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PHARMACOGENETIC ANALYSIS OF INTER-ETHNIC VARIABILITY IN THE UPTAKE TRANSPORTER *SLCO1B1* GENE IN COLOMBIAN, MOZAMBICAN, AND PORTUGUESE POPULATIONS

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Vera Linda Ribeiro Marques (PhD)

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DECLARATION OF AUTHORSHIP

I declare that I am the author of this work, which is original. The work cites other authors and works, which are adequately referred in the text and are listed in the bibliography.

Mulata Haile Nega

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I dedicate this thesis
to my Mother and my Sisters

RESUMO

As atividades de enzimas metabólicos e transportadores de fármacos determinam, em larga medida, a resposta ao fármaco e a sua eliminação. A etnia é uma variável demográfica importante que contribui para a variabilidade interindividual no metabolismo e transporte de fármacos. Como resultado de diferenças étnicas na prevalência de polimorfismos de nucleótido único (SNP), pode ocorrer maior risco de eventos adversos, falha do tratamento ou prevalência de patologias em algumas populações. Os transportadores de influxo são um dos principais factores que contribuem para o processo de eliminação de fármacos. Foi demonstrado que os genes que codificam esses transportadores de influxo são polimórficos. O OATP1B1 é um transportador de influxo conhecido por mediar a captação de vários compostos endógenos e xenobióticos. Várias variantes foram descritas no gene *SLCO1B1* que codifica o OATP1B1. Entre estas, a variante mais estudada, c.521T> C, foi descrita como estando significativamente associada ao risco de miopatias induzidas pela terapêutica com estatinas. Apesar da clara relevância clínica desta variante polimórfica, um número reduzido de populações foram caracterizadas. Até o momento, não existem dados epidemiológicos para a variante *SLCO1B1* c.521T> C nas populações colombiana, moçambicana e portuguesa.

Este estudo teve, assim, como objetivo, avaliar as frequências genotípicas e alélicas da variante *SLCO1B1* c.521T> C em voluntários saudáveis colombianos, moçambicanos e portugueses. O DNA genómico isolado de amostras de sangue obtidas, sob consentimento informado, de 67 indivíduos colombianos, 53 moçambicanos e 61 portugueses foi analisado para este polimorfismo através de um novo método de genotipagem por PCR-RFLP.

Um total de 47 (70,15%), 12 (17,91%) e 8 (11,94%) dos 67 indivíduos colombianos, 47 (88,68%), 5 (9,43%), 1 (1,89%) dos 53 indivíduos moçambicanos e 40 (65,57%), 16 (26,23%), 5 (8,20%), dos 61 indivíduos portugueses eram portadores do genótipo T / T-homo, C / T-hetero e C / C-homozigota, respectivamente . Em termos de frequências alélicas, observou-se um total de 106 alelos T (79,1%) e 28 alelos C (20,9%) em indivíduos colombianos; 99 alelos T (93,4%) e 7 alelos C (6,6%) C em moçambicanos e 96 alelos T (78,7%) e 26 alelos C (21,3%) em indivíduos portugueses. As frequências observadas em moçambicanos mostram uma diferença estatisticamente significativa em relação às observadas em colombianos (p=0,0017) e portugueses (p=0,0022). Por outro lado, as frequências observadas na amostra da população colombiana não são significativamente diferentes (p=1.000) em relação às observadas em Portugal.

Em conclusão, os nossos dados demonstraram que o genótipo homozigota T / T é altamente prevalente, o genótipo homozigota variante, C / C, apresenta a menor frequência em moçambicanos, sendo por outro lado relativamente frequente em populações colombianas e portuguesas. Como resultado, indivíduos (principalmente caucasianos) com o genótipo homozigótico variante c.521C/C podem ser altamente suscetíveis a efeitos adversos relacionados à estatina do que aqueles com o genótipo c.521TT (referência) para o SNP c.521T> C.

Palavras-chave; SNP, *SLCO1B1*, estatinas, transportadores de influxo

ABSTRACT

The activities of drug metabolizing enzymes and transporters greatly determine drug disposition and response. Ethnicity is an important demographic variable contributing to inter-individual variability in drug metabolism and transport. Influx transporters are one of the key contributors to the process of drug disposition. It has been demonstrated that the genes coding for these influx transporters are polymorphic. OATP1B1 is an influx transporter known to mediate the uptake of various endogenous compounds and xenobiotics. Several sequence variations have been discovered in the *SLCO1B1* gene encoding OATP1B1. Statin-induced myopathy is reported to be significantly associated with the c.521T>C polymorphism. To date, there is no *SLCO1B1* c.521T>C epidemiologic data for the Colombian, Mozambican and Portuguese populations.

Therefore, this study aimed at assessing the genotype and allele frequencies of the *SLCO1B1* c.521T>C variant in Colombian, Mozambican and Portuguese healthy volunteers. Genomic DNA isolated from blood samples obtained from 67 Colombian, 53 Mozambican and 61 Portuguese healthy individuals under informed consent was analyzed for the *SLCO1B1* c.521T>C polymorphism using a novel PCR-RFLP genotyping method. A total of 47 (70.15%), 12 (17.91%) and 8 (11.94%) out of 67 Colombian individuals, 47(88.68%), 5(9.43%), 1(1.89%) out of 53 Mozambican individuals, and 40(65.57%), 16(26.23%), 5(8.20%), out of 61 Portuguese individuals were *SLCO1B1* c.521T>C T/T-homo (reference), C/T-hetero, and C/C-homozygote genotypes, respectively. And the allelic frequency of the populations shows a total of 106 (79.1%) T and 28 (20.9%) C alleles of Colombian individuals, 99 (93.4%) T and 7 (6.6%) C alleles of Mozambican, and 96 (78.7%) T and 26 (21.3%) C alleles of Portuguese individuals. The frequency observed in Mozambicans shows statistically significant difference from the ones observed in Colombians ($p=0.0017$) and Portuguese ($p=0.0022$). Whereas the frequency observed in Colombians was not statistically significant ($p=1.000$) from the ones observed in Portugal.

In conclusion, our data demonstrated that the homozygote T/T genotype is highly prevalent, and the homozygote C/C variant is very less frequent in Mozambicans, whereas the homozygote C/C variant was relatively frequent in Colombians and Portuguese populations. As a result, subjects (mainly Caucasians) with the homozygous variant c.521CC genotype may be highly susceptible to statin related adverse effects than those with the c.521TT genotype of the c.521T>C SNP.

Key words; SNP, *SLCO1B1*, statins, influx transporters

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LIST OF ABBREVIATIONS

3'UTR	3'-untranslated region
AA	Amino Acid
ABC	ATP-binding cassette
ACAT	Acyl-coenzyme A: cholesterol acyltransferase
ACE	Angiotensin-converting enzyme
Apo	Apolipoprotein
ASBT	Apical sodium dependent bile acid transporter
ATP	Adenosine triphosphate
AUC	Area under the concentration-time curve
AUC_{0-t}	AUC from time 0 to t hours
Bamet	Bile acid-cisplatin derivative
BBMRI	Biobanking and Biomolecular Resources Research Infrastructure.
BCRP	Breast cancer resistance protein
BMI	Body mass index
Bp	Base pair
BSEP	Bile salt export pump
BSP	Bromosulphophtalein
CA	Cholic acid
CDCA	Chenodeoxycholic acid
cDNA	Coding deoxyribonucleic acid
CHD	Coronary heart disease
CHO	Chinese hamster ovary
CI	Confidence interval
CK	Creatine kinase
CL	Clearance
C_{max}	Peak plasma concentration
CoA	Coenzyme A
CPIC	Clinical Pharmacogenetics Implementation Consortium

CPIC	Clinical Pharmacogenetics Implementation Consortium
CV	Coefficient of variation
CVD	Cardiovascular disease
CYP	Cytochrome P450
CYP7A1	Cholesterol 7α–hydroxylase
CYP8B1	Sterol 12α–hydroxylase
dbSNP	NCBI single nucleotide polymorphism database
DHEAS	Dehydroepiandrosterone sulphate
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
FGF-19	Fibroblast growth factor-19
FXR	Farnesoid X receptor
gDNA	Genomic deoxyribonucleic acid
GLC	Gas-liquid chromatography
HDL	High-density lipoprotein
HEK293	Human embryonic kidney cells
HeLa	Human cervical carcinoma cells, name derived from Henrietta Lacks
HGDP	Human Genome Diversity Project
HGP	Human Genome Project
HIV	Human immunodeficiency virus
HMG	3-hydroxy-3-methylglutaryl
HMG-CoAR	HMG-CoA reductase
Ht	Haplotype
IBABP	Ileal bile acid binding protein
IDL	Intermediate-density lipoprotein
kDa	Kilo Dalton
K_e	Elimination rate constant
kel	Constant rate of elimination

Kg	Kilogram
K_m	Michaelis-Menten kinetic constant
LCL	Lymphoblastoid cell line
LD	Linkage disequilibrium
LD	Linkage disequilibrium
LDL	Low-density lipoprotein
LDL-C	LDL-cholesterol
LST	Liver-specific transporter
LXR	Liver X receptor
MATE	Multidrug and toxin extrusion transporter
MDR1	Multidrug resistance transporter
mRNA	Messenger ribonucleic acid
MRP	Multidrug resistance-associated protein
MRT	Mean residence time
NCBI	National Center for Biotechnology Information
NPC1L1	Niemann-Pick C1-like 1 protein
NTCP	Sodium-dependent taurocholate co-transporting protein
OAT	Organic anion transporter
OATP	Organic anion-transporting polypeptide
OATP1B1	Organic anion transporting polypeptide 1B1
OCT	Organic cation transporter
OCTN	Organic cation/carnitine transporter
OST	Organic solute transporter
PAH	Para-aminohippuric acid
PCR	Polymerase chain reaction
PD	Pharmacodynamic
PEPT	Peptide transporter
PK	Pharmacokinetics
PXR	Pregnane X receptor
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid

SLC	Solute carrier
SLCO	Solute carrier organic anion transporter
SLCO1B1	Solute carrier organic anion transporter family member 1B1
SNP	Single nucleotide polymorphism
SREBP	Sterol regulatory element binding protein
$t_{1/2}$	Elimination half-life
TAE	Tris-Acetate-EDTA Buffer
TM	Transmembrane domain
T_{max}	Time to peak plasma concentration
UGT	Uridine diphosphate-glucuronosyltransferase
UNG	Uracil-N-glycosylase
UV	Ultraviolet
Vd	Volume of distribution
VLDL	Very-low-density lipoprotein

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1. INTRODUCTION

Genetics is a biological science that studies heredity. The passing on of traits from parents to their offspring; either through asexual reproduction or sexual reproduction is known as heredity. The offspring cells or organisms get the genetic information from their parents. Variations among individuals can accumulate and cause species to evolve by natural selection. Inherited traits are controlled by genes and the complete set of genes within an organism's genome is called its genotype (Pearson, 2006).

The interaction of the genotypes with the environment leads to the development of these traits (Visscher, et al., 2008). Consequently, many features of an organism's phenotype are not inherited. For example, the interaction between a person's phenotype and sunlight results in a suntanned skin (Shoag, et al., 2013). Thus, suntans are not passed on to people's children. However, due to differences in their genotype, some people tan more easily than others as in the case of inherited trait of albinism who do not tan at all and are very sensitive to sunburn (Pho & Leachman, 2010). The nucleus of each human somatic cell carries 46 chromosomes: two copies of each of the 22 autosomal chromosomes, and either two copies of the X-chromosome (in females), or one copy each of the X and Y chromosomes (in males). Each cell also contains numerous mitochondria, which have their own ˜16 kb (kilobase pair) genome (Oetting, et al., 1996), (Lander, et al., 2001), (Venter, et al., 2001).

DNA (Deoxyribonucleic Acid) is a complex double stranded molecule composed of four different nucleotides linked by phosphate diester covalent bonds, associated with histone proteins, that encodes genetic information by which heritable traits are known to be passed from one generation to the next (Pearson, 2006); whereas a gene is a portion of DNA molecule that specifies a single functional unit. Genes are different by their sequences of bases. The condensed structures of long DNA molecules within a cell are called chromosomes. The genetic material is therefore inherited from parents in the form of homologous chromosomes that contains a distinct combination of DNA sequences that code for genes. The specific site of a DNA sequence within a chromosome is known as a *locus*. In other words, the term *locus* refers to the chromosomal location of a gene or DNA marker. If the DNA sequence at a specific *locus* varies among individuals, the different forms of this sequence are called alleles. Mutations can change DNA sequences, producing new alleles. The new allele produced by a mutation within a gene may affect the trait that the gene controls, modifying the phenotype of the organism (Futuyma, 2005).

A single copy of the human nuclear genome (one copy each of chromosomes 1-22, X and Y) is 3.2 gigabase pairs in length and has been estimated to contain approximately 30,000–35,000 protein-coding genes; approximately 1.5% of our DNA is protein-coding sequence, hidden within a large excess of non-coding DNA (Lander, et al., 2001) (Venter, et al., 2001). This non-coding DNA contains introns, as well as genes that produce non-coding RNAs, including transfer RNA, ribosomal RNA, small nuclear RNA, small nucleolar RNA, microRNAs, small interfering mRNAs, and small temporal RNAs (Eddy, 2001) (Moss, 2001).

Non-coding DNA also contains numerous other functionally important sequences, including those necessary for maintenance of chromosome ends, proper segregation of chromosomes during cell division, and initiation and regulation of gene expression. Much of our genome has no currently known function; this sequence includes pseudogenes, and the roughly 48% of our DNA that consists of interspersed repetitive elements. This latter group includes many types of transposon-derived repeats, as well as simple sequence repeats (Oetting, et al., 1996). Over 90% of genes contain at least one and usually several alternative splice variants, in which the exons are combined in different ways to produce 2 or more gene products from the same *locus*.

1.1. Genetic Variation

Variation, in biology is, any difference between cells, individual organisms, or groups of organisms of any species caused either by genetic differences (genotypic variation) or by the effect of environmental factors on the expression of the genetic potentials (phenotypic variation). Variation may be shown in physical appearance, metabolism, fertility, mode of reproduction, behavior, learning and mental ability, and other obvious or measurable characters. Genotypic variations are caused by differences in number or structure of chromosomes or by differences in the genes carried by the chromosomes. Eye color, body form, and disease resistance are genotypic variations. Environmentally caused variations may result from one factor or the combined effects of several factors, such as climate, food supply, and actions of other organisms. Phenotypic variations also include stages in an organism's life cycle and seasonal variations in an individual. These variations do not involve any hereditary alteration and in general are not transmitted to future generations; consequently, they are not significant in the process of evolution (The Editors of Encyclopaedia Britannica, 2018).

Genetic variation results in different forms, or alleles, of genes. Any variation that occurs will be due to the genes inherited from our parents. For example, eye color, skin tone and face shape are

all determined by our genes. In contrast, although weight is partly influenced by our genetics, it is strongly influenced by our environment. For example, how much we eat and how often we exercise. Genetic variation can also explain some differences in disease susceptibility and how people react to drugs (The Editors of yourgenome, 2015).

Nevertheless, most traits are more complex and are controlled by multiple interacting genes within and among organisms (Phillips, 2008) (Wu & Lin, 2006). Genetic variation defines the difference in DNA among individuals. It measures the variation that exists in the genetic makeup of individuals within population. Genetic variation is caused by variation in the order of bases in the nucleotides in genes (EMBL-EBI Training, 2017).

Genetic variation can be identified from observations of phenotypic variation in either quantitative traits (e.g., height) or discrete traits (e.g., color). It can also be identified by examining variation at the level of proteins using protein electrophoresis.

Nowadays, the new technologies allow scientists to directly sequence DNA. Analysis of DNA has shown genetic variation in both coding regions and in the non-coding intron region of genes. Phenotypic variation is the result of genetic variation in the nucleotides in the DNA sequence which in turn results in a difference in the amino acids in proteins coded by that DNA sequence, and if the consequential differences in amino acid sequence influences the shape, and thus the function of the enzyme (Pavlopoulos, et al., 2013). For a given genome of a multicellular organism, genetic variation may be acquired in somatic cells or inherited through the germline cells.

There are numerous sources of genetic variation, including mutation, and genetic recombination (crossing over between chromatids of homologous chromosomes during meiosis) (EMBL-EBI Training, 2017). Random mutations are the ultimate source of genetic variation. Mutations are likely to be rare and most of them are neutral or deleterious, but in some occasions, the new alleles can be favored by natural selection. Genetic variation is important in evolution. Evolution relies on genetic variation that is passed down from one generation to the next. Favorable characteristics are 'selected' for, survive and are passed on. This is known as natural selection (The Editors of yourgenome, 2015). Genetic variation is advantageous to a population because it enables some individuals to adapt to the environment while maintaining the survival of the population (Lumen Online Homework Manager, 2017). During meiotic cell division, crossing over (genetic

recombination) and random segregation can result in the production of new alleles or new combinations of alleles. Furthermore, random fertilization and random mating between organisms also contributes to variation (Lumen Online Homework Manager, 2017) (Pavlopoulos, et al., 2013).

The Human Genome Project (HGP) was an international scientific research project with the goal of determining the sequence of nucleotide base pairs that make up human DNA, and of identifying and mapping all of the genes of the human genome from both a physical and a functional standpoint (WGBH, 2003). The project formally launched in 1990 and was declared complete on April 14, 2003 (National Human Genome Research Institute, 2018). Funding came from the US government through the National Institutes of Health (NIH) as well as numerous other groups from around the world. Most of the government-sponsored sequencing was performed in twenty universities and research centers in the United States, the United Kingdom, Japan, France, Germany and China (National Human Genome Research Institute, 2018).

The Human Genome Project (HGP) was declared complete in April 2003. An initial rough draft of the human genome was available in June 2000 and by February 2001 a working draft had been completed and published followed by the final sequencing mapping of the human genome on April 14, 2003. Although this was reported to cover 99% of the euchromatic human genome with 99.99% accuracy, a major quality assessment of the human genome sequence was published on May 27, 2004 indicating over 92% of sampling exceeded 99.99% accuracy which was within the intended goal. (Schmutz, et al., 2004) Further analyses and papers on the HGP continue to occur (U.S. Department of Energy, 2019). There are approximately 22,300 protein-coding genes in human beings, the same range as in other mammals (Mihaela & Steven, 2010).

The sequence of the DNA is stored in databases available to anyone on the Internet. The U.S. National Center for Biotechnology Information (and sister organizations in Europe and Japan) house the gene sequence in a database known as GenBank, along with sequences of known and hypothetical genes and proteins. Other organizations, such as the UCSC Genome Browser at the University of California, Santa Cruz, (Bentley, 2000) and Ensemble (James, et al., 2004) present additional data and annotation and powerful tools for visualizing and searching it.

In 2002 the National Institutes of Health started a $138 million project called the HapMap to catalog the common variants in European, East Asian and African genomes) (Naidoo, et al., 2011). Although the main sequencing phase of the HGP has been completed, studies of DNA variation

continued in the International HapMap Project, whose goal was to identify patterns of single-nucleotide polymorphism (SNP) groups (called haplotypes, or “haps”). The genome published by the HGP does not represent the sequence of every individual's genome. It is the combined mosaic of a small number of anonymous donors, all European origin. The HGP genome is a scaffold for future work in identifying differences among individuals. Subsequent projects sequenced the genomes of multiple distinct ethnic groups, though as of today there is still only one "reference genome”.

1.2. Pharmacogenetics

Pharmacogenetics, related to pharmacology, is the study of genetic factors influencing drug response. In broad terms, it’s the study of the genetic variation between individuals that affects their response to drugs/pharmaceuticals and other xenobiotics, both therapeutically and in terms of adverse effects (side effects). Personalized medicine is the use of pharmacogenetics to understand how an individual can benefit from specific drugs (Springer Nature , 2019). It analyzes how the genetic makeup of an individual affects his/her response to drugs (Gennady, 2015), and it deals with the influence of acquired and inherited genetic variation on drug response in patients by correlating gene expression or single-nucleotide polymorphisms (SNPs) with pharmacokinetics (drug absorption, distribution, metabolism, and elimination) and pharmacodynamics (effects mediated through a drug's biological targets) (UNC: Eshelman School of Pharmacy, 2014) (Julie, 2003) (Kelan & Scott, 2014).

The term pharmacogenomics is often used interchangeably with pharmacogenetics. Although both terms relate to drug response based on genetic influences, pharmacogenetics is the study of interindividual variations in DNA sequence related to drug response, while pharmacogenomics is the study of the variability of the expression of individual genes relevant to disease susceptibility as well as drug response at cellular, tissue, individual or population level (European Medicines Agency, 2002).

Pharmacogenomics aims to develop rational means to optimize drug therapy, with respect to the patients' genotype, to ensure maximum efficiency with minimal adverse effects (Becquemont, 2009). Pharmacogenomics also attempts to eliminate the trial-and-error method of prescribing, allowing physicians to take into consideration their patient's genes, the functionality of these genes, and how this may affect the efficacy of the patient's current or future treatments (and where applicable, provide an explanation for the failure of past treatments) (Sheffield & Phillimore, 2009)

(Hauser, et al., 2018). Such approaches promise the advent of precision medicine and even personalized medicine, in which drugs and drug combinations are optimized for narrow subsets of patients or even for each individual's unique genetic makeup (U.S. Food and Drug Administration, 2005) (Squassina, et al., 2010). Whether used to explain a patient's response or lack thereof to a treatment, or act as a predictive tool, it hopes to achieve better treatment outcomes, greater efficacy, minimization of the occurrence of drug toxicities and adverse drug reactions (ADRs).

Usually patient genotypes are categorized into four predicted phenotypes:

- Ultra-rapid metabolizer: patients with substantially increased metabolic activity
- Extensive metabolizer: normal metabolic activity
- Intermediate metabolizer: patients with reduced metabolic activity
- Poor metabolizer: patients with little to no functional metabolic activity

The two extremes of this spectrum are the poor metabolizers and ultra-rapid metabolizers. Efficacy of a medication is not only based on the above metabolic statuses, but also the type of drug consumed. Drugs can be classified into two main groups: active drugs and prodrugs. Active drugs refer to drugs that are inactivated during metabolism, and prodrugs are inactive until they are metabolized (Cohen, 2008).

Some of the commonly known applications of pharmacogenomics include: Improve drug safety and reduce ADRs; Tailor treatments to meet patients' unique genetic pre-disposition, identifying optimal dosing; Improve drug discovery targeted to human disease; and Improve proof of principle for efficacy trials (Cohen, 2008).

1.3. Genetic Polymorphism

Polymorphism is a combination of the Greek words *poly-* (meaning multiple) and *morph-* (meaning form), polymorphism is a term used in genetics to describe multiple forms of a single gene that exists in an individual or among a group of individuals (Phillips T. , 2019). Where monomorphism means having only one form and dimorphism means there are only two forms, in genetics and biology, the term polymorphism is a very specific term, relating to the multiple forms of a gene that can exist. The term does not extend to heritable character traits with a continuous variation such as height. Instead, polymorphism refers to forms that are discontinuous (have

discrete variation), bimodal (having or involving two modes), or polymodal (multiple modes). For example, earlobes are either attached, or they are not, it is an either/or situation and not like height, which is not a set number or another (Phillips T. , 2019).

1.4. Single Nucleotide Polymorphisms (SNPs)

A gene is said to be polymorphic if more than one allele occupies that gene's locus within a population. In addition to having more than one allele at a specific locus, each allele must also occur in the population at a rate of at least 1% to generally be considered polymorphic (Biology Online Dictionary, 2019) (Ford, Ecological Genetics , 1975).

Put simply, polymorphism is when there are two or more possibilities of a trait on a gene. The most common type of genetic variation amongst people is the single nucleotide polymorphisms (SNPs, pronounced 'snips'). Each single nucleotide polymorphism represents a difference in a single DNA base, adenine (A), guanine (G), thymine (T), or cytosine (C), in a person's DNA molecule. On average they occur once in every 300 bases and are often found in the DNA between genes (Ford, Polymorphism and Taxonomy , 1940) (National Human Genome Research Institute, 2017).

Gene polymorphisms can occur in any region of the genome. In fact, roughly 90% of the genetic variation that exists between humans is the result of SNPs, although most polymorphisms are silent, do not alter cellular function and thus have no effect. Some polymorphisms have a detectable impact on function and have been discovered to contribute to the development of diseases such as cancer and to influence physiological responses to drugs (Chanock, 2007).

A polymorphic variant of a gene can lead to the abnormal expression or to the production of an abnormal form of the protein; this abnormality may cause or be associated with disease. For example, a polymorphic variant of the enzyme CYP4A11 in which thymidine replaces cytosine at the gene's nucleotide 8590 position encodes a CYP4A11 protein that substitutes phenylalanine with serine at the protein's amino acid position 434. This variant protein has reduced enzyme activity in metabolizing arachidonic acid to the blood pressure-regulating eicosanoid, 20-hydroxyeicosatetraenoic acid. A study has shown that humans bearing this variant in one or both of their CYP4A11 genes have an increased incidence of hypertension, ischemic stroke, and coronary artery disease (Wu, et al., 2014).

SNPs act as chromosomal tags to specific regions of DNA, and these regions can be scanned for variations that may be involved in a human disease or disorder. SNPs found to be associated with disease may be useful for diagnostic purposes. In addition, identifying which variations are involved in altering responses to drugs could facilitate the development of personalized medicine. This approach to treatment is based on the concept that genetic screening for specific SNPs in a person's genome can be used to select drugs or adjust dosages most appropriate for that individual. Personalized medicine could be used to avoid potentially dangerous drug responses that are the result of altered cellular metabolism caused by a specific SNP (The Editors of Encyclopaedia Britannica, 2019).

A mutation may create a polymorphism in the population if the resulting variant form is transmitted to subsequent generations without causing major defects in biological functions.

Mutations originate from unrepaired DNA damage caused by replication errors and lesions from endogenous or exogenous mutagens as well as from insertion or deletion of DNA segments of DNA by mobile elements. Mutant proteins or nucleic acids that causes inherited diseases are considered mutations rather than polymorphisms even though they may exist in the population at significant levels – e.g. higher than the 1% level noted.

Polymorphism is related to population diversity. Mutation can be germ line or somatic. Germline mutation is the cause of polymorphism in sexual reproductive population of a species.

All polymorphisms result from mutations but not all mutations will go on to become polymorphisms. A mutation (in DNA) is a change in DNA sequence away from the "reference", not the "normal", because in real biological populations or in the wild, there is really no "normal" or "wild-type", only varying frequencies of different alleles, i.e. polymorphism due to natural variation. Mutations by themselves do not classify as polymorphisms. A polymorphism is a DNA sequence variation that is common in the population. A mutation, on the other hand, is any change in a DNA sequence away from normal (implying that there is a normal allele running through the population and that the mutation changes this normal allele to a rare and abnormal variant) Polymorphism is common in nature; it is related to biodiversity, genetic variation, and adaptation. Continued research into gene-environment interactions may lead to more specialized treatment plans based on an individual's surroundings (Theresa, 2019) (Ford, Ecological Genetics , 1975) (Ford, Polymorphism and Taxonomy , 1940) (Sheppard, 1975).

Common polymorphisms include tandem repeated segments, large (copy number variants) and small segmental deletions/insertions/duplications, and substitutions (SNPs). SNPs are an exceedingly common form of polymorphism that may account for approximately 90% of all known sequence variation (Kerb, 2006) (Collins, et al., 1998). Functional SNPs that lead to changes in gene expression occur in all regions of the genome. SNPs in the coding (exonic) regions of transporter genes can be nonsynonymous, meaning that they lead to a change in encoded amino acids, or synonymous (no amino acid change). Although both kinds of SNPs can have an impact on transporter activity, it is widely accepted that nonsynonymous SNPs are more likely to have functional consequences. Nonsynonymous SNPs can lead to protein misfolding, polarity shift or improper phosphorylation. SNPs in the noncoding regions of transporter genes are less predictable and include variants in the intronic, promoter and 3'-untranslated region (3'UTR). These variants can affect the splicing or regulation of transporter genes. SNPs in the promoter region may modify transporter expression by altering the binding sites for transcription factors (Chorley, et al., 2008) (Gradhand & Kim, 2008).

Polymorphism in drug transporters plays a major role in interindividual differences in drug disposition. Polymorphism leading to functional changes in drug transporters can affect the pharmacokinetics and subsequent pharmacodynamics and toxicological effects of drugs. It can also affect susceptibility to certain diseases (Ho & Kim, 2005). Although detailed information on genetic variability in drug transporter genes is available, our knowledge on identifying those genetic variants that have functional significance and how they contribute to interindividual variability in drug response is still limited.

Polymorphisms in transporter genes can have profound effects on statin pharmacokinetics. A common genetic variant of organic anion-transporting polypeptide 1B1 (OATP 1B1) reduces the hepatic uptake of many statins, increasing the risk of statin-induced myopathy. Similarly, genetically impaired adenosine triphosphate (ATP)-binding cassette G2 (ABC G2) transporter efflux activity, results in a marked increase in systemic exposure to various statins. Importantly, the effects of these genetic polymorphisms differ depending on the specific statin that is used. This provides a rational basis for the individualization of lipid-lowering therapy (Niemi, 2010).

1.5. Membrane Transporters

In addition to the physicochemical properties of a compound such as charge, molecular size, lipophilicity and solubility, carrier-mediated processes or transporters also affect the

transmembrane passage of substrates. Transporters are usually separated into two major classes - uptake and efflux transporters (Figure 1.1); it has been estimated that approximately 2000 genes encode transport proteins in humans (Giacomini & Sugiyama, 2006). The polarized expression of transporters in targeted organs such as the intestine, kidney and liver and the dynamic interplay between uptake and efflux transporters, often with overlapping substrate capabilities, in the cell membrane of epithelial cells, together determine the direction and extent of flow of a number of xenobiotic and endobiotic substances (Kerb, 2006). Moreover, their expression on the interfaces of different parts of the body contributes to the maintenance of several important structural barriers, e.g. the blood-brain barrier and the blood-placental barrier (Graff & Pollack, 2004) (Syme, et al., 2004) (Molsa, et al., 2005). Transporters mediate important physiologic functions via influx and efflux of endogenous substrates such as amino acids, bile acids, steroids, sugars, lipids and hormones that are critical for normal homeostasis. They also play a significant role in mediating the absorption, tissue distribution and elimination of many environmental toxins and drugs (Ho & Kim, 2005) (Shitara & Sugiyama, 2006).

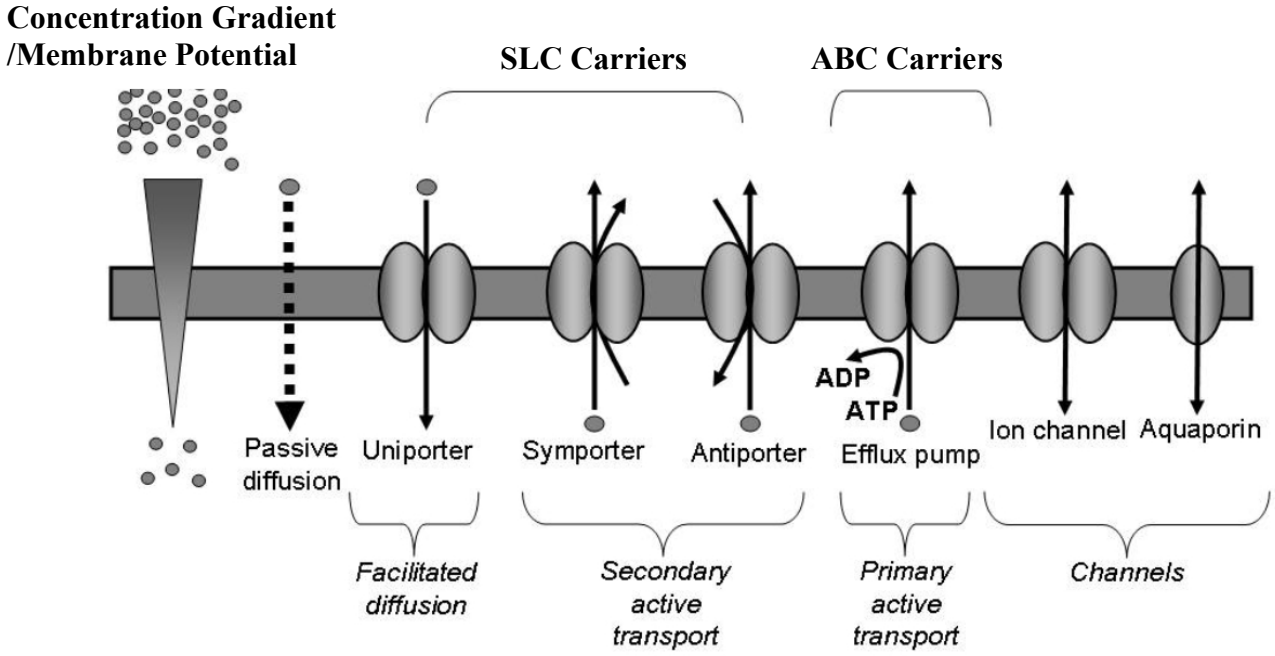

Figure 1.1. Different modes of transport mediated by the solute carriers (SLCs), ATP-binding cassette (ABC) transporters and channels in the plasma membrane (Petzinger & Geyer, 2006)

Influx (uptake) transporters facilitate the entry of drugs into cells. Uptake transporters include the organic anion-transporting polypeptides (OATPs, *SLCO*), organic anion transporters (OATs,

SLC22A), organic cation transporters (OCTs, *SLC22A*), organic cation/carnitine transporters (OCTNs, *SLC22A*), peptide transporters (PEPTs, *SLC15A*) and sodium-dependent taurocholate co-transporting protein (NTCP, *SLC10A1*) (Ho & Kim, 2005) (Zair, 2008). Multidrug and toxin extrusion transporter 1 (MATE1, *SLC47A1*) and multidrug and toxin extrusion transporter 2-K (MATE2-K, *SLC47A2*) also participate into the uptake of compounds into the cell. All these transporters belong to the superfamily of solute carriers (SLCs) (Hagenbuch & Meier, 2004) (Hediger, et al., 2004) (Seithel, et al., 2008a).

1.6. Role of Transporters in Drug Disposition

In the gut, drug absorption from the intestinal lumen is facilitated by uptake transporters and limited by efflux transporters, both expressed in the brush-border membrane of enterocytes (Figure 1.2). Following uptake, drugs are translocated via the basolateral membrane into the portal blood circulation by efflux transporters such as the multidrug resistance-associated proteins (MRPs). Many drugs also pass through the enterocytes by passive diffusion and some drugs already undergo extensive metabolism in the enterocytes (Kullak-Ublick, et al., 2004) (Glaeser, et al., 2007) (Meier, et al., 2007).

Uptake transporters like organic anion transporting polypeptide 1B1 (OATP 1B1) expressed on the basolateral (sinusoidal) membrane of hepatocytes facilitate the influx of compounds into the liver from the portal blood (Figure 1.2). Drugs taken into the hepatocytes often undergo metabolic transformation and/or conjugation, or they may be excreted unchanged (Pang & Rowland, 1977). Transport of the drugs and their metabolites out of the hepatocyte via the canalicular membrane into the bile or via the basolateral membrane back into the portal blood may be mediated by efflux transporters (Shitara & Sugiyama, 2006).

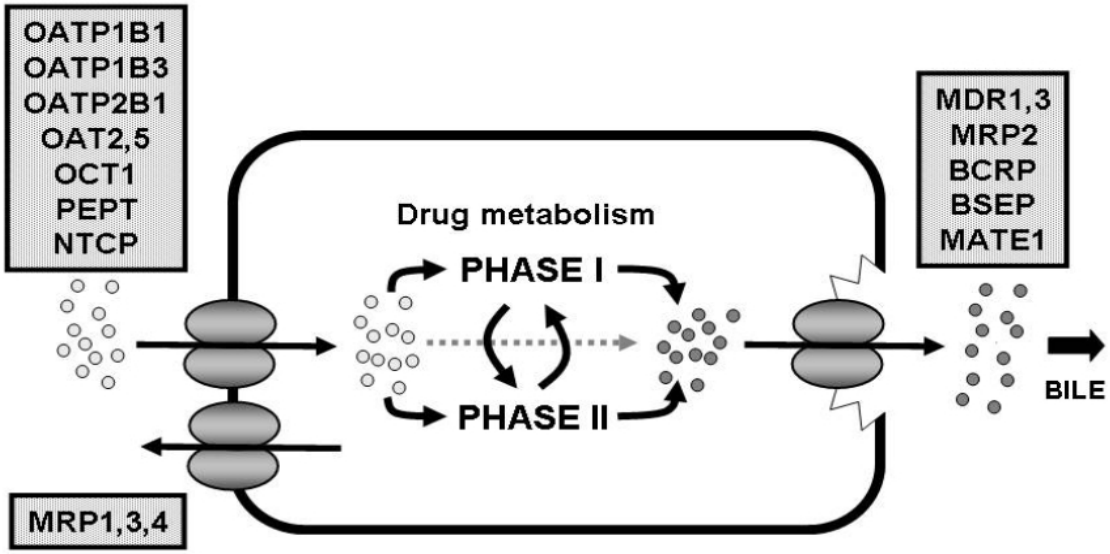

Figure 1.2. Drug elimination in the liver involves uptake into hepatocytes via transporters and/or passive diffusion, followed by metabolism and efflux to the bile or blood (Pasanen M. , 2008).

1.7. Pharmacogenomics of Statins

Statins are reversible competitive inhibitors of the HMG-CoA reductase enzyme, which catalyzes the first and rate-limiting step from HMG-CoA to mevalonate in *de novo* cholesterol synthesis. Figure 1.3 depicts the biosynthetic pathway of cholesterol and other cometabolites.

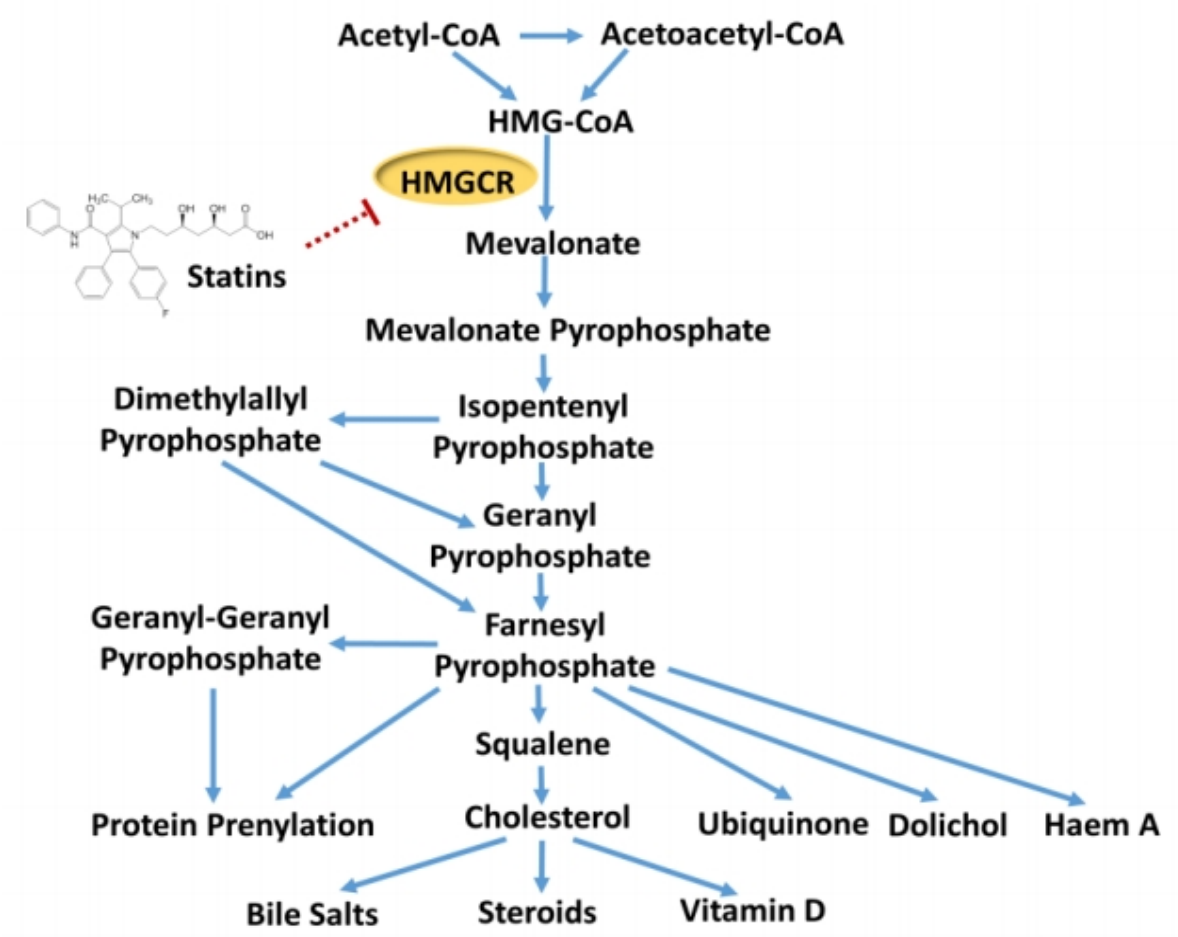

Figure 1.3. Simplified scheme of the biosynthetic pathway of cholesterol and other cometabolites (Turner & Pirmohamed, 2018)

The pharmacogenomics of statins involves many different genes, including those involved in the pharmacokinetics (PK) effects of the drug transport and metabolism (Figure 1.4) and those involved in mediating the pharmacodynamic (PD) effects of statins on plasma lipoprotein metabolism (Whirl-Carrillo, et al., 2012) (Figure 1.5).

Statins (atorvastatin, lovastatin and simvastatin, fluvastatin, pravastatin and rosuvastatin) are dosed orally and enter the systemic circulation through intestinal cells both passively and by active transport via the ABC and SLC gene family transporters (Figure 1.4). The major organs of metabolism and elimination include the liver and, to a lesser extent, the kidney. Metabolism is catalyzed by CYP and UGT gene family enzymes. The main pathway of elimination is ABC transporter mediated biliary excretion (Whirl-Carrillo, et al., 2012).

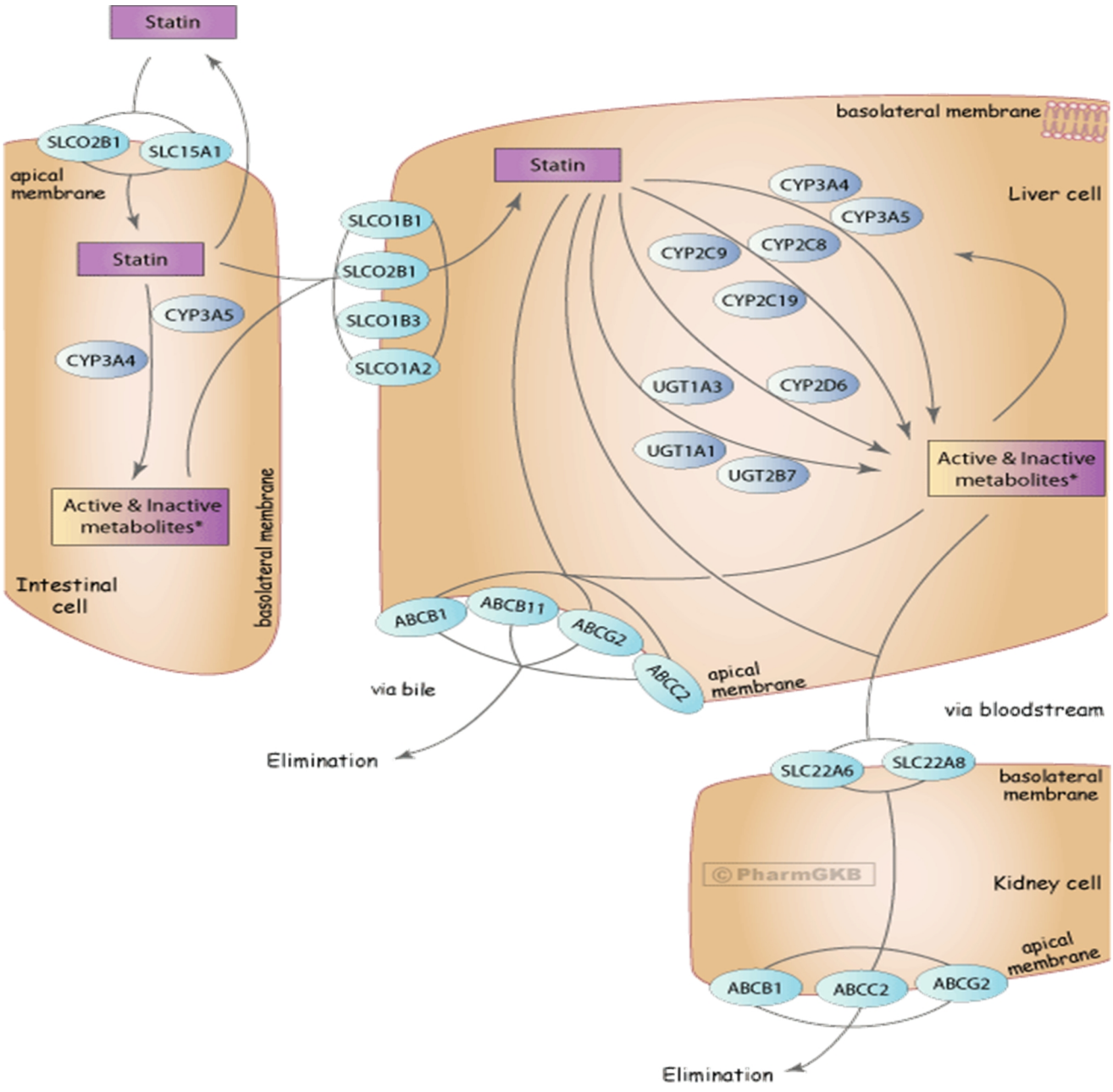

Figure 1.4. Representation of the superset of all genes involved in the transport, metabolism and clearance of statin class drugs (Whirl-Carrillo, et al., 2012)..

Inhibition of HMG CoA reductase reduces cholesterol synthesis in the hepatocytes, leading to a reduction in intracellular cholesterol concentration and a responsive increase in LDL receptor expression on the hepatocyte cell surface. This results in increased extraction of LDL-C from the

blood and decreased circulating total cholesterol and LDL-C concentrations. A meta-analysis showed that statins can lower LDL-C by an average of 1.8 mmol/l, which reduces the risk of CHD events by about 60% and stroke by 17% (Seithel, et al., 2007).

In addition to lowering plasma LDL-C and total cholesterol levels, statins also moderately reduce triglyceride levels and increase HDL-cholesterol levels (Kreisberg & Oberman, 2002). Secondary mechanisms by which statins may reduce lipoprotein levels include inhibition of hepatic synthesis of apoB100 and reduction in synthesis and secretion of triglyceride-rich lipoproteins (VLDL and IDL). In addition, the inhibition of synthesis of nonsteroidal isoprenoid compounds (also produced from mevalonate) (Figure 1.5) may explain the pleiotropic properties of statins leading to beneficial cardiovascular effects independent of their lipid-modifying properties. These include restoration of endothelial cell function, modification of inflammatory responses, antithrombotic and antioxidant effects, as well as reduction of smooth muscle cell proliferation and cholesterol accumulation. These biological effects beyond LDL reduction may differ among statins (Rosenson & Tangney, 1998) (Chong, et al., 2001) (Bonetti, et al., 2003). Statins have been shown to slow the progression or even promote regression of coronary atherosclerosis, possibly due to shrinkage of the lipid core of the atherosclerotic plaque, avoiding plaque rupture that would otherwise trigger intramural hemorrhage and intraluminal thrombosis (Kreisberg & Oberman, 2002) (Liao, 2002).

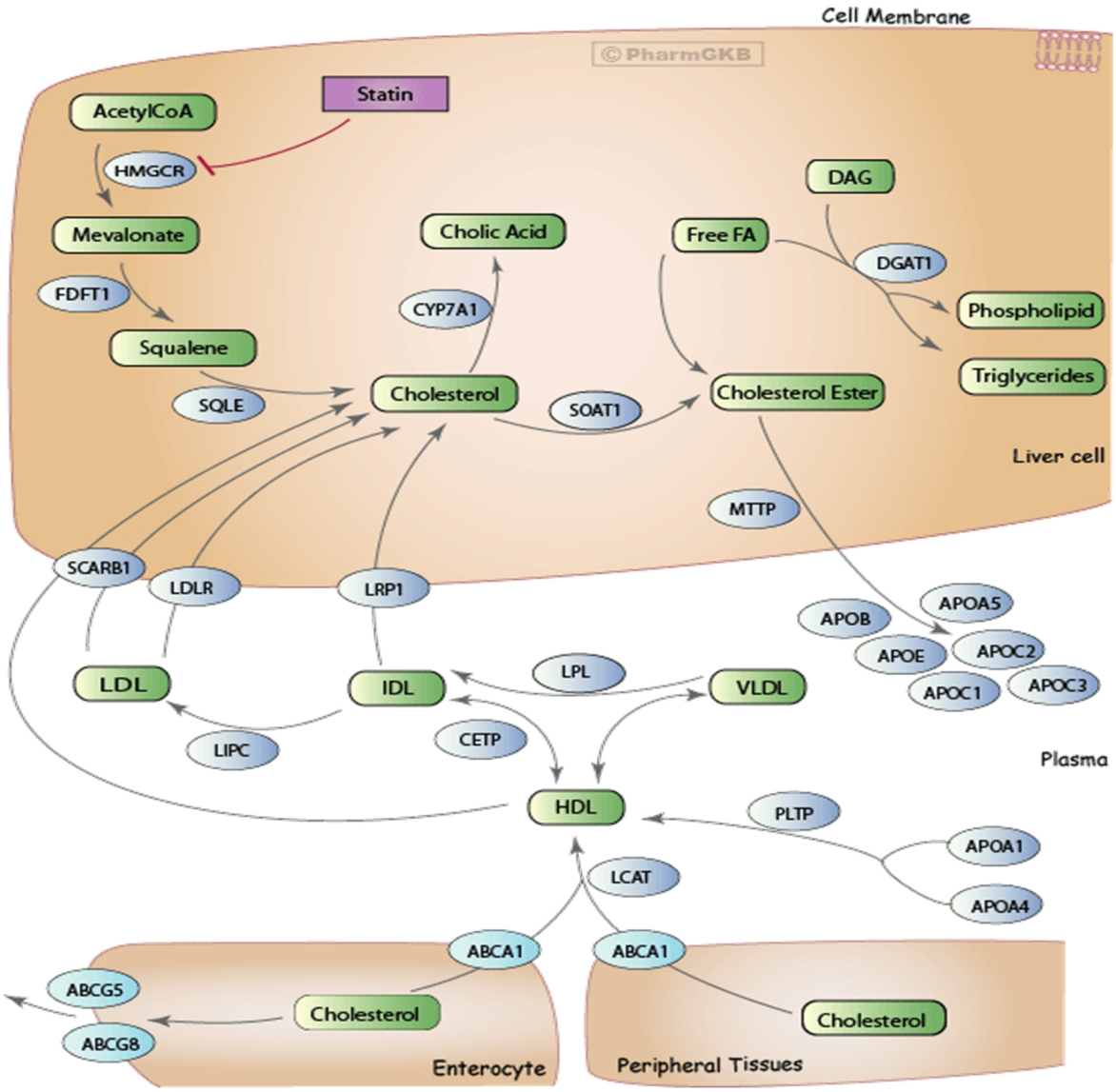

Figure 1.5. Genes involved in mediating statin effects on hepatic cholesterol metabolism and consequent effects on plasma lipoprotein transport (Whirl-Carrillo, et al., 2012).

Interindividual variation in the response to statins limits the beneficial effects of statin therapy. Genetic differences in drug metabolizing enzymes or drug transporters can also predispose to statin myotoxicity. A study analyzed two sets of patient and control groups from large trials involving approximately 12 000 and 20 000 participants, treated with 80 mg and 40 mg of simvastatin per day, respectively (Pasanen M. , 2008). A strong association was found between myopathy and two tightly linked variants in the influx transporter gene *SLCO1B1*, one of them being the c.521T>C variant. The odds ratio for myopathy was 4.5 per copy of the c.521C allele, and 16.9 in c.521CC, compared with the c.521TT homozygotes. In this large-scale study, approximately 60% of the

simvastatin-induced myopathy cases were attributable to the c.521 variant allele. In a previous Japanese study, the *SLCO1B1*15* haplotype also containing the c.521T>C SNP (c.388G-c.521G) was associated with pravastatin-induced myopathy (Morimoto, et al., 2004) (Morimoto, et al., 2005).

1.8. Organic Anion Transporting Polypeptides (OATPs)

Organic anion transporting polypeptides (OATPs (rodents: Oatps; human: OATPs)) are basolateral plasma membrane transport proteins that mediate the sodium-independent uptake of amphipathic compounds including bile salts, steroid conjugates, thyroid hormones, anionic oligopeptides and conjugated and unconjugated bilirubin (Hagenbuch & Meier, 2004).

Their transport mechanism is electroneutral exchange, coupling the cellular uptake of organic compounds with the efflux of neutralizing anions such as bicarbonate, glutathione or glutathione-S-conjugates (Satlin, et al., 1997) (Li, et al., 1998). OATPs have been identified at least in the intestine, liver, kidney, lung, testis, placenta and blood-brain barrier. Genes encoding the OATP uptake transporters form a large family of solute carrier organic anion transporters (*SLCO*) within the *SLC* superfamily (Hagenbuch & Meier, 2004). OATPs in the OATP/*SLCO* superfamily are subdivided into families indicated by a number for > 40% amino acid sequence identity (e.g. OATP1/*SLCO1*), into subfamilies indicated by a letter for >60% amino acid sequence identity (e.g. OATP1B /*SLCO1B*) and into individual genes and gene products indicated by an additional number (e.g. OATP1B1/*SLCO1B1*), according to the phylogenetic relationships and chronology of identification (Hagenbuch & Meier, 2004). OATP1A2 (previously known as OATP-A) was the first member to be described, followed by the discovery of OATP1B1 in 1999 (Kullak-Ublick, et al., 1995) (Abe, et al., 1999) (Hsiang, et al., 1999) (Konig, et al., 2000). Up to now, 36 mammalian OATPs and 11 human OATPs have been identified (Hagenbuch & Meier, 2003) (Mikkaichi, et al., 2004). OATP1B1, encoded by the gene *SLCO1B1*, is one of the main sodium-independent bile acid and organic anion transporters in the liver (Hsiang, et al., 1999) (Konig, et al., 2000).

In a recent study, OATP1B1 was also found at the messenger ribonucleic acid (mRNA) level in small intestinal enterocytes (Glaeser, et al., 2007). In addition to the physiological function, OATPs have been found to transport an increasing number of frequently used drugs, such as several HMG CoA reductase inhibitors (statins), fexofenadine, benzylpenicillin, repaglinide,

valsartan, and temocaprilat (Maeda, et al., 2006). OATPs can be inhibited by cyclosporine, rifampicin, gemfibrozil and macrolides (Niemi, 2007).

OATP1B1 (previously known as OATP-C, OATP2, *SLC21A6*, and liver specific transporter 1 LST-1) is one of the most important influx transporter proteins in the OATP family of transporters (Hagenbuch & Meier, 2004). OATP1B1 is expressed in the sinusoidal membrane of hepatocytes and participate in the active uptake of compounds from the portal blood into the liver (Hagenbuch & Meier, 2004) (Niemi, 2007).

Numerous genetic polymorphisms have been identified in the OATP1B1encoding gene. Indeed, polymorphisms in *SLCO1B1* has recently been associated with altered pharmacokinetics of pravastatin, pitavastatin, simvastatin, atorvastatin, rosuvastatin, repaglinide, nateglinide, fexofenadine, atrasentan, paclitaxel, digoxin and also with variable changes in cholesterol levels following statin administration (Hedman, et al., 2006) (Pasanen, et al., 2006) (Niemi, et al., 2005) (Maeda, et al., 2006)

1.8.1. Structure and Function

Bioinformatics analysis using various topology models has revealed that OATP1B1 is a 691-amino acid glycoprotein with 12 putative membrane-spanning domains (currently accepted structure based on hydropathy analyses) and a large fifth extracellular loop (Pasanen, et al., 2006) (Chang, et al., 2005) (Figure 1.6). Common to all OATP proteins, OATP1B1 carries N-glycosylation sites in extracellular loops 2 and 5. It has two potential phosphorylation sites and transmembrane domain 10 (TM10) is critical for its function. Its apparent molecular mass is 84 kDa, which is reduced after deglycosylation to 54 kDa (Konig, et al., 2000). It's almost exclusive expression in the human liver suggests that it plays a crucial role in the hepatic uptake and clearance of amphipathic organic compounds.

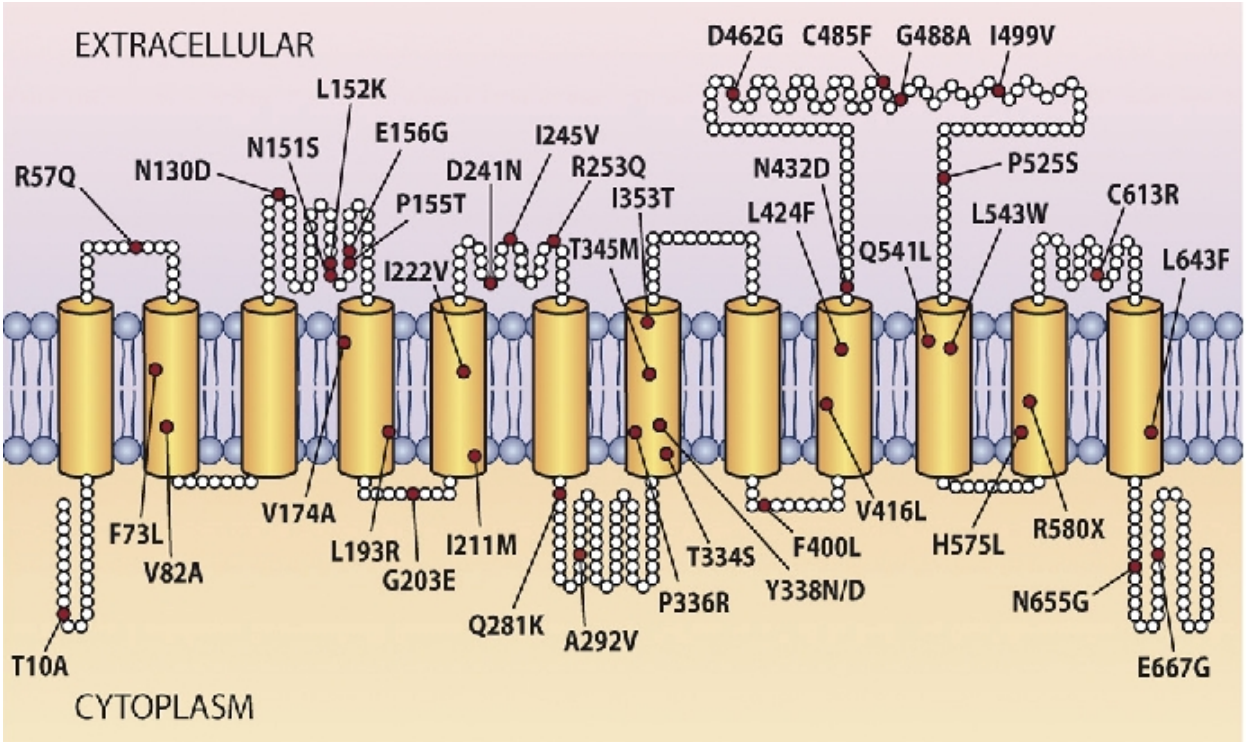

Figure 1.6. The predicted transmembrane secondary structure of OATP1B1, depicting the positions of nonsynonymous single nucleotide polymorphisms (Mikko, et al., 2011).

The mechanism or driving force for transport of substrate transport is not completely understood, although it has been suggested that OATPs translocate their substrates through a central, positively charged pore in a so-called rocker-switch type of mechanism (Meier-Abt, et al., 2005). However, a report demonstrated that the transporter is influenced in different ways by both pH and the membrane potential (Martinez-Becerra, et al., 2011). This transport is independent of sodium, chloride and potassium gradients, membrane potential and ATP levels. The phosphorylation state of at least the rat Oatp1a1 is important for transport, since extracellular ATP reduces Oatp1a1-mediated bromosulphophtalein (BSP) transport in rat hepatocytes via phosphorylation of intracellular serine residues (Glavy, et al., 2000). No higher solution structures are currently known for OATP1B1, but one recent study using three-dimensional quantitative structure-activity relationship models showed that OATP1B1 substrates produce a pharmacophore containing two hydrogen bond acceptors, one hydrogen bond donor and two hydrophobic regions (Hagenbuch & Meier, 2004). In another study using a meta-pharmacophore approach combining limited datasets from different laboratories, cell types and species, a meta-model for OATP1B1was generated in

which the hydrophobic features are centrally located, and hydrogen bond features located at the extremities (Chang, et al., 2005).

1.8.2. Substrates

In general, OATP substrates appear to be anionic amphipathic molecules with a rather high molecular weight (> 450) and a high degree of albumin binding under physiological conditions (Hagenbuch & Meier, 2004). A variety of endogenous compounds such as bile acids, steroid conjugates, bilirubin glucuronides, thyroid hormones, eicosanoids, cyclic peptides and BSP are substrates of OATP1B1. Substrate drugs include the ACE inhibitors enalapril and temocapril, the angiotensin II receptor antagonists olmesartan and valsartan, the antifungal agent caspofungin, the antibiotics rifampicin and benzylpenicillin, methotrexate, the endothelin receptor antagonists atrasentan and bosentan, troglitazone sulphate, the active SN-38 metabolite of the anticancer agent irinotecan, fexofenadine, the glucuronide of the cholesterol-lowering drug ezetimibe and the statins atorvastatin, cerivastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin acid. The pharmacokinetics of repaglinide has been associated with OATP1B1 transport function; although there is no *in vitro* evidence showing that it is an OATP1B1 substrate (Niemi, et al., 2005a) (Kalliokoski, et al., 2008b). In addition, the natural toxins microcystin and phalloidin are substrates of OATP1B1 (Hagenbuch & Meier, 2003). Reports on unbound bilirubin being a substrate of OATP1B1 are somewhat controversial, since it has been shown to be transported by this transporter in both HEK293 (human embryonic kidney) cells and *Xenopus laevis* oocytes, but no transport was observed in another study with HeLa (human cervical carcinoma) and HEK293 cells (Cui, et al., 2001) (Briz, et al., 2003) (Wang, et al., 2003).

1.8.3. Inhibitors

It is widely agreed that inhibition of OATP1B1-mediated hepatic uptake of substrate drugs contributes to these clinically observed drug interactions (Shitara, et al., 2003). Gemfibrozil and its glucuronide metabolite also inhibit OATP1B1 and significantly increase the plasma concentrations of several OATP1B1 substrate drugs including simvastatin acid (Backman, et al., 2000), lovastatin acid and pravastatin (Kyrklund, et al., 2003) (Kyrklund, et al., 2001), cerivastatin (Backman, et al., 2002), repaglinide (Niemi, et al., 2003b), rosuvastatin (Schneck, et al., 2004) and atorvastatin (Backman, et al., 2005). Rifampicin, a strong inducer of drug-metabolizing enzymes, has increased the expression of OATP1B1 in cultured human hepatocytes *in vitro* (Niemi, et al.,

2003a) (Jigorel, et al., 2006). This may explain the slight decrease in plasma pravastatin concentrations when administered concomitantly with rifampicin (Kyrklund, et al., 2004). On the other hand, rifampicin is a relatively potent inhibitor of OATP1B1 and OATP1B3 *in vitro* (Vavricka, et al., 2002) In addition, the antimicrobial drugs clarithromycin, erythromycin, roxithromycin and telithromycin as well as the HIV protease inhibitors indinavir, ritonavir and saquinavir have been identified as OATP1B1 inhibitors *in vitro* (Campbell, et al., 2004) (Hirano, et al., 2006) (Seithel, et al., 2007).

1.9. Pharmacogenetics of *SLCO1B1*

The organic anion transporting polypeptide 1B1 (OATP1B1) is a genetically polymorphic influx transporter expressed on the sinusoidal membrane of human hepatocytes, and it mediates the hepatic uptake of many endogenous compounds and xenobiotics. Studies have demonstrated that OATP1B1 plays a major, clinically important role in the hepatic uptake of many drugs.

The *SLCO1B1* gene is located in chromosome 12 (gene locus 12p12). Many SNPs, both nonsynonymous and synonymous, have been discovered in the *SLCO1B1* gene, and several of these affect transport function *in vitro* and *in vivo*. Most of the SNPs associated with altered transport function span the transmembrane domains or extracellular loop 5 of OATP1B1. The effects of certain *SLCO1B1* polymorphisms on transport function appear to be substrate dependent (Tirona et al., 2001).

Cytogenetic location	12p12.2
Physical location	21.284.127 - 21.392.728
Exons	15
bp	2800
AA	691
Category	Transport carrier
Superfamily/Family	solute carriers (*SLC21A*)/ OATP
Conserved AAs	R57, K361 and R580
Subcellular localization	Basolateral membrane of hepatocytes
Physiological process	Active transport
Regulated by	HNF1A
Transmembrane domains (helices)	12

HGNC id	10959
Haplotypes	*SLCO1B1*(*1A, *1B, *5 and *15)
Common variants	190

Table 1.1. Description of the *SLCO1B1* gene (Whirl-Carrillo, et al., 2012) (NCBI, 2016)

The pharmacogenetics of solute carrier organic anion transporter family member 1B1 (*SLCO1B)* has been the topic of several studies. Two common nonsynonymous *SLCO1B1*variants have been well characterized: rs2306283 (*SLCO1B1*: 492A>G on NM_0064464, previously referred to as 388A>G; encoding OATP1B1:N130D) and rs4149056 (*SLCO1B1* 625T>C on NM_006446.4; commonly referred to as T521C, encoding OATP1B1:V174A). These two variants are in partial linkage disequilibrium. Consequently, there are four important haplotypes with these variants: *SLCO1B1**1A, containing neither variant, *SLCO1B1**1B (rs2306238), *SLCO1B1**5 (rs4149056) and *SLCO1B1**15 (with both variants) (Oshiro, et al., 2010) (Turner & Pirmohamed, 2014). *SLCO1B1* single nucleotide polymorphisms and haplotypes have been implicated in altered pharmacokinetic handling and pharmacodynamic response for several major drug classes. In cellular studies, OATP1B1-Ala174 and associated haplotypes, particularly *SLCO1B1**15, have shown reduced transport activity in comparison with OATP1B1-Val174. This may be a result of intracellular protein sequestration and reduced surface expression (Rommel, et al., 2001) (Kameyama, et al., 2005).

The rs4149056 SNP (DNA c.521T>C, protein p.V174A) in the *SLCO1B1* gene encoding OATP1B1 decreases the transporting activity of OATP1B1, resulting in markedly increased plasma concentrations of, for example, many statins, particularly of active simvastatin acid. The variant thereby enhances the risk of statin-induced myopathy and decreases the therapeutic indexes of statins. Consequently, the importance of *SLCO1B1* genetic variants in clinical pharmacogenetics is underlined by the fact that the US Food and Drug Administration recommendations for some drugs (e.g. simvastatin) have already incorporated information regarding pharmacogenetic information about *SLCO1B1* in their drug label (www.fda.gov). Extensive literature and FDA warning labels indicate increased risk for myopathy in patients with specific genetic differences on the *SLCO1B1* gene. Guidelines regarding the use of pharmacogenomic tests in dosing for simvastatin have been published in Clinical Pharmacology

and Therapeutics by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (Wilke, et al., 2012).

However, the *SLCO1B1* c.521T>C variant can have a different effect on different statins. The same variant also distinctly affects the pharmacokinetics of several other drugs. For example, an enhanced clearance of methotrexate, which increases the risk of gastrointestinal toxicity in the treatment of children with acute lymphoblastic leukemia is associated with certain *SLCO1B1* variants. some drugs (e.g., cyclosporine) potently inhibit OATP1B1, causing clinically significant drug interactions. Thus, OATP1B1 plays a major role in the hepatic uptake of drugs, and genetic variants and drug interactions affecting OATP1B1 activity are important determinants of individual drug responses (Niemi, et al, 2011).

One of the most characterized SNPs in *SLCO1B1*, the c.521T>C SNP, predicts the substitution of alanine for valine at amino acid 174 (Val174Ala). Another prevalent SNP, c.388A>G, causes an amino acid change at position 130 (Asn130Asp). When the effects of SNPs on the respective phenotype are assessed, the underlying haplotype structure should be taken into account. The c.388A>G and the c.521T>C variants are in linkage disequilibrium (LD) and exist in variable *SLCO1B1* haplotypes together. The c.388A-c.521T haplotype is known as **1A* (reference haplotype), c.388G-c.521T as **1B*, c.388A-c.521C as **5* and c.388G-c.521C as **15*. Moreover, at least in Europeans, the haplotypes comprising c.388A>G and c.521T>C can be further subclassified by two promoter region SNPs g.-11187G>A and g.-10499A>C into two other distinctive and potentially functional significant haplotypes: **16* and **17* (Figure 7) (Niemi, et al., 2004). The c.521C allele and the haplotypes **5* and **15* (containing the c.521T>C SNP) have been associated with markedly reduced uptake activity *in vitro* of the OATP1B1 substrates oestrone 3-sulphate, oestradiol-17-D-glucuronide, atorvastatin, cerivastatin, pravastatin, the SN-38 metabolite of irinotecan and rifampicin (Tirona, et al, 2001) (Tirona, et al, 2003) (Iwai, et al, 2004) (Kameyama, et al., 2005)(Nozawa, et al., 2005).

As mentioned previously, OATP1B1-dependent transport is an important step in mediating hepatic clearance of statins. The minor allele of *SLCO1B1* T521C (present in *5, *15, *16, *17 haplotypes) has been consistently associated with elevated circulating concentrations of statins, as measured by plasma area under the curve (AUC) values or Cmax implying reduced hepatic access. Because statins act primarily through hepatic mechanisms, reduced hepatic statin availability associated

with *SLCO1B1* T521C may also influence statin efficacy (Kivistö & Niemi, 2007) (Yohei, et al., 2003) (Pasanen, et al., 2007).

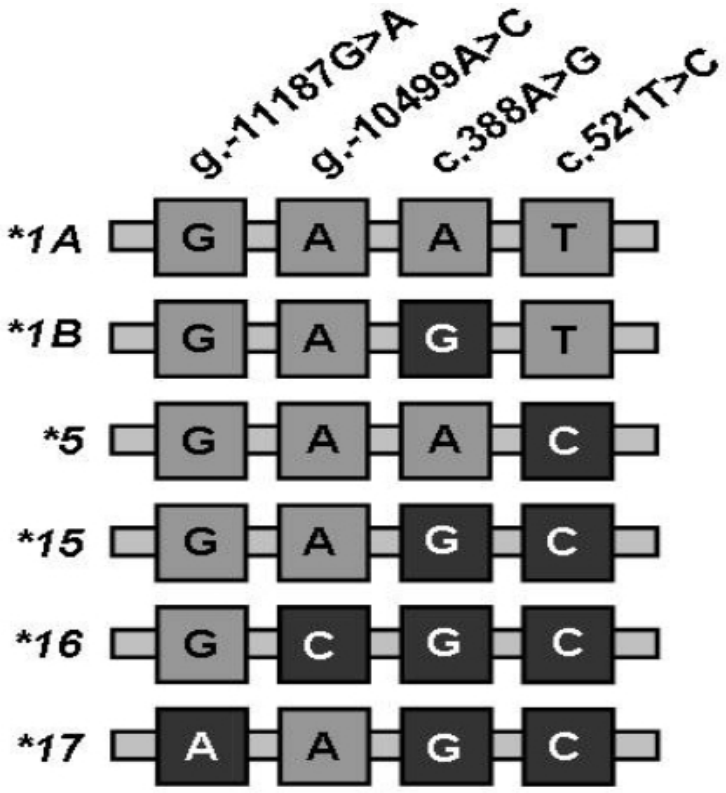

Figure 1.7. Schematic representation of functionally distinctive *SLCO1B1* haplotypes (Pasanen M. , 2008).

OATP-C (-11187G>A) is another SNP in the promoter region of the gene. The c.521T>C variant is generally referred to as a low-activity variant because it has been associated with markedly reduced uptake activity of OATP1B1 substrates *in vitro* and markedly increased plasma concentrations and reduced oral clearances *in vivo* compared with the **1A* (reference) haplotype in a variety of studies (Pasanen M. , 2008). However, it was unclear what the frequency was of **15* compared with the **5* haplotype in other populations. In addition, the frequencies of other, potentially functional, SNPs had been poorly elucidated in any population (Pasanen M. , 2008).

1.10. Global Prevalence of The SNP (c.521T>C)

Regarding the high clinical importance of *SLCO1B1* pharmacogene in drug dosing of statins, it is noteworthy to evaluate the *SLCO1B1* genotype not only of single patients, but also, the general frequency of the polymorphisms in the whole populations of different ethnic backgrounds. Studies showed that the global prevalence of the *SLCO1B1* polymorphism (c.521T>C) is significantly large and that considerable differences are observed in the prevalence of polymorphisms in *SLCO1B1* among populations from different ethnic origins (Mwinyi, et al., 2008) (Jada, et al., 2007) (Table 1.2).

Ethnic group	**n**	**c.521C allele (%)**	**Reference**
Finnish Caucasian	468	20	(Pasanen, et al, 2006)
Caucasian (European)	151	18	(Pasanen, et al, 2008)
German Caucasian	300	15	Mwinyi et al. 2008
Caucasian (Singapore)	36	22	Lee et al. 2005a
Native American	64	24	(Pasanen, et al, 2008)
European American	49	14	Tirona et al. 2001
African American	22	2.3	irona et al. 2001
Sub-Saharan African	105	1.9	(Pasanen, et al, 2008)
North African (Algerian)	29	17	(Pasanen, et al, 2008)
African (Ugandan)	115	3.9	Mwinyi et al. 2008
Japanese	27	19	(Oshiro, et al, 2010)
Korean	22	25	Chung et al. 2005
Chinese	100	13	Jada et al. 2007
Malay	35	13	Lee et al. 2005a
Asian-Indian	35	7.1	Lee et al. 2005a
Indian	100	6.5	Jada et al. 2007
Turkish	94	12	Mwinyi et al. 2008
Pakistani	192	9.4	(Pasanen, et al, 2008)
Israeli	133	20	(Pasanen, et al, 2008)
Oceanian	28	0.0	(Pasanen, et al, 2008)

Table 1.2. Allelic frequencies (%) of SLCO1B1 variant in different populations.

The studies show that large differences in allele frequencies exist between different populations globally. The highest frequencies of the variant c.521C allele were found in America (24%; 95% CI, 18–32%), Middle East (20%; 95% CI, 15–25%) and Europe (18%; 95% CI, 14–23%); the smallest frequency was found in Sub-Saharan Africa (1.9%; 95% CI, 0.7–4.8%). The frequency of c.521C was 12% (95% CI, 9.5–15%) in East Asia and 9.4% (95% CI, 6.9–13%) in Central/South Asia. No carriers of the variant allele were found in Oceania.

The *SLCO1B1**1A & *SLCO1B1**1B are the haplotypes of T, and the *SLCO1B1**5 & *SLCO1B1**15 are the haplotypes of C alleles in *SLCO1B1* gene respectively. And the prevalence of both haplotypes globally is tabulated in table 1.3 below.

Region	**T**		**Allelic Sum (T) (%)**	**C**		**Allelic Sum (C) (%)**
	***1A (%)**	***1B (%)**		***5(%)**	***15(%)**	
Europe	56	26	82	2	16	18
America	37	39	76	-	24	24
Sub-Saharan Africa	21	77	98	-	2	2
North Africa	34	48	82	2	16	18
Middle East	49	31	80	5	15	20
South/Central Asia	52	39	91	-	9	9
East Asia	25	63	88	-	12	12
Oceania	34	66	100	-	-	0

Table 1.3. Global distribution of the haplotypes formed by the c.388A>G and c.521T>C SNPs (**1A, *1B, *5, *15*) in *SLCO1B1* gene (Pasanen M. , 2008).

Considering that these transporters play a key role in the distribution of many drugs and in the transport of endogenous compounds, such as cholesterol and bile acids, inter-individual variability in disease risk and drug response may be explained by the differential prevalence of genetic variants. In spite of its relevance, some populations have not been characterized yet for this SNP.

In the present study we examined the allelic frequencies of the SNP in *SLCO1B1* gene that may play an important role in drug disposition, in populations from three different ethnic/geographic origins. Namely, Native Africans (Mozambican), Latin American (Colombian) and European (Portuguese) populations which are not frequently the target of pharmacogenetic studies, and this is an important issue when considering bridging of drug dosages and regimens used in different populations.

1.11. Aims of The Study

Several sequence variations have been discovered recently in the OATP1B1-encoding *SLCO1B1* gene, some associated with altered transporter activity *in vitro* and *in vivo*. However, the frequency of the *SLCO1B1* gene sequence variations in individual populations have not been systematically investigated. The objective of this study was to determine the frequencies of *SLCO1B1* variant alleles in Latin American (Colombian), Native African (Mozambican) and European (Portuguese) populations.

1.11.1. Specific Aims of the Study

1. To investigate the genotypic and allelic frequencies of the *SLCO1B1*exonic polymorphismT521C (V174A) rs4149056 in populations of different ethnic backgrounds, Latin American (Colombian), Native African (Mozambican) and European (Portuguese) as a basis for future genetic association studies.
2. To compare our data to other populations globally

2. MATERIALS AND METHODS

2.1. Subjects and Study Design

A novel PCR-RFLP genotyping method was developed and optimized to analyse a polymorphic variant of *SLCO1B1* (OATP-C) gene, i.e., T521C. The DNA template was extracted from blood samples obtained under informed consent, from populations of Colombia, Mozambique and Portugal. 67 from Colombian, 53 from Portuguese and 61 from Mozambican healthy volunteers were participated in the study. Colombian subjects were from the North-West region, mainly from Antioquia and Chocó departments. This study followed the recommendations of the Declaration of Helsinki promulgated in 1964 (http://ohsr.od.nih.gov/helsinki.php3). One blood sample was obtained from each participant for DNA extraction. The study was fully carried out in the Pharmacogenomics and Molecular Toxicology Laboratory, CCMAR/CBMR, Universidade do Algarve, Faro, Portugal.

Blood sampling and genomic DNA extraction were carried out by taking 10-ml blood sample from each participant in a tube containing ethylenediaminetetraacetic acid (EDTA) and stored at -20 ºC prior to DNA extraction. Genomic DNA was extracted with standard methods (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). Alternatively, in cases when small amounts of blood were available, DNA was prepared using a quick protocol(Rudbeck & Dissing, 1998).

2.2. Genotyping

The reference sequences were obtained from the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) database (http://www.ncbi.nlm.nih.gov/). Pharmacogenetic analysis of the specific polymorphic variant of the *SLCO1B1* SNP (c.T521C) analyzed in this study (reference sequences, GenBank accession no. NC_000012.10) was performed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The reaction volume in polymerase chain reactions (PCR) was 25 µl containing assay-specific primers, dNTPs, MgCl2, Taq polymerase enzyme, PCR GoTaq Buffer Mix, water and genomic DNA and PCR included 40 cycles at 94ºC for denaturation of the genomic DNA and activation of the Taq Polymerase enzyme, 55ºC for annealing of the primers, and 72ºC for extension (Table 2.1 & 2.2). The PCR assay was performed using Tpersonal Themocycler, Biometra, and finally digestion of amplified products with a specific restriction endonuclease (*Bsp*1286l), followed by electrophoretic separation on 2% (W/V) agarose gel, with a running time of 90 min at 80 V in 1X TAE buffer

(Tris-acetate-EDTA buffer; 40 mM Tris, 20mM acetate, 1 mM EDTA),and visualization of the gel-separated PCR products with Green Safe (NZYTech) staining under UV light (AlphaImager, AlphaInnotech).

For primer design, Primer-BLAST (Ye, et al., 2012) tools were used; the sequences deposited into the GenBank (http://www.ncbi.nlm.nih.gov/genbank/) to design pairs of allele-specific forward primers that overlap with the *SLCO1B1* allelic variant and its corresponding single reverse primers. The primers employed in performing the PCR for the analyzed SNP, c.T521C, were Forward primer (5'>3'); GTTAAATTTGTAATAGAAATGC, and Reverse Primer (5'>3'); GTAGACAAAGGGAAAGTGATCATA.

Ten µl of PCR products were digested with Bsp1286I (Sdul) restriction enzyme (Thermo Scientific), at 37 °C for 2 hours. Digested PCR products were separated by electrophoresis using a 2 % agarose gel stained with green safe and visualized by an UV transilluminator. Some samples were randomly selected for sequencing for confirmation.

In the 260-bp long PCR product of the T521C SNP obtained from TT homozygotes the recognition site of the enzyme was missing, resulting in no digestion. In the samples with CC genotype the enzyme recognition site was present, being therefore digested into 153 and 107-bp long fragments. In samples with TC genotype, a mixed pattern was observed, with 153 -bp, 107-bp and 260-bp fragments.

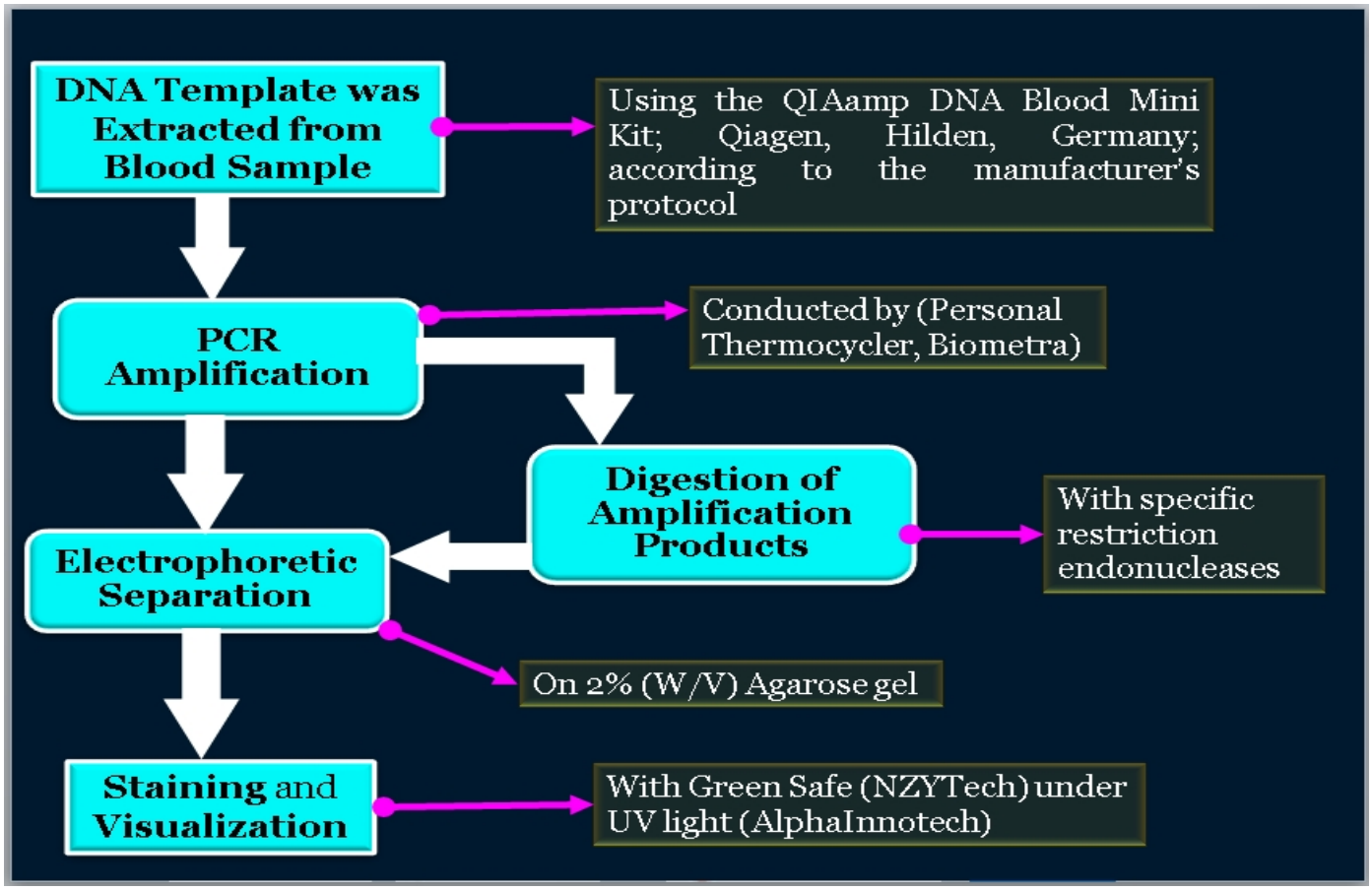

Figure 2.1. Schematic representation of the steps of the genotyping process using PCR-RFLP.

Variables	**Recommended concentration**	**Optimized Concentration**
Forward Primer	0.05 – 1 µM	1 µM
Reverse Primer	0.05 – 1 µM	1 µM
dNTPs	0.02 – 0.2 mM	0.3 mM
MgCl2	0.5 – 5 mM	3 mM
PCR GoTaq Buffer Mix.	1x	1x
Taq polymerase Enzyme	1 - 2.5 U	1.5 U
Target DNA	≤ 1 µg	≅ 1 µg

Table 2.1. Optimized reaction mixture of the final PCR.

Variables	Recommended Temperature	Optimized Temperature	Recommended Duration	Optimized Time
1st DNA Denaturation	92 – 95 °C	94 °C	2 – 5 min	2 min
2nd DNA Denaturation	92 – 95 °C	94 °C	10 – 60 sec	40 sec
Primer Annealing	55 – 70 °C	55 °C	30 – 60 sec	40 sec
1st Extension	72 °C	72 °C	1 min	1 min
No Cycles	20 – 40	40	-	-
Final Extension	72 °C	72°C	5 – 10 min	3 min
Cooling	4 °C	-	-	-

Table 2.2. Optimized reaction condition of the final PCR

2.3. Hardy – Weinberg Equilibrium

The Hardy-Weinberg Law states both the allele frequencies and the genotype frequencies are constant from generation to generation in a large, random-mating population that is not affected by the evolutionary processes of mutation, migration, selection, finite population size (genetic drift), or other forces, so equilibrium is reached. Thus, it is possible to calculate the expected frequencies of genotypes in a population if the frequency of the different alleles in a sample of population is known. The genotype frequencies are calculated using the square expansion of the allele frequencies ($(p + q)^2 = p^2 + 2pq + q^2 = 1$) (The American Phytopathological Society, 2019). As the genotype frequencies must sum to one, summing the elements of the binomial expansion, we obtain the expected genotype proportions among the offspring after a single generation. These frequencies define the Hardy–Weinberg equilibrium (The American Phytopathological Society, 2019).

In the absence of other factors, keeping allele and genotype frequencies the same, you can imagine this process repeating over and over, generation after generation. However, a population in Hardy-Weinberg equilibrium is, by definition, not evolving, since evolution is a change in allele frequencies in a population over generations. Populations are usually not in Hardy-Weinberg equilibrium (at least, not for all the genes in their genome). Instead, populations tend to evolve: the allele frequencies of at least some of their genes change from one generation to the next. Population geneticists often check to see if a population is in Hardy-Weinberg equilibrium because they suspect other forces may be at work (Khanacademy, 2019).

The mechanisms that effect changes in allele frequencies are selection, mutation, migration, and genetic drift, and when one or more of these forces are acting, the population violates Hardy-Weinberg assumptions, and evolution occurs. The Hardy-Weinberg Theorem thus constitutes a null model for the discipline of population genetics and is fundamental to the study of evolution (Nature Education, 2014).

2.4. Statistical Analysis of Data

The method used for calculation of the Hardy-Weinberg equilibrium was described by G. H. Hardy and W. Weinberg in 1908 (Hardy, 1908). Statistical significance ($p < 0.05$) was assessed by $\chi 2$ test to compare the differences between expected and observed frequencies. Fisher's exact test was used to compare results in different studied populations groups.

3. RESULTS

A total of 181 samples were genotyped for the *SLCO1B1* gene variant, T521C (V174A) rs4149056, from subjects of different ethnic groups, 67 samples from Colombian, 53 from Mozambican and 61 from Portuguese populations. The genotypic and allelic frequencies of the populations under study are tabulated in table 3.2 and 3.3.

Population	No. of Subjects	Genotypes			Alleles		%TT	%TC	%CC	%T	%C
		TT	TC	CC	T	C					
Colombia	67	47	12	8	106	28	70.15	17.91	11.94	79.1	20.9
Mozambique	53	47	5	1	99	7	88.68	9.43	1.89	93.4	6.6
Portugal	61	40	16	5	96	26	65.57	26.23	8.20	78.7	21.3

Table 3.1. The genotypic and allelic distribution of the *SLCO1B1* exonic polymorphism T521C (V174A) rs4149056 in healthy Colombian, Mozambican, and Portuguese populations, analysed in this study.

The frequency of homozygote T genotype was observed comparable (p=1.000) in Portuguese (65.6%) and Colombian (70.2%) while the Mozambican population showed the highest frequency among the three populations (88.7%), differing significantly from both the Portuguese (p=0.0022) and Colombian (p=0.0017). The frequency of homozygote C genotype was generally low in all populations. Particularly, the lowest (1.89%) was found in Mozambicans. Whereas the frequency of heterozygote TC genotype was found to be similar in Portuguese (26.23%) and Colombian (17.91%), it as lowest in Mozambique.

The percent allelic frequency of T and C in the T521C (rs4149056) variant was similar in both Colombia and Portugal populations. The prevalence of the variant allele, that leads to reduced function, corresponds to *circa* 20% of the analysed alleles in Portugal and Colombia, being lower in Mozambique.

3.1. Genotypic Frequencies for the SNP 521T>C in Different Population Studies

Table 3.2 lists the information available on the prevalence of the exonic polymorphism *SLCO1B1* (OATP-C), variant T521C, from this study and the literature.

Population	**No. of Subjects.**	**Genotypic frequency (%)**			**Study**
		TT	TC	CC	
Colombians	67	70.15	17.91	11.94	This Study
Mozambicans	53	88.68	9.43	1.89	This Study
Portuguese	61	65.57	26.23	8.20	This Study
Emirates	282	78.72	18.44	2.84	(Saber-Ayad, et al., 2018)
Europeans	236	69.92	25.85	4.24	(Saber-Ayad, et al., 2018)
Tanzanians	364	94.23	5.77	0.00	(Saber-Ayad, et al., 2018)
Roma	470	67.02	31.49	1.49	(Nagy, et al., 2015)
Hungarian	442	65.16	31.90	2.94	(Nagy, et al., 2015)
Finnish	468	63.89	31.84	4.27	(Santos, et al., 2012)
Brazilian	143	74.13	23.78	2.10	(Li, et al., 2012)
Indian (Singapore)	100	87.00	13.00	0.00	(Melo, et al, 2015)
Chinese (Singapore)	100	75.00	24.00	1.00	(Melo, et al, 2015)
Chinese (Han)	111	73.87	24.32	1.80	(Hubacek JA, 2015)
Malays (Singapore)	100	79.00	20.00	1.00	(Melo, et al, 2015)

Table 3.2. The genotypic frequency of the *SLCO1B1* exonic polymorphism T521C (V174A) rs4149056 in healthy Colombian, Mozambicans, and Portuguese population and other different ethnic origins.

3.2. Allelic Frequencies for the SNP 521T>C in Different Population Studies

The allelic frequencies for the 521T>C SNP found in this study and in other populations up to date are listed in table 3.3.

Population	Allelic Frequency (%)		Study
	T	C	
Colombians	79	21	This Study
Mozambicans	93	7	This Study
Portuguese	79	21	This Study
Emirates	88	12	(Saber-Ayad, et al., 2018)
Europeans	83	17	(Saber-Ayad, et al., 2018)
Tanzanians	97	3	(Saber-Ayad, et al., 2018)
Roma	83	17	(Nagy, et al., 2015)
Hungarian	81	19	(Nagy, et al., 2015)
Finnish	80	20	(Santos, et al., 2012)
Brazilian	86	14	(Li, et al., 2012)
Indian (Singapore)	94	6	(Melo, et al, 2015)
Chinese (Singapore)	87	13	(Melo, et al, 2015)
Chinese (Han)	86	14	(Hubacek JA, 2015)
Malays (Singapore)	89	11	(Melo, et al, 2015)

Table 3.3. The allelic frequencies for the *SLCO1B1* exonic polymorphism T521C (V174A) rs4149056 in healthy Colombian, Mozambicans, and Portuguese population and other different ethnic origins.

3.3. Hardy – Weinberg Equilibrium

To evaluate if the studied populations obey the Hardy-Weinberg equilibrium, the expected genotypic frequencies were calculated from the allelic frequencies determined experimentally, and then compared to the observed genotypic frequencies. If, in our case, p = allelic frequency of T, and q= allelic frequency of C, then the expected genotypic frequencies of T/T, T/C and C/C correspond to p2, 2pq, and q2. Table 3.4 shows the results of this analysis for the populations characterized in this study, and for the other populations previously described in the literature.

Population	Genotype	Observed frequency		Expected frequency	P-value
Colombians	TT	47	0.702	0.626	0.0009
	TC	12	0.179	0.331	
	CC	8	0.119	0.044	
Mozambicans	TT	47	0.887	0.872	0.1924
	TC	5	0.094	0.123	
	CC	1	0.019	0.004	
Portuguese	TT	40	0.656	0.619	0.2290
	TC	16	0.262	0.335	
	CC	5	0.082	0.045	
Emirates	TT	222	0.787	0.773	0.1082
	TC	52	0.184	0.212	
	CC	8	0.028	0.015	
Europeans	TT	165	0.699	0.686	0.3577
	TC	61	0.259	0.284	
	CC	10	0.042	0.029	
Tanzanians	TT	343	0.942	0.943	0.8258
	TC	21	0.058	0.056	
	CC	0	0	0.001	
Roma	TT	315	0.670	0.685	0.0743
	TC	148	0.315	0.285	
	CC	7	0.015	0.030	
Hungarian	TT	288	0.652	0.658	0.6687
	TC	141	0.319	0.306	
	CC	13	0.029	0.036	
Finnish	TT	299	0.639	0.637	0.9724
	TC	149	0.318	0.322	
	CC	20	0.043	0.041	
Brazilian	TT	106	0.741	0.740	0.9933

	TC	34	0.238	0.241	
	CC	3	0.021	0.020	
Indian (Singapore)	TT	87	0.870	0.874	0.7968
	TC	13	0.130	0.122	
	CC	0	0	0.004	
Chinese (Singapore)	TT	75	0.750	0.757	0.8264
	TC	24	0.240	0.226	
	CC	1	0.010	0.017	
Chinese (Han)	TT	82	0.739	0.740	0.9946
	TC	27	0.243	0.240	
	CC	2	0.018	0.019	
Malays (Singapore)	TT	79	0.790	0.792	0.9792
	TC	20	0.200	0.196	
	CC	1	0.010	0.012	

Table 3.4. Hardy – Weinberg Equilibrium test of *SLCO1B1* exonic polymorphism; T521C (V174A) rs4149056 in healthy Colombian, Mozambicans, and Portuguese populations and other different ethnic origins.

The two-tailed P-value for the Colombians is less than 0.05, which indicates that there is significant difference between the observed and expected values and therefore the analyzed sample of the Colombian population doesn't follow Hardy-Weinberg equilibrium. Whereas for Mozambique and Portugal population the P-value is greater than 0.05, which means, the difference between the expected and observed genotypic frequencies is not significant and the analyzed sample of the two populations follows Hardy-Weinberg equilibrium. The same can be said for all other populations previously characterized in the literature.

3.4. Comparison of the Populations Analyzed with Other Populations

To evaluate the similarity between the populations analysed in the study to each other, and to others previously studied, the distribution of alleles for the 521T>C SNP in the different populations were compared using a table of contingency. The results are shown in table 3.5 below.

	Colombian	Mozambican	Portuguese
Colombian	-	0.0017*	1.0000
Mozambican	0.0017*	-	0.0022*
Portuguese	1.0000	0.0022*	-
Emirates	0.0115*	0.1296	0.0128*
Europeans	0.3113	0.0044*	0.2921
Tanzanians	0.0001*	0.0743	0.0001*
Roma	0.3325	0.0032*	0.2588
Hungarian	0.5581	0.0010*	0.5399
Finnish	0.8192	0.0003*	0.8109
Brazilian	0.0878	0.0536	0.0779
Indian (Singapore)	0.0001*	1.0000	0.0002*
Chinese (Singapore)	0.0684	0.1201	0.0608
Chinese (Han)	0.1055	0.0643	0.0953
Malays (Singapore)	0.0183*	0.3047	0.0152*

* $p<0.05$; statistically significant difference in observed frequencies

Table 3.5. Comparison of the allele distribution observed in Colombian, Mozambican and Portugal population against different populations by their two-tailed P value

From the above contingency table, the frequencies observed in Colombians are different from the ones observed in Mozambicans, Emirates, Tanzanians, Indians (Singapore), and Malays (Singapore) populations, and that these differences are statistically significant ($p<0.05$). However, frequencies observed in Colombian are similar with those found in Portuguese, Europeans, Roma, Hungarians, Finnish, Brazilians, Chinese (Singapore) and Chinese (Han), since the observed differences are not statistically significant ($p>0.05$).

The frequencies observed in Mozambicans are significantly different from the ones observed in Colombians, Portuguese, Europeans, Roma, Hungarians, and Finnish populations ($p<0.05$). However, the distribution in Mozambicans is similar with Emirates, Tanzanians, Brazilians,

Indians (Singapore), Chinese (Singapore), Chinese (Han) and Malays (Singapore) ($p>0.05$). The Portuguese population showed frequencies that are different from the ones observed in Mozambicans, Emirates, Tanzanians, Indians (Singapore) and Malays (Singapore), populations, and that these differences are statistically extremely significant ($p<0.05$). However, frequencies observed in Portugal are similar with Colombians, Europeans, Roma, Hungarians, Finnish, Brazilians, Chinese (Singapore) and Chinese (Han).

4. DISCUSSION

The disposition of many drugs such as antidislipidemic, antitumor or antidiabetic has been demonstrated to be affected by the chemical properties and structural conformations of influx transporters. This association in turn affects efficacy and/or safety of the drugs. The variability in the response to a large range of drugs may be derived from the pharmacogenetic diversity of these transporters. Therefore, evaluation of SNP frequencies among different populations with variable ethnic background will certainly be very useful as a tool to optimize therapeutics according to variable predicted pharmacokinetics. Many studies reported that statin-induced myopathy is significantly associated with the *SCLO1B1* influx transporter gene c.521T>C (rs4149056) polymorphism. We analyzed the genotypic and allelic frequencies of this polymorphism in Colombian, Mozambican, and Portuguese subjects.

At the best of our knowledge, this study is the first to explore the prevalence of *SLCO1B1* gene polymorphism T521C (rs4149056), in these three populations. In our study, we found that the most dominant genotype in all populations under study was the homozygous T/T, 70.15%, 88.68%, and 65.57% respectively. Individuals with the homozygote variant genotype C/C, which are predicted to show the most significant clinical impact, were found at frequencies of 8.2%, 1.9% and 11.9% in Portuguese, Mozambicans and Colombians, respectively.

The highest frequency of the reference T allele was found in Mozambique (93%) is in agreement with a previous study in another African ancestry, Tanzanians (97%) (Saber-Ayad, et al., 2018), and Indian (Singapore) (94%) and Malays (Singapore) (89%) (Melo, et al., 2015). However, this allelic frequency is relatively higher than Colombian (79%) and Portuguese (79%) subjects (this study), which is also consistent with another studies in Europeans (Germany) (83%) (Saber-Ayad, et al., 2018), Roma (83%), Hungarian (81%) (Nagy, et al., 2015), Austrian (82.3%) (Enko, et al., 2018) and Finnish populations (80%) (Santos, et al., 2012). This discrepancy may be caused by differences in sample sizes between these studies and in the origin of the samples. The differences observed in the frequencies among Mozambique, Colombia and Portugal populations clearly indicates a possible therapeutic failure of the "one-dose-fits-all" approach among these populations.

Recent knowledge suggests that the *SLCO1B1* polymorphisms may have particularly important effects on the pharmacokinetic profile of statin drugs (e.g., atorvastatin) and most studies are mainly focused on the influence of c.521T > C polymorphism. One study shows that higher Cmax,

$AUC_{0\text{-}48h}$, $AUC_{0\text{-}\infty}$, MRT_{expo} and lower T_{max}, Vd and CL for atorvastatin in in SLCO1B1 c.521CC compared to the other genotype group of the c.521T > C (Daka, et al., 2015). In another study, significantly larger (144%) mean AUC $_{0\text{-}48h}$ has been observed in subjects (n = 4) with *SLCO1B1* c.521CC genotype than the subjects (n = 16) with c.521TT (reference) genotype and 61% larger than the subjects (n = 12) with c.521TC genotype. Theoretically speaking, these findings attest that reduced OATP1B1 function could reduce the clearance of atorvastatin, because of the decreased entry into the liver, the main site of metabolism and elimination of atorvastatin (Pasanen, et al., 2007).

Numerous other clinical studies have also confirmed that subjects, who are carriers of *SLCO1B1* 521C allele, had increased plasma concentrations of other OATP1B1 substrates (e.g. pravastatin and repaglinide) compared to the subjects who were reference homozygotes (*SLCO1B1* 521T allele) (Aquilante, et al., 2008) (Kivistö & Niemi, 2007) (Niemi, et al., 2005a) (Kalliokoski, et al., 2008b).

Generally, the myopathy risk for statin users carrying the homozygous C/C genotype is 17-fold higher when compared to the carriers of reference homozygotes, while heterozygous TC subjects show a 5-fold increase in risk (SNPedia: a wiki supporting personal genome annotation, interpretation and analysis, 2019).

The fact that the relatively high frequencies of the low-activity variants in the European (Caucasian) ancestry signposts higher risk of statin induced side effects. Similarly, Ho et al. 2007 reported that European-Americans have significantly higher plasma pravastatin AUCs than African Americans (Ho, et al., 2007). This polymorphism was also associated with an increased risk of cardiovascular adverse effects in individuals taking simvastatin (Voora, et al., 2009).

In the other side, the *SLCO1B1* c.521CC variant genotype was associated with an increased baseline cholesterol synthesis rate. Higher plasma desmosterol to cholesterol ratios and higher plasma desmosterol concentrations had been observed in individuals with the c.521CC genotype than those with the c.521TT genotype (Pasanen M. , 2008). This functional link between cholesterol homeostasis and OATP1B1 could be through bile acid homeostasis, as the regulation of bile acid synthesis and cholesterol homeostasis is tightly linked, with OATP1B1 playing a major role in the uptake of certain bile acids. The bile acid concentration in the liver could be decreased as a result of impaired activity of OATP1B1 by limiting the access of bile acids from the portal

blood into the liver. Basolateral OATP transporters are thought to be mediators of the approximately 20% hepatic uptake of bile acids.

Therefore, the identification and characterization of these genetic variations may help in the development of *SLCO1B1* genotype-based prescription or more personalized drug therapies to achieve the benefits of statin therapy more safely and effectively.

5. CONCLUSIONS

Genetic variants of influx transporters are of key relevance, as it has become evident that these influx transporter proteins play a central role in drug disposition, contributing to variable efficacy and/or safety, as well as in the control of endogenous mediators such as cholesterol, bile acids or vasodilators. The pharmacogenetic results from the current study and many studies in the literature suggests that the influx transporter *SLCO1B1* gene SNPs shows very high population specificity. The functionally detrimental homozygous variant c.521CC genotype is relatively more prevalent in Caucasians than in other African and Asian origin ethnic backgrounds. As a result, subjects (mainly Caucasians) with the homozygous variant c.521CC genotype had shown considerably higher plasma concentrations of statins than those with the c.521TT (reference) genotype. *SLCO1B1* genotyping may have clinical value for adjusting doses of statin therapy to reduce the risk of myopathy development and to aid in the prediction of differential susceptibility to adverse effects. The results obtained in this study may contribute to variable disease risk and therapeutic outcomes in individuals from Colombia, Mozambique and Portugal.

Therefore, the investigation of clinically relevant polymorphisms such as *SLCO1B1* gene, especially, in the populations substantially less characterized for pharmacogenetic variability is very important, since more information is needed before an adequate bridging of therapeutic dosages and regimens.

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