibition (Bahar, 2017). Fluconazole (at a dosage of less than 200 mg) may inhibit the remaining metabolic pathways of flurbiprofen (CYP2C19), therefore there was still an inhibition of fluconazole in PMs (Bahar, 2017).

The frequencies of CYP2C9 alleles in the Palestinian population are similar to the global except for rs9332239 and rs28371685. The frequencies of rs9332239 and rs28371685 Ref alleles in this study (1 for both) are slightly higher than the global frequency (0.997647, and 0.997368, respectively). While the frequencies of rs9332239 and rs28371685 Alt alleles in this study (0 for both) are slightly lower than the global frequency (0.002353, and 0.002632, respectively). The frequency of rs9332239 and rs28371685 Ref allele increases rapidly in the direction of Asia, being very low in Latin American 1 populations (0.994, and 0.9961, respectively). rs9332239 and rs28371685 shows the highest frequency among Asians, other Asian, and east Asian populations (1 for each).

The impact of CYP2C9 genetic diversity on warfarin therapeutic dose requirements has been extensively researched (Seven, 2014). The rs9332239 allele is defined by a missense variant in exon 9 (NM_000771.3:c.1465C>T, p.Pro489Ser, rs9332239), it is also known as CYP2C9*12 (O'Brien, 2013). An inquiry into this variant was motivated by a finding that it produced an enzyme with lower activity towards tolbutamide in vitro (O'Brien, 2013) (Seven, 2014). To our knowledge, there have been no reports of this single nucleotide polymorphism being linked to any other CYP2C9 substrates in clinical samples (Seven, 2014). The absence of clinical data on

rs9332239 is most likely due to its uncommon occurrence in the general population (O'Brien, 2013).

The rs28371685 polymorphism derives from a C1003T mutation in exon 7, resulting in an Arg335Trp substitution, also known as CYP2C9*11 (Allabi, 2004) (Sangkuhl, 2021). Although the functional significance of CYP2C9*11 has yet to be determined, it is believed that CYP2C9 activity in heterozygous genotypes is lower than in homozygous wild-type genotypes (Allabi, 2004).

5.2 CYP2C19

CYP2C19-mediated DDIs engage a variable degree of interaction in different genotypes (Bahar, 2017). The first was a competitive inhibition between two CYP2C19 substrates caused by metabolic route segregation in normal CYP2C19 metabolizers but not in slow CYP2C19 metabolizers (Bahar, 2017). The metabolic activity of CYP2C19 was reduced due to polymorphism in the IMs and PMs. As a result, there was little evidence of competitive binding to this isoenzyme (Bahar, 2017). It was discovered when PPIs and clopidogrel were used together (Bahar, 2017). Clopidogrel effectiveness was considerably impacted by omeprazole and rabeprazole, but not by lansoprazole, in NMs but not in IMs or PMs (Bahar, 2017).

Because lansoprazole has a lesser affinity for CYP2C19 than omeprazole, it did not show a similar gene-dependent interaction (Bahar, 2017). As a result, it had a lower inhibitory efficacy against clopidogrel bioconversion (Bahar, 2017). Rabeprazole, which has a low affinity for CYP2C19, exhibited a potency that was equivalent to omeprazole (Bahar, 2017). This might be because rabeprazole, together with its nonenzymatic metabolite, rabeprazole thioether, was metabolized by CYP2C19, resulting in a higher competitive inhibition of clopidogrel than lansoprazole (Bahar, 2017).

Furthermore, PPIs reduced the clopidogrel impact in CYP2C19*17 homozygotes carriers more than CYP2C19*2 and CYP2C19*1 homozygotes carriers (Bahar, 2017). Because CYP2C19*17 is linked to increased activity, it appears that the greater the involvement of CYP2C19 in clopidogrel bioconversion, the greater the change caused by PPIs (Bahar, 2017). The PPI–warfarin interaction was also discovered to have a competitive inhibitory mechanism (Bahar,

2017). Hemorrhagic problems were more common in CYP2C19 IM patients who took lansoprazole and warfarin at the same time as in NMs and PMs (Bahar, 2017). The combination of polymorphism and lansoprazole inhibition resulted in a decrease in warfarin metabolism, which raised the risk of bleeding (Bahar, 2017). Furthermore, bahar et al. found that omeprazole decreased R-warfarin metabolism in NMs but not S-warfarin (CYP2C9 substrate) metabolism (Bahar, 2017). As a result, CYP2C19 is one of the elements that determines how effective warfarin is (Bahar, 2017).

The second was ticlopidine and omeprazole interaction-induced phenoconversion of the CYP2C19 NM genotype to the PM phenotype (Bahar, 2017). Ticlopidine significantly reduced omeprazole metabolism in NMs and IMs, resulting in pharmacokinetic values comparable to PMs. PMs, on the other hand, were unaffected (Bahar, 2017). Moclobemide and omeprazole, which showed a significant interaction in NMs but not in PMs, similarly demonstrated the gene-dependent interaction (Bahar, 2017).

Oral contraceptives (OCs) and omeprazole interaction revealed that CYP2C19 inhibition mostly affected NMs, with less pronounced CYP2C19 isoenzymes with higher activity (Bahar, 2017). OC inhibited omeprazole metabolism in CYP2C19*1/*1, but not in CYP2C19*1/*17 or CYP2C19*17/*17, by significantly raising the metabolic ratio of omeprazole/5hydroxyomeprazole (Bahar, 2017). It was thought that CYP2C19*17 provided resistance to the inhibitory impact of OC (Bahar, 2017).

The frequencies of CYP2C19 alleles in the Palestinian population are similar to the global except for rs28399504 and rs41291556. The frequencies of rs28399504 and rs41291556 Ref alleles in this study (1 for both) are slightly higher than the global frequency (0.99681, and 0.997506, respectively). While the frequencies of rs28399504 and rs41291556 Alt alleles in this study (0 for both) are slightly lower than the global frequency (0.00319, and 0.002494, respectively). The frequency of rs28399504 and rs41291556 Ref allele increases rapidly in African others population being very low in European population (0.996702, and 0.997328, respectively). rs28399504 and rs41291556 shows the highest frequency among Asian, South Asian, other Asian, East Asian, and African others populations (1 for each).

The rs28399504 and rs41291556 variants cause loss of function. The rs28399504 polymorphism derives from a (c.A1G; transition in the initiation codon). rs28399504 also known as CYP2C19*4 (Santos, 2011). However, rs41291556 is a missense variant affecting the Clopidogrel response; Synonyms include poor metabolism of; Plavix response (Scott, 2011).

Although the clinical significance of the CYP2C19 polymorphism has yet to be determined, it is possible that PMs are more prone to have adverse effects from medicines that are substrates for the enzyme if administered at regular dosages (Seven, 2014). It's also possible that medications that are bioactivated to therapeutically active metabolites, such as proguanil to cycloguanil, will provide less therapeutic effect to PMs (Xu, 2018). The most prevalent variations among Palestinians are rs4986893, rs28399504, and rs41291556 (Goldstein, 1994). It's worth noting that this defect occurs at a comparable and reasonably high frequency in multiple ethnic groups, suggesting that this harmful mutation is rather old and happened before the Black, East and Southeast Asian, and Caucasian racial groupings divided (Dorji, 2019). The lack of rs4986893 in our analysis, on the other hand, emphasizes the ethnic divide between Caucasian and East and Southeast Asian groups, supporting the Asian uniqueness of this allelic variation, whose frequency is very low or nonexistent in various Caucasian communities (Céspedes-Garro, 2016; Dorji, 2019). These data strongly imply that the rs4986893 mutation happened recently, just after the Caucasian and Oriental ethnic groups split apart (Céspedes-Garro, 2016). Because both variations are inactivating, this has implications for the administration of medications like clopidogrel, which rely on CYP2C19 for activation (Bergmeijer, 2018).

In Mediterranean-South Europeans and Middle Easterners, rs12248560 (CYP2C19*17) was shown to be more common than in East Asians (Dorji, 2019). Although rs12248560 and are only expected to predict UM, it has been shown to increase the rate of metabolism of medication substrates such as omeprazole and various antidepressants in UM patients (Pratt, 2018; Koopmans, 2021). The rs12248560 allele was found to be strongly related with an elevated risk of bleeding in individuals using clopidogrel, with rs12248560 homozygous patients having the highest risk (Ahmad, 2017).

Although the Kendall rank correlation coefficient for CYP2C19 between the Palestinian population and the global is not high correlation (Clint= 0.34), but this value is meaningless as there are no statistically significant differences between the Palestinian population and the global. This value could be a result for taking some variants of the gene, and not the entire gene.

5.3 CYP2D6

Different DDI magnitudes are disrupted in CYP2D6 with polymorphism than in normal metabolic activity (Bahar, 2017). First, as shown in dextromethorphan and CYP2D6 inhibitors (terbinafine, perhexiline, and quinidine) interactions, the interaction between CYP2D6 inhibitors and substrates primarily included individuals with functioning CYP2D6 (Bahar, 2017). Similar to the impact of genetic impairment, CYP2D6 inhibitors raised the metabolic ratio of dextromethorphan/dextromethorphan in NMs and IMs but not in PMs (Bahar, 2017). Individuals with one functional allele phenoconverted NM and IM genotypes to PM phenotypes at a greater rate than those with at least two functional alleles (Bahar, 2017). Quinidine was also discovered to inhibit genes selectively when combined with propafenone, venlafaxine, mexiletine, brofaromine, methoxyphenamine, encainide, procainamide, and flecainide (Bahar, 2017).

Metoprolol and several CYP2D6 inhibitors created other genetically determined DDIs (Bahar, 2017). In NMs and IMs, diphenhydramine and dronedarone significantly slowed metoprolol metabolism, but not in PMs (Bahar, 2017). As a result, they only had a substantial impact on metoprolol-treated NMs and IMs' heart rate profile and systolic blood pressure (Bahar, 2017). Furthermore, in their interactions with metoprolol, amiodarone and celecoxib revealed that NMs are more deeply influenced by the DDIs than IMs (Bahar, 2017). Furthermore, diphenhydramine and other CP2D6 inhibitors, such as thioridazine and propafenone, inhibited venlafaxine, mianserin, and mexiletine metabolism to a higher amount in CYP2D6 with completely functioning alleles than in those with decreased or defective alleles (Bahar, 2017).

CYP2D6 inhibitors reduced prodrug bioconversion to a higher extent in those who had two normal function alleles than in people who only had one (Bahar, 2017). Interactions between CYP2D6 inhibitors (paroxetine, amiodarone, cimetidine, and ranitidine) and tramadol, as well as

levomepromazine and codein, shown this (Bahar, 2017). In UMs, NMs, and IMs, CYP2D6 inhibitors significantly reduced the generation of (+)O-desmethyl tramadol, tramadol's active metabolite (Bahar, 2017). The concentration of (+)O-desmethyl tramadol was found to be lower in NMs than in IMs (Bahar, 2017). This meant that the more active alleles there were, the more inhibition there was (Bahar, 2017). The results of the UMs, on the other hand, did not support this notion. This was most likely due to the fact that the research only included two UMs (Bahar, 2017). Furthermore, the second encounter yielded similar outcomes (Bahar, 2017). In NMs, but not in IMs, levomepromazine strongly blocked the O-demethylation of codeine from producing morphine (Bahar, 2017).

CYP2D6 is one of the most well-studied polymorphism genes in the field of pharmacology, and its clinical importance has been well researched. The frequencies of CYP2D6 alleles in the Palestinian population are similar to the global. CYP2D6 has the most various variables between the CYP450 enzyme in the global and the Palestinian population. rs1065852 has the most various frequencies between the populations, ranging between 0.41 in other Asian population and 0.948 in African others population. These changes can contribute to disparities in the pharmacological action of CYP2D6 substrate medications among the other Asian groups since the enzyme activity of the rs1065852 allele is greatly reduced.

The rs1135840 variant's Ref allele is (G). The (C) variation indicates the presence of a non-wild type CYP2D6 variant; however it can't be used to detect the presence of any specific variant because it exists in so many distinct versions (Del Tredici, 2018). The frequency of the Ref allele in the Palestinian population is 0.425373134 which is a lower frequency than the Alt allele. . rs1135840 has low frequencies in all different populations, ranging between 0.28 in Asian population and 0.577 in Latin American 2 population. This might be a Silent mutation, which have no effect.

Recent breakthroughs in the molecular genetics of drug metabolism in general, and CYPs in particular, have raised expectations for the application of pharmacogenetics testing in medication treatment optimization (Seven, 2014). While the pharmacokinetic effects of polymorphic metabolism are fairly well understood for many medicines, research on its relevance in terms of

therapeutic response and dose is lacking (de Leon, 2006). For 14 distinct antidepressant medications, the first tentative average dosage suggestions based on CYP2D6 or CYP2C19 genotypes have been published (Sim, 2006). The guidelines are based on a thorough analysis of research that included pharmacokinetic data on several antidepressants in PM, EM, and UM patients (Sim, 2006). This is the first time pharmacogenetics information has been applied to actual dosage recommendations. To test these guidelines, prospective clinical trials with large patient populations and accurate outcome monitoring are necessary.

In the current study, the pharmacogenetics of drug metabolizing CYPs among Palestinian community was investigated. All participants in the study were Palestinian citizens from various regions in Palestine and were good representations of the Palestinian people. There is a scarcity of information on the frequency of CYPs polymorphisms among Palestinians. We presented data on the frequencies of several factors for CYP2C9, CYP2C9, and CYP2D6 for the first time in this study. Our findings indicate the frequency of the polymorphisms under investigation is similar to those reported globally in the 1000 Genomes database.

6 Conclusion:

Interethnic disparities in medication pharmacokinetics might have therapeutic implications, therefore understanding the underlying processes are crucial. Different ethnic groups have different frequencies of functionally relevant alleles of the well-researched drug-metabolizing enzymes CYP2C9, CYP2C19, and CYP2D6 (Tables 1,2,5). Many therapeutically relevant medicines are eliminated by these enzymes, and interethnic variations in pharmacokinetics have been identified for some drug substrates. Interethnic variations in other variables of drug pharmacokinetics, on the other hand, can be key causes of exposure disparities among groups.

Polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters, drug receptors, and drug targets all contributed to drug response variability. Detection of all of these drug response-related genetic variants (at least tens of thousands) leads to the identification of the best treatment and dose for each individual, and may eventually lead to personal medicine. The determination of allele distribution pattern might be useful in the optimization of

pharmacological therapy when considering the pharmacological consequences of the CYP2C9, CYP2C19, or CYP2D6 genetic polymorphism. As a result, we first assessed the genotype profiles of the CYP2C9, CYP2C19, and CYP2D6 in a random Palestinian community by screening for the major allelic variations and comparing their frequency to earlier results in other groups throughout the world. The frequency of the CYP2C9, and CYP2D6 between the Palestinian population and the global population shows a high correlation (0.95, and 0.96 respectively), and a low correlation for the CYP2C19 (0.34).

To conclude, our data supports the presence of interethnic variations in the allele and genotype frequencies of the CYP2C9, CYP2C19, and CYP2D6. Further studies are needed to determine the therapeutic importance of these changes in terms of treatment outcome and medication dose for medicines metabolized by these polymorphic enzymes. Identification of people with altered pharmacokinetics for the CYP2C9, CYP2C19, or CYP2D6 substrates is potentially clinically important so that proper dose regimens for these medications may be established and adverse drug responses avoided.

7 Limitations:

Our findings contributed to the development of personalized medicine in the Palestinian population by allowing for the genotyping of specific variants. There were a few flaws in our research. For starters, we only had a small number of samples, which may or may not be representative of the community. Furthermore, a comparative investigation of the frequency of variations in various Palestinians would be helpful in determining if mixing samples from all Palestinians is appropriate. We intend to expand the number of samples in future research and evaluate additional variations and their enzymatic activity. In addition, Limitation of the number of the studied variants considered as a Study restriction.

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