THE ASSOCIATION OF *CYP3A4, CYP3A5* AND *MTHFR* GENES POLYMORPHISM ON THE EFFICACY AND SAFETY OF ATORVASTATIN AMONG EGYPTIAN POPULATION

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by

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LIST OF SYMBOLS

a	The intercept
b	The slope
C	Plasma concentration
D	Dose
F	Oral bioavailability
J	Youden index
k	Elimination rate constant
ka	Absorption rate constant
Ke	elimination rate constant
T	Dosing interval (hours)
$t_{½}$	The terminal plasma half-life
Vd	Volume of distribution
x	The concentration of atorvastatin
*	Statistically significant

LIST OF ABBREVIATIONS

ACC	The American College of Cardiology
AE buffer	Elution buffer
AHA	The American Heart Association
AL buffer	Lysis buffer
ALT	Alanine aminotransferase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of variance
ASCVD	Atherosclerotic cardiovascular disease
AST	Aspartate aminotransferase
ATV	Atorvastatin
AUC	Area under the concentration-time curve
$AUC_{(0\text{-}inf)}$	Area under the plasma concentration-time curve from time zero to infinity
$AUC_{(0-\tau)}$	Area under each drug concentration-time curve from dosing to the end of the dosing interval (τ =24 h)
AUROC	Area under the receiver operating characteristic curve
AUP	Area under the peak
AW1 buffer, AW2 buffer	Washing buffers
β	Regression Coefficient
BMI	Body mass index
BSA	Bovine serum albumin
CAD	Coronary artery disease
CE	Collision energy
CHD	Coronary heart disease
CHO	Cholesterol
95% CI	95% Confidence interval
CK	Creatine kinase
CL	Drug Clearance
CL/F	Apparent oral clearance
Cmax	Maximum plasma concentration
CML	Chronic myeloid leukemia
CPIC	Clinical Pharmacogenetics Implementation Consortium

CV	Cardiovascular
CV%	Coefficient of variation
CVDs	Cardiovascular diseases
CXP	Collision cell exit potential
CYPs	Cytochromes P450 enzymes
DP	Declustering potential
DRESS	Drug rash with eosinophilia and systemic symptom
EP	Entrance potential
ESI	Electrospray ionization
FDA	US Food and Drug Administration
FH	Familial hypercholesterolemia
FLD	Fatty liver disease
GM	Geometric mean
HbA1c	Glycated hemoglobin
Hcy	Homocysteine
HDL-C	High-density lipoprotein cholesterol
HMG-CoA	Hydroxymethylglutaryl-CoA
HMOX1	Heme oxygenase-1 gene
HQC	Higher quality control
HWE	Hardy-Weinberg Equilibrium
IQR	Interquartile range
IS	Internal standard
LDL-C	low-density lipoprotein cholesterol
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LQC	Lower quality control
MGB	Minor groove binder
Min – Max	Minimum value - Maximum value
MLR	Multivariate regression
MQC	Middle quality control
MRM	Multi-reaction monitoring
MTHFR	Methylenetetrahydrofolate reductase
MVA	Mevalonate
NA	Not applicable

NFQ	Non-fluorescent quencher
NR	Not reported
OATP	Organic anion transporter polypeptides
OR	Odds ratio
PAD	Peripheral artery disease
PASS	Power Analysis and Sample Size Software
POR*28	P450 oxidoreductase *28
QC	Quality control
r	Correlation coefficient
R^2	Coefficient of determination
ROC	Receiver operating characteristic curve
ROX	Carboxy-X-rhodamine
RSD	Relative standard deviation
RT-PCR	Real-time quantitative polymerase chain reaction
SAM	S-adenosylmethionine
SD	Standard deviation
SEM	Standard error of the mean
SLCO	Solute carrier organic anion 'superfamily of transporters'
SNP	Single-nucleotide polymorphism
S/N	Signal-to-noise ratio
SPSS	Statistical Package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp)
SRM	Selected reaction monitoring
TB	Total bilirubin
TC	Total cholesterol
TG	Triglyceride
TIA	Transient ischemic attack
Tm	Melting temperature
Tris-HCl	Tris(hydroxymethyl)aminomethane
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
UP	Ultra-pure
USPSTF	United States Preventive Services Task Force
UTIs	Urinary tract infections

LIST OF APPENDICES

HUBUNGKAIT POLIMORFISME GEN CYP3A4, CYP3A5, DAN MTHFR KE ATAS KEBERKESANAN DAN KESELAMATAN ATORVASTATIN DALAM KALANGAN POPULASI MESIR

ABSTRAK

Dislipidemia meningkatkan risiko penyakit kardiovaskular (CVD) dalam kalangan rakyat Mesir. Penyesuaian rawatan atorvastatin berdasarkan genotip dapat meningkatkan keberkesanan dan mengurangkan kesan sampingan, sekali gus menyokong usaha Projek Genom Mesir dalam meningkatkan hasil kesihatan. Kajian terdahulu tidak dapat membuktikan secara konklusif tentang hubungkait di antara tindak balas atorvastatin dengan polimorfisme genetik *CYP3A4* dan *CYP3A5*. Penyelidikan lanjut diperlukan untuk mengesahkan variasi gen *MTHFR* sebagai indikator kepada tindakbalas terhadap atorvastatin. Tiada kajian terdahulu mengenai kesan polimorfisme genetik terhadap atorvastatin dijalankan dalam kalangan penduduk Mesir. Justeru, kajian ini bertujuan untuk menyiasat kesan polimorfisme *CYP3A4*1B* (*rs2740574* C/T), *CYP3A4*22* (*rs35599367* G/A), *CYP3A5*3* (*rs776746* T/C), dan *MTHFR* (*rs1801133* G/A) terhadap rawatan atorvastatin dalam kalangan penduduk Mesir. Dalam kajian kohort prospektif ini, 100 subjek telah digenotipkan untuk alel ini. Semua subjek disaring untuk profil serum lipid, enzim hati, jumlah bilirubin dan Kreatin kinase sebelum dan selepas 4 minggu rawatan dengan atorvastatin 40 mg. Paras plasma atorvastatin dinilai 15 jam selepas dos terakhir pada setiap subjek. Farmakokinetik atorvastatin dinilai pada lima metabolizer yang lemah untuk CYP3A4 dan CYP3A5 yang membawa genotip *CYP3A4*1B* (T/T) dan *CYP3A5*3* (C/C). Frekuesi alel *CYP3A4*1B, CYP3A4*22,*

*CYP3A5*3*, dan *MTHFR* adalah masing-masing 86%, 3%, 83%, dan 30.5%. Genotip *CYP3A4*1B* (T/T), *CYP3A4*22* (G/A), dan *CYP3A5*3* (C/C) menunjukkan peningkatan yang signifikan pada serum trigliserida (TG) ($p < 0.05$). Selepas rawatan, pembawa genotip *MTHFR* (A/A) mempunyai jumlah kolesterol (TC) dan kolesterol lipoprotein ketumpatan tinggi (HDL-C) yang lebih rendah berbanding genotip (G/A) dan (G/G) ($p < 0.05$). Selepas rawatan atorvastatin, berlaku peningkatan secara signifikan bagi paras bilirubin pada genotip tertentu seperti genotip *CYP3A4*1B* (T/T), *CYP3A4*22* (G/G), *CYP3A5*3* (C/C), dan *MTHFR* (A/A) ($P < 0.001$). Paras plasma atorvastatin adalah lebih tinggi bagi pembawa genotip *CYP3A4*1B* (T/T) ($p < 0.05$) dan *CYP3A5*3* (C/C) ($p < 0.001$). Ujian regresi linear menunjukkan bahawa pembawa genotip ini lebih cenderung untuk mengalami peningkatan paras plasma atorvastatin (ng/ml). Pekali regresi β adalah 4.22 dan 4.79 pada genotip *CYP3A4*1B* (T/T) dan *CYP3A5*3* (C/C) ($P < 0.001$). Genotip *CYP3A4*22* ((G/G) dan (G/A)) dan tiga genotip *MTHFR* tidak menunjukkan perkaitan signifikan dengan kepekatan plasma atorvastatin dalam kalangan subjek. Alel *CYP3A4*1B* dan *CYP3A5*3* memberi kesan secara signifikan pada farmakokinetik atorvastatin. Dapatan ini boleh membantu pakar perubatan untuk memahami kesan genetik pada tindak balas atorvastatin di kalangan pesakit di Mesir.

THE ASSOCIATION OF CYP3A4, CYP3A5 AND MTHFR GENES POLYMORPHISM ON THE EFFICACY AND SAFETY OF ATORVASTATIN AMONG EGYPTIAN POPULATION

ABSTRACT

Dyslipidemia raises cardiovascular diseases (CVDs) risk in Egyptians. Tailoring atorvastatin treatment based on genotype can enhance efficacy and reduce adverse effects, contributing to the Egyptian Genome project's efforts to improve health outcomes. Previous studies have not provided conclusive evidence regarding the association between response to atorvastatin and *CYP3A4* and *CYP3A5* genetic polymorphisms. Further research is necessary to confirm the *MTHFR* gene variation as a predictive biomarker for responsiveness to atorvastatin. The effects of genetic polymorphisms on atorvastatin therapy were not previously studied among the Egyptians. This research aimed to investigate the effects of *CYP3A4*1B* (*rs2740574* C/T), *CYP3A4*22* (*rs35599367* G/A), *CYP3A5*3* (*rs776746* T/C), and *MTHFR* (*rs1801133* G/A) polymorphisms on atorvastatin treatment in Egyptians. In this prospective cohort study, 100 subjects were genotyped for these alleles. All participants were screened for serum lipid profiles, liver enzymes, total bilirubin (TB), and creatine kinase (CK) before and after a four-week therapy with 40 mg of atorvastatin. Atorvastatin plasma levels were assessed 15 hours after the last dose for each patient. Atorvastatin pharmacokinetics was evaluated in five poor metabolizers for CYP3A4 and CYP3A5 who carried the *CYP3A4*1B* (T/T) and *CYP3A5*3* (C/C) genotypes. *CYP3A4*1B*, *CYP3A4*22*, *CYP3A5*3*, and *MTHFR* allele frequencies were 86%, 3%, 83%, and 30.5% respectively. *CYP3A4*1B* (T/T), *CYP3A4*22* (G/A), and *CYP3A5*3* (C/C) genotypes revealed significant

improvement in serum triglyceride (TG) ($p < 0.05$). *MTHFR* SNP (A/A) genotype carriers had lower post-treatment total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) compared to (G/A) and (G/G) genotypes, respectively ($p < 0.05$). Atorvastatin significantly increased the post-treatment TB levels in particular genotypes such as the *CYP3A4*1B* (T/T) genotype, the *CYP3A4*22* (G/G) genotype, the *CYP3A5*3* (C/C) genotype, and the *MTHFR SNP* (A/A) genotype ($P < 0.001$). Atorvastatin plasma levels were higher in *CYP3A4*1B* (T/T) ($p < 0.05$) and *CYP3A5*3* (C/C) ($p < 0.001$) genotype carriers. Simple linear regression revealed that carrying these genotypes increased atorvastatin plasma levels in (ng/ml). The regression coefficients β were 4.22 and 4.79 in the case of the *CYP3A4*1B* (T/T) genotype and *CYP3A5*3* (C/C) genotype, respectively ($P < 0.001$). The *CYP3A4*22* ((G/G) and (G/A)) genotypes and the three *MTHFR* SNP genotypes did not show significant associations with the plasma atorvastatin concentrations among the subjects. *CYP3A4*1B* and *CYP3A5*3* alleles significantly affected atorvastatin pharmacokinetics. These findings can help clinicians understand the genetic influences on atorvastatin response in Egyptian patients.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Pharmacogenetics is a valuable approach that predicts sub-therapeutic responses to pharmacologic treatments and increased risks of adverse drug reactions (Wake, Ilbawi, Dunnenberger, & Hulick, 2019). Genetic variations occurring naturally in human genes lead to differences in responses to drug therapy (Hauser et al., 2018). Pharmacogenetics has a noteworthy role in assessing the influence of genetic mutations on responses to pharmacologic treatments (Gray, Adhikary, & Janicki, 2018). Many clinically used medications are metabolized by Cytochromes P450 enzymes (CYPs) (Y. Song et al., 2021). Any noticeable decrease or increase in the metabolite levels could occur due to genetic polymorphisms of these vital enzymes, resulting in changes in drug therapeutic outcomes (Chowdhury et al., 2021; Y. Zhang et al., 2024). The metabolism of drugs is considered an essential process that determines responses to medications, adverse effects, and pharmacokinetics (Nakajima, 2017).

The CYP450 enzymes are essential for human health, contributing significantly to xenobiotic metabolism and drug response (Hossam Abdelmonem et al., 2024). In addition to metabolizing medications, they also play a crucial role in the breakdown of endogenous compounds, including bile acids and steroid hormones. Furthermore, these enzymes are actively involved in the biosynthesis of both bile acids and steroids (Hossam Abdelmonem et al., 2024). The CYPs or cytochrome P450 enzyme family is a vast group of heme-containing proteins (Talevi & Bellera, 2021). These enzymes possess the organic cofactor heme, which serves as an essential prosthetic group for their catalytic function. The name of this

superfamily originates from their distinct spectrophotometric absorption peak at 450 nm when exposed to reduced carbon monoxide (Talevi & Bellera, 2021). Moreover, CYPs are more than 50 different enzymes, but the isoenzymes classified under the CYP1, CYP2, and CYP3 families are essential for the metabolism of nearly 80% of the clinically used medications (M. Zhao et al., 2021). CYPs are primarily expressed in the liver. In addition, they are present in the kidneys, placenta, skin, and gastrointestinal tract. CYPs play a role in over 90% of the reported enzymatic reactions (M. Zhao et al., 2021).

A definite gene encodes for every CYP. Each person inherits only one genetic allele from each parent. Alleles are considered to be "wild type" or "variant". The wild form presents frequently in the general population. A normal metabolizer is called an "extensive metabolizer," which has received two copies of the wild alleles. Genetic variations occur when a genetic variant substitutes only one or both wild alleles. Genetic variants usually encode specific CYPs with no function, reduced function, (Hossam Abdelmonem et al., 2024) or increased function (Lee et al., 2021; Marin et al., 2020). People who carry two copies of genetic variants with little to no enzyme activity are called "poor metabolizers" (Hossam Abdelmonem et al., 2024). People who inherit one allele of each kind exhibit lower enzymatic activity and are categorized as "intermediate metabolizers" (Hossam Abdelmonem et al., 2024). Individuals known as "ultra-rapid metabolizers" carry multiple copies of the wild genetic form (Hossam Abdelmonem et al., 2024) or two copies of the variant that enhances enzyme activity (Lee et al., 2021). Carriers of one allele for increased enzyme activity and one normal-function allele are referred to as "rapid metabolizers" (Lee et al., 2021). Precision medicine strategies necessitate screening genotypes and phenotypes (Agache & Akdis, 2019).

CYP genetic polymorphisms lead to variations in responses to medications among populations from different ethnicities (Ahmed et al., 2021; Alali et al., 2022; Dehbozorgi et al., 2018; Liao, Liu, Ai, Yu, & Li, 2018). For instance, the *CYP2D6*4 (rs3892097)* variant is most commonly found in Europeans, indicating a higher frequency of the "poor metabolizer" phenotype within these people (Alali et al., 2022). This CYP enzyme metabolizes about 25% of the currently widely used medications, such as antiarrhythmics, antidepressants, antipsychotics, opioids, and beta-blockers (Alali et al., 2022). Moreover, in East Asian populations, the frequency of CYP2C19 loss-of-function alleles is significantly higher than in individuals from other regions (Y.-W. Chen et al., 2022). This enzyme plays a crucial role in the metabolism of several medications, including proton pump inhibitors (e.g., omeprazole), anticonvulsants (such as phenytoin), and antiplatelet drugs (like clopidogrel) (Elbe et al., 2022). A study examined the impact of *CYP2C19* genetic variations on the pharmacologic effect of omeprazole in Iranian individuals with erosive reflux esophagitis (Zendehdel et al., 2010). The study found that *CYP2C19* genetic polymorphism influences omeprazole therapy, affecting clinical response and esophagitis endoscopic healing based on different genotypes (Zendehdel et al., 2010). Recent research indicates a link between *CYP2C19* polymorphisms, plasma levels of proton pump inhibitors, and the likelihood of adverse drug reactions (Ibrahim, Yusuff, Awaisu, & Elewa, 2025). Due to this concern, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has recommended genotype-guided dosing for proton pump inhibitors. According to CPIC guidelines, individuals classified as intermediate or poor metabolizers face a greater risk of adverse effects. To mitigate this risk, dose reductions are advised for these patients (Ibrahim et al., 2025). Regarding phenytoin metabolism, CYP2C19 and CYP2C9 are crucial for this

biological process (Yaşar, 2018). CYP2C9 is a genetically polymorphic enzyme. *CYP2C9*2* and **3* variants are associated with reducing the metabolism of various medications (Yaşar, 2018). The *CYP2C9*2* and **3* single-nucleotide polymorphisms (SNPs) are prevalent particularly in Caucasian and Asian subjects, respectively (Y. Zhou, Nevosadová, Eliasson, & Lauschke, 2023). The CPIC guidelines offer a framework for tailoring phenytoin treatment based on *CYP2C9* genetic data to improve patient safety (Whirl-Carrillo et al., 2021). Conducting pharmacogenetic testing for *CYP2C9* variations prior to treatment could enhance the accuracy of phenytoin dosing and increase safety (Chang, Hung, Carleton, & Chung, 2020; Fohner et al., 2020). These SNPs influence the pharmacokinetics of phenytoin and could result in adverse drug reactions such as drug rash with eosinophilia and systemic symptom (DRESS) syndrome (Yaşar, 2018). Therefore, standard doses of medications could result in toxicities due to the raised serum levels of medications in the case of patients regarded as poor metabolizers (Ibrahim et al., 2025; Shubbar et al., 2024). Furthermore, some medications, like losartan or tramadol, are not effective as a pharmacologic treatment before their metabolism to active forms (Mahajna et al., 2024; Park, Song, Yee, Yoon, & Gwak, 2021). These medicines are called prodrugs. In case of poor metabolizers of these prodrugs, treatment failure could occur due to the reduced or no production of the active form of the medications (Mahajna et al., 2024; Park et al., 2021).

The activity of CYP plays a significant role in the variability of medication pharmacokinetics (Magliocco, Thomas, Desmeules, & Daali, 2019). Much research has been done to study the influence of CYP genetic variations on the pharmacokinetics of drugs (Gong et al., 2025; Nakajima, 2017). For example, the time needed to reach the appropriate therapeutic trough level of tacrolimus was

significantly longer for individuals classified as CYP3A extensive metabolizers than those classified as CYP3A poor metabolizers (Calabrese et al., 2018). In addition, clopidogrel requires activation by the CYP2C19 enzyme, which is genetically polymorphic (Pereira et al., 2024). Loss-of-function variants in *CYP2C19* can affect pharmacokinetics response; lead to reduced active metabolite levels, increased platelet aggregation, and a higher risk of ischemic events during clopidogrel therapy. Genetic testing for *CYP2C19* can identify affected patients, allowing for alternative treatment options (Pereira et al., 2024). On this point, genetic polymorphisms in metabolic enzymes such as CYP3A4 and CYP3A5 were associated with variations in the pharmacokinetics of statins (Maslub, Radwan, Daud, & Sha'aban, 2023).

Atorvastatin is regarded as one of the most frequently prescribed drugs and the most widely used statin globally (Adams, Tsang, & Wright, 2015; Maslub et al., 2023). It is currently recommended to manage dyslipidaemia and hypercholesterolaemia (Shawish, Bagheri, Musini, Adams, & Wright, 2021). However, it is associated with several adverse reactions, like nausea, nasopharyngitis, insomnia, urinary tract infections, elevation in hepatic enzymes, diarrhoea, dyspepsia, myalgia, and arthralgia (Maslub et al., 2023; Parke-Davis, 2009).

Both CYP3A4 and CYP3A5 enzymes are responsible for atorvastatin metabolism (Figure 1.1). Therefore, *CYP3A4* or *CYP3A5* genetic polymorphisms lead to interindividual differences in the metabolism mediated by the corresponding enzymes (Maslub et al., 2023). In light of these polymorphisms, Binbin Xia et al. study of 187 Chinese subjects showed that atorvastatin maximum plasma concentration (Cmax) was significantly elevated (high atorvastatin exposure) in the

carriers of specific genes, including *CYP3A4*1B* (*rs2740574*) in addition to other genes involved in atorvastatin metabolism and transport in vivo. Moreover, one subject was terminated during the study due to atorvastatin intolerance (Xia et al., 2018). Likely, the *CYP3A4*22* variant is linked to a lower statin dosage required (D. Wang, Guo, Wrighton, Cooke, & Sadee, 2011b). Clinical research validated the relationship between *CYP3A4*22* and the pharmacokinetics of statins (Werk & Cascorbi, 2014). Despite some inconsistent data (Ragia et al., 2015), *CYP3A4*22* appears to be the most clinically significant common variation of *CYP3A4* (Werk & Cascorbi, 2014; Zanger & Schwab, 2013). Regarding the *CYP3A5* gene polymorphism, individuals who express CYP3A5 are anticipated to have a greater metabolic capacity, which would likely lead to reduced bioavailability of atorvastatin (Zubiaur et al., 2021). Accordingly, *CYP3A5* non-expressers (*CYP3A5*3/*3* genotype carriers) were associated with an increased risk of experiencing myalgia and muscle damage in comparison to those with the **1/*3* genotype (Zubiaur et al., 2021). Within this context, *CYP3A4* and *CYP3A5* genetic variations result in differences in atorvastatin metabolism (Maslub et al., 2023).

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme involved in homocysteine (Hcy) metabolism (Zarembska, Ślusarczyk, & Wrzosek, 2024). MTHFR irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is needed for the remethylation of Hcy to methionine. Methionine is then converted to S-adenosylmethionine (SAM), a universal methyl donor that functions in several trans-methylation processes, including DNA methylation, proteins, lipids, and polyamines. This conversion is essential for regulating intracellular Hcy levels and maintaining appropriate SAM levels (Kucukhuseyin et al., 2013; Zarembska et al., 2024). Hyperhomocysteinemia

is one of the classical risk factors for CVDs in addition to hyperlipidemia, genetics, smoking, age, hypertension, diabetes mellitus, family history, and obesity (Kathiresan & Srivastava, 2012; Zarembska et al., 2024). Hence, MTHFR deficiency is frequently associated with increased plasma Hcy levels that elevate the risk of CVDs (Izmirli M., 2014; Zarembska et al., 2024).

The *MTHFR* genetic polymorphism was found to have a significant effect on atorvastatin pharmacokinetics in a pharmacogenetics Mexican pilot study (León-Cachón et al., 2016). Individuals who carry the variant allele for *MTHFR (rs1801133)* showed a reduced Cmax and area under the curve (AUC) values, along with a higher apparent oral clearance (CL/F). This pharmacokinetic pattern indicates increased clearance activity and lower levels of atorvastatin in the system, which may lead to a less effective response to atorvastatin treatment (León-Cachón et al., 2016). This pilot study is the first to report an effect of the *MTHFR (rs1801133)* SNP on statin pharmacokinetics. However, the study did not provide a clear interpretation of the association between the *MTHFR* SNP and atorvastatin pharmacokinetics. It concluded that the mechanism by which the variant allele of *MTHFR (rs1801133)* enhances atorvastatin clearance, leading to a diminished drug response, remains to be clarified (León-Cachón et al., 2016).

The recent emphasis on personalized medication dosages underscores the importance of understanding genetic factors in pharmacotherapy (Principi, Petropulacos, & Esposito, 2023). Research indicates that genetic variations can account for up to 95% of the variability in individual responses to medications (Principi et al., 2023). Recent studies have highlighted the significant impact of pharmacogenetics on individual responses to atorvastatin therapy (Dagli-Hernandez

et al., 2022; Ruiz-Iruela et al., 2018). Consequently, the occurrence of polymorphisms in genes implicated in the metabolic pathway of atorvastatin may account for its pharmacokinetics variability (Eichelbaum, Ingelman-Sundberg, & Evans, 2006; Kim et al., 2022; Maslub, Daud, Radwan, Sha'aban, & Ibrahim, 2024; Maslub et al., 2023), given that their prevalence and effects vary between distinct ethnic groups (Hirschhorn & Daly, 2005; Kim et al., 2022; Maslub et al., 2024). Hence, pharmacogenomic studies seek to optimize the usage of pharmacologic treatments in clinical practice settings (Gong et al., 2025).

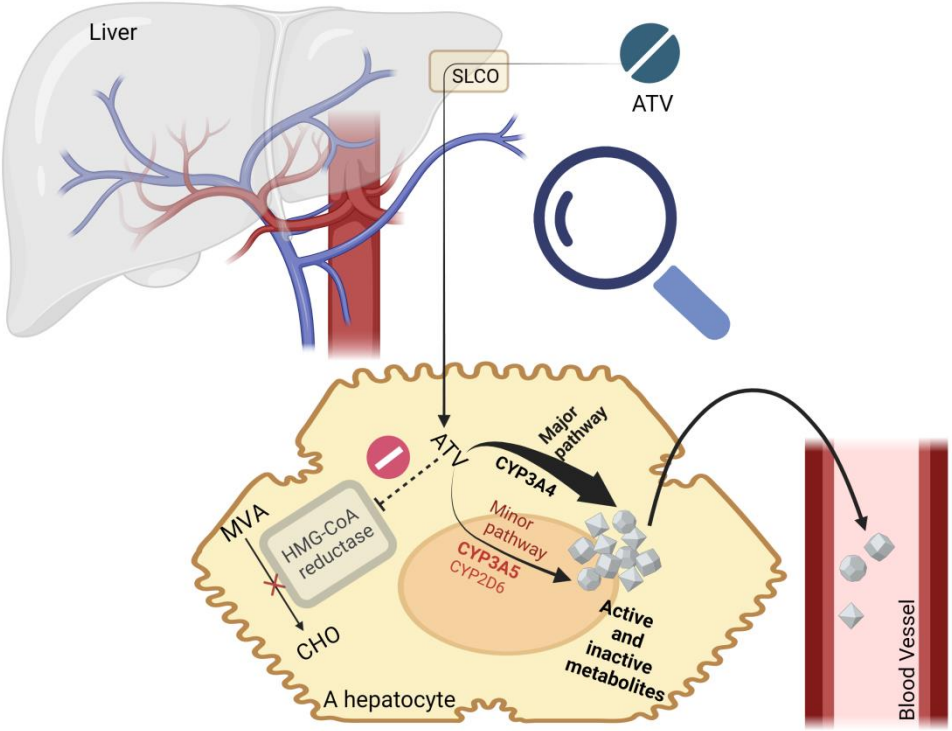

Figure 1.1 CYP3A4 mainly metabolizes atorvastatin through major pathways and minor pathways via CYP3A5 and CYP2D6. This figure was created using BioRender.com.
ATV = atorvastatin, CHO = cholesterol, CYP = cytochrome P450, MVA = mevalonate, SLCO = solute carrier organic anion 'superfamily of transporters'

1.2 Problem statement & Study rationale

In Egypt, 46% of overall deaths are related to CVDs (Mach et al., 2020; Taha et al., 2021). Dyslipidemia increases the risk of CVDs. Several studies showed that high blood cholesterol has been found in 37% of Egyptians (Farag et al., 2017; Reda, Abdel-Rehim, Etman, & Afifi, 2014; Taha et al., 2021). Statins are the first-line treatment for dyslipidemia. Responses to statin therapy show apparent interpersonal deviations in the expected lipid-lowering efficacy. Atorvastatin is an example; polymorphisms in genes responsible for metabolism, distribution, and uptake might substantially modulate therapeutic outcomes (Rosales et al., 2012). These variations in responses develop a significant clinical problem (Nakajima, 2017; Sarlis & Gourgiotis, 2005). Differences in the pharmacokinetics of statins were related to genetic mutations in metabolic enzymes such as CYP3A4 and CYP3A5 (Willrich et al., 2013). CYP3A4 metabolizes 45% to 60% of drugs presently recommended in clinical practice (Lv et al., 2018). CYP3A5 isoform is also regarded as the significant subfamily of the hepatic metabolic enzymes. CYP3A5 genetic polymorphism is linked to variations among people and ethnic groups in medication pharmacokinetics and therapeutic effects (Ang et al., 2018).

Atorvastatin is considered one of the most commonly recommended medications and the most extensively prescribed statin worldwide (Adams et al., 2015). The outcomes of association studies of CYP3A4 and CYP3A5 with response to atorvastatin were inconsistent. Some research has linked polymorphisms of these enzymes to positive clinical consequences after atorvastatin therapy, and some linked them to negative therapeutic outcomes (Kee, Chin, Kennedy, & Maggo, 2020). The issue is that genetic polymorphisms lead to differences in responses to drug therapy (Hauser et al., 2018). These dissimilar responses are significantly apparent among

different populations (Ahmed et al., 2021; Dehbozorgi et al., 2018; Liao et al., 2018). Moreover, the effects of genetic variations of *CYP3A4*1B* and *CYP3A5*3* on atorvastatin therapy were not previously studied among the Egyptian population.

CYP genetic polymorphisms lead to variations in response to medications among populations from different ethnicities. The efficacy and safety of medications could be modulated due to CYP polymorphisms (Ahmed et al., 2021; Dehbozorgi et al., 2018; Liao et al., 2018). Regarding *CYP3A4* genetic variations in diverse ethnicities, a study on Chilean subjects illustrated that the G allele of the variant *rs2740574* in the *CYP3A4* was linked to a better response to atorvastatin. This polymorphism resulted in a decrease in the metabolic activity of CYP3A4 and an enhancement in atorvastatin efficacy (Rosales et al., 2012). A different study on Indian individuals has shown a dissimilar result. It demonstrated no significant association between the *rs2740574* variant and low values of low-density lipoprotein cholesterol (LDL-C) as a response to atorvastatin pharmacotherapy (Ahangari et al., 2020; Poduri et al., 2010). Moreover, the variant CY3A4*1B (*rs2740574*) influenced statin intolerance (Melhem et al., 2021). In Dutch individuals, the G allele of this variant reduced the risk of atorvastatin's adverse effects due to its association with low plasma levels of atorvastatin (Becker et al., 2010; Kee et al., 2020). On the other hand, a study that recruited African Americans and Caucasians demonstrated that CY3A4*1B (*rs2740574*) was not linked to atorvastatin therapy intolerance (Kee et al., 2020; Voora et al., 2009).

Concerning *CYP3A5* polymorphism in different ethnicities, a study involving Han Chinese subjects showed that *CYP3A5*3* genetic variation could result in inter-individual variations in response to atorvastatin treatment (Wei & Zhang, 2015).

However, a study on Chilean patients showed that the G and A alleles of the *rs776746* variant had no influence on response to atorvastatin therapy (Rosales et al., 2012). Moreover, a meta-analysis showed the effect of *CYP3A5*3* genetic variations on the risk of intolerance to statin therapy, including atorvastatin. Furthermore, it concluded that genotyping may help in identifying patients at risk for adverse drug reactions (Yee et al., 2021). However, the Han Chinese cases showed that the G allele of the *rs776746* variant was not linked to adverse effects (J. E. Liu et al., 2017).

More research in different ethnic populations is needed to confirm that the intron 6 SNP (*rs35599367*, *CYP3A4*22*) significantly influences CYP3A4 expression and is a biomarker for predicting responsiveness to atorvastatin (D. Wang et al., 2011b). In addition, further studies are necessary to verify that the *MTHFR* (*rs1801133*) polymorphism is a predictive biomarker for responsiveness to atorvastatin (León-Cachón et al., 2016).

To the best of our knowledge, both alleles C and T of the variant *rs2740574* in the *CYP3A4* gene were not studied before in the Egyptian population. In addition, the effects of genetic polymorphisms of *CYP3A4* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), *CYP3A4*22* (*rs35599367* G/A) and *MTHFR* (*rs1801133* G/A) on atorvastatin plasma concentration and its induced adverse effects were not previously studied among Egyptians.

Consequently, this study will assist a significant number of Egyptians suffering from hypercholesterolemia and are at high risk of CVDs complications by tailoring of atorvastatin treatment based on the patient's genotype. The findings would enhance the efficacy and reduce the risk of atorvastatin-induced adverse

effects and treatment costs. The findings of our study will be part of the efforts made by the Egyptian government through the tremendous national scientific project called "Egyptian Genome" (University, 2020; Wohlers et al., 2020).

1.3 Research Question(s)

1.3.1 What are the allele frequencies of *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), *CYP3A4*22* (*rs35599367* G/A), and *MTHFR* (*rs1801133* G/A) among the study Egyptian population?

1.3.2 Would atorvastatin efficacy (serum lipid and lipoprotein levels) be affected by the genetic polymorphisms of these enzymes?

1.3.3 Would atorvastatin plasma levels be affected by the genetic polymorphisms of these enzymes?

1.3.4 Would atorvastatin safety (liver enzymes, TB, and CK levels) be affected by the genetic polymorphisms of these enzymes after atorvastatin four-week treatment?

1.3.5 Would the pharmacokinetics profile of atorvastatin be affected in carriers of the homozygous mutant genotypes of *CYP3A4*1B (rs2740574 C/T)* and *CYP3A5*3 (rs776746 T/C)* SNPs? (The investigation will focus solely on the *CYP3A4*1B* (Al-Eitan, 2020) and *CYP3A5*3* (Mutawi et al., 2021) variants. Literature data suggest that these variants have a higher prevalence compared to *CYP3A4*22 (rs35599367 G/A)* (Ebid, Ismail, Lotfy, Mahmoud, & M, 2022) and *MTHFR (rs1801133 G/A)* (El-Khawaga et al., 2024) SNPs in similar populations. Additionally, the study is constrained by the principal investigator's personal funding, which limited the exploration of the associations between other SNPs and atorvastatin pharmacokinetics.)

1.4 Research hypotheses

1.4.1 The allele frequencies of *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), *CYP3A4*22* (*rs35599367* G/A), and *MTHFR* (*rs1801133* G/A) among the study Egyptian population would be similar to Caucasians.

1.4.2 The genetic polymorphisms of these enzymes affect serum lipid and lipoprotein levels (atorvastatin efficacy). The *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), and *CYP3A4*22* (*rs35599367* G/A) are associated with improving the lipid profile. However, the *MTHFR* (*rs1801133* G/A) is associated with worsening the lipid profile.

1.4.3 The genetic polymorphisms of these enzymes impact atorvastatin plasma levels. The *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), and *CYP3A4*22* (*rs35599367* G/A) are associated with high atorvastatin plasma levels. However, the *MTHFR* (*rs1801133* G/A) is associated with low atorvastatin plasma levels.

1.4.4 The genetic polymorphisms of these enzymes affect liver enzyme levels, TB, and CK levels after atorvastatin treatment. The *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), and *CYP3A4*22* (*rs35599367* G/A) are associated with high liver enzyme levels, TB, and CK levels. However, the *MTHFR* (*rs1801133* G/A) is associated with low liver enzyme levels, TB, and CK levels.

1.4.5 In carriers of the homozygous mutant genotypes of the *CYP3A4*1B* (*rs2740574* C/T) and *CYP3A5*3* (*rs776746* T/C) alleles, the pharmacokinetics profile of atorvastatin is affected.

1.5 Research aim and objectives

1.5.1 General

This research aims to investigate the association between *CYP3A4*, *CYP3A5*, or *MTHFR* polymorphisms and atorvastatin efficacy and safety in the Egyptian population.

To minimize potential confounding factors, strict eligibility criteria were applied. In order to better control for confounders, individuals taking medications that could interact with atorvastatin, including recent lipid-lowering agents and insulin therapy, were excluded. Participants with comorbidities such as uncontrolled hypothyroidism, poor liver function, or other significant clinical conditions,

including stroke, were also not included. Additionally, all individuals refrained from consuming alcohol both prior to and throughout the study. While some may have been smokers, they were required to abstain entirely from smoking throughout the research period. All subjects also avoided strenuous physical activity starting one week before the study and throughout its duration.

1.5.2 Specific

1.5.2(a) Objective 1

To determine the allele frequencies of *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), *CYP3A4*22* (*rs35599367* G/A), and *MTHFR* (*rs1801133* G/A) alleles among the Egyptian study participants.

1.5.2(b) Objective 2

To determine the association between *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), *CYP3A4*22* (*rs35599367* G/A), or *MTHFR* (*rs1801133* G/A) genetic polymorphisms and changes in serum lipid and lipoprotein levels after atorvastatin treatment among the Egyptian study participants.

1.5.2(c) Objective 3

To determine the relationships between atorvastatin plasma levels among the Egyptian study participants and the genetic polymorphisms of *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), *CYP3A4*22* (*rs35599367* G/A) or *MTHFR* (*rs1801133* G/A).

1.5.2(d) Objective 4

To determine the association between *CYP3A4*1B* (*rs2740574* C/T), *CYP3A5*3* (*rs776746* T/C), *CYP3A4*22* (*rs35599367* G/A) or *MTHFR* (*rs1801133*

G/A) alleles and changes in liver enzymes, TB and CK levels after atorvastatin treatment among the Egyptian study participants.

1.5.2(e) Objective 5

To determine the pharmacokinetics profile of atorvastatin in carriers of the homozygous mutant genotypes of *CYP3A4*1B* (*rs2740574* C/T) and *CYP3A5*3* (*rs776746* T/C) SNPs.

CHAPTER 2

LITERATURE REVIEW

2.1 Genetic polymorphisms that affect atorvastatin response

Several genetic polymorphisms affect atorvastatin's response. Pharmacokinetic variation is a primary source of interpersonal dissimilarities in the pharmacologic effect and response of medications metabolized by the CYP3A pathway (Hohmann, Haefeli, & Mikus, 2016). CYP3A4 isoform metabolizes more clinically used medications than any other metabolic enzyme found in human beings (Werk & Cascorbi, 2014). Atorvastatin is metabolized to active metabolites by CYP3A4 (Zubiaur et al., 2021). Moreover, a previous study showed that CYP3A4 and CYP3A5 were responsible for 85% and 15% of atorvastatin metabolism, respectively (Figure 1.1). Inter-personal variations in CYP3A metabolic pathways are also pronounced, with 20- and 40-fold variability in metabolism. These differences may be associated with polymorphisms in *CYP3A4* or *CYP3A5* (Kitzmiller, Sullivan, Phelps, Wang, & Sadee, 2013). In the same context, atorvastatin is known to be a substrate of the organic anion transporter polypeptides (OATP1B1) and (OATP1B3), encoded by SLCO1B1 and SLCO1B3 genes, respectively. Consequently, genetic polymorphisms are linked to variations in atorvastatin efficacy and safety profile (Zubiaur et al., 2021). In addition to genetic variations in these transporters, The MTHFR enzyme, crucial for Hcy metabolism (Zarembska et al., 2024), has been studied for its potential impact on atorvastatin pharmacokinetics due to its polymorphism (León-Cachón et al., 2016), as shown in Table 2.1.

2.2 Worldwide allele frequencies of *CYP3A4*1B, CYP3A4*22, CYP3A5*3,* and *MTHFR* SNPs across ethnicities

The *CYP3A4*1B* (*rs2740574*) SNP is linked to decreased enzymatic activity (Rosales et al., 2012). *CYP3A4*1B* allele frequency varies among ethnicities (C.-e. Wang, Lu, Chang, Guo, & Qiao, 2018). The frequency of the *CYP3A4*1B* allele varies from 0 to 4% in Asian and European populations, and it can be as high as 82% in African populations (Q. Zhou et al., 2011).

The intronic SNP (*rs35599367*, *CYP3A4*22*) decreases CYP3A4 mRNA and protein expression (D. Wang et al., 2011b). The frequency of the *CYP3A4*22* allele is 5.0% among Europeans, 2.6% in the mixed American population, and less than 1% in the Asian and African populations (van Eerden et al., 2023).

The *CYP3A5*3* SNP leads to a truncated malfunctioning protein in homozygous cases (nonexpressors) (Milane et al., 2021). The frequency of the *CYP3A5*3* allele differs notably across various populations. It is most common in Europeans (94%) and admixed Americans (80%) and least common in the African population (18%) (Galaviz-Hernández et al., 2020).

The *MTHFR rs1801133* variant interferes with the folate metabolic pathway by reducing the activity and thermostability of the MTHFR enzyme, thereby disrupting the methylation process (Chiu et al., 2023). The interference in folate metabolism is associated with higher plasma Hcy (Chiu et al., 2023). The *MTHFR* (*rs1801133)* allele was found to occur in 24-40% of Europeans, 40% of Koreans, and 26-37% of Japanese individuals (L. Zhao et al., 2022).

2.3 Influence of CYP3A polymorphisms on atorvastatin efficacy

2.3.1 Influence of *CYP3A4*1B* polymorphism

CYP3A4 is involved in the metabolism of the lipid-lowering agent atorvastatin. It has been demonstrated that CYP3A4 activity can vary up to 10-fold amongst carriers of genetic variants for this isoenzyme (Ahangari et al., 2020; Hirota, Fujita, & Ieiri, 2020; Lennernäs, 2003). This enzyme could predict the response to statin treatment and adverse effects (Ahangari et al., 2020). Research on the Chilean population showed that the G allele of the variant *rs2740574* in the *CYP3A4* was associated with a better response to atorvastatin. This genetic mutation is linked to a reduced CYP3A4 function, leading to an increased atorvastatin effect (Rosales et al., 2012).

Research on the Indian population has demonstrated a different finding (Kadam, Ashavaid, Ponde, & Rajani, 2016). Kadam et al. found that the wild genotype of the *rs2740574* variant was associated with low values of LDL-C in comparison to the mutant G-allele associated with higher values (Kadam et al., 2016). Another research on the Indian population has shown no significant relationship between the *rs2740574* variant and low values of LDL-C as a response to atorvastatin therapy (Ahangari et al., 2020; Poduri et al., 2010). The reasons behind these different findings could be related to genetic polymorphisms, which can have varying frequencies and effects across populations (Kim et al., 2022; Maslub et al., 2024). Additionally, study design and sample size are critical factors influencing the findings of genetic studies, particularly those focusing on SNPs (Bralić & Buljan, 2023; Politi, Roumeliotis, Tripepi, & Spoto, 2023).

2.3.2 Influence of *CYP3A4*22* polymorphism

Wang et al., 2011 genotyped seven polymorphisms in *CYP3A4/3A5* using gDNA from 273 individuals. Participants were part of the Coronary Artery Disease (CAD) Study at Ohio State University (D. Wang et al., 2011b). Patients using stable dosages (same dose for at least six months) of a hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitor (statins) for cholesterol management were chosen for this research (n=142; atorvastatin). Each patient's statin dose was adjusted to achieve established cholesterol control targets. Lipid levels were assessed at enrolment and after achieving a steady statin dosage. After joining the study, individuals did not take any other lipid-lowering medications. Intron 6 SNP (*rs35599367*, C>T) significantly impacts CYP3A4 expression and might be a biomarker for predicting responsiveness to CYP3A4-metabolized medications. Carriers of the T allele required considerably lower statin dosages (0.2–0.6-fold, P = 0.019) than non-T carriers to achieve optimum lipid control in patients receiving stable doses of atorvastatin for lipid management (D. Wang et al., 2011b).

Ragia et al., 2015 examined the relationship between the *CYP3A4*22* allele and atorvastatin responsiveness. The trial comprised 207 Greek people with primary hypercholesterolemia who were treated with atorvastatin. TC and LDL-C were assessed at the beginning of therapy and six months afterwards. TaqMan test was used to study the *CYP3A4*22* allele. Eighteen people in the overall cohort group possessed the *CYP3A4*22* allele. The *CYP3A4*22* allele was not linked with an atorvastatin lipid-lowering response. Significant confounding or uncontrolled variables, such as polypharmacy and coexisting medical conditions, may obscure the effect of the *CYP3A4*22* allele on the lipid-lowering effect of atorvastatin; consequently, more population-based research is necessary (Ragia et al., 2015).

In a trial conducted in Athens, 105 unrelated children and adolescents with familial hypercholesterolemia (FH) were treated with atorvastatin at dosages from 10 to 40 mg (Drogari et al., 2014). Total cholesterol and LDL-C levels of these patients were evaluated at baseline and six months after therapy initiation. The study showed that *CYP3A4*22* genotype was not linked with lipid reductions in 105 children and adolescents treated with atorvastatin for familial hypercholesterolemia. However, more research in other groups is required to reproduce this finding (Drogari et al., 2014).

These differing findings may be attributed to genetic polymorphisms, which vary in frequency and impact among different populations (Kim et al., 2022; Maslub et al., 2024). Furthermore, the design of the study and the sample size play crucial roles in shaping the outcomes of genetic research (Bralić & Buljan, 2023; Politi et al., 2023).

2.3.3 Influence of *CYP3A5*3* polymorphism

A study involving Han Chinese subjects demonstrated that *CYP3A5*3* genetic mutation could result in person-to-person variations in response to atorvastatin therapy. The study found that polymorphisms of P450 oxidoreductase *28 (POR*28) are linked to more significant increases in the effect on plasma lipids in carriers of *CYP3A5*3/*3* (Wei & Zhang, 2015). However, a research on Chilean patients showed that the G and A alleles of the *rs776746* variant did not affect response to atorvastatin treatment (Rosales et al., 2012).

The variation in findings could be explained by genetic polymorphisms, which differ in their frequency and effects across various populations (Kim et al., 2022; Maslub et al., 2024). Additionally, factors such as the study's design and the sample

size are essential in determining the results of genetic studies (Bralić & Buljan, 2023; Politi et al., 2023).

2.4 Influence of CYP3A polymorphisms on atorvastatin safety profile

2.4.1 Influence of CY3A4*1B polymorphism

Furthermore, statin intolerance is influenced by the variant CY3A4*1B (*rs2740574*) (Melhem et al., 2021). In the Dutch population, especially in females, the G allele of this variant was linked to a reduced risk of raised plasma concentrations of statins, which could lead to adverse drug reactions (Becker et al., 2010; Kee et al., 2020). However, an open-label randomized study illustrated that CY3A4*1B (*rs2740574*) was not associated with atorvastatin therapy intolerance, elevation of CK, or muscle pain in Caucasian as well as African American subjects (Kee et al., 2020; Voora et al., 2009). Another case-control study showed a similar result in Caucasian subjects (Kee et al., 2020; Wilke, Moore, & Burmester, 2005). Consequently, the response to statin treatment significantly differs among populations (Rosales et al., 2012). The differences in research outcomes may be attributed to genetic polymorphisms, as their prevalence and impact vary among different populations (Kim et al., 2022; Maslub et al., 2024). Furthermore, elements like study design and sample size play a crucial role in shaping the findings of genetic research (Bralić & Buljan, 2023; Politi et al., 2023).

2.4.2 Influence of *CYP3A4*22* polymorphism

Potential safety concerns have been raised by a study involving healthy Spanish individuals, which found that the *CYP3A4*22* SNP was associated with increased atorvastatin exposure, as indicated by a high AUC. This suggested

decreased metabolic activity in carriers of this genetic variant. (Saiz-Rodríguez et al., 2020).

2.4.3 Influence of *CYP3A5*3* polymorphism

Regarding intolerance to atorvastatin therapy, the level of serum CK was 25% higher in carriers of the genotype *CYP3A5*3/3* (non-expressors) than in other genotypes (Kee et al., 2020; Wilke et al., 2005). Furthermore, a meta-analysis illustrated the significant influence of *CYP3A5*3* genetic variations on the risk of adverse effects due to statin therapy, including atorvastatin. Moreover, it concluded that *CYP3A5* genotyping could help predict drug-induced toxicities (Yee et al., 2021). On the other hand, research on the Indian population (from south India) showed that the frequency of the genotype *CYP3A5*3/3* of the variant *rs776746* was higher in the cases who suffered myopathy. However, the study could not find any relation between the *CYP3A5* mutation and atorvastatin therapy (Kee et al., 2020; Ramakumari, Indumathi, Katkam, & Kutala, 2018). Another study involving Han Chinese patients demonstrated that the G allele of the *rs776746* variant was not associated with myotoxicity as an adverse effect of atorvastatin therapy (J. E. Liu et al., 2017). A case-control study demonstrated the same result in which indigenous American, sub-Saharan, East Asian, and European subjects were recruited (Frudakis et al., 2007; Kee et al., 2020). Variations in research results can be linked to genetic polymorphisms, as their frequency and effects differ across diverse populations (Kim et al., 2022; Maslub et al., 2024). Additionally, factors such as study methodology and the number of participants significantly influence the outcomes of genetic studies (Bralić & Buljan, 2023; Politi et al., 2023).

2.5 *CYP3A4* polymorphism among Egyptians

Both alleles C and T of the variant *rs2740574* in the *CYP3A4* gene were not studied before in the Egyptian population. In April 2021, pharmacogenomic research was done at the pediatric hospital, Faculty of Medicine, Mansoura University, Mansoura Governorate, Egypt (Mutawi et al., 2021). The research has been conducted to investigate major allelic variants of certain CYPs. It has shown that the frequency of a different variant, *rs35599367*, in the gene *CYP3A4*22* was 2%. It is the first study illustrating the *CYP3A4*22* variant frequency in the Egyptian population (Mutawi et al., 2021). Furthermore, the frequency of an additional variant *rs4646437* in the *CYP3A4* gene was reported in another Egyptian study to be 20%. The study included 50 Egyptian patients after kidney transplantation at the Renal Transplantation Unit of Alexandria University Hospital, Alexandria Governorate, Egypt. The study was designated to demonstrate the influence of *CYP3A4* polymorphism on cyclosporine dosing in a group of renal transplant Egyptian recipients. The study showed that *CYP3A4 rs4646437*C>T had a significant impact on cyclosporine pharmacokinetics; the T carriers required higher cyclosporine dosing (Sharaki, Zeid, Moez, Zakaria, & Nassar, 2015).

2.6 *CYP3A5*3* polymorphism among Egyptians

Concerning *CYP3A5*3* polymorphism among Egyptians, a study was held at the adult oncology department, National Cancer Institute, Cairo University, Giza Governorate, Egypt. It showed that the frequency of *CYP3A5*3* was higher (81.5%) in acute myeloid leukemia cases compared to controls (Abd El Wahab et al., 2017). Another Egyptian study, held at the Urology and Nephrology Center, Mansoura University Hospital, Mansoura, Egypt, reported an allele frequency of 85.53% for

*CYP3A5*3* among 76 renal transplantation recipients. This study was the first Egyptian research focusing on the personalization of tacrolimus doses in Egyptian cases according to the *CYP3A5* genotypes (Mendrinou et al., 2020). In addition, another study on Egyptian cases of chronic myeloid leukemia (CML) was conducted at the Hematology Department, Medical Research Institute, Alexandria University, Alexandria Governorate, Egypt. This research was designed to illustrate the role of *CYP3A5*3* polymorphism in determining the response to imatinib in 86 CML Egyptian cases. The result showed that the frequency of *CYP3A5*3* was 53% and 69% in seventy-eight (early chronic phase) cases and eight (accelerated phase) cases, respectively. *CYP3A5*3* genetic variation influenced imatinib efficacy and was linked to inferior outcomes (Bedewy & El-Maghraby, 2013).

Moreover, additional pharmacogenetic Egyptian research on 130 epileptic children was held at the Pediatrics Department, Menoufia University Hospital, Menoufia Governorate, Egypt. This research aimed to show the role of *CYP3A5*3* genetic variation in predicting resistance to antiepileptic medications in Egyptian epileptic pediatric cases. The prevalence of *CYP3A5*3* was 76.9% and 77.7% in epileptic (n=130) and control (n=65) participants, respectively. Children with epilepsy did not exhibit any pharmaco-resistance due to *CYP3A5*3* polymorphism (Abo El Fotoh, Abd El Naby, Habib, AA, & Kasemy, 2016). Due to the little data about pharmacogenes of significant clinical importance, 145 healthy, unrelated Egyptian children were also included in the research at Mansoura University Children's Hospital, Mansoura Governorate, Egypt. This pharmacogenomic research aimed to screen common genetic variants in certain CYPs, including *CYP3A5*, among the Egyptian population. The prevalence of the variant, 6986A>G (*rs776746*), in the gene *CYP3A5* was 86.2% (Mutawi et al., 2021). Consequently, recent evolution in