UNIVERSIDADE DE SÃO PAULO
Faculdade de Ciências Farmacêuticas
Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia)
Área de Fisiopatologia

Análise farmacogenômica em indivíduos com hipercolesterolemia familial

Carolina Dagli Hernandez

Tese para obtenção do Título de DOUTOR

Orientadora: Profa. Dra. Rosario Dominguez Crespo Hirata

São Paulo

2021

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We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is a proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become, like the radium, a benefit for mankind.

(Marie Skłodowska-Curie)

RESUMO

DAGLI-HERNANDEZ, C. **Análise farmacogenômica em indivíduos com hipercolesterolemia familial**. 2021. 180f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo.

Introdução: A hipercolesterolemia familiar (HF) é uma dislipidemia monogênica com alto risco de desenvolvimento de doença aterosclerótica precoce. As estatinas são o tratamento de primeira linha para pacientes com HF. As estatinas reduzem substancialmente o colesterol da lipoproteína de baixa densidade (LDL-c) e têm uma boa eficácia e perfil de segurança. No entanto, alguns pacientes não respondem adequadamente, enquanto outros apresentam eventos adversos relacionados às estatinas (SRAE). Fatores genéticos e não genéticos contribuem para a variabilidade na resposta às estatinas, mas existem poucos estudos sobre fatores farmacogenômicos na população brasileira.
Objetivo: Explorar a associação de variantes genéticas com a resposta aos hipolipemiantes e SRAE em pacientes brasileiros com HF.
Pacientes e Métodos: Pacientes adultos com HF (n=114) foram selecionados e dados clínicos e farmacoterapêuticos foram obtidos. A resposta ao tratamento com estatinas foi considerada como atingindo uma redução do LDL-c de 50%. Amostras de sangue foram obtidas para exames laboratoriais e extração de DNA genômico. Um painel de 84 genes (relacionados a HF e farmacogenes) foi analisado por sequenciamento de genes direcionados a exon (ETGS). Os dados de sequenciamento de DNA foram analisados usando um pipeline de descoberta de variantes. O impacto funcional das variantes em genes relacionados à farmacocinética (PK) e farmacodinâmica (PD) foi avaliado usando um escore de predição de funcionalidade (FPS) e outras ferramentas *in silico*. A resposta do LDL-c a estatinas e ao risco de SRAE foi analisada em portadores de variantes deletérias nos genes PK e PD, com frequência de alelo raro > 5,0% ou 10%, usando análises de regressão linear univariada e multivariada. A análise de modelagem molecular foi usada para explorar o efeito funcional *in silico* de variantes deletérias.
Resultados: Cinquenta e oito (50,8%) dos pacientes com HF responderam às estatinas e 24 (21,0) apresentam SRAE. Obesidade e consumo de álcool foram mais frequentes no grupo de não respondedores (NRE) ($p<0,05$), enquanto o uso concomitante de ezetimiba e SRAE foram mais prevalentes no grupo de respondedores (RE) ($p<0,05$). A redução do LDL-c foi maior no grupo RE e nos pacientes com *SRAE ($p<0,05$).* ETGS revelou variantes patogênicas em genes relacionados a FH (19 *LDLR*, 1 *APOB* e 1 *PCSK9*), 402 variantes em 23 genes relacionados a PK (186 *missense*, 2 *stop-gain*, 1 *stop-loss*, 10 *frameshift indel*, 5 deleções *in-frame*, 16 em sítios de *splicing*, 29 na região 5´UTR e 153 na região 3´UTR), e 752 variantes em 33 genes relacionados com PD (249 *missense*, 1 *stop-gain*, 9 *start-loss*, 5 *frameshift indel*, 9 *inframe indel*, 26 em sítios de *splicing*, 67 na região 5´UTR e 386 na região 3´UTR). A análise de predição funcional revelou que 21 variantes *missense*, 1 *stop-loss*, 7 *splice-site* e 10 *frameshift / inframe* em genes PK são deletérias. A análise de regressão multivariada de 16 variantes em transportadores ABC e SLC e enzimas que metabolizam CYP com MAF > 10,0% e ajuste para covariáveis não genéticas, revelou que as variantes *ABCC1* rs45511401 (c.2012G>T, p.Gly671Val) e *SLCO1B*3 rs60140950 (c.683G>C) aumentam a redução do LDL-c ao tratamento com estatina ($p<0,05$). A análise de modelagem molecular revelou que Val671 aumenta a interação de ABCC1 com estatinas em comparação com a proteína de referência (Gly671). Em genes relacionados ao PD, 93 *missense*, 1 *start-loss*, 3 *stop-g*ain, 10 *splice-site* and 4 *frameshift* foram considerados deletérios. A variante missense *LPA* rs76062330 (c.5468G>T) foi associada a maior redução do LDL-c, mesmo após as correções (p ajustado=0,001). A análise de regressão linear multivariada mostrou que a variante *KIF6* rs20455 (c.2155T>C) reduziu a resposta do LDL-c à atorvastatina (p=0,014), enquanto a regressão logística multivariada revelou associação de *LPA* rs3124784 (c.6046C>T) com resposta aumentada às estatinas (p=0,022). Variantes deletérias em genes relacionados a PK e PD não foram associadas ao aumento do risco de SRAE em pacientes com FH.
Conclusões: As variantes deletérias *ABCC1* c.2012G>T, *SLCO1B*3 c.683G>C, *LPA* c.5468G>T e *LPA* c.6046C>T aumentaram a redução do LDL-c. *KIF6* rs20455 (c.2155T>C), uma variante neutra, diminuiu a redução de LDL-c à atorvastatina. Variantes deletérias não foram associadas ao aumento de risco de SRAE.

Palavras-chave: Hipercolesterolemia Familial, estatina, farmacogenética, eventos adversos a medicamentos, mialgia.

ABSTRACT

DAGLI-HERNANDEZ, C. **Pharmacogenomic analysis in patients with familial hypercholesterolemia**. 2021. 180p. PhD Thesis – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo.

Introduction: Familial hypercholesterolemia (FH) is a monogenic dyslipidemia with a high risk of developing early atherosclerotic disease. Statins are the first-line treatment of FH patients. Statins substantially reduce low-density lipoprotein cholesterol (LDL-c) and have a good efficacy and safety profile. However, some patients do not respond adequately whereas others experience statin-related adverse events (SRAE). Genetic and non-genetic factors contribute to the variability in the response to statins, but there are few studies on pharmacogenomic factors in the Brazilian population.
Objective: To explore the association of genetic variants with the response to lipid-lowering drugs and SRAE in Brazilian FH patients
Patients and Methods: Adult FH patients (n=114) were selected and clinical and pharmacotherapeutic data were obtained. The response to statin treatment was considered as LDL-c reduction of at least 50%. Blood samples were obtained for laboratory testing and genomic DNA extraction. A panel of 84 genes (related to HF and pharmacogenes) was analyzed by exon-targeted gene sequencing (ETGS). The DNA sequencing data was analyzed using a variant discovery pipeline. The functional impact of variants in pharmacokinetics (PK)- and pharmacodynamics (PD)-related genes was assessed using a functionality prediction score (FPS) and other *in silico* tools. LDL-c response to statin and SRAE risk was in carriers of deleterious variants in PK and PD genes, with minor allele frequency (MAF) > 5.0% or 10%, using univariate and multivariate linear regression analyses. Molecular modeling analysis was used to explore the functional effect in silico of deleterious variants.
Results: Fifty-eight (50.8%) of the FH patients responded to statins and 24 (21.0) had SRAE. Obesity and alcohol consumption were more frequent in the non-responder (NRE) group ($p<0.05$), whereas the concomitant use of ezetimibe and SRAE were more prevalent in the responder (RE) group ($p<0.05$). LDL-c reduction was higher in RE group and in patients with SRAE ($p<0.05$). The ETGS revealed 21 pathogenic variants in FH-related genes (19 *LDLR*, 1 *APOB* and 1 *PCSK9*), 402 variants in 23 PK-related genes (186 missense, 2 stop-gain, 1 stop-loss, 10 frameshift indel, 5 inframe deletions, 16 in splicing region, 29 in the 5´UTR region, and 153 in the 3´UTR region), and 752 variants in 33 PD-related genes (249 missense, 1 stop-gain, 9 start-loss, 5 frameshift indel, 9 inframe indel, 26 in splice-sites, 67 in the 5´UTR region, and 386 in the 3´UTR region). Functional prediction analysis revealed 21 missense, 1 stop-loss, 7 splice and 10 frameshift/inframe variants in PK genes are deleterious. Multivariate regression analysis of 16 variants in ABC and SLC transporters and CYP metabolizing enzymes with MAF > 10.0% and adjustment for non-genetic covariates, revealed that *ABCC1* rs45511401 (c.2012G>T, p.Gly671Val) and *SLCO1B3* rs60140950 (c.683G>C) increased LDL-c reduction to statin treatment ($p<0.05$). Molecular modeling analysis revealed that Val671 enhance the interaction of ABCC1 with statins compared with reference protein (Gly671). In PD-related genes, 93 missense, 1 start-loss, 3 stop-gain, 10 splice-site and 4 frameshift variants were predicted to be deleterious. The missense variant *LPA* rs76062330 (c.5468G>T) was associated with higher LDL-c reduction, even after corrections (Adjusted $p=0.001$). Multivariate linear regression analysis showed that the variant *KIF6* rs20455 (c.2155T>C) reduced the LDL-c response to atorvastatin ($p=0.014$), whereas multivariate logistic regression revealed association of *LPA* rs3124784 (c.6046C>T) with increased response to statins ($p=0.022$). Deleterious variants in PK- and PD- related genes were not associated with increased risk of SRAE in FH patients.
Conclusions: The deleterious variants *ABCC1* c.2012G>T, *SLCO1B*3 c.683G>C, *LPA* c.5468G>T and *LPA* c.6046C>T enhanced LDL-c reduction in FH patients. *KIF6* rs20455 (c.2155T>C), a neutral variant, decreased LDL-c reduction to atorvastatin. Deleterious variants in PK and PD genes were not associated with increased risk of SRAE.

Keywords: Familial hypercholesterolemia, statin, pharmacogenetics, adverse drug events, myalgia.

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ABC	ATP-binding cassette
ACMG	American College of Medical Genetics and Genomics
ADME	Absorption, distribution, metabolism and excretion
ALT	Alanine aminotransferase
AMI	Acute myocardial infarction
Apo A	Apolipoprotein A
Apo B	Apolipoprotein B
APOE	Apolipoprotein E
AST	Aspartate aminotransferase
BCRP	Breast cancer resistance protein
BMI	Body mass index
CAD	Coronary artery disease
CVD	Cardiovascular disease
CI	Confidence interval
CK	Creatine kinase
CPIC	Clinical Pharmacogenetics Implementation Consortium
CVD	Cardiovascular disease
CYP	Cytochrome P450
DLCN	Dutch Lipid Clinic Network
EAS	European Atherosclerosis Society
ESC	European Society of Cardiology
ETGS	Exon-targeted gene sequencing
FH	Familial hypercholesterolemia
FHBGEP	Familial hypercholesterolemia genomics, epigenomics and pharmacogenomics
GFR	Glomerular filtration rate
GOF	Gain-of-function
HbA1c	Glycated hemoglobin
HDL-c	High-density lipoprotein cholesterol
HMG-CoA	3-hidroxi-3-methylglutaryl-CoA
HMGCR	HMG-CoA reductase gene
HMGR	HMG-CoA reductase
hsCRP	High-sensitivity C-reactive protein
HWE	Hardy-Weinberg equilibrium
IDPC	Institute Dante Pazzanese of Cardiology
KIF6	Kinesin Family Member 6 gene
LDL-c	Low-density lipoprotein cholesterol
LDLR	LDL receptor gene
LOF	Loss-of-function
LP(a)	Lipoprotein(a)
MAF	Minor allele frequency
MDR1	Multi-drug resistance protein 1
MRP2	Multi-drug resistance protein 2

NICE	National Institute for Health and Care Excellence
NRE	Non-responder
OATP	Organic anion transporting polypeptide
OR	Odds ratio
PCSK9	Proprotein convertase subtilisin/kexin type 9
PD	Pharmacodynamics
PFS	Prediction framework score
PK	Pharmacokinetics
RE	Responder
SAMS	Statin-associated muscle symptoms
SLC	Solute carrier
SLCO	Solute carrier organic anion transporter family
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variation
SRAE	Statin-related adverse event
SUS	Unified Health System (*Sistema Único de Saúde*)
T4	Thyroxine
TG	Triglycerides
TSH	Thyroid-stimulating hormone
UFRN	Federal University of Rio Grande do Norte
UGT	Uridine 5'-diphospho-glucuronosyltransferase
USP	University of Sao Paulo
UTR	Untranslated region
VLDL-c	Very low-density lipoprotein cholesterol

INDEX

1 INTRODUCTION

1.1 Dyslipidemias

Dyslipidemias are metabolic disorders that cause abnormal concentrations of circulating lipids and lipoproteins, such as increased total and low-density lipoprotein (LDL) cholesterol and/or triglycerides, and/or decreased high-density lipoprotein (HDL) cholesterol (NI et al., 2015). They can be caused by mutations in key genes involved in lipid homeostasis (i.e., familial hypercholesterolemia [FH]) or secondary to a poor lifestyle, medications or comorbidities (HEGELE, 2009).

Extensive evidence has shown that abnormal plasma concentrations of lipoproteins, especially LDL and other apolipoprotein B (apo B)-containing lipoproteins, are the major cause of atherosclerotic cardiovascular disease (CVD), increasing the risk of cardiovascular events, such as ischemic stroke and acute myocardial infarction (AMI) (KAMSTRUP et al., 2008; MACMAHON et al., 2007; YUSUF et al., 2004; ZHANG et al., 2003). Early detection and adequate treatment of dyslipidemias are essential for the correct prevention and control of progression. The CVD mortality rate in Brazil was 372 per 100,000 inhabitants in 2012, showing a downward trend in recent years (MANSUR; FAVARATO, 2016).

Dyslipidemias are classified as primary and secondary. Primary dyslipidemias are caused by mutations in genes involved in the synthesis, metabolism, or plasma removal of lipoproteins. Secondary dyslipidemias can be caused by the effects of other diseases, medications and some lifestyle habits, such as smoking and high-fat diets (XAVIER *et al*., 2013).

Primary dyslipidemias can be diagnosed by phenotypic (clinical and laboratory parameters) or molecular (search for mutations in disease-causing genes) methods. Monogenic dyslipidemias result from mutations in a single gene, while polygenic ones are caused by associations of multiple mutations that alone do not have great repercussion (GARCÍA-GIUSTINIANI; STEIN, 2016; HEGELE et al., 2015).

1.2 Familial hypercholesterolemia

FH is a primary dyslipidemia with frequent monogenic inheritance and autosomal dominant transmission (GOLDSTEIN; BROWN, 2009). FH prevalence was estimated at 1:313 in the heterozygous form and 1:400,000 in the homozygous form, which implies in more than 30 million affected individuals worldwide. (BEHESHTI et al., 2020). The prevalence varies according to ethnicity, as in the case of South Africans (1:72) and Lebanese (1:85) (BRAUTBAR et al., 2015; TURGEON; BARRY; PEARSON, 2016; VALLEJO-VAZ et al., 2015).

One of the main characteristics of FH is elevated plasma concentrations of LDL cholesterol (LDL-c) and early coronary artery disease (CAD) (IZAR et al., 2021). Untreated heterozygous individuals have a higher risk of early CAD than unaffected individuals; in the case of homozygotes when untreated, usually survival does not exceed 30 years of age. (TURGEON; BARRY; PEARSON, 2016; VALLEJO-VAZ et al., 2015). Thus, the cumulative risk of a cardiovascular event at age 50 is up to 44% in men and 20% in women, being a much higher risk than estimated for patients with other dyslipidemias, which justifies not recommending traditional methods to estimate cardiovascular risk (TURGEON; BARRY; PEARSON, 2016).

Patients carrying homozygous mutations in the LDL receptor gene (*LDLR*) have extremely high plasma LDL-c concentrations, reaching values between 600 and 1,200 mg/dL. Heterozygotes have LDL-c values between 300 and 440 mg/dL (IZAR et al., 2021). This increase in plasma cholesterol results in accelerated cholesterol infiltration into some tissues, leading to clinical manifestations such as corneal arch, xanthelasmas, tuberous and tendinous xanthomas (BRAUTBAR *et al*., 2015).

To diagnose FH, phenotypic criteria are used, evaluating the presence of xanthomas and corneal arch, early CAD and high LDL-c concentrations, as well as cascade screening according to family history (IZAR et al., 2021). Clinical signs are not always present in heterozygotes, which makes diagnosis challenging (IZAR et al., 2021). It has also been discussed that the increasingly frequent use of statins has reduced the incidence of CVD and delayed the appearance of typical clinical signs of FH in patients and their families, which in turn jeopardized FH diagnosis using only clinical signs. It is estimated that less than 5% of the affected population is diagnosed, and late diagnosis may be associated with a worse prognosis (VALLEJO-VAZ *et al*., 2015).

In Brazil, the diagnostic criteria are based on the recommendations of the National Institute for Health and Care Excellence (NICE), a public agency of the United Kingdom Department of Health, and the Dutch Lipid Clinic Network (DLCN). These recommendations were incorporated in the Brazilian Guideline on Familial Hypercholesterolemia together with the analysis of mutations in genes encoding LDLR (*LDLR*), apolipoprotein B (*APOB*) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes for molecular diagnosis (IZAR et al., 2021).

FH has an autosomal dominant inheritance and results from functional mutations in *LDLR*, *APOB* and *PCSK9*, proteins that regulate cholesterol homeostasis. LDLRs are expressed in all cells, mainly in hepatocytes, and are responsible for the uptake of LDL by endocytosis. Apolipoprotein B (Apo B) is the constitutive apolipoprotein of LDL and is responsible for the interaction of LDL with the receptor, so that the uptake of LDL particles occurs. PCSK9 has the function of degrading the LDLR. Mutations in the genes of these proteins cause reduced plasma LDL uptake, its accumulation in plasma and, in the long term, in tissues (IZAR et al., 2021; TURGEON; BARRY; PEARSON, 2016).

There are also autosomal recessive forms of FH that are rare (estimated frequency 1:5,000,000). An example arises from mutations in the gene that encodes the LDLR adapter protein 1 (*LDLRAP1*), a protein responsible for associating LDL receptors with clathrins, in the lined clefts of the cell surface membrane. Mutations in *LDLRAP1* lead to loss of protein function, resulting in an increase in LDL (BRAUTBAR et al., 2015; IZAR et al., 2021; SANTOS; MARANHAO, 2014).

Molecular diagnosis of FH is based on the search for functional mutations in genes causing autosomal dominant FH. However, although several functional mutations associated with FH have already been described in the *LDLR, APOB* and *PCSK9* genes, in the last decade other genes involved in cholesterol metabolic pathways have also been associated, characterizing polygenic FH (HUBACEK et al., 2017; JANNES et al., 2015). A risk score for polygenic FH was tested, which could help in determining cardiovascular risk and choosing a personalized treatment (PAQUETTE et al., 2017).

The UK NICE guideline, published in 2008, recommends the diagnosis of FH by genetic testing and confirmation of the presence of mutations in up to third degree relatives (WIERZBICKI; HUMPHRIES; MINHAS, 2008). Similarly, the modified DLCN criteria recommend the inclusion of genetic tests for the diagnosis of FH (IZAR et al., 2021).

1.3 Pharmacological treatment of FH patients

Although lifestyle factors, such as diet and physical activity, play a role in plasma LDL balance, it is mostly affected by cholesterol *de novo* biosynthesis and metabolism (AFONSO et al., 2018). Cholesterol *de novo* biosynthesis occurs substantially in the liver and the rate-limiting step is the conversion of 3-hidroxi-3-methylglutaryl-CoA (HMG-CoA) to mevalonic acid, a reaction catalyzed by HMG-CoA reductase (HMGR, encoded by *HMGCR*). Endogenous and exogenous cholesterol is transferred to very-low-density lipoprotein (VLDL), which in the bloodstream is converted to LDL. LDL clearance depends on the interaction between apo B, the structural protein of LDL and VLDL, and the LDLR, a transmembrane protein present in the cell surface (LUO; YANG; SONG, 2020).

High plasma LDL-c is associated with atherosclerosis due to LDL retention and plaque formation in the arterial intima. LDL modifications, especially oxidation, disturb vascular homeostasis and facilitating the infiltration of pro-inflammatory cells by multiple mechanisms (TALL; WESTERTERP, 2019). Macrophages recognize and internalize modified LDL particles, becoming foam cells that mark the process of plaque formation. The rupture of the atherosclerotic plaque in advanced stages may cause the formation of thrombi that can result in cardiovascular events, such as myocardial infarction and stroke (ZMYSŁOWSKI; SZTERK, 2017).

The pharmacological treatment of FH aims to reduce the concentration of LDL-c and therefore prevent the development of CAD. Due to the seriousness of the disease, treatment should be started as soon as possible and maintained for the long term, in order to reduce the time of exposure to high LDL-c concentrations and, consequently, the incidence of resulting cardiovascular events.

Currently, statins are the first-line treatment for FH, as they are highly effective in reducing plasma LDL-c. Statin treatment has shown to decrease the incidence of cardiovascular events in many clinical trials (AWAN et al., 2012; CANNON et al., 2004; LAROSA et al., 2005; PEDERSEN et al., 2004; RIDKER et al., 2008, 2009; SACKS et al., 1996; SHEPHERD et al., 1995). The 2019 European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) guidelines recommend statin treatment to reduce LDL-c in patients at high cardiovascular risk, such as FH patients (MACH et al., 2020).

Mechanistically, statins act via inhibition of HMGR, mainly in hepatocytes, decreasing cholesterol *de novo* biosynthesis and inducing sterol regulatory element-binding protein (SREBP) 2 activation. SREBP-2 is a transcription factor that regulates the expression of genes

involved in cholesterol metabolism, including *LDLR*. Elevated LDLR expression on the surface of hepatocytes increases LDL uptake by up to 55%, further enhancing the statin response (WARD; WATTS; ECKEL, 2019). The expression of other genes involved in cholesterol metabolism is also induced, including those encoding the proprotein convertase subtilisin/kexin type 9 (*PCSK9*), apo B (*APOB*) and apo E (*APOE*) (NOZUE, 2017).

The therapeutic goal for FH patients is a reduction of at least 50% of plasma LDL-c; however, patients with two or more risk factors (such as male gender, smoking, presence of premature CAD in family members, low concentrations of HDL-cholesterol) should have the regimen intensified (IZAR et al., 2021).

Despite the great advances in the treatment of hypercholesterolemia, FH is treated insufficiently or late. There is great inter-individual variability of response, with an estimate of only approximately 20% of patients achieving therapeutic goals (VALLEJO-VAZ *et al*., 2015). To improve the response of these patients, other lipid-lowering drugs are being used, such as cholesterol absorption inhibitors (ezetimibe), bile acid sequestrant resins and microsomal triglyceride transfer protein inhibitor (MTP). Other classes are in development such as squalene synthase inhibitor, PCSK9 inhibitors, and thyroid hormone analogues. (HIRATA *et al*., 2013).

Many non-genetic factors, including gender, age, smoking status, diabetes, ethnicity, have been reported as predictors of statin response. Male gender and younger age have been described as predictors of lower LDL-c reductions upon simvastatin, atorvastatin and rosuvastatin (KARLSON et al., 2017; ONI-ORISAN et al., 2018). Diabetes and smoking also worsen statin response, while East Asians showed better statin response (ONI-ORISAN et al., 2018).

In addition to non-genetic factors, genetic variants have shown to affect statin response and the risk of adverse drug events (MAXWELL et al., 2017; ONI-ORISAN et al., 2018). In the meta-analysis of total genome-wide association studies (GWAS) variants in *APOE* (rs445925), *SLCO1B1* (rs2900478), *LPA* (rs10455872) and *SORT1/CELSR2/PSRC1* (rs646776) contributed to up to 5.1% of the variability in LDL-c reduction resulting from the use of statins (POSTMUS et al., 2014).

Statin-related adverse events (SRAE) have also been widely studied. The SRAE most frequently reported are statin-associated muscular events (SAMS), with a frequency in non-blinded observational studies ranging from 7 to 29% (MACH et al., 2018; STROES et al., 2015). Other SRAE reported also include increased glucose levels, with the development of

new-onset diabetes mellitus (1 per 1000 patients per year of exposure); mild proteinuria; and elevation of liver transaminases (MACH et al., 2018).

SAMS occur due to the blockage of mevalonate pathway caused by statins, which not only reduces cholesterol production, but also other final products, such as ubiquinone. Ubiquinone, also called coenzyme Q10 (CoQ10), is critical for mitochondrial production of energy in the muscle (KEE et al., 2020). The lack of these products leads to mitochondrial dysfunction and other intracellular events, leading to muscle pain, myotoxicity, or even muscle cell apoptosis (KEE et al., 2020).

SAMS are classified as four clinical presentations, according to the National Lipid Association: 1) Myalgia, that includes muscle aches, soreness, stiffness, tenderness and cramps with normal creatine kinase (CK) levels; 2) Myopathy, manifested by muscle weakness, not necessarily accompanied by pain or high CK levels; 3) Myositis, with muscle inflammation; 4) Myonecrosis, with hyperCKemia; and 5) Myonecrosis with myoglobinuria or acute renal failure, also named rhabdomyolysis (ROSENSON et al., 2014). The incidence tends to vary with the statin used and the dose, with simvastatin showing the highest rates of SAMS (TOTH et al., 2018).

SAMS have many predisposing factors, such as older age (over 75 years), female gender, hypothyroidism, abuse of alcohol, and drug interactions that inhibit enzymes responsible for statin metabolism, such as amiodarone, that inhibits CYP3A4. Genetic variants are also important contributors to the incidence of SAMS (TOTH et al., 2018).

Importantly, SRAE, especially SAMS, affect statin adherence. Myalgia has been reported as the main reason for treatment interruption (60%), followed by cost (16%) and lack of efficacy (13%) (WEI et al., 2013). Patient education is also fundamental for adherence. In one study, it was observed that 25% of FH patients were unaware of the risk of CAD resulting from the disease and 10% did not know the reason for using the medication, which could influence treatment adherence (HOLLMAN; OLSSON; EK, 2006). In the study by McGinnis et al., 2007, non-adherent patients were also shown to have less knowledge about the benefits of statins than adherent patients (MCGINNIS et al., 2007).

1.4 Statin pharmacogenetics

1.4.1 Pharmacokinetics genes

Genetic alterations can compromise pharmacokinetic parameters, by modifying the activity of enzymes responsible for drug metabolism, and pharmacodynamics, by modifying the affinity of a receptor for agonists and antagonists. In this way, the response to lipid-lowering drugs can be reduced or the risks of adverse effects can be increased (HIRATA et al., 2013; SANTOS et al., 2012b).

Most genetic associations with statin response have been found in genes involved in statin absorption, distribution, metabolism and excretion (ADME), such as drug-metabolizing enzymes of the cytochrome P450 (*CYP*) family or influx/efflux transporters that actively participate in its pharmacokinetics and variants in these genes can impact statin plasma levels and, consequently, its efficacy and safety. Statins are absorbed in the intestine by passive diffusion or active transport by the organic anion transporting polypeptide (OATP) 2B1 (OATB2B1, encoded by *SLCO2B1*), a member of solute carrier (*SLC*) transporter family. Statins are further carried through the portal vein to the liver and their hepatic uptake is mediated by OATP1B1 (*SLCO1B1*), with minor contributions of OATP1B3 (*SLCO1B3*) for rosuvastatin and fluvastatin (ROCHA; PEREIRA; RODRIGUES, 2018).

In the liver, atorvastatin and simvastatin undergo first-pass metabolism by CYP3A4 and to a lesser extent by CYP3A5, generating both active and inactive metabolites. Fluvastatin is metabolized by CYP2C9, which also plays minor roles in the hydrolysis of rosuvastatin. However, rosuvastatin pharmacokinetics depend mostly on influx and efflux transporters (HIROTA; FUJITA; IEIRI, 2020).

Statin excretion occurs via bile and depends on the activity of the efflux transporters of the ATP-binding cassette (ABC) superfamily that are present in the canalicular membrane of hepatocytes, namely multi-drug resistance protein 1 (MDR1, encoded by *ABCB1*), multi-drug resistance protein 2 (MRP2, encoded by *ABCC2*), and breast cancer resistance protein (BCRP, encoded by *ABCG2*). These transporters are also present in the apical membrane of enterocytes where they mediate the intestinal efflux of statins (ROCHA; PEREIRA; RODRIGUES, 2018). MRP1, encoded by *ABCC1*, is present in the basolateral membrane of hepatocytes and is involved in statin efflux to the bloodstream. MRP1, MOAT-B (*ABCC4*), MOAT-C (*ABCC5*) and OATP2B1 are also present in myocytes and the balance of their activities are suggested to be involved in statin myotoxicity (KNAUER et al., 2010).

Polymorphisms in key genes have been related to the presence of adverse events to statins. The concentration of statins in the blood, especially simvastatin and atorvastatin, may be related both to the response and to the presence of myalgia and other adverse events; therefore, variants in these transporters can also influence treatment.

SLCO1B1 is the most studied gene involved in statin pharmacokinetics. Variants of the *SLCO1B1* have been linked to lower doses of statins to reach the therapeutic goal and the development of myopathy (HIRATA *et al*., 2013; PATEL *et al*., 2014; REINER, 2014). *SLCO1B1*5* (rs4149056, c.521T>C) is a particularly well-described decreased function variant. Extensive evidence demonstrates an association between this variant and simvastatin-induced myopathy (HOU et al., 2015; THE SEARCH COLLABORATIVE GROUP, 2008). It was suggested that *SLCO1B1*5* interfered with plasma statin concentrations and increased the risk of myalgia, reaching a frequency 50% in homozygous individuals against 19% in those without the mutated allele (VOORA et al., 2009). Consequently, *SLCO1B1*5* was included in international guidelines as a risk allele for myopathy, together with two haplotypes containing **5* C allele, namely *SLCO1B1*15* (**5* C and rs2306283 G alleles) and *SLCO1B1*17* (**5* C, rs2306283 G, and rs4149015 A alleles) (RAMSEY et al., 2014).

*CYP3A5*3* polymorphism also showed an association with SAMS. Homozygous carriers of **3* allele showed greater muscle damage resulting from the use of atorvastatin compared to heterozygotes (WILKE; MOORE; BURMESTER, 2005). Another important gene in this mechanism is *ABCB1*, whose T allele of the c.3435C>T polymorphism was more frequent in patients with myalgia (GLUBA-BRZOZKA et al., 2016).

There are also studies that focus on understanding the impact of genetic variants in LDL-c reduction. Drug transporters, such as *SLCO1B1* and *ABCB1*, have been widely studied. *SLCO1B1* rs2306283 (c.388A>G), for example, was associated with a more pronounced reduction in LDL-c after treatment with atorvastatin and may be a predictor of therapeutic response (RODRIGUES *et al*., 2011). We recently suggested that *SLCO1B1*15* and variants in *SLCO1B3* and *ABCB11* delayed rosuvastatin response in an FH patient, without jeopardizing LDL-c reduction after 12 weeks of treatment (DAGLI-HERNANDEZ et al., 2020). *ABCB1* rs1045642 (c.3435C>T) was also associated with better statin response in some studies (HOENIG et al., 2011; SU et al., 2015). Two independent Brazilian cohorts also showed that *ABCB1* rs2032582 (c.2677T>G/A) GG genotype and A allele increased total cholesterol reduction and LDL-c reduction to simvastatin (REBECCHI et al., 2009) and atorvastatin (FIEGENBAUM et al., 2005a), respectively.

In CYP enzymes, *CYP3A4*22*, for example, has been associated with higher LDL-c reduction in simvastatin users (ELENS et al., 2011), but this result was not observed in Brazilian hypercholesterolemic patients using simvastatin (FIEGENBAUM et al., 2005a) or atorvastatin (RODRIGUES et al., 2013; WILLRICH et al., 2013). Our group reported an association between *CYP3A5*3* (rs776746) and lower reduction of total cholesterol, LDL-c and HDL cholesterol (HDL-c) after atorvastatin treatment in non-African descendants (WILLRICH et al., 2008), but this result still remains controversial, since other studies did not find any association (ROSALES et al., 2012). We later observed that changes in cholesterolemia promoted by atorvastatin influence the regulation of mRNA expression in *CYP3A4* and *CYP3A5* in peripheral blood mononuclear cells. The presence of the *CYP3A5*3* polymorphism (AGT haplotype) also contributes to the variability of *CYP3A5* mRNA expression (WILLRICH *et al*., 2013).

1.4.2 Pharmacodynamics genes

The variability of the response to statins can be partly attributed to polymorphisms in more than 30 genes. Among these are several variants associated with LDL-c metabolism such as *LDLR, PCSK9, APOE* and *HMGCR* (HIRATA *et al*., 2013).

Studies performed by our group evaluated the relationship of several genes with the response to statins and ezetimibe in individuals with hypercholesterolemia. Common polymorphisms of *LDLR, APOB, APOE, APOA1* and *SCARB1* genes were associated with variability in response to statins in Brazilian individuals with polygenic hypercholesterolemia (CERDA et al., 2010; GUZMÁN et al., 2000; HIRATA et al., 2013; SALAZAR et al., 2000a, 2000b).

Another study investigated the pharmacogenetics of simvastatin and/or ezetimibe and reported that the *LDLR* rs879255000 (p.Trp556Arg) variant, in homozygosis, is associated with failure to respond to statins and with lower response (15%) to ezetimibe (SCHAEFER *et al*., 2012).

APOE has also shown to be associated with statin response. In a meta-analysis, it was shown that low frequency alleles *APOE* c.-2189G>A and *SORT1/CELSR2/PSRC1* c.*1859C>T polymorphisms were associated with a higher statin response, while *LPA* rs10455872 (g.161010118A>G) and *SLCO1B1* rs2900478 (c.1498-1256T>A) were associated with a lower response (POSTMUS et al., 2014). Additionally, *APOE* variants, especially the ε2 and ε4

alleles, have also been associated with increased and decreased LDL-c reduction, respectively, when treated with statin (GUAN et al., 2019).

HMGCR c.451-174A>T and other variants can also lead to resistance to statin treatment. One of the proposed mechanisms is an alternative processing of mRNA and production of an isoform less sensitive to inhibition by statins (HIRATA *et al*., 2013). Another example is *HMGCR* rs17244841 (g.331648A>T), whose T allele decreased LDL-c response to simvastatin 40 mg/d in African-American hypercholesterolemic (HC) patients (KRAUSS et al., 2008; MANGRAVITE et al., 2010). *HMGCR* rs3846662 (c.1564-106A>G) A allele also decreased LDL-c response to statins in patients with dyslipidemia and FH (CANO-CORRES et al., 2018; LEDUC et al., 2016).

1.4.3 Pharmacogenetics of statins in the Brazilian population

Statin pharmacogenetics studies have been performed in Brazilian cohorts and have brought important contributions, as we discussed in a recent review (DAGLI-HERNANDEZ et al., 2021). Most studies were performed with simvastatin and atorvastatin, and several genes and outcomes – including statin anti-inflammatory effects – have been explored. The main focus of these studies was lipid changes after statin treatment, and most associations were found with genes involved in statin pharmacodynamics, such as *HMGCR, LDLR, APOB, SCARB1,* and others (DAGLI-HERNANDEZ et al., 2021).

However, some of the findings were not consistent with the literature. For example, only one study including Brazilian patients explored the association between SAMS and of *SLCO1B1*5* or **15* and no association was found (SANTOS et al., 2012a). Nevertheless, there is strong evidence in the literature that *SLCO1B1*5, *15* and **17* cause SAMS, which is why those are the only variants present in the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for increasing the risk simvastatin-induced myotoxicity (RAMSEY et al., 2014). Due to this lack of evidence, the Brazilian guidelines for dyslipidemia management have not included a recommendation of genotyping *SLCO1B1*5* and haplotypes in simvastatin users (FALUDI et al., 2017; IZAR et al., 2021).

These differences could be due to a series of factors related to the population of study (DAGLI-HERNANDEZ et al., 2021). Most international pharmacogenetic studies that found relevant associations were performed with non-admixed populations, such as Caucasians or Asians. The Brazilian population, on the other hand, is highly heterogeneous, with a very particular admixture between several ethnicities that include Caucasians, Africans, East Asians,

and Amerindians. The coexistence of variants that are frequent in different ethnicities can mask or potentialize the outcome to be observed, which therefore could bring more confounding factors to the pharmacogenetic studies. In this way, the sequencing of target genes could bring new associations by adjusting these confounding factors.

Additionally, there are few pharmacogenetic studies with Brazilian FH patients. Most studies focused mainly on hypercholesterolemic patients (DAGLI-HERNANDEZ et al., 2021). It is important to study the pharmacogenetics of Brazilian FH patients for multiple reasons. The presence of pathogenic FH-related variants can modify treatment response in these patients, and treatment response is crucial for these high-risk patients. Also, those patients are exposed to higher statin doses and therefore are more susceptible to SRAE. In order to fill this gap, we studied the influence of pharmacogenetic variants detected by exon-targeted gene sequencing in FH patients.

2 OBJECTIVES

2.1 General objective

This thesis aimed to explore the association of genetic variants with the response to lipid-lowering drugs in Brazilian FH patients.

2.2 Specific objectives

1) To identify variants in genes involved in statin pharmacokinetics (PK) and pharmacodynamics (PD) in a Brazilian cohort of FH patients.
2) To explore functional effect using *in silico* algorithms and molecular modeling analysis for deleterious variants.
3) To evaluate the influence of deleterious variants in PK and PD genes on lipid-lowering response in a Brazilian cohort in FH patients.
4) To explore the association of variants in PK and PD genes with the predisposition to SRAE in FH patients.

3 METHODS

3.1 Study design and patients

This study is a part of the FHBGEP project that aims to investigate genomic, epigenomic, and pharmacogenomic factors associated with FH in the Brazilian population (BORGES et al., 2021). Two-hundred unrelated adult FH patients were recruited at three Brazilian Medical Centers from October 2014 to January 2020. FH was clinically diagnosed as possible (3-5 points), probable (6-8 points) or definite (>8 points) according to Dutch Lipid Clinic Network (DLCN) modified criteria (IZAR et al., 2021; WORLD HEALTH ORGANIZATION, 1998).

Patients with the following comorbidities were excluded: liver failure, severe chronic kidney disease (estimated glomerular filtration rate, GFR < 30 mL/min/1.73m^2) and/or nephrotic syndrome, clinically uncontrolled neoplasms, positive serology for human immunodeficiency virus (HIV), hypothyroidism, and/or Cushing's syndrome. Patients who withdrew from the study, aged less than 18 years old, without medical records available or with no history of statin treatment were also excluded from the pharmacogenetics analysis.

3.2 Ethical aspects

The study protocol was approved by the Ethics Committees of the Institute Dante Pazzanese of Cardiology (IDPC) (CAAE #24618713.0.1001.5462, #24618713.0.1001.5462) and #05234918.4.0000.5462, School of Pharmaceutical Sciences (CAAE #24618713.0.3001.0067) of the University of Sao Paulo (USP), and Federal University of Rio Grande do Norte (CAAE #24618713.0.2001.5292), Brazil. The study was conducted according to good clinical practices and the Declaration of Helsinki guidelines (as revised in 2013). All subjects signed an approved written informed consent before enrollment.

3.3 Blood samples and laboratory testing

Blood samples were obtained from fastened patients (at least 8 h) for DNA sequencing and laboratory testing: serum lipid profile (total cholesterol and fractions, triglycerides, apolipoproteins AI and B); glycemic profile (glucose, glycated hemoglobin and insulin); thyroid-related hormones (thyroid-stimulating hormone and thyroxine); liver (alanine and aspartate aminotransferases) and muscle (creatine kinase) enzymes; creatinine; and high sensitivity C-reactive protein (hsCRP).

Plasma glucose, triglycerides, total cholesterol, HDL-c were determined by colorimetric enzymatic methods. LDL-c and VLDL cholesterol levels were calculated using Friedwald's formula (FRIEDWALD; LEVY; FREDNICKSON, 1972). Urea, creatinine, CK, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by kinetic methods. Uric acid was determined by the modified kinetic method of Bulger and Johns. Apolipoprotein A (apo A), apo B and hsCRP were determined by immunoturbidimetry. Glycated hemoglobin (HbA1c) was determined by high-throughput liquid chromatograpy (HPLC). These determinations described above were made by a Dimension RXL automatic analyzer (Siemens, Munich, Germany) following the manufacturer's instructions.

Thyroid-stimulating hormone (TSH), thyroxine (T4) and insulin were determined by sandwich-type enzymatic immunoassays, with detection by electrochemiluminescence, using a CENTAURO automatic analyzer (Siemens, Munique, Alemanha).

Laboratory external quality control was performed by the program of quality control of the Brazilian Society of Clinical Pathology.

3.4 Exon-targeted gene sequencing

Genetic analyses were performed as previously described (BORGES et al., 2021). Briefly, genomic DNA was extracted from whole blood samples using QIAamp® DNA Blood Maxi Kit (QIAGEN, Hilden, Germany). DNA quantification, purity (A260/A280 ratio), and integrity were analyzed using the QUBIT® 2.0 fluorometer (Life Technologies, Forest City, IA, USA), NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and 2200 TapeStation® system (Agilent Technologies, Santa Clara, CA, USA).

FH- and pharmacogenetics-related genes were analyzed from a panel with 84 genes using an exon-targeted gene sequencing strategy (BORGES et al., 2021). Briefly, exons and regulatory regions were selected using Illumina's Design Studio tools

(https://accounts.illumina.com/). Good-quality genomic DNA was used for library construction using the Nextera Rapid Capture Custom Enrichment Kit (Illumina, San Diego, CA, USA). Clustering and paired-end sequencing reactions were performed using MiSeq® Reagent kit V2 (300-cycles) in the MiSeq® system (Illumina, San Diego, CA, USA). PhiX (1%) was used as library clustering and diversity controls. Sequencing data was analyzed using a variant discovery pipeline previously described (BORGES et al., 2021).

The molecular diagnosis of FH was carried out by identifying variants previously associated with FH, such as gain-of-function variants in *PCSK9*, or variants classified as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines (RICHARDS et al., 2015).

3.5 Clinical and pharmacotherapy data

Clinical and biodemographic data, including patient medical history, lifestyle information, medications in use and adverse events, were obtained using a questionnaire and clinical examination, as previously described (Borges et al. 2020).

Information on pharmacotherapy and laboratory tests was also obtained from medical records. To mitigate information bias, the time between the medical visit and the corresponding laboratory test was set to a maximum of 30 days. Baseline LDL-c was considered the highest plasma level without statin treatment for at least 30 days when clearly indicated in the medical record. On-treatment LDL-c was defined as the lowest level with statin treatment.

Patients were considered responders (RE) if they reached an LDL-c reduction of at least 50% and non-responders (NRE) if they did not reach the therapy target (GOLDBERG et al., 2011; IZAR et al., 2021). Absolute LDL-c target was set according to the CAD risk stratification defined by the Update of the Brazilian Guideline for FH (IZAR et al., 2021): 1) Very high risk: patients carrying manifested CAD (history of AMI, angina *pectoris*, previous myocardial revascularization, or ischemic or transitory cerebrovascular event); 2) High risk: primary prevention with baseline LDL-c > 400 mg/dL, or baseline LDL-c > 310 mg/dL with one high-risk factor (tobacco smoking, male gender, or HDL-c < 40 mg/dL), or baseline LDL-c > 190 mg/dL with two high-risk factors; 3) Intermediate risk: Primary prevention without high-risk factors. The therapy target for each risk group was the following: 1) Very high risk: LDL-c reduction ≥ 50% and on-treatment LDL-c < 50 mg/dL; 2) High risk: LDL-c reduction ≥ 50% and on-treatment LDL-c < 70 mg/dL; 3) Intermediate risk: LDL-c reduction ≥ 50% and on-treatment LDL-c < 70 mg/dL.

FH patients were grouped according to the type and intensity of the statin therapy and the clinical response. Treatment intensity was established according to the American College of Cardiology/American Heart Association and the Brazilian guideline criteria, where moderate intensity were the following doses: simvastatin 20-40 mg, atorvastatin 10-20 mg or rosuvastatin 5-10 mg; and high intensity: simvastatin 80 mg + ezetimibe 10 mg, atorvastatin 40-80 mg or rosuvastatin 20-40 mg (CHOU et al., 2016; IZAR et al., 2021). Drug-drug interactions were annotated when a concomitant medication could inhibit or induce enzymatic activity and affect statin response (BELLOSTA; CORSINI, 2018). SRAE were considered when clearly stated by the cardiologist as associated with statin therapy and were followed by dose reduction or change of statin (IZAR et al., 2021). Reduced adherence was considered for patients who reported at least one event of non-adherence to statin or ezetimibe (BORETZKI et al., 2017).

3.6 Pharmacogenetic analysis

3.6.1 Pharmacokinetics genes

A total of 23 genes involved in pharmacokinetics (PK) of statins, including cytochrome P450 (CYP) and uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes, as well as ABC and SLC transporters, were analyzed (**Supplementary table 1**).

An optimized prediction model was used to evaluate functional impact of variants in PK-related genes (ZHOU et al., 2018). Briefly, missense, stop-gain, and stop-loss variants were analyzed using ANNOVAR (WANG; LI; HAKONARSON, 2010) to assess the pathogenicity scores of five algorithms (LRT, Mutation Assessor, PROVEAN, VEST3 and CADD). Next, the PK optimized prediction model was used and variants were classified according to the functionality prediction score (FPS) as neutral (FPS < 0.5), deleterious (FPS > 0.5) or loss-of-function (LOF) (FPS = 1.0). Splicing site and frameshift variants were considered deleterious when they were classified as pathogenic or with decreased or increased activity in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and PharmVar (https://www.pharmvar.org/). Also, the functional impact of variants in splice sites was performed using ANNOVAR's dbNSFP v4.2 *in silico* algorithm, followed by manual checking for the proximity to the branch point. Frameshift variants were considered deleterious.

3.6.2 Pharmacodynamics genes

A total of 34 genes involved in the pharmacodynamics (PD) of statins were included in the analysis (**Supplementary table 1**). Variants in genes associated with FH (*LDLR, APOB, PCSK9, LDLRAP1*), cholesterol homeostasis and metabolism (*HMGCR, MYLIP,* and others), reverse transport of cholesterol, apolipoproteins and other genes that were previously associated with statin response were analyzed.

The functionality of missense, stop gain and stop loss variants was assessed using the following *in silico* prediction algorithms: PolyPhen-2, Mutation Assessor, SIFT, PROVEAN, CADD, DANN, and FATHMM. If the majority of the algorithms predicted the variant as deleterious, it was annotated as deleterious. The functional impact of variants in splice sites was predicted using dbNSFP v4.2 *in silico* algorithm, followed by manual checking for the proximity to the branch point. Frameshift variants were considered deleterious.

3.7 Molecular Modeling Analysis

The impact of deleterious genetic variants on the interaction between the protein and the statin ligands (simvastatin, atorvastatin and rosuvastatin) was assessed using molecular modeling analysis as previously described (BORGES et al., 2021).

Briefly, amino acid sequences of reference proteins were downloaded from the Uniprot database (https://www.uniprot.org/help/uniprotkb) and tri-dimensional models were generated using AlphaFold2 pipeline (https://github.com/deepmind/alphafold). Protein models (reference and variants) were prepared by adding hydrogen atoms, fixing missing side chains, removing sulphate ions and other crystallization buffer molecules such as glycerol and minimizing by Biopolymer in Sybyl X suite (https://www.certara.com/). The molecular modeling analysis was was performed with the help of Dr. Glaucio Monteiro Ferreira.

3.8 Statistical analyses

Statistical analyses were performed using RStudio V 4.0.3 (RStudio, Inc, Boston, MA, USA) and GraphPad Prism V8 (Sigma, San Diego, CA, USA). A cut-off of p-value < 0.05 was used for statistical significance.

The distribution of the continuous variables was evaluated by the Kolmogorov-Smirnov test and those with normal distribution are shown as mean and SD and were compared using *t*-test. Continuous variables with skewed distribution are shown as median and interquartile range and were compared using Mann-Whitney. For comparisons of continuous variables, Benjamini-Hochberg correction was used to adjust p-values, considering a false discovery rate (FDR) of 10%. Categorical variables were compared by chi-square or Fisher's exact tests.

SNPassoc R package version 2.7 was used to analyze genotype and allele frequencies of the variants and Hardy-Weinberg equilibrium (HWE).

Univariate and multivariate linear and logistic regression analyses were performed to investigate the influence of deleterious genetic variants on statin response and SRAE in FH patients. In univariate regression analyses, p-values were corrected using Benjamini-Hochberg correction for multiple tests. In multivariate regression analyses models, BMI, baseline LDL-c, treatment intensity, ezetimibe use, and SRAE (for analysis of statin response only) were used as covariates.

4 RESULTS

4.1 Characteristics of the individuals and molecular diagnosis

Of the 200 FH patients selected for this study, 86 were excluded due lack of information from medical records: 19 did not use lipid-lowering medication; 55 did not have baseline laboratory data; 6 did not have on-treatment data; and 6 did not have a medical record available.

Biodemographic and clinical characteristics of 114 FH patients are described in the **Table 1.** Most patients were white (53.5%) and women (71.9%), and clinically diagnosed FH as defined (41.2%), probable (27.2%) and possible (31.6%) according to the modified DCLN criteria. Most patients were at very high risk (56.1%) and high risk (9.7 %) of CAD. The molecular diagnosis was confirmed for 35 (30.7%) patients, who carried pathogenic or likely pathogenic variants in *LDLR* (32) and *APOB* (1), according to ACMG classification, and a GOF variant in *PCSK9* (2) previously associated with FH. No pathogenic or likely pathogenic variants were found in *LDLRAP1* in this cohort (**Table 2**) (BORGES, 2019).

4.2 Statin response

4.2.1 Therapy targets

A total of 58 (50.8%) FH patients were considered RE and 56 (49.2%) were considered NRE to statin treatment. Clinical and molecular diagnosis of FH variables had similar results between RE and NRE groups, with exception of median BMI and frequency of obesity and alcohol consumption that were higher in the NRE group ($p<0.05$) (**Table 1**). No difference was observed in FH clinical diagnosis between NRE and RE groups. Most patients were of very high risk (56.1%), intermediate risk (34.2%) and high risk (9.7%). The risk was similarly distributed in RE and NRE groups.

Most patients were treated with atorvastatin (79.8%), followed by simvastatin (10.5%) and rosuvastatin (9.6%). The type and intensity of statin did not differ between RE and NRE groups ($p>0.05$), but association of statin with ezetimibe was more prescribed in RE group ($p=0.046$). Regarding drug interactions, total of 10 (8.8%) patients were taking amlodipine, an inhibitor of CYP3A4, but no difference was observed between RE and NRE ($p>0.05$). One patient was also taking carbamazepine, which is an inducer of CYP3A4. Reduced adherence to therapy was similar between RE and NRE patients ($p>0.05$). Myalgia and other SRAE were more frequent in RE than NRE patients ($p=0.001$).

Table 1 Biodemographic and clinical data of FH patients grouped according to statin response

Variable[a]		**Total** (114)	**RE** (58)	**NRE** (56)	***p-value***
Age, years		57.1 (37.9-76.3)	54.9 (34.7 - 75.1)	57.6 (41.9 - 73.3)	*0.261*
Gender (female), %		71.9 (82)	69.0 (40)	75.0 (42)	*0.611*
Ethnics, %	White	53.5 (54)	58.5 (31)	48.9 (23)	*0.326*
	Brown	31.7 (32)	24.5 (13)	38.3 (18)	
	Black	14.9 (15)	17.0 (9)	12.8 (6)	
Xanthomas, %		12.3 (14)	13.8 (8)	10.7 (6)	*0.830*
Arcus cornealis, %		17.9 (20)	14.0 (8)	21.8 (12)	*0.407*
FH clinical diagnosis[b], %	Defined or Probable	68.4 (78)	75.9 (44)	60.7 (34)	*0.124*
	Possible	31.6 (36)	24.1 (14)	39.3 (22)	
FH molecular	FH-variants	30.7 (35)	34.5 (20)	26.8 (15)	*0.491*
diagnosis,%	*APOB*	0.9 (1)	0.0 (0)	1.8 (1)	*0.166*
	LDLR	28.3 (32)	34.5 (20)	21.4 (12)	
	PCSK9	1.8 (2)	0.0 (0)	3.6 (2)	
	LDLRAP1	0.0 (0)	0.0 (0)	0.0 (0)	
Hypertension, %		62.5 (70)	60.3 (35)	64.8 (35)	*0.770*
Type 2 diabetes, %		21.6 (24)	26.3 (15)	16.7 (9)	*0.316*
Obesity, %		28.6 (32)	17.2 (10)	40.7 (22)	***0.011***
BMI, kg/cm²		27.7 (22.5-32.9)	26.3 (21.4 - 31.2)	28.2 (22.5 - 33.9)	***0.011***
Medical history, %	AMI	29.2 (33)	28.1 (16)	30.4 (17)	*0.952*
	CAD	40.0 (42)	44.0 (22)	36.4 (20)	*0.550*
	CVE	6.0 (6)	3.9 (2)	8.2 (4)	*0.637*
Alcohol consumption, %		25.0 (22)	14.6 (7)	37.5 (15)	***0.007***
Tobacco smoking, %		14.3 (16)	17.2 (10)	11.1 (6)	*0.510*
CAD risk, %	Very high risk	56.1 (64)	53.4 (31)	58.9 (33)	*0.095*
	High risk	9.7 (11)	15.5 (9)	3.6 (2)	
	Intermediate risk	34.2 (39)	31.0 (18)	37.5 (21)	
Lipid-lowering	Atorvastatin	79.8 (91)	77.6 (45)	82.1 (46)	*0.275*
treatment, %	Simvastatin	10.5 (12)	8.6 (5)	12.5 (7)	
	Rosuvastatin	9.6 (11)	13.8 (8)	5.4 (3)	
	Statins + Eze	36.8 (42)	46.6 (27)	26.8 (15)	***0.046***
Statin intensity, %	Moderate	14.0 (16)	6.9 (4)	21.4 (12)	*0.050*
	High	86.0 (98)	93.1 (54)	78.6 (44)	
Drug interactions, %	CYP3A4 inhibitors[c]	10 (8.8)	7 (12.1)	3 (5.3)	*0.349*
	CYP3A4 inhibitors + inducers[d]	1 (0.01)	0 (0.0)	1 (1.9)	-
Reduced adherence, %	Statins	15.9 (18)	17.2 (10)	14.5 (8)	*0.893*
	Ezetimibe	10.6 (12)	13.8 (8)	7.3 (4)	*0.413*
SRAE, %	Myalgia	16.8 (19)	29.3 (17)	3.6 (2)	***0.001***
	Others[e]	21.2 (24)	34.5 (20)	7.3 (4)	***0.001***

Number of patients in brackets. Patients ≥ 50% LDL cholesterol reduction on statin treatment were classified as responders. Categorical variables were compared by chi-square test. Continuous variables are shown as median and interquartile range and were compared by Mann-Whitney test. AMI: acute myocardial infarction; BMI: body mass index; CAD: coronary artery disease; CVE: cerebrovascular event; Eze: ezetimibe; NRE: non responder; RE: responder; SRAE: statin-related adverse events
[a] Data were not available for ethnics (13 patients), arcus cornealis (2), hypertension (2), diabetes (3), BMI (4), obesity (2), history of infarction (1), CAD (9), CVE (14), tobacco smoking (2), alcohol consumption (26), age (2). [b] DCLN modified criteria. [c] All patients in this category used the CYP3A4 inhibitor amlodipine. [d] All patients in this category used the CYP3A4 inhibitor amlodipine and the CYP3A4 inducer carbamazepine. [e] Including also stomach pain (4), diarrhea (1), urinary tract infection (1), increased hepatic enzymes (1) and joint pain (1).

Table 2 FH-related pathogenic variants in FH patients (n=114).

Gene	dbSNP code	Variant	Amino acid change	Type	*In silico* analysis[a]	ACMG Classification	Number of patients (zigosity)
APOB	rs61744153	c.11477C>T	p.Thr3826Met	Missense	P	LP	1 (He)
LDLR	rs112029328	c.313+1G>A	-	Splice-site	NA	P	2 (He)
	rs121908026	c.530C>T	p.Ser177Leu	Missense	P	P	2 (He)
	rs875989902	c.533A>T	p.Asp178Val	Missense	P	LP	1 (He)
	rs121908039	c.551G>A	p.Cys184Tyr	Missense	P	P	1 (He)
	rs879254797	c.1118G>A	p.Gly373Asp	Missense	P	LP	2 (He)
	rs28942078	c.1285G>A	p.Val429Met	Missense	P	P	1 (He)
	rs28942079	c.1291G>A	p.Ala431Thr	Missense	P	P	1 (He)
	rs879254913	c.1463T>C	p.Ile488Thr	Missense	P	LP	2 (He)
	rs373646964	c.1474G>A	p.Asp492Asn	Missense	P	LP	1 (He)
	rs28941776	c.1646G>A	p.Gly549Asp	Missense	P	P	2 (He)
	rs137929307	c.1775G>A	p.Gly592Glu	Missense	P	LP	2 (He)
	rs753707206	c.1801G>C	p.Asp601His	Missense	P	LP	2 (He)
	rs879254687	c.818-2A>G	-	Splice-site	NA	P	1 (He)
	rs1135402774	c.1474del	p.Asp492fs	*InDel*	NA	P	1 (He)
	rs121908031	c.2043C>A	p.Cys681*	Stop-gain	P	P	6 (He)
	rs752596535	c.501C>G	p.Cys167*	Stop-gain	P	P	2 (He)
	rs1135402768	c.487C>T	p.Gln163*	Stop-gain	P	P	1 (He)
	rs875989887	c.-140C>A	-	5'UTR	NA	LP	1 (Ho)
	rs387906307	c.-138del-T	-	5'UTR	NA	LP	1 (He)
*PCSK9**	rs141502002	c.1405C>T	p.Arg469Trp	Missense	LB	Conflict*	2 (He)

[a] The functionality of missense, stop-gain and stop-loss variants was assessed using the following *in silico* prediction algorithms: PolyPhen-2, Mutation Assessor, SIFT, PROVEAN, CADD, DANN, and FATHMM. ACMG: American College of Medical Genetics and Genomics; He: heterozygous; Ho: homozygous; LB: likely benign; LP: likely pathogenic; NA: not applicable; P: pathogenic; UTR: untranslated region.
Data obtained from BORGES, 2019.

When considering the absolute therapy target, 100 FH patients (87.7%) did not achieve optimum LDL-c levels after therapy. None of the patients of the CAD very high-risk group reached an on-treatment LDL-c < 50 mg/dL. Also, only two patients (18.2%) of the CAD high risk group reached an LDL-c < 70 mg/dL and 12 (30.8%) of the CAD intermediate risk group reached an LDL-c < 100 mg/dL (**Supplementary table 2**).

Baseline and post-treatment values of serum lipid profile in RE and NRE groups are shown in the **Figure 1**. RE group had higher baseline total cholesterol and LDL-c than the NRE patients, and lower on-treatment concentrations ($p<0.05$) (**Supplementary table 3**). As expected, RE patients had higher reduction of total cholesterol (absolute and % change), LDL-c (absolute and % change) and triglycerides (% change) after treatment than NRE group ($p<0.05$) (**Figure 1** and **Supplementary table 3**). Apo AI, apo B, glucose and insulin concentrations on treatment were higher in NRE than in RE groups ($p<0.05$), whereas other variables were not significantly different between the groups (**Supplementary table 4**).

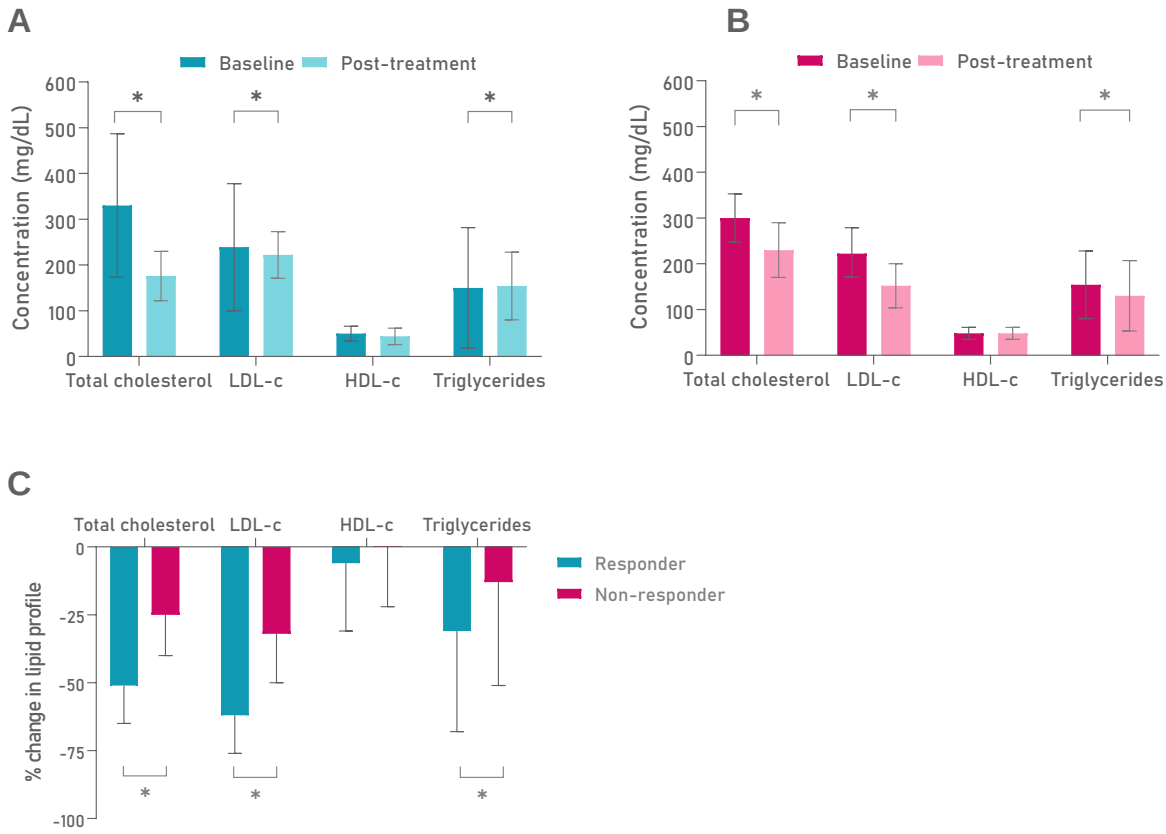

Figure 1 Serum lipid profile in FH patients treated with lipid-lowering drugs.
A. Baseline and post-treatment mean values and SD in responders (LDL-c reduction ≥ 50%). **B.** Baseline and post-treatment l mean values and SD in non-responders (LDL-c reduction < 50%). **C.** Serum lipid response (mean values and SD of % change) in responder and non-responder groups. *p<0.05 (compared by *t*-test).

Individuals on high intensity treatment showed lower post-treatment total cholesterol (p=0.011) and triglycerides (p=0.004) than individuals on moderate intensity treatment, but no difference was observed in other lipid parameters (**Supplementary table 5**). As expected, the reductions (% change) in total cholesterol, LDL-c and triglycerides, and HDL-c increase, were markedly higher, in individuals receiving high intensity treatment. In patients taking ezetimibe in combination with statin, ezetimibe users had higher baseline total cholesterol and LDL-c concentrations (p<0.05) and higher total cholesterol and LDL-c reductions (p<0.05) than non-users (**Supplementary table 5**).

4.2.2 Statin-related adverse events

A total of 24 (21.0%) patients experienced SRAE, which included myalgia (19, 79.1%), stomach pain (4, 16.7%), diarrhea (1, 4.2%), urinary tract infection (1, 4.2%), increased hepatic enzymes (1, 4.2%) and joint pain (1, 4.2%). Biodemographic characteristics of these patients are shown in the **Table 3**. SRAE group had higher frequency of xanthomas, FH-related pathogenic variants, with a higher frequency of pathogenic variants in *LDLR*, and reduced adherence to statins and ezetimibe ($p<0.05$). Interestingly, the prevalence of NRE patients was lower in SRAE group compared to no-SRAE ($p=0.001$).

Differences were also observed in the lipid profile of FH patients who experience or not SRAE (**Figure 2** and **Supplementary table 6**). SRAE group showed higher total cholesterol and LDL-c reductions compared to the no SRAE group ($p<0.05$). Baseline total cholesterol and LDL-c were also higher in SRAE group, while the on-treatment total cholesterol was lower (**Supplementary table 6**).

Table 3 Biodemographic characteristics of FH patients with SRAE (n=114).

Variable[a]		**Total** (114)	**No SRAE** (90)	**SRAE** (24)	***p-value***
Age, years		57.1 (37.9-76.3)	57.3 (38.3 - 76.3)	56.9 (38.9 - 74.9)	*0.830*
Gender (female), %		71.9 (82)	72.2 (65)	70.8 (17)	*1.000*
Ethnics, %	White	53.5 (54)	53.2 (41)	54.2 (13)	*0.538*
	Brown	31.7 (31)	29.9 (23)	37.5 (9)	
	Black	14.9 (15)	16.9 (13)	8.3 (2)	
Xanthomas, %		12.3 (14)	6.7 (6)	33.3 (8)	***0.002***
Arcus cornealis, %		17.9 (20)	16.9 (15)	21.7 (5)	*0.810*
FH clinical diagnosis[b], %	Definite or probable	68.4 (78)	64.4 (58)	83.3 (20)	*0.128*
	Possible	31.6 (36)	35.6 (32)	16.7 (4)	
FH molecular	FH variants	30.7 (35)	24.4 (22)	54.2 (13)	***0.011***
diagnosis, %	*APOB*	0.9 (1)	0.0 (0)	4.2 (1)	***0.006***
	LDLR	28.3 (32)	22.2 (20)	50.0 (12)	
	PCSK9	1.8 (2)	2.2 (2)	0.0 (0)	
	LDLRAP1	0.0 (0)	0.0 (0)	0.0 (0)	
Hypertension, %		62.5 (70)	61.4 (54)	66.7 (16)	*0.812*
Type 2 diabetes, %		21.6 (24)	18.4 (16)	33.3 (8)	*0.196*
Obesity, %		28.6 (32)	33.0 (29)	12.5 (3)	*0.087*
BMI, kg/cm²		27.7 (22.5-32.9)	27.9 (22 - 33.8)	25.9 (22.1 - 29.7)	*0.126*
Medical history, %	AMI	29.2 (33)	28.9 (26)	30.4 (7)	*1.000*
	CAD	40.0 (42)	36.5 (31)	55.0 (11)	*0.205*
	CVE	6.0 (6)	6.4 (5)	4.5 (1)	*1.000*
Alcohol consumption, %		25.0 (22)	28.8 (19)	13.6 (3)	*0.281*
Tobacco smoking, %		14.3 (16)	14.8 (13)	12.5 (3)	*0.918*
Lipid-lowering	Atorvastatin	79.8 (91)	83.3 (75)	66.7 (16)	*0.192*
treatment, %	Simvastatin	10.5 (12)	8.9 (8)	16.7 (4)	
	Rosuvastatin	9.6 (11)	7.8 (7)	16.7 (4)	
	Statins + Eze	36.8 (42)	33.3 (30)	50.0 (12)	*0.206*
Statin intensity, %	Moderate	14.0 (16)	15.6 (14)	8.3 (2)	*0.566*
	High	86.0 (98)	84.4 (76)	91.7 (22)	
Statin response	RE	50.9 (58)	42.2 (38)	83.3 (20)	***0.001***
	NRE	49.1 (56)	57.8 (52)	16.7 (4)	
Drug interactions	CYP3A4 inhibitors[c]	10 (8.8)	5.6 (5)	20.8 (5)	*0.052*
	CYP3A4 inhibitors + inducers[d]	1 (0.01)	0 (0.0)	1.0 (1.9)	-
Reduced adherence, %	Statins	15.9 (18)	10.1 (9)	37.5 (9)	***0.003***
	Ezetimibe	10.6 (12)	5.6 (5)	29.2 (7)	***0.003***

Number of patients in brackets. SRAE, group included patients that experienced all SRAE, including myalgia (19), stomach pain (4), diarrhea (1), urinary tract infection (1), increased hepatic enzymes (1) and joint pain (1). Categorical variables were compared by chi-square test. Continuous variables are shown as median and interquartile range and were compared by Mann-Whitney test. AMI: acute myocardial infarction; BMI: body mass index; CAD: coronary artery disease; CVE: cerebrovascular event; Eze: ezetimibe; NRE: non responder; RE: responder; SRAE: statin-related adverse events

[a] Data were not available for ethnics (13 patients), *arcus cornealis* (2), hypertension (2), diabetes (3), BMI (4), obesity (2), history of infarction (1), CAD (9), CVE (14), tobacco smoking (2), alcohol consumption (26), age (2). [b] DCLN modified criteria. [c] All patients in this category used the CYP3A4 inhibitor amlodipine. [d] All patients in this category used the CYP3A4 inhibitor amlodipine and the CYP3A4 inducer carbamazepine.

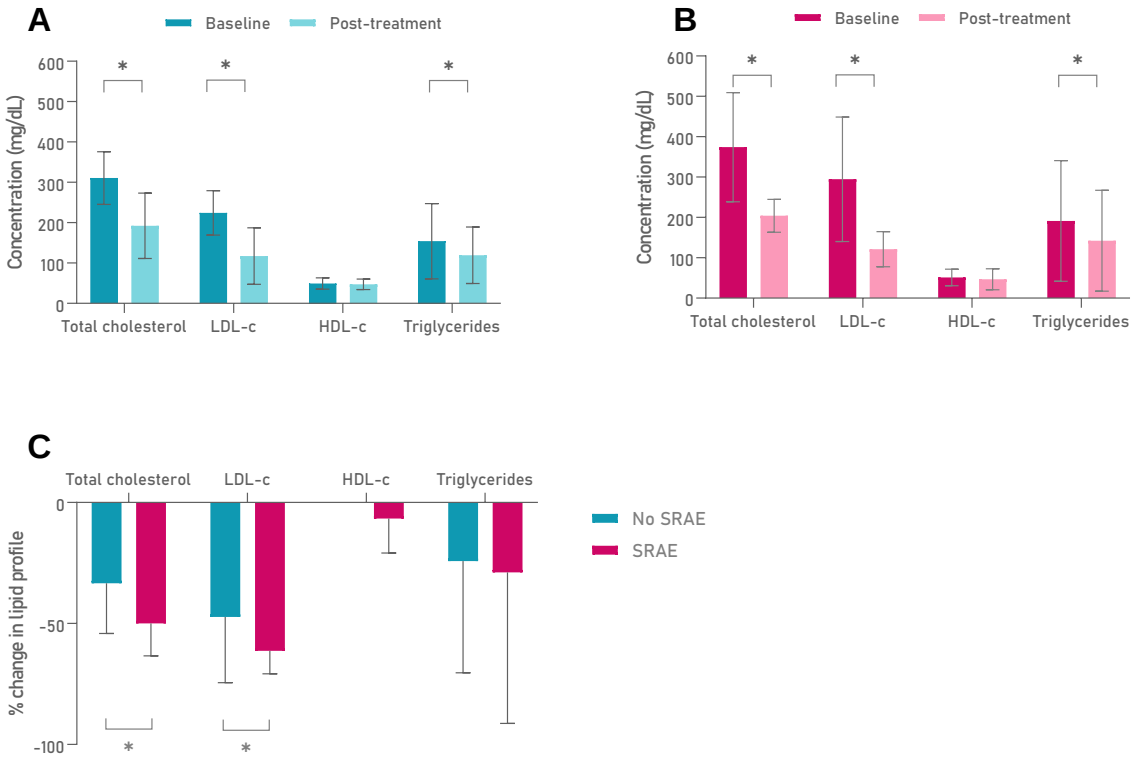

Figure 2 Serum lipid profile of FH patients with statin-related adverse events (SRAE).
A. Mean values and SD in patients without SRAE. **B.** Mean values and SD in patients with SRAE. **C.** Serum lipid response (Mean values and SD of % change) in patients with and without SRAE. *p<0.05 (compared by *t*-test).

4.3 Pharmacokinetics genes

4.3.1 Variants in PK-related genes

ETGS analysis identified 402 variants in 23 PK-related genes: 186 missense, 2 stop-gain, 1 stop-loss, 10 frameshift indel, 5 inframe deletions, 16 in splicing region, 29 in the 5´UTR region, and 153 in the 3´UTR region (**Supplementary table 7**). Of the total variants identified, 36 are novel, as they are not reported at dbSNP database (https://www.ncbi.nlm.nih.gov/snp/). Data on these novel variants were submitted to the NCBI (https://www.ncbi.nlm.nih.gov/sra/PRJNA662090). Most of the variants in PK genes were in Hardy-Weinberg equilibrium, except for 30 variants.

4.3.2 Functionality prediction of variants in PK genes

The FPS was calculated for missense and stop-loss variants in PK genes, and deleterious FPS (>0.5) of 61 variants with MAF > 1.0% are shown in **Table 4**. The most frequent variants were *SLCO1B3* rs60140950 (c.767G>C, LOF) (MAF: 14.7%); *SLCO1B1* rs4149056 (c.521T>C) (MAF: 11.0%), *CYP2C9*2* rs1799853 (c.430C>T, LOF) (MAF: 8.8%), *CYP2D6* rs1065852 (c.941G>A, LOF) (MAF: 6.0%), and *ABCC3* rs11568591 (c.3890G>A) (MAF: 6.5%). Five novel deleterious variants were detected, but their FPS score could not be calculated due to the lack of prediction by the functionality prediction algorithms used; therefore, they were considered deleterious, with a FPS score of 1.0 (**Supplementary table 8**).

A total of 16 splice-site variants in PK genes were considered deleterious according to the functional prediction algorithm (located at splice donor or splice acceptor regions) (**Table 5**). Two known deleterious splice variants, *CYP3A5*3* and *CYP3A5*6,* were detected in FH patients. *CYP3A5*3* (MAF: 49.6%) and *SLC22A1* rs35854239 (c.275_1276del) (MAF: 45.7%) were the most frequent variants.

In silico functional analysis of frameshift and inframe variants in PK-related genes, 3 inframe variants were considered as likely deleterious and 7 frameshift variants were considered deleterious, including the novel variant *ABCC1* c.66del (**Table 6**).

Table 4 Missense and stop-loss variants in PK-related genes (MAF > 1.0%) with deleterious functionality prediction score (FPS>0.5).

Gene	Variant	NT change	AA change	Type	MAF (%)	FPS
CYP2C8	rs1058930	c.486C>G	p.Ile162Met	missense	4.9	0.6
CYP2C9	rs1799853 (*CYP2C9*2)*	c.430C>T	p.Arg144Cys	missense	8.8	1
	rs2256871 (*CYP2C9*9)*	c.752A>G	p.His251Arg	missense	2.2	0.8
CYP2C19	rs17884712 (*CYP2C19**9)	c.431G>A	p.Arg144His	missense	2.2	0.8
CYP2D6	rs1065852	c.100C>T	p.Pro34Ser	missense	6.0	1
	rs28371703	c.271C>A	p.Leu91Met	missense	1.1	0.6
	rs1058172	c.941G>A	p.Arg314His	missense	4.9	1
CYP3A5	rs6977165	c.423A>G	p.X141Trp	stoploss	5.7	1
UGT1A3	rs45449995	c.808A>G	p.Met270Val	missense	2.2	0.75
ABCC1	rs45511401	c.2012G>T	p.Gly671Val	missense	3.8	0.8
ABCC2	rs8187692	c.3542G>T	p.Arg1181Leu	missense	2.7	0.8
	rs17216317	c.3872C>T	p.Pro1291Leu	missense	3.3	0.8
ABCC3	rs11568591	c.3890G>A	p.Arg1297His	missense	6.5	0.8
	rs141856639	c.3971G>A	p.Arg1324His	missense	1.1	1
SLC15A1	rs8187820	c.364G>A	p.Val122Met	missense	1.6	0.6
SLC22A1	rs2282143	c.1022C>T	p.Pro341Leu	missense	1.1	0.8
	rs35888596	c.113G>A	p.Gly38Asp	missense	2.2	1
	rs34059508	c.1393G>A	p.Gly465Arg	missense	1.1	0.8
	rs12208357	c.181C>T	p.Arg61Cys	missense	3.8	0.6
SLCO1B1	rs59502379	c.1463G>C	p.Gly488Ala	missense	1.8	0.8
	rs4149056 (*SLCO1B1*5)*	c.521T>C	p.Val174Ala	missense	11.0	0.8
SLCO1B3	rs60140950	c.767G>C	p.Gly228Ala	missense	14.7	1

AA: amino acid; FPS: functionality prediction score; MAF: minor allele frequency; NT: nucleotide; PK: pharmacokinetics. [a] Only variants with MAF> 1.0% were considered.

Table 5 *In silico* functional prediction of splice-site variants in PK-related genes.

Gene	Variant	NT change[b]	Type	MAF (%)	Prediction [a]
ABCC1	rs8187856	g.16146576C>G	Splice region	1.1	B
ABCC2	rs533334893	g.101552117G>A	Splice donor	0.5	D
ABCC3	rs11568607	g.48745787G>A	Splice region	2.2	B
ABCG2	rs34124189	g.89053790G>A	Splice region	0.5	B
CYP1A2	rs1288558234	g.75041241del	Splice region	0.5	B
	rs913188841	g.75041242C>G	Splice region	0.5	B
CYP2C8	rs11572078	g.96827126dup	Splice region	17.4	B
	rs2071426	g.5932A>G	Splice donor	23.9	D
CYP2D6	rs3892097	g.6866G>A	Splice acceptor	2.2	D
CYP3A5	rs776746 (*CYP3A5*3*)	g.12083G>A	Splice acceptor	49.6	D
	rs10264272 (*CYP3A5*6*)	g.19787G>A	Splice defect	3.1	D
SLC15A1	rs8187827	g.99354731T>C	Splice region	0.5	B
SLC22A1	rs35854239	c.275_1276del	Splice acceptor	45.7	D
SLCO1B1	rs77271279	g.21329832G>T	Splice donor	0.9	D
SLCO1B3	rs3764009	g.21013948C>T	Splice region	16.3	B
	rs958332597	g.21032366C>T	Splice region	0.5	B

B: benign; D: deleterious; MAF: minor allele frequency; NT: nucleotide; PK: pharmacokinetics. [a] Functionality prediction was made using dbNSFP v4.2 *in silico* prediction algorithm. [b] Genomic placement is described using the GRCh37 (hg19) version of the reference genome.

Table 6 *In silico* functional prediction of frameshift and inframe variants in PK-related genes.

Gene	Variant	NT change	Type	MAF (%)	Prediction [a]
ABCC1	Novel	c.66del	Frameshift variant	0.5	D
CYP2D6	rs5030656	c.88_690del	Inframe deletion	0.5	LD
	rs5030655	c.54del	Frameshift truncation	1.1	D
CYP3A5	rs200579169	c.2dup	Frameshift truncation	0.4	D
	rs41303343	c.035dup	Frameshift variant	1.8	D
	rs547253411	c.372del	Frameshift truncation	0.4	D
SLC22A1	rs72552763	c.258_1260del	Disruptive inframe deletion	18.5	LD
SLCO1B3	rs780598056	c.333del	Frameshift truncation	0.5	D
	rs558592800	c.19_120insAATT	Frameshift elongation	0.5	D
SLCO2B1	rs60113013	c._14del	Inframe insertion	1.6	LD

D: deleterious; LD: likely deleterious; MAF: minor allele frequency. [a] Functionality prediction was made using manually considering the region of the variant. Inframe variants were considered likely deleterious while frameshift variants were considered as deleterious.

4.3.3 Association study between variants in PK genes and response

4.3.3.1 LDL-c reduction

To assess the influence of variants in PK genes on statin response, 24 deleterious variants detected at least in three carriers were analyzed. FH patients carrying the homozygous form of the minor allele were grouped with the heterozygous carriers and compared with non-carriers (dominant inheritance model). **Figure 3** and **Supplementary table 9** show the results for deleterious variants in PK genes with MAF > 5%.

Carriers of the deleterious variant *ABCC1* rs45511401 (c.2012G>T) T allele had greater on-treatment LDL-c reduction with either all statins or atorvastatin treatment ($p<0.001$, adjusted $p<0.10$). One patient was considered an outlier because her LDL-c increased after statin treatment. *SLCO1B1* rs4146056 c.521C allele, a known deleterious variant, and *CYP3A5*3*, a non-functional splicing variant, were not associated with statin response (**Supplementary table 9**).

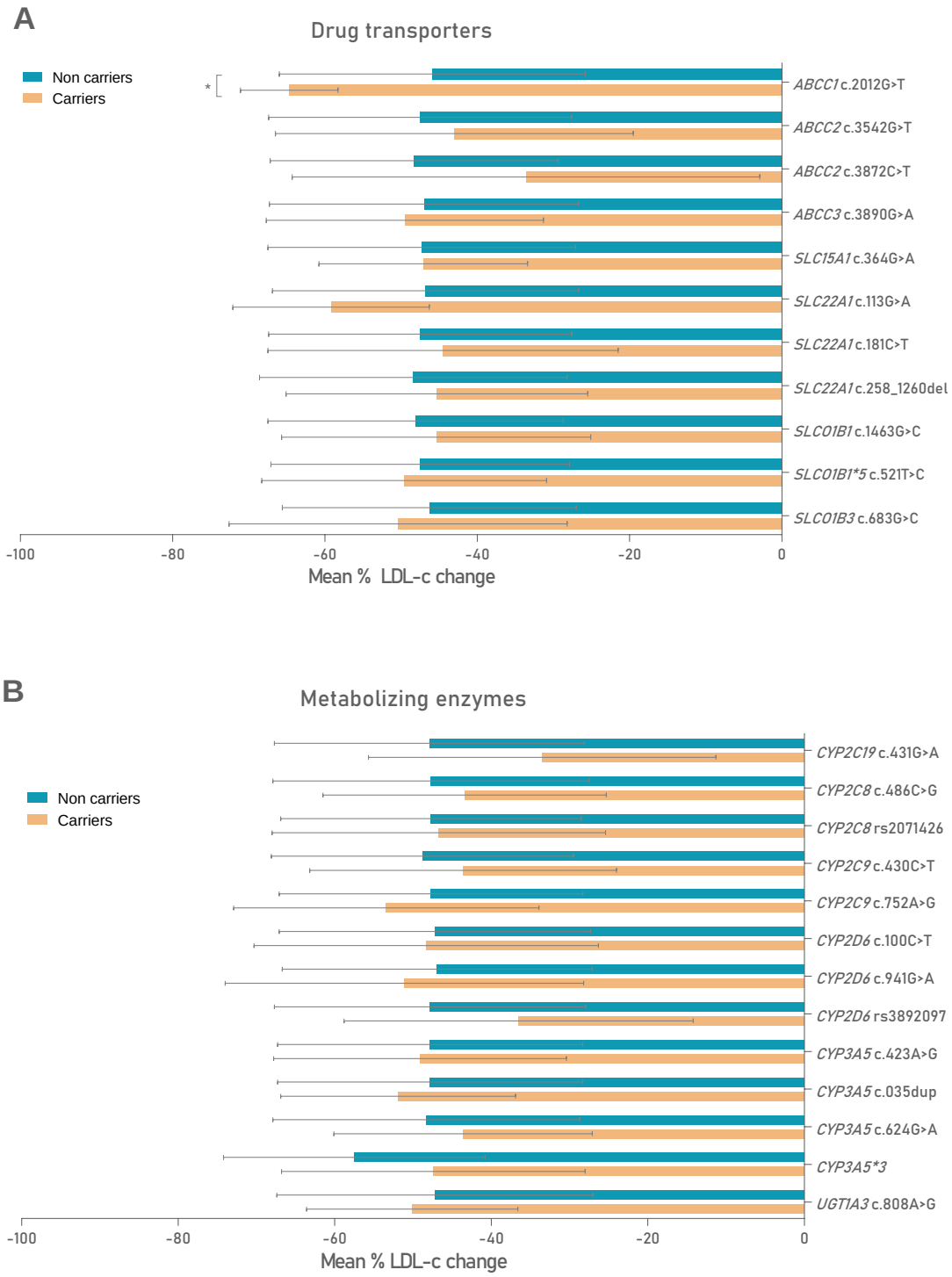

Figure 3 Mean LDL cholesterol response (% change) after lipid-lowering treatment in FH patients carrying deleterious- variants in PK genes (MAF>5.0%).

A. Variants in ABC and SLC transporters. **B.** Variants in CYP and UGT metabolizing enzymes. *p<0.05 (compared by *t*-test).

Univariate linear regression analysis showed that *ABCC1* c.2012T allele contributed for an additional reduction of 18.8% in LDL-c after statin therapy (p=0.016, adjusted p = 0.096) (**Supplementary table 10**). Baseline LDL-c and therapy intensity also enhanced LDL-c reduction, whereas BMI had an opposite effect (p-adjusted<0.05). Multivariate linear regression analysis of variants in PK-related genes with MAF > 1.0% was performed adjusting each model only with non-genetic covariates (body mass index, baseline LDL-c, therapy intensity, and presence of SRAE). This analysis showed no association between *ABCC1* c.2012G>T or other variants and enhanced LDL-c reduction (**Supplementary table 11**).

Next, we performed a multivariate linear regression analysis by including all deleterious variants with MAF >10% in the model and adjusting for non-genetic covariates using a dominant model (**Table 7**). In this model, *ABCC1* c.2012T allele enhanced LDL-c reduction by 13.8% after statin therapy (p=0.046) and *SLCO1B3* c.683C by 8.9% (p=0.047).

Table 7 Influence of deleterious variants (MAF >10%) on LDL-c response to statins in FH patients: Multivariate linear regression analysis.

Variant		**n**	**β**	**SE**	***p-value***
CYP2C8 g.5932A>G	G allele	92	2.8	3.7	*0.447*
*CYP3A5*3* g.12083G>A	A allele	114	12.4	7.6	*0.106*
ABCC1 c.2012G>T	T allele	92	-13.8	6.8	***0.046***
SLC22A1 c.1260_1262del	Deletion	92	-8.1	9.1	*0.378*
SLCO1B1 c.521T>C	C allele	114	-3.4	4.5	*0.449*
SLCO1B3 c.767G>C	C allele	92	-8.9	4.4	***0.047***

The model was adjusted with the following covariates: body mass index, baseline LDL-c, therapy intensity, and presence of SRAE. n: number of patients; β: linear coefficient; SE: standard error; LDL-c: low-density lipoprotein cholesterol; FH: familial hypercholesterolemia; SRAE: statin-related adverse events.

Univariate logistic regression analysis of with variants in PK-related genes and non-genetic variables showed that higher baseline LDL-c, ezetimibe use, manifestation of SRAE or myopathy and lower BMI were associated with higher likelihood of being responder to statin (p<0.05) (**Supplementary table 12**). However, the association with ezetimibe use and BMI was not sustained after correction (p>0.05).

Multivariate logistic regression analysis showed that variants in PK-related genes were not associated with the likelihood of being responder to statins, even after adjustment with non-genetic covariates (**Table 8**).

Table 8 Association of deleterious variants (MAF> 1.0%) in PK-related genes with statin response in FH patients: Multivariate logistic regression analysis.

Variable		**RE, % (58)**	**NRE, % (56)**	**OR (95%CI)**	***p-value***
Deleterious variants					
CYP2C19 c.431G>A	A allele	2.2 (1)	6.4 (3)	2.58 (0.30 - 54.78)	*0.428*
CYP2C8 c.486C>G	A allele	4.4 (2)	12.8 (6)	1.81 (0.33 - 14.3)	*0.520*
CYP2C8 g.5932A>G	G allele	44.4 (20)	42.6 (20)	0.87 (0.33 - 2.26)	*0.772*
*CYP2C9*2* c.430C>T	T allele	12.1 (7)	21.4 (12)	1.65 (0.48 - 6.14)	*0.437*
*CYP2C9*9* c.752A>G	G allele	5.2 (3)	3.6 (2)	4.49 (0.38 - 56.46)	*0.225*
CYP2D6 c.941G>A	A allele	11.1 (5)	8.5 (4)	0.41 (0.07 - 2.1)	*0.287*
CYP2D6 c.100C>T	T allele	11.1 (5)	10.6 (5)	0.4 (0.08 - 1.99)	*0.260*
CYP2D6 g.6866G>A	A allele	2.2 (1)	6.4 (3)	1.09 (0.1 - 25.79)	*0.948*
CYP3A5 c.624G>A	A allele	3.4 (2)	3.6 (2)	0.95 (0.08 - 12.82)	*0.968*
CYP3A5 c.423A>G	G allele	10.3 (6)	12.5 (7)	0.99 (0.25 - 4.01)	*0.986*
*CYP3A5*3* g.12083G>A	A allele	93.1 (54)	94.6 (53)	0.86 (0.11 - 6.38)	*0.881*
UGT1A3 c.808A>G	G allele	4.4 (2)	2.1 (1)	0.13 (0 - 1.95)	*0.166*
ABCC1 c.2012G>T	T allele	15.6 (7)	0.0 (0)	NR	-
ABCC2 c.3872C>T	T allele	2.2 (1)	10.6 (5)	7.58 (0.81 - 199.03)	*0.123*
ABCC2 c.3542G>T	T allele	4.4 (2)	6.4 (3)	1.15 (0.13 - 12.74)	*0.903*
ABCC3 c.3890G>A	A allele	13.3 (6)	12.8 (6)	0.95 (0.24 - 3.96)	*0.946*
SLC15A1 c.364G>A	A allele	4.4 (2)	2.1 (1)	0.1 (0 - 1.33)	*0.098*
SLC22A1 c.181C>T	T allele	4.4 (2)	8.5 (4)	1.34 (0.19 - 12.57)	*0.776*
SLC22A1 c.113G>A	A allele	6.7 (3)	2.1 (1)	0.44 (0.02 - 4.02)	*0.504*
SLC22A1 c.1260_1262del	Deletion	31.1 (14)	38.3 (18)	0.88 (0.31 - 2.5)	*0.813*
*SLCO1B1*5* c.521T>C	C allele	24.1 (14)	19.6 (11)	0.62 (0.2 - 1.84)	*0.391*
SLCO1B1 c.1463G>C	C allele	3.4 (2)	3.6 (2)	1.75 (0.12 - 22.02)	*0.657*
SLCO1B3 c.767G>C	C allele	26.7 (12)	23.4 (11)	0.62 (0.18 - 1.98)	*0.418*

Each model was adjusted with the following covariates: body mass index, baseline LDL-c, therapy intensity, and presence of SRAE. Number of patients in round brackets. NRE: non-responder; RE: responder; OR: odds ratio; CI: confidence interval; FH: familial hypercholesterolemia; NR: not reported (no patients in NRE group); PK: pharmacokinetics; SRAE: statin-related adverse events.

4.3.3.2 Molecular modeling results

Molecular modeling analysis was performed to explore the influence of the missense variant *ABCC1* rs45511401 (c.2012G>T, p.Gly671Val) on amino acid interactions with statin ligands. In this way, the amino acid sequence of reference ABCC1 was downloaded from the Uniprot database (code: P33527) and the tri-dimensional model was generated by AlphaFold2 pipeline. ABCC1 reference (Gly671) and variant (Val671) models were prepared by adding hydrogen atoms, fixing missing side chains, removing sulphate ions and other crystallization buffer molecules such as glycerol and minimizing by Biopolymer in Sybyl X suite.

As shown in **Figure 4**, the variant Val671 resulted in shorter distances of ABCC1 interactions with atorvastatin (2.1 Å), rosuvastatin (1.1 Å) and simvastatin (1.7 Å) compared to the reference Gly671 (4.1 Å, 3.7 Å, 4.3 Å, respectively). These results indicate that the amino acid change from Glycine to Valine in position 671 enhance the interaction of ABCC1 with statins, possibly reducing the efflux rate in the basolateral membrane of the hepatocytes. In this way, the variant would cause more retention of statins within the liver increasing the LDL-c response.

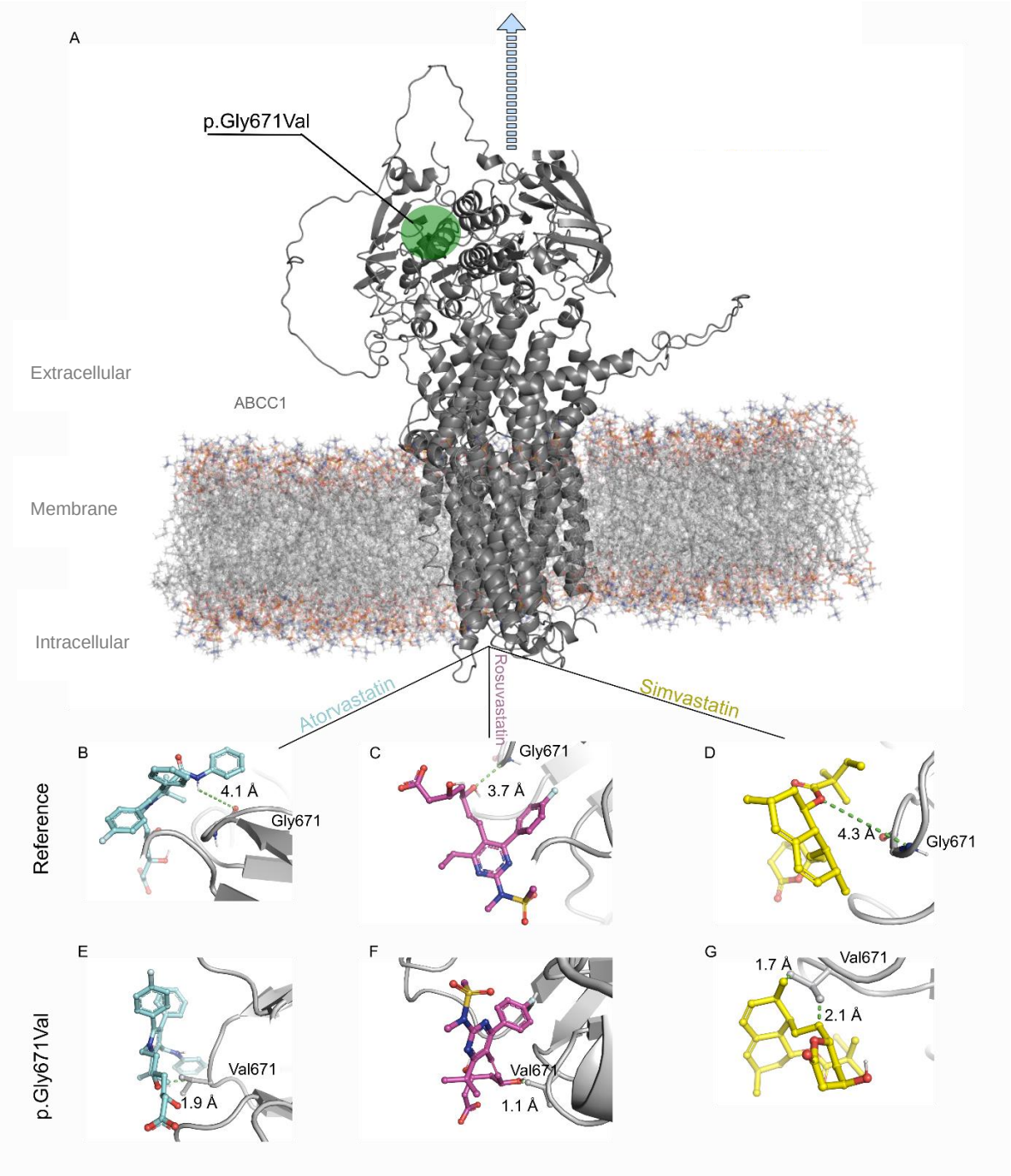

Figure 4 Molecular Modeling Analysis. Influence of *ABCC1* rs45511401 (c.2012G>T, p.Gly671Val) on amino acid interaction with statins.

A. Representation of ABCC1 anchored in the basolateral membrane of a hepatocyte. The arrow indicates the sense of statin efflux. **B**, **C**, and **D**. Interactions between ABCC1 reference (Gly671) and atorvastatin, rosuvastatin and simvastatin, respectively. **E**, **F**, and **G**. Interactions between ABCC1 variant (Val671) and atorvastatin, rosuvastatin and simvastatin, respectively.

4.3.3.3 Statin-related adverse events

The association of deleterious variants in PK genes with MAF > 1.0% and non-genetic variables with SRAE was also assessed by univariate logistic regression analysis. Higher baseline LDL-c increased the risk of SRAE ($p<0.05$). Reduced adherence, drug interaction with CYP3A4 inhibitor and FH-related variants were also predictors of SRAE, but these associations were not maintained after corrections (adjusted $p>=0.05$) (**Supplementary table 13**). Deleterious variants in PK genes were not associated with SRAE according to univariate logistic regression analysis (**Supplementary table 13**) or multivariate logistic regression analysis after adjustment with non-genetic covariates ($p=0.067$) (**Table 9**).

Table 9 Association of deleterious variants (MAF > 1.0%) in PK-related genes with SRAE in FH patients: Multivariate logistic regression analysis.

Variable		**No SRAE , % (90)**	**SRAE, % (24)**	**OR (95%CI)**	***p-value***
CYP2C8 c.486C>G	A allele	45.5 (35)	35.7 (5)	0.70 (0.19 - 2.37)	*0.574*
CYP2C9 c.430C>T	T allele	16.9 (15)	12.5 (3)	0.54 (0.1 - 2.2)	*0.428*
CYP2C9 c.752A>G	G allele	2.2 (2)	12.5 (3)	3.03 (0.35 - 29.74)	*0.309*
CYP3A5 c.624G>A	A allele	3.4 (3)	4.2 (1)	1.34 (0.06 - 13.48)	*0.817*
CYP3A5 c.423A>G	G allele	11.2 (10)	12.5 (3)	1.11 (0.22 - 4.44)	*0.886*
*CYP3A5*3* g.12083G>A	A allele	93.3 (83)	95.8 (23)	2.7 (0.33 - 60.01)	*0.418*
ABCC1 c.2012G>T	T allele	6.5 (5)	14.3 (2)	1.65 (0.2 - 9.46)	*0.594*
ABCC2 c.3872C>T	T allele	5.2 (4)	14.3 (2)	6.12 (0.72 - 41.6)	*0.067*
ABCC2 c.3542G>T	T allele	5.2 (4)	7.1 (1)	1.28 (0.06 - 11.08)	*0.841*
ABCC3 c.3890G>A	A allele	13 (10)	14.3 (2)	0.72 (0.07 - 4.06)	*0.734*
SLC22A1 c.113G>A	A allele	3.9 (3)	7.1 (1)	3.44 (0.16 - 32.63)	*0.317*
SLC22A1 c.1260_1262del	Deletion	37.7 (29)	14.3 (2)	0.27 (0.04 - 1.19)	*0.122*
SLCO1B1 c.521T>C	C allele	21.3 (19)	25.0 (6)	1.23 (0.36 - 3.85)	*0.727*
SLCO1B1 c.1463G>C	C allele	3.4 (3)	4.2 (1)	2.4 (0.11 - 22.59)	*0.479*
SLCO1B3 c.767G>C	C allele	26 (20)	14.3 (2)	0.36 (0.05 - 1.68)	*0.252*

Each model was adjusted with the following covariates: baseline LDL-c, presence of FH-related variant and adherence to statin. Number of patients in round brackets. P-value was adjusted using the Benjamini-Hochberg correction. NRE: non-responder; RE: responder; OR: odds ratio; CI: confidence interval; BMI: body mass index; FH: familial hypercholesterolemia; LDL-c: low-density lipoprotein cholesterol; PK: pharmacokinetics; SRAE: statin-related adverse events.

4.4 Pharmacodynamics genes

4.4.1 Variants in PD-related genes

ETGS analysis identified 752 variants in 33 PD-related genes, with 85 novel variants. The variants were of the following types: 249 missense, 1 stop-gain, 9 start-loss, 5 frameshift indel, 9 inframe indel, 26 in splicing region, 67 in the 5´UTR region, and 386 in the 3´UTR region (**Supplementary table 14**). Most of the variants were in Hardy-Weinberg equilibrium, except for 29 variants.

4.4.2 Functionality prediction of variants in PD genes

A total of 111 variants were predicted as deleterious, of which 97 were missense, start -loss or stop -gain, 10 were splice-sites and 4 were frameshift (**Supplementary table 14**). Variants with MAF higher than 1% and their functionality predictions are shown in **Table 10** (missense, start loss and stop gain), **Table 11** (splice-site) and **Table 12** (frameshift and inframe).).

The most frequent deleterious variant was the missense variant *ABCA1* rs2230808 (c.4760G>A, p.Lys1587Arg), with a frequency of 36.4%, followed by *KIF6* rs20455 (c.2155T>C (rs20455, p.Trp719Arg), with a MAF of 44.3%, and *APOB* rs679899 (c.1853C>T (rs679899, p.Ala618Val), with a MAF of 37.3%. In FH-related genes, deleterious variants were found in *APOB, LDLR* and *LDLRAP1*, while only a likely deleterious splice-site variant was found in *PCSK9.* Two novel variants were predicted as deleterious, both frameshift variants: *LDLR* c.454del and c.103del (**Table 12**).

Table 10 Missense, start-loss and stop-gain variants (MAF > 1.0%) in PD-related genes predicted as deleterious.

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction
FH-related genes						
APOB	rs1367117	c.293C>T	p.Thr98Ile	missense	32.0	D
	rs679899	c.1853C>T	p.Ala618Val	missense	37.3	D
	rs6752026	c.433C>T	p.Pro145Ser	missense	2.2	D
	rs676210	c.8216C>T	p.Pro2739Leu	missense	19.3	D
	rs12713675	c.7367C>A	p.Ala2456Asp	missense	2.2	D
	rs12720855	c.9880T>C	p.Ser3294Pro	missense	2.2	D
	rs1801699	c.5741A>G	p.Asn1914Ser	missense	4.4	D
	rs533617	c.5768A>G	p.His1923Arg	missense	1.3	D
LDLR	rs121908031	c.1539C>A	p.Cys513X	Stop-gain	1.8	D
	rs879254913	c.959T>C	p.Ile320Thr	missense	1.3	D
	rs121908031	c.1539C>A	p.Cys513X	Stop-gain	1.8	D
LDLRAP1	rs41291058	c.712C>T	p.Arg238Trp	missense	2.2	D
Cholesterol homeostasis and metabolism						
ABCG4	rs12271907	c.1035C>G	p.Asn345Lys	missense	3.8	D
	rs35060365	c.1055C>T	p.Pro352Leu	missense	1.1	D
APOA4	rs12721041	c.37G>A	p.Val13Met	missense	2.2	D
APOE	rs7412	c.526C>T	p.Arg176Cys	missense	2.6	D
	rs429358	c.388T>C	p.Cys130Arg	missense	12.3	D[b]
CYP7A1	rs8192875	c.1039G>A	p.Asp347Asn	missense	1.1	D
LPA	rs3124784	c.6046C>T	p.Arg2016Cys	missense	27.6	D
	rs139145675	c.5311C>T	p.Arg1771Cys	missense	2.2	D
	rs41267807	c.6068A>G	p.Tyr2023Cys	missense	1.3	D
	rs41272110	c.4195A>C	p.Thr1399Pro	missense	10.5	D
	rs76062330	c.5468G>T	p.Gly1823Val	missense	3.5	D
	rs3798220	c.5673A>G	p.Ile1891Met	missense	3.1	D
	rs41259144	c.2969G>A	p.Arg990Gln	missense	1.8	D
Reverse cholesterol transport						
ABCA1	rs2230808	c.4760G>A	p.Lys1587Arg	missense	36.4	D
	rs9282541	c.688C>T	p.Arg230Cys	missense	1.8	D
CETP	rs5880	c.988G>C	p.Ala330Pro	missense	5.7	D
Cholesterol efflux						
ABCG5	rs6756629	c.148C>T	p.Arg50Cys	missense	6.1	D
ABCG8	rs11887534	c.55G>C	p.Asp19His	missense	6.1	D
	rs4148211	c.161A>G	p.Tyr54Cys	missense	30.3	D
	rs80025980	c.239G>A	p.Cys80Tyr	missense	1.3	D
Associated with statin-related adverse events						
COQ10A	rs60542959	c.3G>T	p.A2_Met44del	start-loss	2.2	D
LPL	rs1801177	c.106G>A	p.Asp36Asn	missense	2.2	D
	rs328	c.1421C>G	p.Ser474X	Stop-gain	7.4	D
Transcription regulators of cholesterogenic genes						
SREBF2	rs2229440	c.1867G>A	p.Val623Met	missense	3.1	D
Other genes						
CLMN	rs61750771	c.2698A>T	p.Ile900Phe	missense	1.8	D
KIF6	rs20455	c.2155T>C	p.Trp719Arg	missense	44.3	D[b]

AA: amino acid; FPS: functionality prediction score; MAF: minor allele frequency; NT: nucleotide; PD: pharmacodynamics. [a] Only variants with MAF> 1.0% were considered. [b] Although this variant was predicted as neutral, it was considered deleterious as it has already been associated with statin response in previous studies.

Table 11 *In silico* functional prediction of splice-site variants in PD-related genes

Gene	Variant	NT change[b]	Type	MAF (%)	Prediction [a]
ABCA1	rs77663187	g.107556811del	splice region	16.1	N
	rs769705621	g.107556792_107556793insA	splice acceptor	9.3	D
	rs769705621	g.107556792_107556793insAA	splice acceptor	21.2	D
	rs769705621	g.107556792_107556793insAAA	splice acceptor	22.0	D
	rs769705621	g.107556792_107556793insAAAA	splice acceptor	7.9	D
	rs769705621	g.107556792_107556793insAAAAA	splice acceptor	3.5	D
	rs769705621	g.107556792_107556793insAAAAAA	splice acceptor	3.5	D
	rs769705621	g.107556792_107556793insAAAAAAA	splice acceptor	3.5	D
ABCG1	rs77603571	g.43627101G>A	splice region	0.5	N
APOA2	rs6413453	g.161192316G>A	splice region	5.3	N
APOC2	rs74500990	g.45451954G>C	splice region	0.9	N
APOC3	rs138326449	g.116701354G>A	splice donor	16.7	D
CLMN	rs5810715	g.95670813del	splice region	0.9	N
LDLR	rs112029328	g.11213463G>A	splice donor	0.9	D
	rs116405216	g.11221324G>A	splice region	0.4	N
	rs879254687	g.11218066A>G	splice acceptor	13.6	D
LIPA	rs2297472	g.90984990G>A	splice region	0.4	N
LPA	rs143431368	g.160969693T>C	splice acceptor	0.5	D
	rs41272114	g.161006077C>T	splice donor	2.6	D
	rs756764319	g.160962134C>T	splice region	0.4	N
PCSK9	rs2495477	g.55518467A>G	splice region	18.4	N
SREBF1	rs45567732	g.17718146C>A	splice region	0.4	N

MAF: minor allele frequency; NT: nucleotide; PD: pharmacodynamics. [a] Functionality prediction was made using dbNSFP v4.2 *in silico* prediction algorithm. [b] Genomic placement is described using the GRCh37 (hg19) version of the reference genome.

Table 12 *In silico* functional prediction of frameshift and inframe variants in PD-related genes

Gene	Variant	NT change	Type	MAF (%)	Prediction [a]
APOA4	rs539176882	c.145_1146insACAGCAGCAGG	Disruptive inframe insertion	1.3	LD
APOB	rs17240441	c.5_43del	Disruptive inframe deletion	2.2	LD
	rs562574661	c.3480_13482del	Inframe deletion	0.4	LD
LDLR	Novel	c.454del	Frameshift variant	0.4	D
	rs879255131	c.573_1574del	Frameshift variant	0.4	D
	Novel	c.103del	Frameshift variant	0.4	D
	rs1135402774	c.70del	Frameshift variant	0.4	D
PCSK9	rs35574083	c.2_43insCTG	Disruptive inframe insertion	14.0	LD
SREBF2	rs143615881	c.03_205del	Disruptive inframe deletion	0.4	LD
	rs779626156	c.85_193del	Disruptive inframe deletion	0.4	LD

D: deleterious; LD: likely deleterious; MAF: minor allele frequency; NT: nucleotide; PD: pharmacodynamics. [a] Functionality prediction was made using manually considering the region of the variant. Inframe variants were considered likely deleterious while frameshift variants were considered as deleterious.

4.4.3 Association study between variants in PD genes and response to statins

4.4.3.1 LDL-c reduction

A total of 40 deleterious variants with MAF > 1.0% were selected for the association analysis with LDL-c reduction after statin (**Table 13**) and atorvastatin (**Table 14**) treatment.

For this analysis, we considered 5 possible variations of *ABCA1* rs769705621, with the insertion of one to seven adenines in splice-site region. Since the three last variations, g.107556792_107556793insAAAAA/AAAAAA/AAAAAAA, were in complete linkage disequilibrium ($R^2 = 0.998$), we only considered the insertion of five adenines as a marker of these indels.

ABCA1 rs769705621 (g.107556792_107556793insAAAAA) was associated with lower LDL-c reduction in carriers ($p<0.001$, adjusted $p=0.003$) (**Table 13**). Interestingly, the insertion of one to four adenines in the same site did not result in any differences in LDL-c change between carriers and non-carriers ($p>0.05$).

The missense variants *KIF6* rs20455 (c.508T>C) and *LPA* rs41267807 (c.6068A>G) were also associated with lower LDL-c reduction ($p<0.05$), but these significances were dropped after adjustment (adjusted $p>0.05$). On the other hand, the missense variant *LPL* rs1801177 (c.106G>A) was associated with higher statin response ($p=0.022$), which was not maintained after multiple testing adjustment (adjusted $p=0.296$) (**Table 13**).

When only the response to atorvastatin was analyzed (**Table 14**), *ABCA1* g.107556792_107556793insAAAAA and *KIF6* rs20455 (c.508T>C) were associated with lower atorvastatin response ($p<0.05$), but these associations were not maintained after corrections ($p>0.05$). On the other hand, the missense variant *LPA* rs76062330 (c.5468G>T) was associated with higher LDL-c reduction, even after corrections (adjusted $p=0.001$).

Table 13 Influence of deleterious variants (MAF > 1.0%) in PD-related genes on LDL-c reduction in FH patients on statin treatment.

Gene	rs code	NT change	Type	LDL-c reduction, %			Adjusted
				Non carriers	Carriers	p-*value*	p-value
All statins							
ABCA1	rs769705621	g.107556792_107556793 insA	splice-site	-45.6 ± 19.6 (70)	-48.6 ± 17.6 (16)	*0.555*	*0.910*
	rs769705621	g.107556792_107556793 insAA	splice-site	-48.7 ± 19.8 (64)	-48.2 ± 19.2 (40)	*0.902*	*0.973*
	rs769705621	g.107556792_107556793 insAAA	splice-site	-48 ± 20.3 (64)	-47.3 ± 18.5 (43)	*0.848*	*1.000*
	rs769705621	g.107556792_107556793 insAAAA	splice-site	-48 ± 19.6 (79)	-46.2 ± 16.7 (10)	*0.748*	*0.989*
	rs769705621	g.107556792_107556793 insAAAAA	splice-site	-48.5 ± 19.5 (110)	-33.6 ± 3.6 (4)	***<0.001***	***0.003***
	rs9282541	c.688C>T	missense	-48.5 ± 19.5 (110)	-34 ± 10.6 (4)	*0.065*	*0.534*
	rs2230808	c.4760A>G	missense	-45.1 ± 18.1 (16)	-48.4 ± 19.6 (98)	*0.504*	*0.939*
ABCG4	rs12271907	c.1035C>G	missense	-47.6 ± 20.2 (86)	-42.2 ± 17.3 (6)	*0.491*	*1.000*
ABCG5	rs6756629	c.148C>T	missense	-48.2 ± 19.6 (100)	-46.6 ± 18 (14)	*0.768*	*0.955*
ABCG8	rs4148211	c.161A>G	missense	-50.7 ± 17.4 (54)	-45.6 ± 20.9 (60)	*0.157*	*0.717*
	rs11887534	c.55G>C	missense	-47.6 ± 19.5 (100)	-50.4 ± 18.8 (14)	*0.618*	*0.975*
	rs80025980	c.239G>A	missense	-47.9 ± 19.5 (111)	-52.5 ± 15.3 (3)	*0.658*	*0.899*
APOA4	rs12721041	c.37G>A	missense	-47.7 ± 19.5 (109)	-53.5 ± 18.4 (5)	*0.526*	*0.938*
	rs12713675	c.7367C>A	missense	-48.5 ± 19 (109)	-37.1 ± 26.2 (5)	*0.391*	*1.000*
	rs12720855	c.9880T>C	missense	-48.5 ± 19 (109)	-37.1 ± 26.2 (5)	*0.391*	*1.000*
APOB	rs6752026	c.433C>T	missense	-47.8 ± 19.7 (109)	-52.8 ± 6.7 (5)	*0.191*	*0.713*
	rs1801699	c.5741A>G	missense	-48.3 ± 19.9 (104)	-44.5 ± 12.6 (10)	*0.400*	*0.965*
	rs1367117	c.293C>T	missense	-46.7 ± 20.6 (50)	-48.9 ± 18.5 (64)	*0.552*	*0.943*
	rs679899	c.1853C>T	missense	-46.9 ± 17.6 (43)	-48.7 ± 20.4 (71)	*0.619*	*0.940*
	rs533617	c.5768A>G	missense	-47.9 ± 19.3 (111)	-50.6 ± 27.6 (3)	*0.881*	*1.000*
	rs676210	c.8216C>T	missense	-48.1 ± 18.1 (75)	-47.7 ± 21.8 (39)	*0.929*	*0.977*
APOE	rs7412	c.526C>T	missense	-47.4 ± 19.5 (108)	-57.8 ± 16 (6)	*0.178*	*0.730*
	rs429358	c.388T>C	missense	-47 ± 19.7 (89)	-51.4 ± 18.1 (25)	*0.307*	*0.820*
CETP	rs5880	c.988G>C	missense	-47.1 ± 19.2 (101)	-54.5 ± 19.9 (13)	*0.226*	*0.773*
CLMN	rs61750771	c.2698A>T	missense	-48 ± 19.6 (110)	-47.2 ± 10.5 (4)	*0.890*	*0.986*
COQ10A	rs60542959	c.3G>T	start-loss	-48 ± 19.7 (109)	-47.5 ± 11.3 (5)	*0.932*	*0.955*
KIF6	rs20455	c.2155T>C	missense	-53.1 ± 18.7 (39)	-45.3 ± 19.3 (75)	***0.038***	*0.391*
LDLR	rs879254913	c.959T>C	missense	-48 ± 19.6 (111)	-47 ± 4.0 (3)	*0.749*	*0.942*
	rs121908031	c.1539C>A	stop-gain	-48 ± 19.5 (110)	-47.4 ± 16.1 (4)	*0.949*	*0.949*
LDLRAP1	rs41291058	c.712C>T	missense	-47.9 ± 19.5 (109)	-49.6 ± 18.4 (5)	*0.851*	*0.976*
LPA	rs41267807	c.6068A>G	missense	-48.5 ± 19.4 (111)	-28.8 ± 5.9 (3)	***0.012***	*0.239*
	rs41272110	c.4195A>C	missense	-49.6 ± 18.9 (90)	-41.8 ± 20.3 (24)	*0.100*	*0.649*
	rs139145675	c.5311C>T	missense	-47.6 ± 19.7 (109)	-55.7 ± 8.6 (5)	*0.106*	*0.591*
	rs3124784	c.6046C>T	missense	-45.5 ± 21.2 (60)	-50.7 ± 16.9 (54)	*0.148*	*0.768*
	rs76062330	c.5468G>T	missense	-47.5 ± 19.4 (107)	-55.5 ± 18.2 (7)	*0.301*	*0.870*
	rs3798220	c.5673A>G	missense	-47.5 ± 19.1 (108)	-56.5 ± 24.3 (6)	*0.410*	*0.970*
	rs41259144	c.2969G>A	missense	-48.5 ± 18.6 (110)	-34.9 ± 35.5 (4)	*0.502*	*0.978*
	rs41272114	g.161006077C>T	splice-site	-48.1 ± 19.6 (108)	-44.9 ± 16 (6)	*0.647*	*0.953*
LPL	rs1801177	c.106G>A	missense	-47.2 ± 19.3 (109)	-65.4 ± 11.7 (5)	***0.022***	*0.296*
	rs328	c.1421C>G	stop-gain	-49 ± 18.7 (97)	-42.2 ± 22.5 (17)	*0.256*	*0.907*
SREBF2	rs2229440	c.1867G>A	missense	-47.7 ± 19.7 (107)	-52 ± 13.8 (7)	*0.468*	*1.000*

Number of patients in round brackets. Data are shown as mean ± SD and compared by t-test. FH: familial hypercholesterolemia; LDL-c: low-density lipoprotein cholesterol; NT: nucleotide; PD: pharmacodynamics.

Table 14 Influence of deleterious variants (MAF > 1.0%) in PD-related genes on LDL-c reduction in FH patients on atorvastatin treatment.

Gene	rs code	NT change	Type	LDL-c reduction, %			Adjusted
				Non carriers	Carriers	p-*value*	p-value
Atorvastatin							
ABCA1	rs9282541	c.688C>T	missense	-48.5 ± 18.3 (94)	-32.4 ± 11.4 (4)	*0.060*	*0.587*
	rs2230808	c.4760A>G	missense	-44.6 ± 18.3 (16)	-48.5 ± 18.3 (82)	*0.404*	*1.000*
	rs769705621	g.107556792_107556793 insA	splice-site	-48.5 ± 17.8 (65)	-44.8 ± 13.4 (9)	*0.472*	*0.995*
	rs769705621	g.107556792_107556793 insAAAA	splice-site	-49 ± 18.1 (52)	-46.2 ± 19.4 (39)	*0.475*	*0.975*
	rs769705621	g.107556792_107556793 insAAA	splice-site	-49.5 ± 17.4 (52)	-46.9 ± 19.7 (36)	*0.516*	*0.959*
	rs769705621	g.107556792_107556793 insAA	splice-site	-45.7 ± 18 (55)	-48 ± 17.3 (16)	*0.641*	*0.961*
	rs769705621	g.107556792_107556793 insAAAAA	splice-site	-48.3 ± 18.4 (95)	-34 ± 4.3 (3)	***0.006***	*0.106*
ABCG4	rs12271907	c.1035C>G	missense	-47.9 ± 18.8 (74)	-45.6 ± 13.3 (4)	*0.754*	*0.933*
ABCG5	rs6756629	c.148C>T	missense	-48.6 ± 18 (84)	-43.4 ± 20.1 (14)	*0.371*	*0.999*
ABCG8	rs4148211	c.161A>G	missense	-51.1 ± 16.7 (49)	-44.6 ± 19.4 (49)	*0.079*	*0.619*
	rs80025980	c.239G>A	missense	-47.7 ± 18.4 (95)	-52.5 ± 15.3 (3)	*0.649*	*0.921*
	rs11887534	c.55G>C	missense	-48 ± 17.9 (84)	-47.1 ± 21.4 (14)	*0.887*	*0.975*
APOA4	rs12721041	c.37G>A	missense	-47.8 ± 18.3 (95)	-50.5 ± 24.1 (3)	*0.865*	*0.978*
APOB	rs6752026	c.433C>T	missense	-47.7 ± 18.6 (94)	-52.1 ± 7.5 (4)	*0.347*	*0.967*
	rs679899	c.1853C>T	missense	-46.1 ± 17.4 (38)	-49 ± 18.9 (60)	*0.442*	*0.985*
	rs1801699	c.5741A>G	missense	-48 ± 19 (90)	-46.4 ± 7.2 (8)	*0.635*	*0.971*
	rs12713675	c.7367C>A	missense	-48.1 ± 18 (94)	-42.6 ± 26.7 (4)	*0.711*	*0.956*
	rs12720855	c.9880T>C	missense	-48.1 ± 18 (94)	-42.6 ± 26.7 (4)	*0.711*	*0.904*
	rs676210	c.8216C>T	missense	-47.4 ± 18 (68)	-48.9 ± 19.2 (30)	*0.732*	*0.951*
	rs1367117	c.293C>T	missense	-47.3 ± 19.6 (45)	-48.3 ± 17.3 (53)	*0.789*	*0.947*
APOE	rs7412	c.526C>T	missense	-47.3 ± 18.3 (93)	-57.9 ± 17.9 (5)	*0.260*	*0.882*
	rs429358	c.388T>C	missense	47.1 ± 18.8 (77)	50.7 ± 16.3 (21)	*0.398*	*0.887*
CETP	rs5880	c.988G>C	missense	-47.1 ± 18.6 (87)	-54 ± 14.6 (11)	*0.174*	*0.755*
CLMN	rs61750771	c.2698A>T	missense	-47.9 ± 18.6 (94)	-47.2 ± 10.5 (4)	*0.905*	*0.954*
COQ10A	rs60542959	c.3G>T	missense	-48.2 ± 18.3 (93)	-41.8 ± 20.1 (5)	*0.519*	*0.942*
KIF6	rs20455	c.2155T>C	missense	-54.8 ± 16.5 (33)	-44.4 ± 18.3 (65)	***0.006***	***0.073***
LDLR	rs879254913	c.959T>C	missense	-48.2 ± 18.4 (95)	-38.6 ± 11.1 (3)	*0.271*	*0.880*
LDLRAP1	rs41291058	c.712C>T	missense	-47.6 ± 18.3 (94)	-53.3 ± 18.8 (4)	*0.591*	*0.960*
LPA	rs76062330	c.5468G>T	missense	-46.9 ± 18.5 (92)	-62.2 ± 4.7 (6)	***<0.001***	***0.001***
	rs139145675	c.5311C>T	missense	-47.4 ± 18.6 (93)	-55.7 ± 8.6 (5)	*0.101*	*0.603*
	rs3124784	c.6046C>T	missense	-45.2 ± 19.1 (49)	-50.5 ± 17.3 (49)	*0.155*	*0.758*
	rs41272110	c.4195A>C	missense	-48.9 ± 18.2 (78)	-43.8 ± 18.8 (20)	*0.287*	*0.861*
	rs41259144	c.2969G>A	missense	-47.8 ± 18.4 (95)	-50.6 ± 20.2 (3)	*0.832*	*0.983*
	rs41272114	g.161006077C>T	splice-site	-47.9 ± 18.5 (93)	-47.2 ± 16.2 (5)	*0.929*	*0.966*
LPL	rs1801177	c.106G>A	missense	-47.2 ± 18.2 (94)	-63.7 ± 13 (4)	*0.080*	*0.492*
	rs328	c.1421C>G	Stop-gain	-48.3 ± 18.3 (84)	-45.2 ± 18.7 (14)	*0.566*	*0.940*
SREBF2	rs2229440	c.1867G>A	missense	-47.5 ± 18.5 (93)	-55.1 ± 13.1 (5)	*0.272*	*0.848*

Number of patients in round brackets. Data are shown as mean ± SD and compared by *t*-test. FH: familial hypercholesterolemia; LDL-c: low-density lipoprotein cholesterol; NT: nucleotide; PD: pharmacodynamics.

Univariate linear regression analysis for LDL-c reduction after treatment with statins showed that *KIF6* rs20455 (c.2155T>C) reduced LDL-c change when considering the treatment with all statins and atorvastatin isolated, whereas *LPA* rs76062330 (c.5468G>T) was associated with higher LDL-c reduction after treatment with atorvastatin. However, these associations were not significant after multiple testing adjustment (adjusted p>0.05) (**Supplementary table 15**).

Results of multivariate linear regression analysis, after adjusting for covariates, showed that deleterious variants in PD-related genes did no influence the response to all statins (**Table 15**). Interestingly, *KIF6* c.2155T>C was associated with lower LDL-c reduction after atorvastatin treatment (p=0.014) (**Table 16**).

In univariate logistic regression analysis, only *LPA* rs3124784 (c.6046C>T) was associated with higher likelihood of being responder to all statins and to atorvastatin (**Supplementary table 16**), but this result was not maintained after multiple testing adjustment (adjusted p>0.05). Multivariate logistic regression analysis with adjustment for non-genetic covariates confirmed the association of *LPA* c.6046C>T with higher likelihood of being responder to all statins (p=0.022) (**Table 17**). Similar result was observed when considering only atorvastatin use (**Table 18**).

Table 15 Influence of deleterious variants (MAF > 1.0%) in PD-related genes on all statins response of FH patients. Multivariate linear regression analysis.

Gene	rs code	NT change	Allele	n	β	SE	p-*value*
All statins							
ABCA1	rs769705621	g.107556792_107556793 insA	A allele	86	1.9	4.8	*0.689*
	rs769705621	g.107556792_107556793 insAAAA	AA allele	104	-0.8	3.2	*0.805*
	rs769705621	g.107556792_107556793 insAAA	AAA allele	107	0.1	3.3	*0.964*
	rs769705621	g.107556792_107556793 insAA	AAAA allele	89	-0.6	5.2	*0.915*
	rs769705621	g.107556792_107556793 insAAAAA	AAAAA allele	114	2.9	8.3	*0.724*
ABCG4	rs12271907	c.1035C>G	G allele	92	2.7	7.4	*0.713*
ABCG5	rs6756629	c.148C>T	A allele	114	-1.1	4.7	*0.813*
ABCG8	rs4148211	c.161A>G	G allele	114	-0.2	3.1	*0.959*
	rs80025980	c.239G>A	A allele	114	0.3	9.5	*0.974*
APOB	rs12713675	c.7367C>A	T allele	114	8.4	7.3	*0.253*
	rs12720855	c.9880T>C	G allele	114	8.4	7.3	*0.253*
	rs1801699	c.5741A>G	C allele	114	-1.3	5.6	*0.822*
	rs533617	c.5768A>G	C allele	114	-2.3	9.3	*0.806*
	rs6752026	c.433C>T	A allele	114	0.1	7.3	*0.992*
	rs676210	c.8216C>T	A allele	114	2.6	3.2	*0.418*
APOE	rs7412	c.526C>T	T allele	114	-5.9	6.7	*0.382*
	rs429358	c.388T>C	C allele	114	-3.7	3.6	*0.312*
CETP	rs5880	c.988G>C	C allele	114	-6.4	4.9	*0.193*
CLMN	rs61750771	c.2698A>T	A allele	114	-2.3	9.3	*0.808*
COQ10A	rs60542959	c.3G>T	T allele	114	-3.5	7.4	*0.641*
KIF6	rs20455	c.2155T>C	G allele	114	6.2	3.2	*0.059*
LDLR	rs121908031	c.2043C>A	A allele	114	5.1	8.2	*0.531*
	rs879254913	c.1463T>C	C allele	114	1.2	9.3	*0.895*
LDLRAP1	rs41291058	c.712C>T	T allele	114	-7.7	7.3	*0.291*
LPA	rs139145675	c.5311C>T	A allele	114	-3.5	7.3	*0.629*
	rs3124784	c.6046C>T	A allele	114	-5.6	3.1	*0.073*
	rs41259144	c.2969G>A	T allele	114	12.7	8.0	*0.118*
	rs41267807	c.6068A>G	C allele	114	8.2	9.4	*0.383*
	rs41272114	g.161006077C>T	T allele	114	3	6.7	*0.649*
	rs76062330	c.5468G>T	A allele	114	-5.1	6.2	*0.414*
LPL	rs328	c.1421C>G	G allele	114	8	4.2	*0.056*
SREBF2	rs2229440	c.1867G>A	A allele	114	-6.5	6.2	*0.294*

Each model was adjusted with the following covariates: body mass index, baseline LDL-c, therapy intensity, and presence of SRAE. n: Number of patients. β: linear coefficient; SE: standard error; FH: familial hypercholesterolemia; NT: nucleotide; PD: pharmacodynamics.

Table 16 Influence of genetic variants (MAF > 1.0%) in PD-related genes on atorvastatin response of FH patients. Multivariate linear regression analysis.

Gene	rs code	NT change	Allele	n	β	SE	p-*value*
Atorvastatin							
ABCA1	rs769705621	g.107556792_107556793 insA	A allele	86	2.8	4.9	*0.566*
	rs769705621	g.107556792_107556793 insAAAA	AA allele	104	1.6	3.4	*0.644*
	rs769705621	g.107556792_107556793 insAAA	AAA allele	107	1.6	3.5	*0.651*
	rs769705621	g.107556792_107556793 insAA	AAAA allele	89	1.3	5.3	*0.811*
	rs769705621	g.107556792_107556793 insAAAAA	AAAAA allele	114	-0.6	9.5	*0.952*
ABCG4	rs12271907	c.1035C>G	G allele	92	4.5	8.9	*0.617*
ABCG5	rs6756629	c.148C>T	A allele	114	2	4.7	*0.667*
ABCG8	rs4148211	c.161A>G	G allele	114	0.7	3.3	*0.844*
	rs80025980	c.239G>A	A allele	114	-1.3	9.4	*0.893*
APOB	rs12713675	c.7367C>A	T allele	114	3	8	*0.705*
	rs12720855	c.9880T>C	G allele	114	3	8	*0.705*
	rs1801699	c.5741A>G	C allele	114	-4.3	6.3	*0.495*
	rs6752026	c.433C>T	A allele	114	3.3	8	*0.608*
	rs676210	c.8216C>T	A allele	114	0.8	3.5	*0.815*
APOE	rs7412	c.526C>T	T allele	114	-4.5	7.2	*0.540*
	rs429358	c.388T>C	C allele	114	-5.1	3.9	*0.192*
CETP	rs5880	c.988G>C	C allele	114	-8.1	5.2	*0.124*
CLMN	rs61750771	c.2698A>T	A allele	114	-2.4	9.1	*0.792*
COQ10A	rs60542959	c.3G>T	T allele	114	2.5	7.3	*0.728*
KIF6	rs20455	c.2155T>C	G allele	114	8.4	3.4	***0.014***
LDLR	rs879254913	c.1463T>C	C allele	114	9.1	9.1	*0.319*
LDLRAP1	rs41291058	c.712C>T	T allele	114	-9.5	8	*0.236*
LPA	rs139145675	c.5311C>T	A allele	114	-4.3	7.2	*0.552*
	rs3124784	c.6046C>T	A allele	114	-4.8	3.3	*0.147*
	rs41259144	c.2969G>A	T allele	114	-2.2	9.2	*0.811*
	rs41272114	g.161006077C>T	T allele	114	0.1	7.3	*0.985*
	rs76062330	c.5468G>T	A allele	114	-11	6.5	*0.096*
LPL	rs328	c.1421C>G	G allele	114	5.8	4.6	*0.207*
SREBF2	rs2229440	c.1867G>A	A allele	114	-8.4	7.2	*0.243*

Each model was adjusted with the following covariates: body mass index, baseline LDL-c, therapy intensity, and presence of SRAE. n: Number of patients. β: linear coefficient; SE: standard error; FH: familial hypercholesterolemia; NT: nucleotide; PD: pharmacodynamics.

Table 17 Association of deleterious variants (MAF > 1.0%) in PD-related genes with all statin response of FH patients. Multivariate logistic regression analysis.

Gene	rs code	NT change	Allele	RE	NRE	OR (95% CI)	*p-value*
All statins							
ABCA1	rs769705621	g.107556793insA	A allele	20.0 (8)	17.4 (8)	1.56 (0.42 - 6.23)	*0.507*
	rs769705621	g.107556793insAA	AA allele	34.5 (19)	42.9 (21)	1.81 (0.67 - 5.09)	*0.245*
	rs769705621	g.107556793insAAA	AAA allele	35.2 (19)	45.3 (24)	1.62 (0.63 - 4.24)	*0.319*
	rs769705621	g.107556793insAAAA	AAAA allele	8.7 (4)	14 (6)	3.52 (0.47 - 42.9)	*0.260*
ABCG4	rs12271907	c.1035C>G	G allele	4.4 (2)	8.5 (4)	1.84 (0.27 - 17.66)	*0.548*
ABCG5	rs6756629	c.148C>T	T allele	10.3 (6)	14.3 (8)	1.69 (0.42 - 7.37)	*0.464*
ABCG8	rs4148211	c.161A>G	G allele	44.8 (26)	60.7 (34)	1.23 (0.5 - 2.99)	*0.649*
	rs80025980	c.239G>A	A allele	1.7 (1)	3.6 (2)	3.95 (0.18 - 156.41)	*0.410*
APOB	rs12713675	c.7367C>A	A allele	3.4 (2)	5.4 (3)	1.62 (0.17 - 18.89)	*0.675*
	rs12720855	c.9880T>C	C allele	3.4 (2)	5.4 (3)	1.62 (0.17 - 18.89)	*0.675*
	rs1801699	c.5741A>G	G allele	5.2 (3)	12.5 (7)	1.63 (0.35 - 9.43)	*0.551*
	rs533617	c.5768A>G	G allele	1.7 (1)	3.6 (2)	1.92 (0.16 - 45.01)	*0.612*
	rs6752026	c.433C>T	T allele	5.2 (3)	3.6 (2)	0.84 (0.09 - 6.54)	*0.871*
	rs676210	c.8216C>T	T allele	31 (18)	37.5 (21)	2.39 (0.89 - 6.9)	*0.093*
APOE	rs7412	c.526C>T	T allele	8.6 (5)	1.8 (1)	0.25 (0.01 - 2.14)	*0.253*
	rs429358	c.388T>C	C allele	20.7 (12)	23.2 (13)	1.2 (0.5 - 2.8)	*0.745*
CETP	rs5880	c.988G>C	C allele	13.8 (8)	8.9 (5)	0.43 (0.1 - 1.84)	*0.258*
CLMN	rs61750771	c.2698A>T	T allele	3.4 (2)	3.6 (2)	0.65 (0.03 - 10.36)	*0.756*
COQ10A	rs60542959	c.3G>T	T allele	5.2 (3)	3.6 (2)	0.33 (0.04 - 2.36)	*0.272*
KIF6	rs20455	c.2155T>C	C allele	58.6 (34)	73.2 (41)	1.59 (0.6 - 4.26)	*0.349*
LDLR	rs121908031	c.2043C>A	A allele	3.4 (2)	3.6 (2)	3.7 (0.25 - 80.77)	*0.363*
	rs879254913	c.1463T>C	C allele	1.7 (1)	3.6 (2)	1.9 (0.16 - 43.93)	*0.617*
LDLRAP1	rs41291058	c.712C>T	T allele	5.2 (3)	3.6 (2)	0.27 (0.03 - 1.97)	*0.201*
LPA	rs139145675	c.5311C>T	T allele	6.9 (4)	1.8 (1)	0.5 (0.02 - 4.55)	*0.575*
	rs3124784	c.6046C>T	T allele	56.9 (33)	37.5 (21)	0.33 (0.12 - 0.84)	***0.022***
	rs41259144	c.2969G>A	A allele	1.7 (1)	5.4 (3)	2.93 (0.3 - 66.9)	*0.393*
	rs41272114	g.161006077C>T	T allele	3.4 (2)	7.1 (4)	4.44 (0.57 - 58.27)	*0.192*
	rs76062330	c.5468G>T	T allele	10.3 (6)	1.8 (1)	0.13 (0.01 - 1.01)	*0.087*
LPL	rs328	c.1421C>G	G allele	13.8 (8)	16.1 (9)	1.92 (0.55 - 7.45)	*0.319*
SREBF2	rs2229440	c.1867G>A	A allele	6.9 (4)	5.4 (3)	0.53 (0.08 - 3.37)	*0.500*

Each model was adjusted with the following covariates: body mass index, baseline LDL-c, therapy intensity, and presence of SRAE. n: Number of patients. OR: odds ratio; CI: confidence interval; FH: familial hypercholesterolemia; NT: nucleotide; PD: pharmacodynamics; RE: responder; NRE: non-responder.

Table 18 Association of variants (MAF > 1.0%) in PD-related genes with atorvastatin response of FH patients. Multivariate logistic regression.

Gene	rs code	NT change	Allele	RE	NRE	OR (95% CI)	p-*value*
Atorvastatin							
ABCA1	rs769705621	g.107556793insA	A allele	22.2 (8)	22.9 (8)	1.61 (0.41 - 7.09)	*0.502*
	rs769705621	g.107556793insAA	AA allele	36 (18)	47.4 (18)	1.7 (0.56 - 5.24)	*0.348*
	rs769705621	g.107556793insAAA	AAA allele	38.8 (19)	47.6 (20)	1.59 (0.57 - 4.56)	*0.379*
	rs769705621	g.107556793insAAAA	AAAA allele	11.9 (5)	12.5 (4)	3.43 (0.28 - 113.8)	*0.390*
	rs769705621	g.107556793insAAAAA	AAAAA allele	1.9 (1)	4.4 (2)	NR	-
ABCG4	rs12271907	c.1035C>G	G allele	2.4 (1)	8.1 (3)	6.25 (0.53 - 241.38)	*0.208*
ABCG5	rs6756629	c.148C>T	T allele	11.3 (6)	17.8 (8)	1.62 (0.36 - 7.97)	*0.532*
ABCG8	rs4148211	c.161A>G	G allele	39.6 (21)	62.2 (28)	1.21 (0.45 - 3.23)	*0.709*
	rs80025980	c.239G>A	A allele	3.8 (2)	2.2 (1)	3.2 (0.11 - 132.62)	*0.496*
APOB	rs12713675	c.7367C>A	A allele	3.8 (2)	4.4 (2)	1.11 (0.05 - 18.91)	*0.939*
	rs12720855	c.9880T>C	C allele	3.8 (2)	4.4 (2)	1.11 (0.05 - 18.91)	*0.939*
	rs1801699	c.5741A>G	G allele	3.8 (2)	13.3 (6)	1.68 (0.31 - 13.11)	*0.569*
	rs6752026	c.433C>T	T allele	3.8 (2)	4.4 (2)	1.94 (0.2 - 19.64)	*0.548*
	rs676210	c.8216C>T	T allele	28.3 (15)	33.3 (15)	2.34 (0.79 - 7.51)	*0.135*
APOE	rs7412	c.526C>T	T allele	7.5 (4)	2.2 (1)	0.55 (0.02 - 5.96)	*0.637*
	rs429358	c.388T>C	C allele	20.8 (11)	22.2 (10)	0.98 (0.3 - 3.2)	*0.977*
CETP	rs5880	c.988G>C	C allele	15.1 (8)	6.7 (3)	0.3 (0.05 - 1.48)	*0.151*
CLMN	rs61750771	c.2698A>T	T allele	3.8 (2)	4.4 (2)	0.68 (0.03 - 12.72)	*0.783*
COQ10A	rs60542959	c.3G>T	T allele	5.7 (3)	4.4 (2)	0.33 (0.04 - 2.35)	*0.271*
KIF6	rs20455	c.2155T>C	C allele	58.5 (31)	75.6 (34)	1.79 (0.62 - 5.37)	*0.283*
LDLR	rs879254913	c.1463T>C	C allele	1.9 (1)	4.4 (2)	1.82 (0.16 - 41.74)	*0.638*
LDLRAP1	rs41291058	c.712C>T	T allele	5.7 (3)	2.2 (1)	0.16 (0.01 - 1.51)	*0.144*
LPA	rs139145675	c.5311C>T	T allele	7.5 (4)	2.2 (1)	0.55 (0.02 - 5.48)	*0.630*
	rs3124784	c.6046C>T	T allele	62.3 (33)	35.6 (16)	0.26 (0.09 - 0.73)	***0.012***
	rs41259144	c.2969G>A	A allele	1.9 (1)	4.4 (2)	2.27 (0.18 - 55.4)	*0.535*
	rs41272114	g.161006077C>T	T allele	3.8 (2)	6.7 (3)	3.25 (0.31 - 85.24)	*0.373*
LPL	rs328	c.1421C>G	G allele	13.2 (7)	15.6 (7)	2.51 (0.59 - 13.45)	*0.235*
SREBF2	rs2229440	c.1867G>A	A allele	5.7 (3)	4.4 (2)	0.63 (0.06 - 6.26)	*0.691*

Each model was adjusted with the following covariates: body mass index, baseline LDL-c, therapy intensity, and presence of SRAE. n: Number of patients. OR: odds ratio; CI: confidence interval; FH: familial hypercholesterolemia; NT: nucleotide; PD: pharmacodynamics; RE: responder; NRE: non-responder; NR: not reported.

4.4.3.2 Statin-related adverse events

Univariate logistic regression analysis of deleterious variants in PD-related genes and SRAE in FH patients showed that carriers of the variant *ABCA1* rs769705621 (g.107556793insA) have higher risk of SRAE (p=0.027), but this association was not maintained after corrections (p=0.648) (**Supplementary table 17**). No significant associations of variants in PD-related genes with SRAE were also found in the multivariate logistic regression analysis (**Table 19**).

Table 19 Association of deleterious variants (MAF > 1.0%) in PD-related genes with SRAE in FH patients. Multivariate logistic regression analysis.

Gene	rs code	NT change	Allele	No SRAE	SRAE	OR (95% CI)	p-*value*
ABCA1	rs769705621	g.107556793insA	A allele	14.3 (10)	40.0 (6)	3.45 (0.77 - 15.39)	*0.098*
	rs769705621	g.107556793insAA	AA allele	35.8 (29)	50.0 (11)	2.24 (0.73 - 7.21)	*0.162*
	rs769705621	g.107556793insAAA	AAA allele	42.4 (36)	33.3 (7)	0.6 (0.19 - 1.72)	*0.353*
	rs769705621	g.107556793insAAAA	AAAA allele	8.8 (6)	20 (4)	2.8 (0.55 - 13.5)	*0.198*
ABCG4	rs12271907	c.1035C>G	G allele	5.2 (4)	14.3 (2)	5.92 (0.7 - 40.07)	*0.072*
ABCG5	rs6756629	c.148C>T	T allele	11.2 (10)	16.7 (4)	1.34 (0.29 - 5.2)	*0.685*
ABCG8	rs4148211	c.161A>G	G allele	56.2 (50)	37.5 (9)	0.44 (0.15 - 1.2)	*0.115*
	rs80025980	c.239G>A	A allele	1.1 (1)	8.3 (2)	12.5 (0.93 - 306.72)	*0.060*
APOB	rs12713675	c.7367C>A	A allele	4.5 (4)	4.2 (1)	2.07 (0.1 - 16.69)	*0.540*
	rs12720855	c.9880T>C	C allele	4.5 (4)	4.2 (1)	2.07 (0.1 - 16.69)	*0.540*
	rs1801699	c.5741A>G	G allele	10.1 (9)	4.2 (1)	0.56 (0.03 - 3.62)	*0.608*
	rs6752026	c.433C>T	T allele	4.5 (4)	4.2 (1)	1.12 (0.05 - 8.98)	*0.923*
	rs676210	c.8216C>T	T allele	31.5 (28)	41.7 (10)	1.24 (0.43 - 3.46)	*0.681*
APOE	rs7412	c.526C>T	T allele	4.5 (4)	8.3 (2)	1.87 (0.19 - 13.01)	*0.545*
	rs429358	c.388T>C	C allele	24.7 (22)	12.5 (3)	0.28 (0.05 - 1.09)	*0.097*
CETP	rs5880	c.988G>C	C allele	12.4 (11)	8.3 (2)	0.57 (0.07 - 2.88)	*0.537*
CLMN	rs61750771	c.2698A>T	T allele	3.4 (3)	4.2 (1)	1.24 (0.05 - 11.91)	*0.865*
KIF6	rs20455	c.2155T>C	C allele	69.7 (62)	54.2 (13)	0.42 (0.14 - 1.21)	*0.108*
LDLR	rs121908031	c.2043C>A	A allele	2.2 (2)	8.3 (2)	1.57 (0.14 - 16.87)	*0.699*
LPA	rs139145675	c.5311C>T	T allele	3.4 (3)	8.3 (2)	4.69 (0.53 - 34.51)	*0.129*
	rs3124784	c.6046C>T	T allele	49.4 (44)	37.5 (9)	0.43 (0.14 - 1.21)	*0.123*
	rs41272114	g.161006077C>T	T allele	4.5 (4)	8.3 (2)	2.53 (0.27 - 17.03)	*0.359*
	rs76062330	c.5468G>T	T allele	5.6 (5)	8.3 (2)	1.63 (0.19 - 9.67)	*0.612*
LPL	rs328	c.1421C>G	G allele	13.5 (12)	20.8 (5)	3.55 (0.86 - 14.18)	*0.071*
SREBF2	rs2229440	c.1867G>A	A allele	6.7 (6)	4.2 (1)	1.28 (0.06 - 9.01)	*0.832*

Each model was adjusted for the following covariates: baseline LDL-c, presence of FH-related variant, and adherence to statin. Number of patients in round brackets. OR: odds ratio; CI: confidence interval; FH: familial hypercholesterolemia; PD: pharmacodynamics; SRAE: statin-related adverse events.

5 DISCUSSION

In this study, very high percentage (49.2%) of FH patients did not achieve the therapy target of LDL-c reduction ≥ 50%. This result is in line with previous studies, in which the percentage of FH individuals who did not achieve the same treatment goal ranged from 48% to 59% (DEGOMA et al., 2016; KORNEVA; KUZNETSOVA; JULIUS, 2019; PIJLMAN et al., 2010).

Only 30.7% of the FH patients carried a pathogenic or likely pathogenic variant in FH-related genes. The association of pathogenic variants in *LDLR, APOB, PCSK9* and *LDLRAP1* with FH in this cohort has been previously discussed elsewhere (BORGES, 2019). However, the molecular diagnosis was performed only with variants considered pathogenic with ACMG criteria, which possibly excluded pathogenic variants that were not previously associated with FH. Still, most patients (68.4%) had a defined or probable clinical diagnosis of FH with DCLN modified criteria (IZAR et al., 2021). No difference was observed in FH diagnosis between RE and NRE groups, showing a balanced sample of FH patients. Importantly, the presence of FH-related variants did not show to influence statin response.

When comparing the characteristics between RE and NRE group, NRE patients showed lower baseline total cholesterol and LDL-c levels and higher frequency alcohol consumption. Similar results were reported in the PROSPER study, that included elderly patients with CVD or with high risk of CVD (TROMPET et al., 2016). In this study, non-responders to pravastatin drank alcohol and smoked tobacco more often, were less likely to have hypertension, and had lower LDL-c levels. This, in turn, could indicate that these patients were more aware of their health and less aware of the disease status, which consequently increased the risk of non-adherence to therapy in non-responders (TROMPET et al., 2016).

Lack of adherence is an important barrier for the effective treatment of FH patients. A study has reported an adherence of 89% by FH patients (GALEMA-BOERS et al., 2014). However, a recent study showed worrying data: only 57% of FH patients with a definite diagnosis were fully adherent to therapy, while 16% were partially adherent and 27% were not adherent (KORNEVA; KUZNETSOVA; JULIUS, 2019).

Although the NRE group showed indicators of lack of adherence (higher alcohol consumption and lower baseline cholesterol level), NRE group probably had comparable adherence to RE group. First, statin or ezetimibe adherence were similar between RE and NRE groups. Although there is a possibility of lack of information about patient adherence from the medical charts, we considered “reduced adherence” as any event of lack of adherence in the

whole history of the medical chart – not only in the visit considered for data collection – to mitigate a possible lack of information about adherence. Second, FH patients tend to be aware of their disease and the possible outcomes resulting from it – more than 76% of FH patients in Japan and the US acknowledged their disease status (BUCHOLZ et al., 2018; TADA et al., 2020), which is an enabler for treatment adherence (KINNEAR et al., 2019). Hence, lack of adherence was not the main factor for classifying patients as NRE.

Many elements indicate that a contributing factor for reduced response in our cohort could be related to the lipid-lowering treatment prescribed to FH patients. Although treatment response was independent of the type of statin used, the addition of ezetimibe and possibly the use of a high intensity treatment showed to be associated with statin response. These results indicate that NRE patients are probably more undertreated compared to RE patients, which might reflect a reality of the Brazilian health system. Although simvastatin and atorvastatin, two extensively used statins, are provided for free by SUS, rosuvastatin and ezetimibe must be bought by the patient (DO NASCIMENTO et al., 2018).

Rosuvastatin 40 mg is currently the strongest statin dosage available. A meta-analysis showed rosuvastatin 40 mg led to a mean LDL-c reduction of -55%; atorvastatin 80 mg, in contrast, led to a mean LDL-c reduction of approximately -45% (KARLSON et al., 2016a). Ezetimibe 10 mg incremental reduction in LDL-c, when associated with a high-intensity statin treatment, was reported to be -14% in another meta-analysis (LEE et al., 2021). Despite these numbers, many patients, especially those treated by the public health system, do not have access to these medications because of their cost, which therefore would contribute to reducing the intensity of the treatment received (DAGLI-HERNANDEZ et al., 2021). Nevertheless, both the use of rosuvastatin 40 mg and/or the addition of ezetimibe 10 mg to statin treatment would be highly beneficial for FH patients, especially those who are unresponsive to treatment and at very high cardiovascular risk.

When considering the absolute LDL-c target level proposed by the Brazilian FH guideline (IZAR et al., 2021), ensuring the best treatment for FH patients is even more crucial. In our sample, the majority of patients, including all of the patients with very high risk of CAD, did not reach optimum LDL-c levels.

Most of FH patients have not achieved these targets in previous studies as well. In the SAFEHEART study, only 4.7% of patients achieved an on-treatment LDL-c < 70 mg/dL after 5 years on high-intensity statin treatment (PEREZ DE ISLA et al., 2016). In the CASCADE study, only 24% and 17% of primary and secondary prevention FH patients, respectively,

achieved the absolute LDL-c goals of <100 mg/dL or <70 mg/dL (DUELL et al., 2019). Therefore, it is important to increase the accessibility to the best lipid-lowering treatment to FH patients by providing ezetimibe 10 mg and rosuvastatin 40 mg through SUS to FH patients.

Besides having lower LDL-c reduction, NRE group also showed lower baseline total cholesterol and LDL-c levels, and higher on-treatment total cholesterol and LDL-c. Furthermore, their mean on-treatment LDL-c levels were higher than the least rigid absolute therapy target of LDL-c <100 mg/dL. These results are in line with previous studies, in which lower baseline LDL-c was a strong predictor of reduced therapy response (KARLSON et al., 2016b; MASSON et al., 2014). A recent study in Japanese patients with *de novo* AMI also observed that hypo-responders (patients with LDL-c reduction ≤ 15%) had a higher baseline LDL-c and this low response was a predictor of heart failure (TSUDA et al., 2020). Thus, it is essential to consider that lower baseline LDL-c does not necessarily mean lower risk or better lipid-lowering response in FH patients.

Despite treatment-related factors previously discussed, most patients in NRE group still received a high intensity treatment. Therefore, their insufficient response could also be due to the presence of pharmacogenetic variants.

In PK-related genes, most associations with statin response described in previous studies were observed with *ABCB1, SLCO1B1, CYP3A4, CYP2C9* and *CYP3A5* variants. For example, *ABCB1* rs2032582 (c.2677T>G/A) and *ABCB1* rs1045642 (c.3435C>T) were associated with better statin response in some studies (FIEGENBAUM et al., 2005a; HOENIG et al., 2011; REBECCHI et al., 2009; SU et al., 2015), as well as *SLCO1B1* rs2306283 (c.388A>G) (RODRIGUES et al., 2011), while *CYP3A5*3* showed to be associated with lower statin response (WILLRICH et al., 2008). However, there is still controversy about these associations and no variant shows strong evidence of influencing statin response.

In our study, *ABCC1* c.2012G>T showed to improve statin response in FH patients.

ABCC1 (or MRP1) is an ABC membrane transporter highly expressed in the thymus, skeletal muscle tissue, kidney, urinary bladder, and gastrointestinal tract according to the Human Protein Atlas (https://www.proteinatlas.org). It promotes the efflux of drugs, including statins and its metabolites, from hepatocytes to the bloodstream (DAGLI-HERNANDEZ et al., 2021). ABCC1 is a highly conserved protein (WANG et al., 2006), but several variants, deleterious or not, have been identified worldwide (ROCHA; PEREIRA; RODRIGUES, 2018).

Previous studies have reported the importance of *ABCC1* c.2012G>T in pharmacogenetics. It has been previously associated with febrile neutropenia in breast cancer

patients undergoing treatment with 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy (VULSTEKE et al., 2013). Also, an *in vitro* study demonstrated that HEK293 overexpressing *ABCC1* c.2012T allele (p.671Val) retained approximately 20% more doxorubicin compared to the reference protein, indicating that it might be related to doxorubicin-associated acute cardiac toxicity (JUNGSUWADEE et al., 2012).

When considering statin response, however, there are conflicting results on the influence of *ABCC1* c.2012G>T. In our study, this variant showed to higher percent LDL-c reduction after treatment with all statins and atorvastatin. In contrast, a study with Iranian hypercholesterolemic patients showed that carriers of c.2012T allele had lower percent reduction of LDL-c and total cholesterol compared to GG carriers when on atorvastatin 10 mg/d treatment (p=0.02), but no difference was observed in patients using atorvastatin 20 or 40 mg (p=0.81) (BEHDAD et al., 2017). Similarly, a previous work from our group showed no association between this variant and LDL-c reduction in Brazilian hypercholesterolemic patients, but *ABCC1* mRNA levels were reduced in mononuclear cells of patients treated with atorvastatin 10 mg/day compared to baseline levels (REBECCHI et al., 2009).

The prediction framework score used in this study showed that *ABCC1* c.2012G>T is potentially deleterious. We also observed a stronger interaction between ABCC1 Val671 and three statins by molecular docking. *ABCC1* rs45511401 (c.2012G>T) causes a change from glycine to valine in position 671 (p.Gly671Val) in the protein. Although both amino acids are nonpolar, an *in silico* characterization study showed this change shifts the free energy of ABCC1, turning it into potentially deleterious (VOHRA et al., 2018). This is possibly due to the special properties of the reference amino acid, glycine. Glycine has a hydrogen in its side chain, differently from other amino acids, that carry a carbon. This confers unique flexibility to glycine, and allows it to be in tight regions of proteins, which is not accessible to other amino acids (BETTS; RUSSELL, 2003). The change to valine, that does not contain these properties, can cause conformational changes in the protein, making this region more accessible to substrates (BETTS; RUSSELL, 2003). This stronger protein-ligand interaction possibly leads to a less efficient statin efflux from hepatocytes by retaining the statins bound in position 671 in ABCC1. Since ABCC1 acts in statin efflux from the liver, a possible mechanism through which p.Gly671Val increased statin response would be that the lower function of this protein led to an increased intracellular statin concentration in hepatocytes. This, in turn, could enhance the inhibition of HMGCR and therefore potentialize the cholesterol-lowering effect.

A similar mechanism was proposed in a case report on a pharmacogenetic analysis of a female FH patient with late rosuvastatin response previously published (DAGLI-HERNANDEZ et al., 2020). The patient underwent a 6-week rosuvastatin wash-out period, after which rosuvastatin 20 mg was reintroduced. However, after 6 weeks of treatment, her lipid profile did not show any changes from baseline, which could only be observed after 12 weeks of rosuvastatin treatment. The patient was a carrier of the deleterious variants *SLCO1B1*15*, *SLCO1B3* rs4149117 and rs7311358, *ABCB11* rs2287622, and LOF variant *CYP3A5*3*. Possibly, the effect of the deleterious variants in the influx proteins SLCO1B1 and SLCO1B3 led to a slow internalization of rosuvastatin by hepatocytes, which led to a lower response in the first 6 weeks. However, the patient still responded to rosuvastatin treatment after 12 weeks. This could be due to an accumulation of rosuvastatin in hepatocytes resulting from the effect of the deleterious variant in the efflux protein ABCB11.

It is noteworthy that the previous studies were performed with lower doses of atorvastatin (10 mg to 40 mg) (BEHDAD et al., 2017; REBECCHI et al., 2009), while the majority of patients in our cohort were on high atorvastatin doses (atorvastatin 40 or 80 mg). It is possible that clearer effects of *ABCC1* c.2012G>T are observed in higher statin doses. However, *in vitro* and *in vivo* studies with larger samples are necessary to clarify these disparities.

Interestingly, the influence of *ABCC1* c.2012G>T on the percent LDL-c change was significant in the multiple regression linear analysis when considering deleterious variants in PK genes with MAF>10%. This result possibly shows that the influence of pharmacogenetic variants is not isolated but depends on the burden of deleterious variants carried by each patient. Therefore, similarly to the discussion presented in our case report (DAGLI-HERNANDEZ et al., 2020), the effect of each variant on statin response could be potentialized or annulated by the interaction with other variants, consequently leading to the phenotype observed. Since we had a limited number of patients, we could not analyze the effect of variants with lower MAF; however, this approach could be used for future pharmacogenetic studies with higher sample sizes in order to understand how these variants interact with each other.

The multivariate linear regression analysis also showed that *SLCO1B3* c.767G>C significantly enhanced statin response.

SLCO1B3 encodes the OATP1B3 influx transporter. It is present in the basal membrane of hepatocytes and has several drug substrates, including atorvastatin, fluvastatin, rosuvastatin and pitavastatin (MAEDA, 2015). Although OATP1B1 (encoded by *SLCO1B1*) has a major

contribution in statin uptake, OATP1B3 also plays an important role. In an *in vivo* study with OATP1B3 knockout mice, knocking in of *SLCO1B3* decreased atorvastatin and simvastatin plasma concentrations by 33% and 27%, respectively, due to their uptake by OATP1B3 (HIGGINS et al., 2014).

SLCO1B3 c.767G>C (p.Gly228Ala) causes a change from Glycine to Alanine in position 228, which is inside OATP1B3 channel in the transmembrane domain of the transporter. Similar to *ABCC1* c.2012G>T, the substitution of the flexible amino acid Glycine to Alanine possibly caused conformational changes to OATP1B3 protein structure. Moreover, Alanine is a hydrophobic amino acid whose side chain is relatively inert (BETTS; RUSSELL, 2003). Therefore, the substitution from Glycine to Alanine in the transmembrane domain could possibly affect any interaction between OATP1B3 p.228 Glycine and its substrates in this position.

To the best of our knowledge, this variant has not been approached in any pharmacokinetic study on statins. However, it was recently shown to increase telmisartan area under curve (AUC) by 22% per allele copy in healthy Finnish volunteers (HIRVENSALO et al., 2020). This evidence shows that this variant is possibly of low function. Moreover, similarly to the discussed in our case report, it is possible that *SLCO1B3* c.767G>C and *ABCC1* c.2012G>T only have significant effects on statin response when both are present and modulate the uptake and efflux of statins.

Other common variants on PK genes did not show to influence statin response in this study. In fact, the remaining variants did not consistently show to impact statin response in previous studies. *CYP3A5*3*, for example, has shown to decrease total cholesterol, LDL-c and HDL-c reduction after atorvastatin treatment in Brazilian hypercholesterolemic patients (WILLRICH et al., 2008), but no differences were observed in another study with Chilean hypercholesterolemic patients (ROSALES et al., 2012). Other variants, such as *SLCO1B1*5*, have shown to increase statin blood levels in previous studies, but did not show to impact statin response, which therefore is in agreement with the results in our study (DAGLI-HERNANDEZ et al., 2021).

In PD-related genes, most associations with statin response described in the literature were observed with genes associated to cholesterol metabolism. Many studies showed associations between statin response and *LDLR, PCSK9, APOB, APOE* variants and other genes (DAGLI-HERNANDEZ et al., 2021). In our study, we observed an association between *KIF6* rs20455 c.2155T>C and lower atorvastatin response.

Kinesin Family Member 6 (KIF6) belongs to the superfamily of kinesins and is involved in the microtubular-dependent intracellular transport of protein complexes, organelles and mRNA (MIKI et al., 2001). KIF6 contains two equal dimers, whose N-terminal domain is responsible for interacting and moving along microtubules. Its C-terminal domain interacts directly or indirectly with the molecules being transported, also called "cargo" (LI et al., 2010).

The *KIF6* c.2155T>C variant is frequent in many populations and C allele has been reported to increase up to 50% the risk of coronary heart disease (CHD) (BARE et al., 2007; IAKOUBOVA et al., 2008a; PENG et al., 2012; RUIZ-RAMOS et al., 2015). It causes an amino acid substitution from tryptophan to arginine in position 719 (p.Trp719Arg), which is close to the domain that interacts with the cargo. Tryptophan is nonpolar and aromatic, while Arginine is polar and positively charged (BETTS; RUSSELL, 2003). This polarity change could cause not only conformational changes in KIF6, but also changes in KIF6-cargo interaction, consequently affecting the transportation of molecules by KIF6.

The mechanism through which KIF6 affects lipid levels or cardiovascular events in response to statins is still unknown. Studies have shown conflicting results concerning the impact of *KIF6* c.2155T>C in LDL-c reduction due to statin treatment. Similarly to our results, a recent study showed that CC genotype carriers had attenuated LDL-c and c-non-HDL-c reduction in atorvastatin, simvastatin and rosuvastatin users; additionally, rosuvastatin users carrying this variant showed an increase in HDL-c (RUIZ-IRUELA et al., 2018). These differences, however, were not observed in other studies (IAKOUBOVA et al., 2008b; LI et al., 2010, 2011).

Paradoxically, previous studies have shown a decrease in the risk of CHD in C-allele carriers using high-dose atorvastatin or pravastatin compared to non-carriers (IAKOUBOVA et al., 2008b; LI et al., 2010). It has been hypothesized that the observed benefit of this variant on cardiovascular outcomes occurs mostly through statin pleiotropic effects, particularly the early plaque-stabilizing effect, rather than LDL-c reduction (IAKOUBOVA et al., 2008b; RUIZ-IRUELA et al., 2018). Moreover, a meta-analysis showed that *KIF6* c.2155T>C changes the influence of LDL-c on the risk of CHD, increasing the vulnerability to the deleterious effects of LDL-c in carriers of this variant. LDL-c reduction could, therefore, reduce CHD risk to C-allele carriers than to TT-carriers (FERENCE et al., 2017).

In this study, a surrogate outcome of LDL-c reduction was used to understand the impact of variants in PD genes on statin efficacy, but not on CAD risk. Also, the studies previously cited were performed with hypercholesterolemic patients, but not FH patients. Therefore, *KIF6*

c.2155T>C possibly does have an impact on LDL-c reduction after atorvastatin treatment in FH patients, but its effect on the risk of CAD in FH patients still remains to be further studied in a prospective study.

LPA rs3124784 (c.6046C>T) and rs76062330 (c.5468G>T) were also associated with increased statin response in this study. *LPA* c.6046C>T increased the likelihood of being responder to all statins and atorvastatin, while *LPA* c.5468G>T influenced atorvastatin response by enhancing LDL-c reduction in T allele carriers.

LPA encodes the apolipoprotein(a) precursor. Apolipoprotein(a) is an apolipoprotein that is linked to apo B100 by disulfide bridge in lipoprotein(a) [Lp(a)], a type of plasma lipoprotein similar to LDL. *LPA* is responsible for >90% of the variance in circulating Lp(a) (ENAS et al., 2019). Lp(a) is synthetized mostly in the liver and binds to LDL receptors with lower affinity when compared to LDL (JANG et al., 2020). Lp(a) is also more susceptible to oxidation than LDL, which in turn facilitates its uptake by macrophages in the arterial wall. Consequently, high Lp(a) levels have been extensively associated with the risk of CAD (MARANHÃO et al., 2014).

Lp(a) levels tend to be constant in an individual's life, but have wide interindividual variability, ranging from <1 mg/dL to >1,000 mg/dL (MARANHÃO et al., 2014). Heterozygous FH patients have higher Lp(a) levels than the general population, which contributes to the higher risk of cardiovascular events (VUORIO et al., 2020). It is estimated that 8 to 20% of LDL-c quantified by Friedewald's formula is in Lp(a), depending on Lp(a) plasma concentration (LI; WILCKEN; DUDMAN, 1994). Statin treatment does not affect Lp(a) levels, but ezetimibe has shown to decrease it by 29% (NOZUE; MICHISHITA; MIZUGUCHI, 2010).

Variants in *LPA* have already been associated with statin response. A large meta-analysis of genome-wide association studies confirmed the association between *LPA* rs10455872 (g.161010118A>G), an intronic variant, and worse statin response, with each G allele attenuating LDL-c reduction by 5.9% (POSTMUS et al., 2014). Other studies have found the same result (CHASMAN et al., 2012; DESHMUKH et al., 2012). This variant is in high linkage disequilibrium with the *LPA* copy number variation kringle IV type 2 (KIV-2), which was shown to be responsible for 30% of Lp(a) level variation. Since Lp(a) levels are not affected by statins, it is possible that apparent non-responders to statins have high concentrations of LDL-c retained in Lp(a), which in turn are measured in Friedewald's formula (DESHMUKH et al., 2012).

To the best of our knowledge, this is the first study to show an association between *LPA* c.6046C>T and c.5468G>T and statin response. Both variants are missense and located in the peptidase S1 domain, which contains an inactive serine protease (BATEMAN et al., 2021; MARANHÃO et al., 2014). We did not find any studies with other *LPA* variants located in this domain. It is likely that these variants, are associated with low plasma Lp(a) levels, which would therefore contribute to the differences in LDL-c response to statins. Therefore, LDL-c reduction would account mostly for the reduction in LDL particles. This could be due to a reduction in apo(a) expression or to increased interaction with receptors that act on the uptake of Lp(a), such as megalin receptors (MARANHÃO et al., 2014). However, this hypothesis has to be confirmed by large populational studies and *in vitro* studies as well.

Other important PD-related variants have been detected in FH patients and were not associated with statin response in our study. For example, variants in *APOE,* such as rs429358 (c.388T>C, ε2) and rs7412 (c.526C>T , ε4), have been associated with better and worse lipid lowering, respectively, to atorvastatin, pravastatin and simvastatin in previous studies (GUAN et al., 2019). Studies with Brazilian hypercholesterolemic patients, however, did not find these associations (CERDA et al., 2011; FIEGENBAUM et al., 2005b; ISSA et al., 2012). Variants in *HMGCR, LDLR, PCSK9,* and *APOB* also did not show any associations with statin response.

The lack of association of variants in most of PD genes with statin response could be due the small sample size of this study, which did not allow the analysis of rare variants. Also FH patients already carry deleterious variants in PD genes or genes involved in cholesterol homeostasis, which already jeopardizes the LDL-c response to statins. Most of our FH patients possibly had polygenic FH, with deleterious variants in more than one gene involved in cholesterol metabolism.

A total of 21% of FH patients experienced SRAE. There is little information about SRAE in FH patients (PANG; CHAN; WATTS, 2020), but a study has reported a frequency of statin intolerance as high as 15% in FH patients (DEGOMA et al., 2016).

Considering that the patients studied used mostly high intensity treatment, a high frequency of SRAE reports is expected in this population, since high statin dosage is a risk factor for SRAE (NGUYEN et al., 2018). In fact, most patients that experienced SRAE were of the RE group. It is known that SRAE, including the most frequent event, SAMS, are drug concentration-dependent (KEE et al., 2020). Therefore, a plausible explanation for the higher frequency of SRAE in responders is that these patients probably have higher plasma levels of statin, which led to a higher risk of SRAE. Importantly, the use of a CYP3A4 inhibitor, such as

amiodarone, could be a predictor of SRAE in this cohort. In fact, a meta-analysis showed that drug-drug interactions with statins are a risk factor for myopathy and rhabdomyolysis (NGUYEN et al., 2018) The inhibition of CYP3A4 by amiodarone is a drug interaction that possibly impacted simvastatin and atorvastatin metabolism, potentially increasing statin levels and consequently the susceptibility to SRAE (BUCSA et al., 2015).

Another interesting observation is that most patients in SRAE group carried an FH-related variant and had xanthomas, a clinical manifestation of FH. Although we did not find any associations between SRAE and the statin used or the intensity of the treatment, one could hypothesize that patients with clinical manifestations of FH tend to follow a stricter statin regimen, which also could have raised the risk of SRAE. This in turn showed that SRAE affect therapy adherence negatively: SRAE group had episodes of reduced adherence to statins and ezetimibe more frequently than no SRAE group, which is in line to what has been observed by therapy adherence studies (WEI et al., 2013).

Other variables were described as risk factors for SAMS. A meta-analysis showed that female gender and age higher than 65 years old are risk factors for myopathy, which we did not observe in our study (NGUYEN et al., 2018). Clinical-related factors include having diabetes mellitus, renal and hepatic impairment, hypothyroidism, and cardiovascular disease. (NGUYEN et al., 2018; TOTH et al., 2018). Lifestyle factors, such as alcohol abuse and physical exercise, were also associated with myalgia risk (TOTH et al., 2018). However, we did not observe these associations in our study. Therefore, the SAMS observed in our study might rather be treatment-related than patient-related.

Importantly, we could not classify the type of myalgia experienced by the patients or evaluate if the SAMS was associated with CK elevations. This is because the FH patients that experienced SAMS usually interrupted their statin treatment on their own, before undergoing a laboratory testing and a medical visit. Therefore, it was only possible to collect the SAMS report from the medical recros, but we could not associate the events with any laboratory measures.

In this study, no deleterious PK- and PD-related variants were significantly associated with increased risk of SRAE. We have previously discussed the lack of association between SRAE and *SLCO1B1*5* and **15*, a well-described variant, in the Brazilian population in a recent review (DAGLI-HERNANDEZ et al., 2021). This is probably due to low sample sizes, which impaired the statistical power of the analysis in previous studies with Brazilian patients (DAGLI-HERNANDEZ et al., 2021). Although SRAE were very frequent in this study, the size of the SRAE group is still small, which therefore makes the association study difficult.

Therefore, it is necessary to increase the sample size in order to study the association between genetic variants and SRAE in FH patients.

Our study has some limitations. First, we have a low sample size, which impaired the association study of deleterious variants, especially those with lower frequency. Second, this is an observational, retrospective study, which is susceptible to some biases, such as information bias. However, we mitigate these biases by establishing a rigorous protocol of medical records review and data selection.

6 CONCLUSIONS

In this thesis, several deleterious variants in PK- and PD-related genes were detected by *in silico* analysis and some impacted statin response in Brazilian FH patients.

In PK-related genes, the deleterious variant *ABCC1* rs45511401 (c.2012G>T) was a major contributor on LDL-c response to statins, and enhanced LDL-c reduction after statin treatment. Molecular docking showed this variant causes stronger interaction between ABCC1 and the statin, impairing statin efflux. *SLCO1B3* rs60140950 *(*c.767G>C), a deleterious variant, also showed to enhance LDL-c reduction.

In PD-related genes, the neutral variant *KIF6* rs20455 *(*c.2155T>C), reduced atorvastatin response in FH patients, whereas The deleterious variants *LPA* rs3124784 (c.6046C>T) and rs76062330 (c.5468G>T) increased statin response.

PK- or PD-variants were not associated with increased risk of SRAE in Brazilian FH patients.

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8 SUPPLEMENTARY TABLES

Supplementary table 1 Panel of statin PK- and PD-related genes sequenced.

PD-related genes	***PK-related genes***
ABCA1	*ABCB1*
ABCG1	*ABCB11*
ABCG4	*ABCC1*
ABCG5	*ABCC2*
ABCG8	*ABCC3*
APOA1	*ABCG2*
APOA2	*CYP1A2*
APOA4	*CYP2C19*
APOA5	*CYP2C8*
APOB	*CYP2C9*
APOC1	*CYP2D6*
APOC2	*CYP3A4*
APOC3	*CYP3A5*
APOC4	*SLC15A1*
APOE	*SLC22A1*
CETP	*SLC22A6*
CLMN	*SLC22A8*
COQ10A	*SLCO1B1*
CYP7A1	*SLCO1B3*
HMGCR	*SLCO2B1*
KIF6	*UGT1A1*
LDLR	*UGT1A3*
LDLRAP1	*UGT2B7*
LIPA	
LIPC	
LPA	
LPL	
MYLIP	
PCSK9	
PON1	
SCAP	

Supplementary table 2 Clinical data of FH patients classified according to CAD risk.

			CAD risk[a]			
		Total	**Very high**	**High**	**Intermediate**	***p value***
		(n=114)	(n = 64)	(n = 11)	(n = 39)	
High risk factors						
Gender	Male	28.1 (32)	34.4% (22)	36.4% (4)	15.4% (6)	*0.093*
Medical history[b], %	AMI	29.2 (33)	51.6 (33)	0.0 (0)	0.0 (0)	***<0.001***
	CAD	40.0 (42)	70.0 (42)	0.0 (0)	0.0 (0)	***<0.001***
	CVE	6.0 (6)	10.3 (6)	0.0 (0)	0.0 (0)	*0.099*
	Angina	40.6 (41)	69.5 (41)	0.0 (0)	0.0 (0)	***<0.001***
	MR	30.9 (34)	54.8 (34)	0.0 (0)	0.0 (0)	***<0.001***
Tobacco smoking[b], %		14.3 (16)	19.4 (12)	27.3 (3)	2.6 (1)	***0.027***
Therapy factors						
LDL-c absolute target, %	<50 mg/dL	2.6 (3)	0.0 (0)	18.2 (2)	2.6 (1)	***0.002***
	<70 mg/dL	9.6 (11)	9.4 (6)	18.2 (2)	7.7 (3)	*0.578*
	< 100 mg/dL	34.2 (39)	31.2 (20)	27.3 (3)	41.0 (16)	*0.525*
LDL-c reduction ≥ 50%			81.8 (9)	48.4 (31)	46.2 (18)	*0.095*
TT reached[c], %		12.3 (14)	0.0 (0)	18.2 (2)	30.8 (12)	***<0.001***

Number of patients in brackets. Categorical variables were compared by chi-square test. AMI: acute myocardial infarction; CAD: coronary artery disease; CVE: cerebrovascular event; LDL-c: low-density lipoprotein cholesterol; MR: myocardial revascularization; TT: therapy target.

[a] The stratification of CAD risk was performed according to the Update of the Brazilian Guideline for FH (IZAR et al., 2021):
1) Very high risk: patients carrying manifested CAD (history of AMI, angina *pectoris*, previous myocardial revascularization or ischemic or transitory CVE);
2) High risk: primary prevention with baseline LDL-c > 400 mg/dL, or baseline LDL-c > 310 mg/dL with one high risk factor (tobacco smoking, male gender or HDL-c < 40 mg/dL), or baseline LDL-c > 190 mg/dL with two high risk factors;
3) Intermediate risk: Primary prevention without high risk factors.

[b] Data were not available for history of AMI (1), CAD (9), CVE (14), tobacco smoking (2).

[c] The therapy target for each risk group was the following:
1) Very high risk: LDL-c reduction ≥ 50% + on-treatment LDL-c < 50 mg/dL;
2) High risk: LDL-c reduction ≥ 50% + on-treatment LDL-c < 70 mg/dL;
3) Intermediate risk: LDL-c reduction ≥ 50% + on-treatment LDL-c < 70 mg/dL.

Supplementary table 3 Influence of lipid-lowering treatment on serum lipids of FH patients.

Variable		**Total** (n=114)	**RE** (n=58)	**NRE** (n=56)	***p-value***
Total cholesterol, mg/dL	Baseline	318 (216 - 420)	330 (173 - 487)	300 (247 - 353)	***0.004***
	On-treatment	197 (133 - 261)	176 (122 - 230)	230 (170 - 290)	***<0.001***
	% change	-36 (-61 - -11)	-51 (-65 - -37)	-25 (-40 - -10)	***<0.001***
	p-value	***<0.001***	***<0.001***	***<0.001***	
LDL cholesterol, mg/dL	Baseline	226 (128 - 324)	239 (100 - 378)	222 (171 - 273)	***0.005***
	On-treatment	118 (51 - 185)	96 (60 - 132)	152 (104 - 200)	***<0.001***
	% change	-51 (-81 - -21)	-62 (-76 - -48)	-32 (-50 - -13)	***<0.001***
	p-value	***<0.001***	***<0.001***	***<0.001***	
HDL cholesterol, mg/dL	Baseline	49 (35 - 63)	50 (34 - 66)	48 (35 - 61)	*0.711*
	On-treatment	47 (30 - 64)	44 (26 - 62)	48 (35 - 61)	*0.473*
	% change	0 (-26 - 26)	-6 (-31 - 19)	0 (-22 - 22)	*0.230*
	p-value	*0.619*	*0.268*	*0.546*	
Triglycerides, mg/dL	Baseline	154 (52 - 256)	150 (18 - 282)	154 (80 - 228)	*0.511*
	On-treatment	122 (43 - 201)	105 (12 - 198)	130 (53 - 207)	*0.073*
	% change	-24 (-72 - 24)	-31 (-68 - 6)	-13 (-57 - 31)	***0.003***
	p-value	***<0.001***	***<0.001***	***0.010***	

Patients with LDL-c reduction of at least 50% after statin treatment were classified as responders. Continuous variables are shown as median and interquartile range and were compared by Mann-Whitney or Wilcoxon test. . n: number of patients; HDL: high-density lipoprotein; LDL: low-density lipoprotein; RE: responder; NRE non responder.

Supplementary table 4 Concentration of laboratory variables on treatment in FH patients grouped according to statin response.

Variable	**Total (n=113)**	**RE (n=58)**	**NRE (n=56)**	***p-value***
Apo AI, mg/dL	147 (112 - 182)	142 (105 - 178)	153 (121 - 185)	***0.036***
Apo B, mg/dL	125 (73 - 177)	119 (75 - 163)	150 (93 - 207)	***0.007***
Glucose, mg/dL	92 (73 - 111)	89 (76 - 102)	95 (74 - 116)	***0.004***
HbA1c, %	6 (5.3 - 6.7)	6.0 (5.2 - 6.8)	5.9 (5.2 - 6.6)	*0.617*
Insulin, µIU/mL	7.9 (1.9 - 13.9)	7.0 (2.1 - 11.9)	9.4 (4.4 - 14.4)	***0.028***
Creatinine, mg/dL	0.8 (0.6 - 1)	0.8 (0.6 - 1)	0.7 (0.4 - 1)	*0.075*
ALT, U/L	32 (11 - 53)	32 (12 - 52)	31.5 (11.3 - 51.7)	*0.446*
AST, U/L	26 (16 - 36)	28 (17 - 39)	24.5 (15.5 - 33.5)	*0.221*
CK, U/L	91.5 (12.3 - 170.7)	94.5 (34.5 - 154.5)	88.5 (6.3 - 170.7)	*0.924*
hsCRP, mg/dL	0.5 (0.2 - 0.8)	0.5 (0.3 - 0.7)	0.6 (0.3 - 0.9)	*0.832*
TSH, µIU/mL	1.6 (0.1 - 3.1)	1.5 (-0.2 - 3.2)	1.7 (0.5 - 2.9)	*0.899*
T4, ng/dL	1.0 (0.8 - 1.2)	1.0 (0.8 - 1.2)	0.9 (0.7 - 1.1)	*0.680*

Patients with LDL-c reduction of at least 50% after statin treatment were classified as responders. Continuous variables are shown as median and interquartile range and were compared by Mann-Whitney test. Information on laboratory data was missing for apo AI (33 patients), apo B (33), glucose (17), HbA1c (27), insulin (32), creatinine (27), ALT (25), AST (25), CK (24), hsCRP (31), TSH (23) and T4 (25). n: number of patients; ALT: alanine aminotransferase; Apo AI: apolipoprotein AI; Apo B: apolipoprotein B; AST: aspartate aminotransferase; CK: creatine kinase; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; T4: thyroxine; TSH: thyroid-stimulating hormone; hsCRP: high sensitivity C-reactive protein; RE: responder; NRE non responder.

Supplementary table 5 Influence of the type of lipid-lowering treatments on lipid levels of FH patients (n=114).

Variable		Statin intensity			Ezetimibe		
		Moderate (n=16)	**High** (n=98)	***p-value***	**Non-users** (n=72)	**Users** (n=42)	***p-value***
TC	Baseline	306 (253-359)	322 (213-431)	*0.109*	304 (239-369)	333 (204-462)	*0.011*
	On-treatment	232 (160-304)	192 (125-259)	*0.011*	203 (121-285)	188 (137-239)	*0.362*
	% change	-22 (-40 – -4)	-40 (-65 – -15)	*<0.001*	-34 (-53– -15)	-47 (-74– -20)	*0.031*
	p-value	*<0.001*	*<0.001*		*<0.001*	*<0.001*	
LDL-c	Baseline	222 (149-295)	230 (123-337)	*0.071*	221 (169-273)	244 (142-346)	*0.001*
	On-treatment	130 (68-192)	116 (58 - 174)	*0.116*	117 (49-185)	122 (65-179)	*0.936*
	% change	-32 (-56 – -8)	-53 (-72 – -31)	*0.002*	-47 (-73–-21)	-61 (-88–-34)	*0.009*
	p-value	*<0.001*	*<0.001*		*<0.001*	*<0.001*	
HDL-c	Baseline	52 (44-60)	48 (33 - 63)	*0.103*	49 (34-64)	49 (34-64)	*0.342*
	On-treatment	53 (36-70)	46 (29-63)	*0.086*	45 (29-61)	48 (32-64)	*0.374*
	% change	-6 (-38 - 26)	0 (-23 - 23)	*0.003*	-1 (-26 - 24)	0 (-24 - 24)	*0.764*
	p-value	*1.000*	*0.680*		*0.837*	*0.581*	
TG	Baseline	162 (100-224)	154 (42 - 66)	*0.831*	157 (41-273)	142 (41-243)	*0.464*
	On-treatment	157 (73-241)	110 (34-186)	*0.004*	130 (30-230)	108 (38-178)	*0.085*
	% change	-4 (-39 - 31)	-27 (-75 - 21)	*0.001*	-24 (-68-20)	-24 (-75-27)	*0.438*
	p-value	*0.391*	*<0.001*		*<0.001*	*<0.001*	

Continuous variables are shown as median and interquartile range and were compared by Mann-Whitney test. FH: familial hypercholesterolemia; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides.

Supplementary table 6 Association between SRAE and serum lipids of FH patients (n=114).

Variable		No SRAE (90)	SRAE (24)	*p-value*
TC	Baseline	310 (245 - 376)	374 (239 - 509)	***0.001***
	On-treatment	192 (111 - 273)	204 (163 - 245)	***0.001***
	% change	-33.4 (-12.7 - -54.1)	-50.0 (-36.6 - -63.4)	***0.001***
	p-value	***<0.001***	***<0.001***	
LDL-c	Baseline	224 (169 - 279)	295 (140 - 449)	***0.007***
	On-treatment	117 (47 - 187)	121 (78 - 165)	*0.784*
	% change	-47.3 (-20.1 - -74.5)	-61.3 (-51.8 - -70.8)	***0.002***
	p-value	***<0.001***	***<0.001***	
HDL-c	Baseline	49 (35 - 63)	51 (30.5 - 71.5)	*0.352*
	On-treatment	47 (34 - 60)	46.5 (20.5 - 72.5)	*0.833*
	% change	0 (-26.3 - 26.3)	-6.6 (-20.9 – 7.7)	*0.325*
	p-value	*0.824*	*0.523*	
TG	Baseline	154 (60.8 - 247.2)	191 (41.5 - 340.5)	*0.242*
	On-treatment	119 (49 - 189)	142 (17 - 267)	*0.385*
	% change	-24.2 (-70.4 – 22.0)	-28.9 (- 91.3 – 33.5)	*0.985*
	p-value	***<0.001***	*0.279*	

Continuous variables are shown as median and interquartile range and were compared by Mann-Whitney test. FH: familial hypercholesterolemia; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides; SRAE: statin-related adverse events.

Supplementary table 7 Variants in PK-related genes identified in FH patients (n=114).

Gene	rs code	NT change	AA change	Type	MAF (%)	*In silico* prediction	HWE p -value
ABCB1	rs2032582	c.2677T>G	p.Ser893Ala	missense	59.2	N	*0.247*
	rs28364277	c.*146G>A		3'UTR	3.1	N	*1.000*
	rs2229107	c.3421T>A	p.Ser1141Thr	missense	1.3	N	*1.000*
	rs2235052	c.*82_*79delTTAC		3'UTR	2.2	N	*1.000*
	rs17064	c.*89A>T		3'UTR	7.9	N	*1.000*
	rs3842	c.*193A>G		3'UTR	12.3	N	*0.213*
	rs9282564	c.61A>G	p.Asn21Asp	missense	3.9	N	*1.000*
	rs3213619	c.-693T>C		5'UTR	4.8	N	*0.224*
	rs2032582	c.2677T>A	p.Ser893Thr	missense	3.1	N	*1.000*
	rs3747802	c.-113086T>C		5'UTR	0.9	N	*1.000*
	rs2229109	c.1199G>A	p.Ser400Asn	missense	3.5	N	*1.000*
	rs28364275	c.*21T>C		3'UTR	1.8	N	*1.000*
	rs28364278	c.*172_*173insGAGAGACA		3'UTR	1.8	N	*1.000*
	rs35023033	c.2005C>T	p.Arg669Cys	missense	0.4	N	*1.000*
	rs35730308	c.3322T>C	p.Trp1108Arg	missense	0.4	N	*1.000*
	rs28364274	c.3751G>A	p.Val1251Ile	missense	0.9	N	*1.000*
	rs57521326	c.3262G>A	p.Asp1088Asn	missense	0.9	N	*1.000*
	rs28364279	c.*252A>C		3'UTR	0.4	N	*1.000*
	rs28364280	c.*316G>A		3'UTR	0.4	N	*1.000*
	rs36008564	c.781A>G	p.Ile261Val	missense	0.4	N	*1.000*
ABCB11	rs2287622	c.1331T>C	p.Val444Ala	missense	58.7	N	*<0.001*
	rs473351	c.*236A>G		3'UTR	63.6	N	*0.009*
	rs495714	c.*368G>A		3'UTR	56.0	N	*0.037*
	rs496550	c.*420A>G		3'UTR	56.0	N	*0.037*
	rs2287622	c.1331T>C	p.Val444Ala	missense	58.7	N	*<0.001*
	rs11568364	c.2029A>G	p.Met677Val	missense	5.4	N	*1.000*
	rs1521808	c.3556G>A	p.Glu1186Lys	missense	0.5	N	*1.000*
	rs766285158	c.3691C>T	p.Arg1231Trp	missense	0.5	N	*1.000*
	Novel	c.*614G>A		3'UTR	0.5	N	*1.000*
	rs11568357	c.616A>G	p.Ile206Val	missense	0.5	N	*1.000*
	rs111482608	c.1636C>A	p.Gln546Lys	missense	0.5	N	*1.000*
	rs11568370	c.1774G>C	p.Glu592Gln	missense	0.5	N	*1.000*
ABCC1	rs129081	c.*801G>C		3'UTR	40.2	N	*0.034*
	rs3743527	c.*543C>T		3'UTR	21.2	N	*0.093*
	rs4148381	c.*1321_*1322insT		3'UTR	51.1	N	*0.000*
	rs8056298	c.*1385T>G		3'UTR	97.8	N	*<0.001*
	rs212090	c.*866T>A		3'UTR	40.2	N	*0.011*
	rs129081	c.*801G>C		3'UTR	40.2	N	*0.034*
	rs113264879	c.*883G>A		3'UTR	0.5	N	*1.000*
	rs16967632	c.*1645G>A		3'UTR	0.5	N	*1.000*
	rs142023064	c.*1293_*1297delGAAAA		3'UTR	2.2%	N	*1.000*
	rs150927043	c.*1759T>A		3'UTR	1.6	N	*1.000*
	rs4148381	c.*1321_*1322insTT		3'UTR	30.2	N	*0.802*
	rs212091	c.*1512T>C		3'UTR	11.4	N	*0.006*
	rs4148356	c.2168G>A	p.Arg723Gln	missense	0.5	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	*In silico* prediction	HWE p -value
	rs4148380	c.*1293G>A		3'UTR	4.9	N	*0.151*
	rs113328089	c.*228G>A		3'UTR	2.2	N	*1.000*
	Novel	c.*1293G>0		3'UTR	1.1	N	*1.000*
	Novel	c.66del5>C		frameshift deletion	0.5	D	*1.000*
	rs45511401	c.2012G>T	p.Gly671Val	missense	3.8	D	*1.000*
	rs139158420	c.*401C>T		3'UTR	0.5	N	*1.000*
	Novel	c.*1752_*1753insA		3'UTR	1.6	N	*1.000*
	rs111601005	c.*1752delA		3'UTR	2.2	N	*1.000*
	rs45492303	c.*1237G>C		3'UTR	2.2	N	*1.000*
	rs74009607	c.*443C>T		3'UTR	2.2	N	*1.000*
	rs80085493	c.*1604C>T		3'UTR	0.5	N	*1.000*
	Novel	c.*1015_*1016delGC		3'UTR	0.5	N	*1.000*
	rs8187856	g.16146576C>G		splicing	1.1	N	*1.000*
	rs146369277	c.*800C>G		3'UTR	0.5	N	*1.000*
	rs183032276	c.4154G>A	p.Arg1385Gln	missense	0.5	N	*1.000*
	rs112282109	c.1898G>A	p.Arg633Gln	missense	0.5	N	*1.000*
	rs557646879	c.-88_-75del-		5'UTR	0.5	N	*1.000*
	rs147785655	c.*1000G>A		3'UTR	0.5	N	*1.000*
	rs45569938	c.*546T>G		3'UTR	0.5	N	*1.000*
	rs13337489	c.3140G>C	p.Cys1047Ser	missense	1.1	N	*1.000*
	rs28706727	c.3436G>A	p.Val1146Ile	missense	0.5	N	*1.000*
	rs143805318	c.*1644C>T		3'UTR	0.5	N	*1.000*
	Novel	c.145T>G		missense	0.5	N	*1.000*
	rs182967563	c.*272G>A		3'UTR	0.5	N	*1.000*
	rs187769078	c.185G>A	p.Arg62Gln	missense	0.5	N	*1.000*
	rs188577026	c.*891A>G		3'UTR	0.5	N	*1.000*
	rs199815778	c.4441G>A	p.Val1481Ile	missense	0.5	N	*1.000*
ABCC2	rs2273697	c.1249G>A	p.Val417Ile	missense	16.8	N	*0.429*
	rs45441199	c.3107T>C	p.Ile1036Thr	missense	1.1	N	*1.000*
	rs927344	c.116A>T	p.Tyr39Phe	missense	98.9	N	*<0.001*
	rs17222723	c.3563T>A	p.Val1188Glu	missense	7.6	N	*0.051*
	rs8187699	c.3817A>G	p.Thr1273Ala	missense	0.5	N	*1.000*
	rs8187710	c.4544G>A	p.Cys1515Tyr	missense	9.8	N	*0.136*
	rs17222617	c.2546T>G	p.Leu849Arg	missense	1.6	N	*1.000*
	rs717620	c.-24C>T		5'UTR	17.9	N	*0.701*
	rs2273697	c.1249G>A	p.Val417Ile	missense	16.8	N	*0.429*
	rs138578110	c.*259G>T		3'UTR	1.1	N	*1.000*
	rs8187692	c.3542G>T	p.Arg1181Leu	missense	2.7	D	*1.000*
	rs7080681	c.1058G>A	p.Arg353His	missense	2.7	N	*1.000*
	rs17216317	c.3872C>T	p.Pro1291Leu	missense	3.3	D	*1.000*
	rs72558199	c.3196C>T	p.Arg1066X	stopgain	0.5	N	*1.000*
	rs141413284	c.1860T>A	p.Asp620Glu	missense	0.5	N	*1.000*
	rs533334893	g.101552117G>A		splicing	0.5	D	*1.000*
ABCC3	rs34926034	c.202C>T	p.His68Tyr	missense	1.1	N	*1.000*
	rs141856639	c.3971G>A	p.Arg1324His	missense	1.1	D	*1.000*
	rs35999272	c.2758C>T	p.Pro920Ser	missense	2.2	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	*In silico* prediction	HWE p -value
	rs34346931	c.1223A>G	p.Glu408Gly	missense	0.5	N	*1.000*
	rs150601692	c.4030A>G	p.Lys1344Glu	missense	0.5	N	*1.000*
	rs11568591	c.3890G>A	p.Arg1297His	missense	6.5	D	*1.000*
	rs200779271	c.980T>C	p.Ile327Thr	missense	0.5	N	*1.000*
	rs201562834	c.871C>T	p.Arg291Trp	missense	0.5	N	*1.000*
	rs1003354	c.1580C>T	p.Thr527Met	missense	0.5	N	*1.000*
	rs143608762	c.694C>T	p.Arg232Trp	missense	0.5	N	*1.000*
	rs35777968	c.296G>A	p.Arg99Gln	missense	0.5	N	*1.000*
	rs139106724	c.2377G>A	p.Val793Ile	missense	1.1	N	*1.000*
	rs200413276	c.2558C>A	p.Ala853Asp	missense	0.5	N	*1.000*
	rs372683132	c.922G>A	p.Gly308Ser	missense	1.1	N	*1.000*
	rs34926034	c.202C>T	p.His68Tyr	missense	1.1	N	*1.000*
	rs11568584	c.2153A>T	p.Lys718Met	missense	0.5	N	*1.000*
	rs11568607	g.48745787G>A		splicing	2.2	N	*1.000*
	rs11568590	c.4094A>G	p.Gln1365Arg	missense	0.5	N	*1.000*
	rs11568608	c.1820G>A	p.Ser607Asn	missense	1.1	N	*1.000*
	rs34291385	c.2293G>C	p.Val765Leu	missense	1.1	N	*1.000*
	rs200903266	c.3401G>A	p.Arg1134Gln	missense	0.5	N	*1.000*
	rs138342952	c.*258G>C		3'UTR	1.1	N	*1.000*
	rs11568588	c.4042C>T	p.Arg1348Cys	missense	1.1	N	*1.000*
	rs148804178	c.205C>G	p.Leu69Val	missense	0.5	N	*1.000*
	rs563802547	c.*140_*141insT		3'UTR	0.5	N	*1.000*
ABCG2	rs45605536	c.1582G>A	p.Ala528Thr	missense	1.1	N	*1.000*
	rs111766106	c.-18485C>T		5'UTR	0.5	N	*1.000*
	rs45605536	c.1582G>A	p.Ala528Thr	missense	1.1	N	*1.000*
	rs45510401	c.*1964T>C		3'UTR	2.2	N	*0.026*
	rs72554040	c.-91177C>T		5'UTR	8.2	N	*0.389*
	rs1448784	c.*1066T>C		3'UTR	1.1	N	*1.000*
	rs2231142	c.421C>A	p.Gln141Lys	missense	6.5	N	*1.000*
	rs2231137	c.34G>A	p.Val12Met	missense	6.0	N	*1.000*
	rs10030206	c.*1295A>T		3'UTR	1.1	N	*1.000*
	rs115770495	c.*1726G>A		3'UTR	2.2	N	*1.000*
	rs1337337886	c.131A>G	p.Tyr44Cys	missense	0.5	N	*1.000*
	rs35965584	c.1624A>G	p.Thr542Ala	missense	0.5	N	*1.000*
	rs45630471	c.-18400A>G		5'UTR	0.5	N	*1.000*
	rs2231135	c.-18847T>C		5'UTR	1.1	N	*1.000*
	Novel	c.1453C>A		missense	0.5	N	*1.000*
	rs138606116	c.1060G>A	p.Gly354Arg	missense	0.5	N	*1.000*
	rs55927234	c.-18436C>G		5'UTR	0.5	N	*1.000*
	rs34783571	c.1858G>A	p.Asp620Asn	missense	0.5	N	*1.000*
	rs34264773	c.1758A>T	p.Lys586Asn	missense	0.5	N	*1.000*
	Novel	c.*1575T>C		3'UTR	0.5	N	*1.000*
	rs34124189	g.89053790G>A		splicing	0.5	N	*1.000*
CYP1A2	rs33923017	c.*360_*361insT		3'UTR	11.4	N	*0.595*
	rs33923017	c.*360_*361insTT		3'UTR	22.8	N	*0.012*
	rs34002060	c.*1034delT		3'UTR	15.2	N	*0.213*

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	rs33923017	c.*360_*361insT		3'UTR	11.4	N	*0.595*
	rs58661304	c.*270A>C		3'UTR	5.4	N	*0.012*
	Novel	c.*1033_*1034insT		3'UTR	6.0	N	*1.000*
	rs1288558234	g.75041241del		splicing	0.5	N	*1.000*
	rs17861157	c.894C>A	p.Ser298Arg	missense	3.3	N	*0.065*
	rs45540640	c.613T>G	p.Phe205Val	missense	0.5	N	*1.000*
	rs913188841	g.75041242C>G		splicing	0.5	N	*1.000*
	rs201763966	c.142T>G	p.Trp48Gly	missense	0.5	N	*1.000*
	Novel	c.*1035_*1036insT		3'UTR	18.5	N	*0.127*
	Novel	c.*1035delT		3'UTR	18.5	N	*0.127*
	Novel	c.*1034_*1035delTT		3'UTR	19.6	N	*0.070*
	Novel	c.*361_*362insT		3'UTR	12.5	N	*0.600*
	Novel	c.*361_*362insTT		3'UTR	12.5	N	*0.600*
	rs201977879	c.*361delT		3'UTR	17.9	N	*0.211*
	Novel	c.*274C>0		3'UTR	1.1	N	*1.000*
	rs11636419	c.*171A>G		3'UTR	6.5	N	*1.000*
	rs150722579	c.*292_*293insC		3'UTR	1.6	N	*1.000*
	rs17861162	c.*1324C>G		3'UTR	8.7	N	*1.000*
	rs201077484	c.*274delC		3'UTR	1.6	N	*1.000*
	rs57295890	c.*282delC		3'UTR	10.3	N	*1.000*
	rs200442208	c.*282C>A		3'UTR	2.2	N	*1.000*
	rs780737808	c.*304_*305insAT		3'UTR	1.6	N	*1.000*
	Novel	c.*1034_*1035insT		3'UTR	1.1%	N	*1.000*
	rs201443593	c.*292A>C		3'UTR	0.5	N	*1.000*
	rs56141902	c.*854G>A		3'UTR	0.5	N	*1.000*
	Novel	c.*271_*274delAAAC		3'UTR	2.7	N	*0.044*
	rs758124536	c.409C>T	p.Arg137Trp	missense	0.5	N	*1.000*
	Novel	c.*283_*284insA		3'UTR	1.1	N	*1.000*
	Novel	c.*282_*283delins0		3'UTR	1.1%	N	*1.000*
	Novel	c.*283delA		3'UTR	1.1	N	*1.000*
	Novel	c.*263_*264insA		3'UTR	0.5	N	*1.000*
	rs200675446	c.*263delA		3'UTR	4.9	N	*1.000*
	rs45564134	c.*974delG		3'UTR	0.5	N	*1.000*
	rs28465265	c.*274C>A		3'UTR	0.5	N	*1.000*
CYP2C19	rs3758581	c.991A>G	p.Ile331Val	missense	43.5	N	*<0.001*
	rs3758581	c.991A>G	p.Ile331Val	missense	43.5	N	*<0.001*
	rs17884712	c.431G>A	p.Arg144His	missense	2.2	D	*1.000*
	rs576823729	c.648C>G	p.Cys216Trp	missense	0.5	N	*1.000*
	rs17882687	c.55A>C	p.Ile19Leu	missense	0.5	N	*1.000*
	rs17878459	c.276G>C	p.Glu92Asp	missense	3.3	N	*1.000*
	rs58973490	c.449G>A	p.Arg150His	missense	1.1	N	*1.000*
CYP2C8	rs1058932	c.*24C>T		3'UTR	23.9	N	*0.006*
	rs11572078	g.96827126dup		splicing	17.4	N	*<0.001*
	rs2071426	g.5932A>G		splicing	23.9	D	*1.000*
	rs1058932	c.*24C>T		3'UTR	23.9	N	*0.006*
	rs11572103	c.499A>T	p.Ile167Phe	missense	3.3	N	*0.065*

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	rs10509681	c.890A>G	p.Lys297Arg	missense	4.9	N	*1.000*
	rs11572080	c.110G>A	p.Arg37Lys	missense	5.4	N	*1.000*
	rs77147096	c.787G>A	p.Gly263Ser	missense	0.5	N	*1.000*
	rs1058930	c.486C>G	p.Ile162Met	missense	4.9	D	*0.151*
	rs369591911	c.65G>A	p.Arg22Gln	missense	0.5	N	*1.000*
	rs11572066	c.-86A>C		5'UTR	0.5	N	*1.000*
	rs143386810	c.844G>A	p.Gly282Ser	missense	0.5	N	*1.000*
CYP2C9	rs1799853	c.430C>T	p.Arg144Cys	missense	8.8	D	*1.000*
	rs9332242	c.*108C>G		3'UTR	8.8	N	*1.000*
	rs1799853	c.430C>T	p.Arg144Cys	missense	8.8	D	*1.000*
	rs28371685	c.1003C>T	p.Arg335Trp	missense	0.9	N	*1.000*
	rs1057910	c.1075A>C	p.Ile359Leu	missense	7.5	N	*0.475*
	rs577147873	c.*60C>T		3'UTR	0.4	N	*1.000*
	rs7900194	c.449G>A	p.Arg150His	missense	1.3	N	*1.000*
	rs9332241	c.*88C>T		3'UTR	1.3	N	*1.000*
	rs2256871	c.752A>G	p.His251Arg	missense	2.2	D	*1.000*
	rs201055266	c.1034T>C	p.Met345Thr	missense	0.4	N	*1.000*
	rs28371686	c.1080C>G	p.Asp360Glu	missense	0.4	N	*1.000*
	rs9332239	c.1465C>T	p.Pro489Ser	missense	0.4	N	*1.000*
CYP2D6	rs16947	c.733C>T	p.Arg245Cys	missense	32.6	N	*0.211*
	rs769258	c.31G>A	p.Val11Met	missense	4.3	N	*0.119*
	rs16947	c.733C>T	p.Arg245Cys	missense	32.6	N	*0.211*
	rs1058172	c.941G>A	p.Arg314His	missense	4.9	D	*1.000*
	rs1065852	c.100C>T	p.Pro34Ser	missense	6.0	D	*0.224*
	Novel	c.551C>T		missense	0.5	N	*1.000*
	rs5030656	c.88_690del	p.Lys230_C442delins	nonframeshift deletion	1.1	LD	*1.000*
	rs28371717	c.556G>T	p.Ala186Ser	missense	0.5	N	*1.000*
	rs28371704	c.281A>G	p.His94Arg	missense	1.6	N	*1.000*
	rs3892097			splicing	2.2	D	*1.000*
	rs28371706	c.320C>T	p.Thr107Ile	missense	2.2	N	*1.000*
	rs139779104	c.482G>A	p.Gly161Glu	missense	0.5	N	*1.000*
	rs5030655	c.54del4>T	p.Trp152Gfs*2	frameshift deletion	0.5	D	*1.000*
	rs140513104	c.821C>T	p.Pro274Leu	missense	0.5	N	*1.000*
	rs59421388	c.859G>A	p.Val287Met	missense	0.5	N	*1.000*
	rs61736512	c.406G>A	p.Val136Met	missense	0.5	N	*1.000*
	rs28371703	c.271C>A	p.Leu91Met	missense	1.1	D	*1.000*
CYP3A4	rs28969391	c.*767delT		3'UTR	18.0	N	*0.757*
	rs28969391	c.*767delT		3'UTR	18.0	N	*0.757*
	rs28988604	c.*683C>T		3'UTR	3.5	N	*1.000*
	rs12721631	c.*329C>T		3'UTR	1.3	N	*1.000*
	rs4986907	c.485G>A	p.Arg162Gln	missense	0.4	N	*1.000*
	rs28371763	c.*948A>T		3'UTR	1.3	N	*1.000*
	rs28988606	c.*1095C>T		3'UTR	0.9	N	*1.000*
CYP3A5	rs15524	c.*14T>C		3'UTR	21.1	N	*0.576*
	rs776746	g.12083G>A		splicing	77.6	D	*0.431*

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	rs41279857	c.299C>A	p.Ser100Tyr	missense	0.4	N	*1.000*
	rs10264272	c.624G>A	p.Lys208Lys	splicing	3.1	N	*1.000*
	rs149664815	c.1378C>T	p.Gln460X	stopgain	0.4	D	*1.000*
	rs15524	c.*14T>C		3'UTR	21.1	N	*0.576*
	rs28371765	c.-3554A>C		5'UTR	0.4	N	*1.000*
	rs28365095	c.-3625G>A		5'UTR	0.4	N	*1.000*
	rs6977165	c.423A>G	p.X141Trp	stoploss	5.7	D	*1.000*
	rs145774441	c.827T>C	p.Ile276Thr	missense	0.4	N	*1.000*
	rs28371764	c.-3613C>T		5'UTR	3.1	N	*1.000*
	rs200579169	c.92dupG	p.Gly31fs	frameshift insertion	0.4	D	*1.000*
	rs28383468	c.88C>T	p.His30Tyr	missense	0.4	N	*1.000*
	rs41303343	c.1035dupT	p.Thr346Yfs*2	frameshift insertion	1.8	D	*1.000*
	rs147489136	c.608T>G	p.Phe203Cys	missense	0.4	N	*1.000*
	rs547253411	c.1372delG	p.Val458Sfs*16	frameshift deletion	0.4	D	*1.000*
	rs41303343	c.1035dupT	p.Thr346fs	frameshift insertion	1.8	D	*1.000*
	rs6957030	c.419T>G	p.Leu140Arg	missense	0.4	N	*1.000*
SLC15A1	rs1289389	c.*688G>A		3'UTR	19.0	N	*0.024*
	rs759932207	c.*178_*177delTT		3'UTR	19.6	N	*0.070*
	rs779338904	c.*178_*176delTTT		3'UTR	2.8	N	*1.000*
	Novel	c.*178_*179insT		3'UTR	1.1	N	*1.000*
	rs1289389	c.*688G>A		3'UTR	19.0	N	*0.024*
	rs4646234	c.*598A>G		3'UTR	12.5	N	*1.000*
	rs2297322	c.350G>A	p.Ser117Asn	missense	18.5	N	*0.003*
	rs7331216	c.*59A>G		3'UTR	9.8	N	*0.517*
	rs113824127	c.*211G>T		3'UTR	1.1	N	*1.000*
	rs8187820	c.364G>A	p.Val122Met	missense	1.6	D	*1.000*
	rs8187838	c.1352C>A	p.Thr451Asn	missense	1.6	N	*1.000*
	Novel	c.*176_*177insT		3'UTR	3.8	N	*1.000*
	Novel	c.*174_*175insT		3'UTR	5.4	N	*1.000*
	Novel	c.*177delT		3'UTR	3.8	N	*1.000*
	Novel	c.*175delT		3'UTR	5.4	N	*1.000*
	Novel	c.*176_*175delTT		3'UTR	5.4	N	*1.000*
	Novel	c.*177_*175delTTT		3'UTR	5.4	N	*1.000*
	Novel	c.*178_*175delTTTT		3'UTR	5.4	N	*1.000*
	Novel	c.*178_*175delTTTT		3'UTR	2.2	N	*1.000*
	rs3783002	c.*224C>T		3'UTR	7.6	N	*0.346*
	rs4646227	c.1256G>C	p.Gly419Ala	missense	4.3	N	*1.000*
	rs2274828	c.1348G>A	p.Val450Ile	missense	0.5	N	*1.000*
	rs572627369	c.*160T>C		3'UTR	0.5	N	*1.000*
	rs578247729	c.*914C>T		3'UTR	0.5	N	*1.000*
	rs8187827	g.99354731T>C		splicing	0.5	N	*1.000*
	rs398037820	c.*178delT		3'UTR	3.9	N	*1.000*
	Novel	c.800A>T		missense	0.5	N	*1.000*
	rs114218227	c.*125G>A		3'UTR	1.1	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	*In silico* prediction	HWE p -value
	rs8187821	c.351C>A	p.Ser117Arg	missense	0.5	N	*1.000*
	Novel	c.*451G>A		3'UTR	0.5	N	*1.000*
	rs79136019	c.*587T>C		3'UTR	1.6	N	*1.000*
	rs8187815	c.-73T>C		5'UTR	1.1	N	*1.000*
	rs146304164	c.1246G>C	p.Val416Leu	missense	0.5	N	*1.000*
	Novel	c.*150_*144delCTTTTTC		3'UTR	0.5	N	*1.000*
	rs4646206	c.-33C>T		5'UTR	0.5	N	*1.000*
	Novel	c.*178_*173delTTTTTT		3'UTR	0.6	N	*1.000*
SLC22A1	rs113569197	c.1275_1276del	p.Pro425fs	frameshift deletion	33.2	N	*<0.001*
	rs628031	c.1222A>G	p.Met408Val	missense	66.8	N	*0.004*
	rs683369	c.480G>C	p.Leu160Phe	missense	85.9	N	*<0.001*
	rs776304541	c.1406G>A	p.Arg469His	missense	0.5	N	*1.000*
	rs35854239	c.275_1276del	p.Pro425fs	splicing	45.7	D	*<0.001*
	rs72552763	c.258_1260del	p.420_420del	nonframeshif t deletion	18.5	LD	*1.000*
	rs34205214	c.1025G>A	p.Arg342His	missense	2.2	N	*1.000*
	rs34447885	c.41C>T	p.Ser14Phe	missense	2.2	N	*1.000*
	rs41267797	c.1390G>A	p.Val464Ile	missense	4.9	N	*0.151*
	rs72552763	c.258_1260del	p.Met420del	nonframeshif t deletion	18.5	D	*1.000*
	rs35270274	c.1463G>T	p.Arg488Met	missense	1.6	N	*1.000*
	rs35888596	c.113G>A	p.Gly38Asp	missense	2.2	D	*1.000*
	rs34059508	c.1393G>A	p.Gly465Arg	missense	1.1	D	*1.000*
	rs2282143	c.1022C>T	p.Pro341Leu	missense	1.1	D	*1.000*
	rs12208357	c.181C>T	p.Arg61Cys	missense	3.8	D	*0.090*
	rs36103319	c.659G>T	p.Gly220Val	missense	0.5	N	*1.000*
	rs78899680	c.1442G>T	p.Gly481Val	missense	0.5	N	*1.000*
	rs34130495	c.1201G>A	p.Gly401Ser	missense	0.5	N	*1.000*
	rs774654623	c.1396C>A	p.Pro466Thr	missense	0.5	N	*1.000*
SLC22A6	rs4149170	c.-127G>A		5'UTR	12.0	N	*0.009*
	rs4149171	c.-20A>G		5'UTR	16.8	N	*0.006*
	rs4149170	c.-127G>A		5'UTR	12.0	N	*0.009*
	rs11568627	c.311C>T	p.Pro104Leu	missense	0.5	N	*1.000*
	rs150811286	c.*46T>C		3'UTR	0.5	N	*1.000*
	rs11568626	c.149G>A	p.Arg50His	missense	0.5	N	*1.000*
	rs181212822	c.*57G>A		3'UTR	0.5	N	*1.000*
SLC22A8	rs145493231	c.-857A>G		5'UTR	0.5	N	*1.000*
	Novel	c.*353C>T		3'UTR	0.5	N	*1.000*
	rs11568481	c.560C>T	p.Ala187Val	missense	0.5	N	*1.000*
	rs4149179	c.-16G>A		5'UTR	3.8	N	*0.090*
	rs45438191	c.473T>C	p.Val158Ala	missense	0.5	N	*1.000*
SLCO1B1	rs2306283	c.388A>G	p.Asn130Asp	missense	47.4	N	*0.354*
	rs4149056	c.521T>C	p.Val174Ala	missense	11.0	D	*0.355*
	rs4149087	c.*439T>G		3'UTR	38.2	N	*0.691*
	rs4149088	c.*463A>G		3'UTR	35.5	N	*1.000*
	rs2306283	c.388A>G	p.Asn130Asp	missense	47.4	N	*0.354*
	rs11045819	c.463C>A	p.Pro155Thr	missense	13.2	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	*In silico* prediction	HWE p -value
	rs11045891	c.*449A>C		3'UTR	15.4	N	*0.725*
	rs11045852	c.733A>G	p.Ile245Val	missense	0.9	N	*1.000*
	rs74064213	c.1495A>G	p.Ile499Val	missense	0.9	N	*1.000*
	rs34671512	c.1929A>C	p.Leu643Phe	missense	5.3	N	*0.263*
	rs59502379	c.1463G>C	p.Gly488Ala	missense	1.8	D	*1.000*
	rs71581985	c.*46T>G		3'UTR	0.9	N	*1.000*
	rs77271279	g.21329832G>T		splicing	0.9	D	*1.000*
	rs61760249	c.*575G>A		3'UTR	0.4	N	*1.000*
	rs79135870	c.664A>G	p.Ile222Val	missense	0.4	N	*1.000*
	rs59113707	c.1200C>G	p.Phe400Leu	missense	0.4	N	*1.000*
	rs72655363	c.*82C>T		3'UTR	0.4	N	*1.000*
SLCO1B3	rs3764009	g.21013948C>T		splicing	16.3	N	*<0.001*
	rs4149117	c.250T>G	p.Ser84Ala	missense	76.1	N	*<0.001*
	rs4149158	c.-7_-4del-		5'UTR	24.5	N	*0.040*
	rs527574443	c.-28_-11del-		5'UTR	24.5	N	*0.040*
	rs7305323	c.-2125C>T		5'UTR	64.1	N	*<0.001*
	rs7311358	c.615G>A	p.Met205Ile	missense	72.8	N	*<0.001*
	rs397689574	c.*347_*348insA		3'UTR	32.6	N	*0.629*
	rs57585902	c.355A>G	p.Thr119Ala	missense	1.1	N	*1.000*
	rs60140950	c.767G>C	p.Gly228Ala	missense	14.7	D	*0.048*
	rs780598056	c.1333delG	p.Val445Sfs*6	frameshift deletion	0.5	D	*1.000*
	rs773176181	c.1247G>C	p.Gly416Ala	missense	0.5	N	*1.000*
	rs150007972	c.233C>A	p.Thr78Asn	missense	0.5	N	*1.000*
	rs61736817	c.1282C>T	p.Leu428Phe	missense	0.5	N	*1.000*
	rs76963574	c.1628C>G	p.Ala543Gly	missense	0.5	N	*1.000*
	rs115227445	c.592C>G	p.Leu198Val	missense	0.5	N	*1.000*
	rs77957556	c.*642G>A		3'UTR	1.1	N	*1.000*
	rs558592800	c.119_120insAATTG	p.Asp42Efs*12	frameshift insertion	0.5	D	*1.000*
	Novel	c.596G>T		missense	0.5	N	*1.000*
	Novel	c.-2107A>T		5'UTR	0.5	N	*1.000*
	rs12299012	c.1595T>C	p.Val532Ala	missense	1.1	N	*1.000*
	rs958332597	g.21032366C>T		splicing	0.5	N	*1.000*
SLCO2B1	rs11236359	c.-2866A>G		5'UTR	75.5	N	*<0.001*
	rs1944612	c.-36A>G		5'UTR	98.9	N	*<0.001*
	rs2851069	c.-71T>C		5'UTR	47.3	N	*0.078*
	rs11236359	c.-2866A>G		5'UTR	75.5	N	*<0.001*
	rs17133818	c.*1386C>T		3'UTR	6.0	N	*1.000*
	rs1801906	c.*1070T>C		3'UTR	9.2	N	*1.000*
	rs2306168	c.1025C>T	p.Ser342Phe	missense	6.5	N	*0.263*
	rs3781727	c.*396T>C		3'UTR	6.5	N	*1.000*
	rs41298121	c.*1222T>C		3'UTR	10.3	N	*0.558*
	rs12422149	c.503G>A	p.Arg168Gln	missense	12.0	N	*0.595*
	Novel	c.*956C>A		3'UTR	0.5	N	*1.000*
	rs41298117	c.*721C>G		3'UTR	3.8	N	*1.000*
	rs78825186	c.485G>A	p.Arg162His	missense	1.1	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	*In silico* prediction	HWE p -value
	rs145875125	c.1206C>A	p.Asn402Lys	missense	0.5	N	*1.000*
	rs185838153	c.*1776T>C		3'UTR	0.5	N	*1.000*
	rs60113013	c._614del	p.Glu4_T6del	nonframeshift deletion	1.6	LD	*1.000*
	rs35199625	c.169G>A	p.Val57Met	missense	1.1	N	*1.000*
UGT1A3	rs6431625	c.140T>C	p.Val47Ala	missense	41.8	N	*0.212*
	rs28898619	c.342G>A	p.Met114Ile	missense	1.1	N	*1.000*
	rs3821242	c.31T>C	p.Trp11Arg	missense	45.7	N	*0.027*
	rs6431625	c.140T>C	p.Val47Ala	missense	41.8	N	*0.212*
	rs61764030	c.473C>T	p.Ala158Val	missense	1.1	N	*1.000*
	rs149324549	c.775G>C	p.Gly259Arg	missense	1.1	N	*1.000*
	rs45449995	c.808A>G	p.Met270Val	missense	2.2	D	*0.026*
	rs61764031	c.523A>T	p.Asn175Tyr	missense	0.5	N	*1.000*
	rs140541315	c.172G>A	p.Ala58Thr	missense	0.5	N	*1.000*
	rs13406898	c.431C>T	p.Thr144Ile	missense	0.5	N	*1.000*
	rs45595237	c.145C>T	p.Arg49Trp	missense	0.5	N	*1.000*
UGT2B7	rs57075995	c.*100_*101insA		3'UTR	17.9	N	*0.122*
	rs7439366	c.802T>C	p.Tyr268His	missense	62.0	N	*<0.001*
	rs57075995	c.*100_*101insA		3'UTR	17.9	N	*0.122*
	Novel	c.*101delA		3'UTR	29.1	N	*<0.001*
	rs111878373	c.-2G>A		5'UTR	1.1	N	*1.000*
	rs140153012	c.321A>T	p.Leu107Phe	missense	1.1	N	*1.000*
	rs57075995	c.*100_*101insAA		3'UTR	2.2	N	*1.000*
	Novel	c.*101_*102insA		3'UTR	22.8	N	*0.012*
	Novel	c.*101_*102insAA		3'UTR	22.8	N	*0.012*
	rs60103519	c.536C>T	p.Thr179Ile	missense	1.1	N	*1.000*
	rs78265585	c.*247C>A		3'UTR	1.6	N	*1.000*

In silico functionality prediction was performed either using the functionality prediction score (FPS) for missense variants or dbNSFP v4.2 *in silico* algorithm for splice variants. Frameshift variants were considered deleterious. Nonframeshift variants were considered potentially deleterious. AA: amino acid; NT nucleotide; D: deleterious; HWE: Hardy-Weinberg equilibrium; LD: likely deleterious;/ MAF: minor allele frequency; N: neutral; PK: pharmacokinetics; UTR: untranslated region.

Supplementary table 8 FPS score of variants in PK-related genes identified in FH patients (n = 114).

Gene	rs code	NT change	AA change	Type	MAF (%)	FPS
ABCB1	rs2032582	c.2677T>G	p.Ser893Ala	missense	59.2	0.2
	rs2229107	c.3421T>A	p.Ser1141Thr	missense	1.3	0.0
	rs9282564	c.61A>G	p.Asn21Asp	missense	3.9	0.0
	rs2032582	c.2677T>A	p.Ser893Thr	missense	3.1	0.4
	rs2229109	c.1199G>A	p.Ser400Asn	missense	3.5	0.2
	rs35023033	c.2005C>T	p.Arg669Cys	missense	0.4	0.6
	rs35730308	c.3322T>C	p.Trp1108Arg	missense	0.4	0.8
	rs28364274	c.3751G>A	p.Val1251Ile	missense	0.9	0.2
	rs57521326	c.3262G>A	p.Asp1088Asn	missense	0.9	0.6
	rs36008564	c.781A>G	p.Ile261Val	missense	0.4	0.2
ABCB11	rs2287622	c.1331T>C	p.Val444Ala	missense	58.7	0.0
	rs11568364	c.2029A>G	p.Met677Val	missense	5.4	0.2
	rs1521808	c.3556G>A	p.Glu1186Lys	missense	0.5	0.4
	rs766285158	c.3691C>T	p.Arg1231Trp	missense	0.5	1.0
	rs11568357	c.616A>G	p.Ile206Val	missense	0.5	0.0
	rs111482608	c.1636C>A	p.Gln546Lys	missense	0.5	0.2
	rs11568370	c.1774G>C	p.Glu592Gln	missense	0.5	0.6
ABCC1	rs4148356	c.2168G>A	p.Arg723Gln	missense	0.5	0.0
	rs45511401	c.2012G>T	p.Gly671Val	missense	3.8	0.8
	rs183032276	c.4154G>A	p.Arg1385Gln	missense	0.5	1.0
	rs112282109	c.1898G>A	p.Arg633Gln	missense	0.5	0.0
	rs13337489	c.3140G>C	p.Cys1047Ser	missense	1.1	0.0
	rs28706727	c.3436G>A	p.Val1146Ile	missense	0.5	0.4
	Novel	c.145T>G	p.Cys49Gly	missense	0.5	NR
	rs187769078	c.185G>A	p.Arg62Gln	missense	0.5	0.4
	rs199815778	c.4441G>A	p.Val1481Ile	missense	0.5	0.4
ABCC2	rs2273697	c.1249G>A	p.Val417Ile	missense	16.8	0.0
	rs45441199	c.3107T>C	p.Ile1036Thr	missense	1.1	0.2
	rs927344	c.116A>T	p.Tyr39Phe	missense	98.9	0.2
	rs17222723	c.3563T>A	p.Val1188Glu	missense	7.6	0.2
	rs8187699	c.3817A>G	p.Thr1273Ala	missense	0.5	0.2
	rs8187710	c.4544G>A	p.Cys1515Tyr	missense	9.8	0.2
	rs17222617	c.2546T>G	p.Leu849Arg	missense	1.6	0.4
	rs8187692	c.3542G>T	p.Arg1181Leu	missense	2.7	0.8
	rs7080681	c.1058G>A	p.Arg353His	missense	2.7	0.0
	rs17216317	c.3872C>T	p.Pro1291Leu	missense	3.3	0.8
	rs72558199	c.3196C>T	p.Arg1066X	stopgain	0.5	0.5
	rs141413284	c.1860T>A	p.Asp620Glu	missense	0.5	0.2
ABCC3	rs34926034	c.202C>T	p.His68Tyr	missense	1.1	0.0
	rs141856639	c.3971G>A	p.Arg1324His	missense	1.1	1.0
	rs35999272	c.2758C>T	p.Pro920Ser	missense	2.2	0.0
	rs34346931	c.1223A>G	p.Glu408Gly	missense	0.5	1.0
	rs150601692	c.4030A>G	p.Lys1344Glu	missense	0.5	0.0
	rs11568591	c.3890G>A	p.Arg1297His	missense	6.5	0.8
	rs200779271	c.980T>C	p.Ile327Thr	missense	0.5	0.0
	rs201562834	c.871C>T	p.Arg291Trp	missense	0.5	0.2
	rs1003354	c.1580C>T	p.Thr527Met	missense	0.5	0.4
	rs143608762	c.694C>T	p.Arg232Trp	missense	0.5	0.8
	rs35777968	c.296G>A	p.Arg99Gln	missense	0.5	0.0
	rs139106724	c.2377G>A	p.Val793Ile	missense	1.1	0.4
	rs200413276	c.2558C>A	p.Ala853Asp	missense	0.5	0.6
	rs372683132	c.922G>A	p.Gly308Ser	missense	1.1	0.4
	rs11568584	c.2153A>T	p.Lys718Met	missense	0.5	0.4
	rs11568590	c.4094A>G	p.Gln1365Arg	missense	0.5	0.0
	rs11568608	c.1820G>A	p.Ser607Asn	missense	1.1	0.0
	rs34291385	c.2293G>C	p.Val765Leu	missense	1.1	0.4
	rs200903266	c.3401G>A	p.Arg1134Gln	missense	0.5	1.0

Gene	rs code	NT change	AA change	Type	MAF (%)	FPS
	rs11568588	c.4042C>T	p.Arg1348Cys	missense	1.1	0.2
	rs148804178	c.205C>G	p.Leu69Val	missense	0.5	0.6
ABCG2	rs45605536	c.1582G>A	p.Ala528Thr	missense	1.1	0.4
	rs2231142	c.421C>A	p.Gln141Lys	missense	6.5	0.2
	rs2231137	c.34G>A	p.Val12Met	missense	6	0.2
	rs1337337886	c.131A>G	p.Tyr44Cys	missense	0.5	0.8
	rs35965584	c.1624A>G	p.Thr542Ala	missense	0.5	0.4
	Novel	c.1453C>A	p.Pro485Thr	missense	0.5	NR
	rs138606116	c.1060G>A	p.Gly354Arg	missense	0.5	0.0
	rs34783571	c.1858G>A	p.Asp620Asn	missense	0.5	0.4
	rs34264773	c.1758A>T	p.Lys586Asn	missense	0.5	0.3
CYP1A2	rs17861157	c.894C>A	p.Ser298Arg	missense	3.3	0.2
	rs45540640	c.613T>G	p.Phe205Val	missense	0.5	1.0
	rs201763966	c.142T>G	p.Trp48Gly	missense	0.5	0.8
	rs758124536	c.409C>T	p.Arg137Trp	missense	0.5	1.0
CYP2C19	rs3758581	c.991G>G	p.Val331Val	missense	43.5	NR
	rs17884712	c.431G>A	p.Arg144His	missense	2.2	0.8
	rs576823729	c.648C>G	p.Cys216Trp	missense	0.5	0.6
	rs17882687	c.55A>C	p.Ile19Leu	missense	0.5	0.0
	rs17878459	c.276G>C	p.Glu92Asp	missense	3.3	0.2
	rs58973490	c.449G>A	p.Arg150His	missense	1.1	0.0
CYP2C8	rs11572103	c.499A>T	p.Ile167Phe	missense	3.3	0.4
	rs10509681	c.890A>G	p.Lys297Arg	missense	4.9	0.2
	rs11572080	c.110G>A	p.Arg37Lys	missense	5.4	0.4
	rs77147096	c.787G>A	p.Gly263Ser	missense	0.5	0.0
	rs1058930	c.486C>G	p.Ile162Met	missense	4.9	0.6
	rs369591911	c.65G>A	p.Arg22Gln	missense	0.5	1.0
	rs143386810	c.844G>A	p.Gly282Ser	missense	0.5	0.8
CYP2C9	rs1799853	c.430C>T	p.Arg144Cys	missense	8.8	1.0
	rs28371685	c.1003C>T	p.Arg335Trp	missense	0.9	0.6
	rs1057910	c.1075A>C	p.Ile359Leu	missense	7.5	0.2
	rs7900194	c.449G>A	p.Arg150His	missense	1.3	0.0
	rs2256871	c.752A>G	p.His251Arg	missense	2.2	0.8
	rs201055266	c.1034T>C	p.Met345Thr	missense	0.4	1.0
	rs28371686	c.1080C>G	p.Asp360Glu	missense	0.4	0.8
	rs9332239	c.1465C>T	p.Pro489Ser	missense	0.4	0.8
CYP2D6	rs16947	c.733T>T	p.Cys245Cys	missense	32.6	NR
	rs769258	c.31G>A	p.Val11Met	missense	4.3	0.0
	rs1058172	c.941G>A	p.Arg314His	missense	4.9	1.0
	rs1065852	c.100C>T	p.Pro34Ser	missense	6	1.0
	Novel	c.551C>T	p.Ala184Val	missense	0.5	
	rs28371717	c.556G>T	p.Ala186Ser	missense	0.5	0.0
	rs28371704	c.281A>G	p.His94Arg	missense	1.6	0.0
	rs28371706	c.320C>T	p.Thr107Ile	missense	2.2	0.0
	rs139779104	c.482G>A	p.Gly161Glu	missense	0.5	0.6
	rs140513104	c.821C>T	p.Pro274Leu	missense	0.5	1.0
	rs59421388	c.859G>A	p.Val287Met	missense	0.5	0.4
	rs61736512	c.406G>A	p.Val136Ile	missense	0.5	0.0
	rs28371703	c.271C>A	p.Leu91Met	missense	1.1	0.6
CYP3A4	rs4986907	c.485G>A	p.Arg162Gln	missense	0.4	0.0
CYP3A5	rs41279857	c.299C>A	p.Ser100Tyr	missense	0.4	0.8
	rs149664815	c.1378C>T	p.Gln460X	stopgain	0.4	1.0
	rs6977165	c.423A>G	p.X141Trp	stoploss	5.7	1.0
	rs145774441	c.827T>C	p.Ile276Thr	missense	0.4	0.6
	rs28383468	c.88C>T	p.His30Tyr	missense	0.4	0.0
	rs147489136	c.608T>G	p.Phe203Cys	missense	0.4	1.0
	rs6957030	c.419T>G	p.Leu140Arg	missense	0.4	0.0
SLC15A1	rs2297322	c.350G>A	p.Ser117Asn	missense	18.5	0.0
	rs8187820	c.364G>A	p.Val122Met	missense	1.6	0.6

Gene	rs code	NT change	AA change	Type	MAF (%)	FPS
	rs8187838	c.1352C>A	p.Thr451Asn	missense	1.6	0.0
	rs4646227	c.1256G>C	p.Gly419Ala	missense	4.3	0.0
	rs2274828	c.1348G>A	p.Val450Ile	missense	0.5	0.0
	Novel	c.800A>T	p.Glu267Val	missense	0.5	NR
	rs8187821	c.351C>A	p.Ser117Arg	missense	0.5	0.0
	rs146304164	c.1246G>C	p.Val416Leu	missense	0.5	0.0
SLC22A1	rs628031	c.1222A>G	p.Met408Val	missense	66.8	0.0
	rs683369	c.480G>C	p.Leu160Phe	missense	85.9	0.0
	rs776304541	c.1406G>A	p.Arg469His	missense	0.5	0.5
	rs34205214	c.1025G>A	p.Arg342His	missense	2.2	0.0
	rs34447885	c.41C>T	p.Ser14Phe	missense	2.2	0.2
	rs41267797	c.1390G>A	p.Val464Ile	missense	4.9	0.0
	rs35270274	c.1463G>T	p.Arg488Met	missense	1.6	0.0
	rs35888596	c.113G>A	p.Gly38Asp	missense	2.2	1.0
	rs34059508	c.1393G>A	p.Gly465Arg	missense	1.1	0.8
	rs2282143	c.1022C>T	p.Pro341Leu	missense	1.1	0.8
	rs12208357	c.181C>T	p.Arg61Cys	missense	3.8	0.6
	rs36103319	c.659G>T	p.Gly220Val	missense	0.5	0.8
	rs78899680	c.1442G>T	p.Gly481Val	missense	0.5	0.3
	rs34130495	c.1201G>A	p.Gly401Ser	missense	0.5	0.8
	rs774654623	c.1396C>A	p.Pro466Thr	missense	0.5	0.0
SLC22A6	rs11568627	c.311C>T	p.Pro104Leu	missense	0.5	0.6
	rs11568626	c.149G>A	p.Arg50His	missense	0.5	0.6
SLC22A8	rs11568481	c.560C>T	p.Ala187Val	missense	0.5	0.0
	rs45438191	c.473T>C	p.Val158Ala	missense	0.5	0.0
SLCO1B1	rs2306283	c.388A>G	p.Asn130Asp	missense	47.4	0.0
	rs4149056	c.521T>C	p.Val174Ala	missense	11	0.8
	rs11045819	c.463C>A	p.Pro155Thr	missense	13.2	0.2
	rs11045852	c.733A>G	p.Ile245Val	missense	0.9	0.2
	rs74064213	c.1495A>G	p.Ile499Val	missense	0.9	0.0
	rs34671512	c.1929A>C	p.Leu643Phe	missense	5.3	0.0
	rs59502379	c.1463G>C	p.Gly488Ala	missense	1.8	0.8
	rs79135870	c.664A>G	p.Ile222Val	missense	0.4	0.0
	rs59113707	c.1200C>G	p.Phe400Leu	missense	0.4	0.0
SLCO1B3	rs4149117	c.250T>G	p.Ser84Ala	missense	76.1	0.2
	rs7311358	c.615G>A	p.Met205Ile	missense	72.8	0.0
	rs57585902	c.355A>G	p.Thr119Ala	missense	1.1	0.0
	rs60140950	c.767G>C	p.Gly228Ala	missense	14.7	1.0
	rs773176181	c.1247G>C	p.Gly416Ala	missense	0.5	0.8
	rs150007972	c.233C>A	p.Thr78Asn	missense	0.5	0.4
	rs61736817	c.1282C>T	p.Leu428Phe	missense	0.5	0.0
	rs76963574	c.1628C>G	p.Ala543Gly	missense	0.5	0.8
	rs115227445	c.592C>G	p.Leu198Val	missense	0.5	0.0
	Novel	c.596G>T	p.Gly199Val	missense	0.5	NR
	rs12299012	c.1595T>C	p.Val532Ala	missense	1.1	0.0
SLCO2B1	rs2306168	c.1025C>T	p.Ser342Phe	missense	6.5	0.0
	rs12422149	c.503G>A	p.Arg168Gln	missense	12	0.2
	rs78825186	c.485G>A	p.Arg162His	missense	1.1	0.0
	rs145875125	c.1206C>A	p.Asn402Lys	missense	0.5	0.0
	rs35199625	c.169G>A	p.Val57Met	missense	1.1	0.4
UGT1A3	rs6431625	c.140T>C	p.Val47Ala	missense	41.8	0.0
	rs28898619	c.342G>A	p.Met114Ile	missense	1.1	0.0
	rs3821242	c.31T>C	p.Trp11Arg	missense	45.7	0.0
	rs61764030	c.473C>T	p.Ala158Val	missense	1.1	0.3
	rs149324549	c.775G>C	p.Gly259Arg	missense	1.1	0.3
	rs45449995	c.808A>G	p.Met270Val	missense	2.2	0.8
	rs61764031	c.523A>T	p.Asn175Tyr	missense	0.5	0.0
	rs140541315	c.172G>A	p.Ala58Thr	missense	0.5	0.0
	rs13406898	c.431C>T	p.Thr144Ile	missense	0.5	0.3

Gene	rs code	NT change	AA change	Type	MAF (%)	FPS
	rs45595237	c.145C>T	p.Arg49Trp	missense	0.5	0.5
UGT2B7	rs7439366	c.802T>C	p.Tyr268His	missense	62	0.3
	rs140153012	c.321A>T	p.Leu107Phe	missense	1.1	0.0
	rs60103519	c.536C>T	p.Thr179Ile	missense	1.1	0.0

AA: amino acid; NT nucleotide; NR: not reported (for variants that did not show any prediction in the 5 algorithms used); FPS: functionality prediction score; MAF: minor allele frequency; PK: pharmacokinetics.

Supplementary table 9 Influence of deleterious variants in PK-related genes on LDL-c reduction in FH patients on statin treatment.

Gene	rs code	NT change	Type	LDL-c reduction (%)			*Adjusted*	Prediction
				Non carriers	Carriers	*p-value*	*p-value*	
All statins								
ABCC1	rs45511401	c.2012G>T	missense	-45.9 ± 20.1 (85)	-64.7 ± 6.4 (7)	*<0.0001*	*0.001*	0.8
ABCC2	rs17216317	c.3872C>T	missense	-48.3 ± 18.9 (86)	-33.6 ± 30.7 (6)	*0.297*	*1.000*	0.8
	rs8187692	c.3542G>T	missense	-47.5 ± 19.9 (87)	-43 ± 23.5 (5)	*0.693*	*0.912*	0.8
ABCC3	rs11568591	c.3890G>A	missense	-47 ± 20.3 (80)	-49.5 ± 18.2 (12)	*0.665*	*0.950*	0.8
CYP2C19	rs17884712	c.431G>A	missense	-47.9 ± 19.8 (88)	-33.5 ± 22.2 (4)	*0.286*	*1.000*	0.8
CYP2C8	rs1058930	c.486C>G	missense	-47.7 ± 20.2 (84)	-43.4 ± 18.1 (8)	*0.548*	*1.000*	0.6
	rs2071426	g.96828323T>C	splicing	-47.7 ± 19.2 (52)	-46.7 ± 21.3 (40)	*0.816*	*0.906*	D
CYP2C9	rs1799853	c.430C>T	missense	-48.8 ± 19.3 (95)	-43.6 ± 19.6 (19)	*0.298*	*1.000*	1.0
	rs2256871	c.752A>G	missense	-47.7 ± 19.4 (109)	-53.4 ± 19.5 (5)	*0.558*	*0.962*	0.8
CYP2D6	rs3892097	g.42524947C>T	splicing	-47.8 ± 19.9 (88)	-36.5 ± 22.3 (4)	*0.387*	*1.000*	D
	rs1058172	c.941G>A	missense	-46.9 ± 19.8 (83)	-51.1 ± 22.9 (9)	*0.608*	*1.000*	1.0
	rs1065852	c.100C>T	missense	-47.2 ± 19.9 (82)	-48.3 ± 22 (10)	*0.875*	*0.931*	1.0
CYP3A5	rs776746	g.99270539C>T	splicing	-57.5 ± 16.7 (7)	-47.4 ± 19.4 (107)	*0.168*	*1.000*	D
	rs41303343	c.035dup	frameshift insertion	-47.8 ± 19.5 (110)	-51.9 ± 15 (4)	*0.634*	*0.990*	D
	rs6977165	c.423A>G	stoploss	-47.8 ± 19.5 (101)	-49.1 ± 18.7 (13)	*0.823*	*0.901*	1.0
SLC15A1	rs8187820	c.364G>A	missense	-47.3 ± 20.2 (89)	-47.1 ± 13.7 (3)	*0.983*	*1.000*	0.6
SLC22A1	rs35888596	c.113G>A	missense	-46.8 ± 20.1 (88)	-59.2 ± 12.9 (4)	*0.147*	*1.000*	1.0
	rs72552763	c.258_1260del	non-frameshift deletion	-48.4 ± 20.2 (60)	-45.3 ± 19.8 (32)	*0.484*	*1.000*	D
	rs12208357	c.181C>T	missense	-47.5 ± 19.9 (86)	-44.5 ± 23 (6)	*0.768*	*0.937*	0.6
SLCO1B1	rs4149056	c.521T>C	missense	-47.5 ± 19.6 (89)	-49.6 ± 18.7 (25)	*0.633*	*1.000*	0.8
	rs59502379	c.1463G>C	missense	-48.1 ± 19.4 (110)	-45.4 ± 20.3 (4)	*0.808*	*0.940*	0.8
SLCO1B3	rs60140950	c.683G767G>C	missense	-46.3 ± 19.3 (69)	-50.4 ± 22.2 (23)	*0.432*	*1.000*	1.0
UGT1A3	rs45449995	c.808A>G	missense	-47.2 ± 20.2 (89)	-50.1 ± 13.5 (3)	*0.753*	*0.942*	0.8
Atorvastatin								
ABCC1	rs45511401	c.2012G>T	missense	-46.3 ± 18.4 (72)	-65.8 ± 6.2 (6)	*0.000*	*0.001*	0.8
ABCC2	rs8187692	c.3542G>T	missense	-48.4 ± 18.1 (73)	-39.6 ± 24.1 (5)	*0.467*	*1.000*	0.8
	rs17216317	c.3872C>T	missense	-48.2 ± 18.3 (73)	-42.7 ± 23.4 (5)	*0.637*	*0.965*	0.8
ABCC3	rs11568591	c.3890G>A	missense	-47.8 ± 18.3 (69)	-47.9 ± 21.3 (9)	*0.988*	*0.988*	0.8
CYP2C19	rs17884712	c.431G>A	missense	-48.6 ± 18.1 (74)	-33.5 ± 22.2 (4)	*0.269*	*1.000*	0.8
CYP2C8	rs2071426	g.96828323T>C	splicing	-46.5 ± 17 (43)	-49.4 ± 20.4 (35)	*0.512*	*1.000*	D
	rs1058930	c.486C>G	missense	-48.1 ± 18.6 (71)	-44.7 ± 19.2 (7)	*0.665*	*0.924*	0.6
CYP2C9	rs1799853	c.430C>T	missense	-48.9 ± 17.8 (81)	-42.8 ± 20.5 (17)	*0.264*	*1.000*	1.0
	rs1799853	c.430C>T	missense	-48.9 ± 17.8 (81)	-42.8 ± 20.5 (17)	*0.264*	*1.000*	1.0
	rs2256871	c.752A>G	missense	-47.6 ± 18.3 (93)	-53.4 ± 19.5 (5)	*0.547*	*1.000*	0.8
CYP2D6	rs3892097	g.42524947C>T	splicing	-48.4 ± 18.3 (74)	-36.5 ± 22.3 (4)	*0.364*	*1.000*	D
	rs1065852	c.100C>T	missense	-48.4 ± 18.7 (69)	-43.6 ± 17.1 (9)	*0.452*	*1.000*	1.0
	rs1058172	c.941G>A	missense	-48.2 ± 18.6 (71)	-43.7 ± 18.5 (7)	*0.553*	*0.988*	1.0

Gene	rs code	NT change	Type	LDL-c reduction (%)			*Adjusted*	Prediction
				Non carriers	Carriers	p-*value*	*p-value*	
CYP3A5	rs776746	NA	splicing	-57.5 ± 16.7 (7)	-47.1 ± 18.3 (91)	*0.160*	*1.000*	D
	rs41303343	c.035dup	frameshift insertion	-47.7 ± 18.3 (95)	-52 ± 20.4 (3)	*0.751*	*0.963*	D
SLC22A1	rs35888596	c.113G>A	missense	-47.2 ± 18.6 (74)	-59.2 ± 12.9 (4)	*0.158*	*1.000*	1.0
	rs12208357	c.181C>T	missense	-47.2 ± 18.5 (75)	-62.3 ± 15.4 (3)	*0.228*	*1.000*	0.6
	rs72552763	c.258_1260de1>l	nonframeshift deletion	-48.5 ± 19.2 (51)	-46.5 ± 17.4 (27)	*0.649*	*0.954*	D
	rs72552763	c.258_1260de1>l	nonframeshift deletion	-48.5 ± 19.2 (51)	-46.5 ± 17.4 (27)	*0.649*	*0.954*	D
SLCO1B1	rs4149056	c.521T>C	missense	-47.2 ± 18.5 (78)	-50.3 ± 17.7 (20)	*0.502*	*1.000*	0.8
	rs59502379	c.1463G>C	missense	-48 ± 18.3 (94)	-45.4 ± 20.3 (4)	*0.815*	*0.927*	0.8
SLCO1B3	rs60140950	c.683G767G>C	missense	-47 ± 17.7 (63)	-51.4 ± 21.8 (15)	*0.473*	*1.000*	1.0
ABCC3	rs11568591	c.3890G>A	missense	-35.8 ± 16.7 (22)	-33.5 ± 23.6 (3)	*0.884*	*0.921*	0.8
CYP2C8	rs2071426	g.96828323T>C	splicing	-36.3 ± 15.2 (15)	-34.3 ± 20.3 (10)	*0.790*	*0.941*	D
CYP2C9	rs1799853	c.430C>T	missense	-37.9 ± 20.2 (30)	-31.2 ± 18.3 (9)	*0.360*	*1.000*	1.0
CYP3A5	rs776746	g.99270539C>T	splicing	-33.8 ± 26 (3)	-36.6 ± 19.6 (36)	*0.870*	*0.946*	D
SLC22A1	rs72552763	c.258_1260de1>l	nonframeshift deletion	-37.4 ± 15.6 (19)	-29.5 ± 21.4 (6)	*0.432*	*1.000*	D
	rs12208357	c.181C>T	missense	-36.4 ± 17.5 (22)	-29.5 ± 14.2 (3)	*0.503*	*1.000*	0.6
SLCO1B1	rs4149056	c.521T>C	missense	-35.4 ± 19.2 (27)	-38.6 ± 21.6 (12)	*0.668*	*0.903*	0.8
SLCO1B3	rs60140950	c.683G767G>C	missense	-37.1 ± 18.4 (20)	-29.3 ± 8.3 (5)	*0.178*	*1.000*	1.0

FH patients carrying the homozygous form of the minor allele (AA) were grouped with the heterozygous carriers (RA) and compared with non-carriers (RR). Continuous variables are shown as mean ± SD and were compared by *t*-test.The p-value was adjusted using the Benjamini-Hochberg correction. *In silico* functionality prediction was performed either using the functionality prediction score (FPS) for missense variants or dbNSFP v4.2 *in silico* algorithm for splice variants. Frameshift variants were considered deleterious. Nonframeshift variants were considered potentially deleterious. NT nucleotide; D: deleterious; FPS: Functionality prediction score; N: neutral.

Supplementary table 10 Influence of variants in PK-related genesgenetic and non-genetic variables on LDL-c reduction in FH patients: Univariate linear regression analysis.

Variant		β	SE	*p-value*	*Adjusted p-value*
Deleterious variants					
*CYP2C19*9* c.431G>A	A allele	14.4	10.2	*0.159*	*0.520*
CYP2C8 c.486C>G	A allele	4.2	7.4	*0.570*	*0.855*
CYP2C8 g.5932A>G	G allele	1	4.2	*0.813*	*0.915*
CYP2C9 c.430C>T	T allele	4.2	7.4	*0.570*	*0.606*
CYP2C9 c.752A>G	G allele	5.2	4.9	*0.286*	*0.859*
CYP2D6 c.941G>A	A allele	-5.7	8.9	*0.525*	*0.862*
CYP2D6 c.100C>T	T allele	-4.2	7.0	*0.551*	*0.940*
CYP2D6 g.6866G>A	A allele	11.3	10.2	*0.270*	*0.608*
CYP3A5 c.624G>A	A allele	-1.2	6.7	*0.862*	*0.912*
CYP3A5 c.423A>G	G allele	-1.3	5.7	*0.827*	*0.924*
*CYP3A5*3* g.12083G>A	A allele	4.6	7.6	*0.684*	*0.504*
UGT1A3 c.808A>G	G allele	-2.9	11.8	*0.182*	*0.504*
ABCC1 c.2012G>T	T allele	-18.8	7.7	***0.016***	***0.096***
ABCC2 c.3872C>T	T allele	14.7	8.3	*0.082*	*0.328*
ABCC2 c.3542G>T	T allele	4.5	9.2	*0.625*	*0.900*
ABCC3 c.3890G>A	A allele	-2.5	6.2	*0.685*	*0.881*
SLC15A1 c.364G>A	A allele	0.2	11.8	*0.987*	*0.987*
SLC22A1 c.181C>T	T allele	3	8.5	*0.726*	*0.901*
SLC22A1 c.113G>A	A allele	-12.5	10.2	*0.224*	*0.538*
SLC22A1 c.1260_1262del	Deletion	3.1	4.4	*0.485*	*0.831*
*SLCO1B1*5* c.521T>C	C allele	-2.1	4.4	*0.641*	*0.888*
SLCO1B1 c.1463G>C	C allele	2.7	9.9	*0.784*	*0.941*
SLCO1B3 c.767G>C	C allele	-4.1	4.8	*0.396*	*0.750*
Treatment					
Baseline LDL-c		-0.1	0.02	***<0.001***	***<0.001***
High intensity treatment		-15.8	5.0	***0.002***	***0.024***
Atorvastatin		-7.3	5.9	*0.218*	*0.561*
Rosuvastatin		-16.4	8.0	***0.043***	*0.193*
Ezetimibe		-8.8	3.7	***0.018***	***0.090***
Drug interactions	CYP3A4 inhibitor	-8.9	6.4	*0.164*	*0.590*
SRAE	Presence	-11.8	4.3	***0.007***	***0.063***
	Myopathy	-11.8	4.7	*0.014*	*0.101*
Reduced adherence		14.4	10.2	*0.159*	*0.919*
Patient characteristics					
Age		0.11	0.1	*0.391*	*0.834*
Gender	Male	-3.3	4.0	*0.413*	*0.834*
Ethnics	Brown	7.2	4.3	*0.099*	*0.317*
	Black	-0.4	5.6	*0.942*	*0.972*
Type 2 diabetes		-2.8	4.5	*0.525*	*0.859*
BMI		1.44	0.4	***<0.001***	***<0.001***
FH-related variant	Carrier	-4.2	3.9	*0.288*	*0.627*

β: linear coefficient; SE: standard error; BMI: body mass index; FH: familial hypercholesterolemia; LDL-c: low-density lipoprotein cholesterol; SRAE: statin-related adverse events. P-value was adjusted using the Benjamini-Hochberg correction.

Supplementary table 11 Influence of deleterious variants (MAF > 1.0%) on LDL-c response to statins in FH patients: Multivariate linear regression analysis

Variant		**n**	**β**	**SE**	***p-value***
CYP2C19 c.431G>A	A allele	92	14.4	8.7	*0.101*
CYP2C8 c.486C>G	A allele	92	-2.2	6.5	*0.737*
CYP2C8 g.5932A>G	G allele	92	0.6	3.7	*0.863*
*CYP2C9*2* c.430C>T	T allele	114	2.3	4.2	*0.595*
*CYP2C9*9* c.752A>G	G allele	114	9	7.5	*0.232*
CYP2D6 c.941G>A	A allele	92	-6.5	6.0	*0.281*
CYP2D6 c.100C>T	T allele	92	-6.4	5.8	*0.272*
CYP2D6 g.6866G>A	A allele	92	0.3	90.	*0.974*
CYP3A5 c.624G>A	A allele	114	-1.3	8.1	*0.873*
CYP3A5 c.423A>G	G allele	114	-2.1	4.9	*0.669*
*CYP3A5*3* g.12083G>A	A allele	114	4.7	6.4	*0.463*
UGT1A3 c.808A>G	G allele	92	-11.3	10.3	*0.274*
ABCC1 c.2012G>T	T allele	92	-11.5	6.7	*0.092*
ABCC2 c.3872C>T	T allele	92	12.2	7.2	*0.095*
ABCC2 c.3542G>T	T allele	92	3.6	8.0	*0.656*
ABCC3 c.3890G>A	A allele	92	-2.0	5.3	*0.710*
SLC15A1 c.364G>A	A allele	92	-11.8	10.3	*0.253*
SLC22A1 c.181C>T	T allele	92	-2.3	7.3	*0.757*
SLC22A1 c.113G>A	A allele	92	-10.3	8.8	*0.247*
SLC22A1 c.1260_1262del	Deletion	92	-1.4	4.0	*0.720*
*SLCO1B1*5* c.521T>C	C allele	114	-3.3	3.7	*0.365*
SLCO1B1 c.1463G>C	C allele	114	3.1	8.1	*0.701*
SLCO1B3 c.767G>C	C allele	92	-6.3	4.3	*0.150*

Each model was adjusted with the following covariates: body mass index, baseline LDL-c, therapy intensity, and presence of SRAE. n: number of patients; β: linear coefficient; SE: standard error; LDL-c: low-density lipoprotein cholesterol; FH: familial hypercholesterolemia; SRAE: statin-related adverse events.

Supplementary table 12 Association of variants in PK-related genes and non-genetic variables with statin response in FH patients: Univariate logistic regression analysis

Variable		RE, % (58)	NRE, % (56)	OR (95%CI)	*p-value*	*Adjusted p-value*
Deleterious variants						
*CYP2C19*9* c.431G>A	A allele	2.2 (1)	6.4 (3)	3.0 (0.4 - 61.9)	*0.349*	*0.785*
CYP2C8 c.486C>G	A allele	4.4 (2)	12.8 (6)	3.1 (0.7 - 22.3)	*0.175*	*0.700*
CYP2C8 g.5932A>G	G allele	44.4 (20)	42.6 (20)	0.9 (0.4 - 2.1)	*0.855*	*0.993*
CYP2C9 c.430C>T	T allele	12.1 (7)	21.4 (12)	2.0 (0.7 - 5.8)	*0.185*	*0.666*
CYP2C9 c.752A>G	G allele	5.2 (3)	3.6 (2)	0.7 (0.1 - 4.3)	*0.678*	*0.939*
CYP2D6 c.941G>A	A allele	11.1 (5)	8.5 (4)	0.7 (0.2 - 3)	*0.676*	*0.973*
CYP2D6 c.100C>T	T allele	11.1 (5)	10.6 (5)	1.0 (0.2 - 3.7)	*0.942*	*1.000*
CYP2D6 g.6866G>A	A allele	2.2 (1)	6.4 (3)	3.0 (0.4 - 61.9)	*0.349*	*0.739*
CYP3A5 c.624G>A	A allele	3.4 (2)	3.6 (2)	1.0 (0.1 - 8.9)	*0.972*	*1.000*
CYP3A5 c.423A>G	G allele	10.3 (6)	12.5 (7)	1.2 (0.4 - 4.1)	*0.718*	*0.909*
*CYP3A5*3* g.12083G>A	A allele	93.1 (54)	94.6 (53)	1.3 (0.3 - 6.9)	*0.733*	*0.880*
UGT1A3 c.808A>G	G allele	4.4 (2)	2.1 (1)	0.5 (0.0 - 5)	*0.541*	*0.885*
ABCC1 c.2012G>T	T allele	15.6 (7)	0.0 (0)	-	-	-
ABCC2 c.3872C>T	T allele	2.2 (1)	10.6 (5)	5.2 (0.8 - 102.6)	*0.138*	*0.475*
ABCC2 c.3542G>T	T allele	4.4 (2)	6.4 (3)	1.5 (0.2 - 11.5)	*0.683*	*0.921*
ABCC3 c.3890G>A	A allele	13.3 (6)	12.8 (6)	1.0 (0.3 - 3.3)	*0.936*	*1.000*
SLC15A1 c.364G>A	A allele	4.4 (2)	2.1 (1)	0.5 (0 - 5)	*0.541*	*0.927*
SLC22A1 c.181C>T	T allele	4.4 (2)	8.5 (4)	2 (0.4 - 15)	*0.437*	*0.874*
SLC22A1 c.113G>A	A allele	6.7 (3)	2.1 (1)	0.3 (0 - 2.5)	*0.311*	*0.746*
SLC22A1 c.1260_1262del	Deletion	31.1 (14)	38.3 (18)	1.4 (0.6 - 3.3)	*0.470*	*0.891*
*SLCO1B1*5* c.521T>C	C allele	24.1 (14)	19.6 (11)	0.8 (0.3 - 1.9)	*0.563*	*0.844*
SLCO1B1 c.1463G>C	C allele	3.4 (2)	3.6 (2)	1 (0.1 - 8.9)	*0.972*	*1.000*
SLCO1B3 c.767G>C	C allele	26.7 (12)	23.4 (11)	0.8 (0.3 - 2.2)	*0.718*	*0.891*
Treatment						
Baseline LDL-c (mg/dL)		275 ± 90	226 ± 61	0.99 (0.98 – 0.99)	***0.002***	***0.024***
High intensity treatment		93.1 (54)	78.6 (44)	0.3 (0.1 - 0.8)	***0.033***	*0.198*
Atorvastatin		77.6 (45)	82.1 (46)	1.3 (0.5 - 3.4)	*0.545*	*0.853*
Rosuvastatin		13.8 (8)	5.4 (3)	0.4 (0.1 - 1.3)	*0.141*	*0.634*
Ezetimibe		46.6 (27)	26.8 (15)	0.4 (0.2 - 0.9)	***0.030***	*0.216*
Drug interaction	CYP3A4 inhibitor	13.2 (7)	6.7 (3)	0.5 (0.1 - 1.8)	*0.295*	*1.000*
SRAE		29.3 (17)	3.6 (2)	0.1 (0 - 0.3)	***0.002***	***0.036***
Myopathy		34.5 (20)	7.3 (4)	0.1 (0 - 0.4)	***0.001***	***0.036***
Reduced adherence		17.2 (10)	14.5 (8)	0.8 (0.3 - 2.2)	*0.696*	*0.895*
Patient characteristics						
Age		53.3 ± 14.9	56.7 ± 13.9	1.0 (0.99 - 1.04)	*0.215*	*0.645*
Male gender		31.0 (18)	25.0 (14)	0.7 (0.3 - 1.7)	*0.474*	*0.853*
Ethnics	Brown + black	46.6 (27)	58.9 (33)	1.5 (0.7 - 3.4)	*0.288*	*0.741*
BMI (kg/cm²)		26.9 ± 3.5	29.2 ± 5.0	1.1 (1.04 - 1.26)	***0.009***	***0.081***
Type 2 diabetes		25.9 (15)	16.7 (9)	0.6 (0.2 - 1.4)	*0.220*	*0.660*
FH-related variant	Carrier	34.5 (20)	26.8 (15)	0.7 (0.3 - 1.5)	*0.374*	*0.792*

Number of patients in round brackets. Categorical variables are expressed as percentage and number between brackets. Continuous variables are expressed as mean and standard deviation. P-value was adjusted using the Benjamini-Hochberg correction with a FDR of 10%. NRE: non-responder; RE: responder; OR: odds ratio; CI: confidence interval; BMI: body mass index; FH: familial hypercholesterolemia; LDL-c: low-density lipoprotein cholesterol; NR: not reported (No patients in NRE group); SRAE: statin-related adverse events.

Supplementary table 13 Association of variants in PK-related genes and non-genetic variables with SRAE in FH patients: Univariate logistic regression analysis.

Variant		No SRAE (n= 89)	SRAE (n=24)	OR (95%CI)	*p-value*	*Adjusted p-value*
Deleterious variants						
CYP2C8 c.486C>G	A allele	45.5 (35)	35.7 (5)	0.7 (0.2 - 2.1)	*0.501*	*0.895*
*CYP2C9*2* c.430C>T	T allele	16.9 (15)	12.5 (3)	0.7 (0.2 - 2.4)	*0.606*	*0.947*
*CYP2C9*3* c.752A>G	G allele	2.2 (2)	12.5 (3)	6.2 (1 - 49.5)	*0.053*	*0.331*
CYP3A5 c.624G>A	A allele	3.4 (3)	4.2 (1)	1.2 (0.1 - 10.3)	*0.852*	*0.926*
CYP3A5 c.423A>G	G allele	11.2 (10)	12.5 (3)	1.1 (0.2 - 4.1)	*0.863*	*0.932*
*CYP3A5*3* g.12083G>A	A allele	93.3 (83)	95.8 (23)	1.7 (0.3 - 32.2)	*0.646*	*0.950*
ABCC1 c.2012G>T	T allele	6.5 (5)	14.3 (2)	2.4 (0.3 - 12.6)	*0.327*	*0.743*
ABCC2 c.3872C>T	T allele	5.2 (4)	14.3 (2)	3.0 (0.4 - 17.5)	*0.227*	*0.568*
ABCC2 c.3542G>T	T allele	5.2 (4)	7.1 (1)	1.4 (0.1 - 10.5)	*0.770*	*1.000*
ABCC3 c.3890G>A	A allele	13 (10)	14.3 (2)	1.1 (0.2 - 5)	*0.895*	*0.932*
SLC22A1 c.113G>A	A allele	3.9 (3)	7.1 (1)	1.9 (0.1 - 16.2)	*0.591*	*0.985*
SLC22A1 c.1260_1262del	Deletion	37.7 (29)	14.3 (2)	0.3 (0 - 1.1)	*0.107*	*0.446*
*SLCO1B1*5* c.521T>C	C allele	21.3 (19)	25 (6)	1.2 (0.4 - 3.4)	*0.702*	*0.975*
SLCO1B1 c.1463G>C	C allele	3.4 (3)	4.2 (1)	1.2 (0.1 - 10.3)	*0.852*	*0.968*
SLCO1B3 c.767G>C	C allele	26 (20)	14.3 (2)	0.5 (0.1 - 1.9)	*0.356*	*0.742*
Treatment						
Baseline LDL-c (mg/dL)		240 ± 75	296 ± 90	1 (1.00 - 1.01)	***0.004***	***0.033****
High intensity treatment		84.3 (75)	91.7 (22)	2.1 (0.5 - 13.7)	*0.365*	*0.702*
Atorvastatin		83.1 (74)	66.7 (16)	0.4 (0.1 - 1.2)	*0.081*	*0.405*
Rosuvastatin		7.9 (7)	16.7 (4)	2.3 (0.6 - 8.6)	*0.207*	*0.575*
Ezetimibe		32.6 (29)	50 (12)	2.1 (0.8 - 5.2)	*0.119*	*0.425*
Drug interaction	CYP3A4 inhibitor	5.6 (5)	20.8 (5)	4.4 (1.1 - 17.4)	***0.029***	*0.196*
Reduced adherence		10.1 (9)	37.5 (9)	5.3 (1.8 - 16)	***0.002***	*0.050*
Patient characteristics						
Age		55.1 ± 14.4	55.9 ± 13.6	1 (0.97- 1.03)	*0.822*	*1.027*
Gender	Male	27 (24)	29.2 (7)	1.1 (0.4 - 2.9)	*0.830*	*0.988*
Ethnics	Brown + Black	46.1 (35)	45.8 (11)	1.0 (0.4 - 2.5)	*0.985*	*0.985*
BMI		28.4 ± 4.7	26.8 ± 3.4	0.9 (0.81 - 1.02)	*0.134*	*0.419*
FH-related variant	Carrier	22.5 (20)	54.2 (13)	4.1 (1.6 - 10.7)	***0.004***	*0.050*

Number of patients in round brackets. P-value was adjusted using the Benjamini-Hochberg correction. NRE: non-responder; RE: responder; OR: odds ratio; CI: confidence interval; BMI: body mass index; FH: familial hypercholesterolemia; LDL-c: low-density lipoprotein cholesterol; NR: not reported (No patients in NRE group); SRAE: statin-related adverse events.

Supplementary table 14 Variants in PD-related genes identified in FH patients (n=114).

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
ABCA1	rs200463326	c.*1466delT		3'UTR	21.9	N	*0.002*
	rs2066718	c.2311G>A	p.Val771Met	missense	9.2	N	*0.049*
	rs2230806	c.656G>A	p.Arg219Lys	missense	35.1	N	*0.684*
	rs2230808	c.4760A>G	p.Lys1587Arg	missense	63.6	D	*0.690*
	rs363717	c.*1896G>A		3'UTR	80.7	N	*0.006*
	rs557492263	c.*321delT		3'UTR	43.9	N	*<0.001*
	rs769705621			splicing	21.2	D	*1.000*
	rs769705621			splicing	7.9	D	*0.000*
	rs1799777	c.-76_-75insG		5'UTR	16.2	N	*0.733*
	rs1800977	c.-24490C>T		5'UTR	37.7	N	*0.549*
	rs1800978	c.-18G>C		5'UTR	15.8	N	*0.735*
	rs73517870	c.*395T>A		3'UTR	7	N	*0.432*
	Novel			splicing	9.6	N	*0.595*
	rs115059464	c.*2213T>C		3'UTR	0.4	N	*1.000*
	rs200463326	c.*1466delT		3'UTR	21.9	N	*0.002*
	rs2066714	c.2649A>G	p.Ile883Met	missense	22.4	N	*0.185*
	rs41432545	c.*1653T>A		3'UTR	6.6	N	*0.389*
	rs77663187	g.107556811del		splicing	7.0	N	*1.000*
	rs142039624	c.*738G>T		3'UTR	0.4	N	*1.000*
	rs4149338	c.*693C>T		3'UTR	32	N	*0.137*
	rs4149339	c.*1440C>T		3'UTR	30.7	N	*0.126*
	rs4149340	c.*1911C>T		3'UTR	3.9	N	*1.000*
	rs769705621	g.107556792_107556793insA		splicing	9.3	D	*0.779*
	rs769705621	g.107556792_107556793insAA		splicing	21.2	D	*1.000*
	rs769705621	g.107556792_107556793insAAA		splicing	22.0	D	*1.000*
	rs769705621	g.107556792_107556793insAAAA		splicing	7.9	D	*1.000*
	rs769705621	g.107556792_107556793insAAAAA		splicing	3.5	D	*1.000*
	rs769705621	g.107556792_107556793insAAAAAA		splicing	3.5	D	*1.000*
	rs769705621	g.107556792_107556793insAAAAAAA		splicing	3.5	D	*1.000*
	rs779989235	c.*1466_*1465delTT		3'UTR	11.7	N	*0.355*
	rs4149341	c.*2311A>G		3'UTR	11	N	*0.355*
	rs2066718	c.2311G>C	p.Val771Leu	missense	0.4	N	*1.000*
	rs75141626	c.*2705G>A		3'UTR	2.2	N	*1.000*
	rs763013834	c.*321_*322insT		3'UTR	1.8	N	*1.000*
	Novel	c.*320_*321insT		3'UTR	20.6	N	*0.003*
	Novel	c.*1464delT		3'UTR	18.9	N	*0.012*
	Novel	c.*1465_*1464delTT		3'UTR	18.9	N	*0.012*
	Novel	c.*1466_*1464delTTT		3'UTR	23	N	*0.006*
	rs373974758	c.*2899_*2897delGTT		3'UTR	6.6	N	*0.389*
	rs35207495	c.2602G>A	p.Glu868Lys	missense	0.4	D	*1.000*
	Novel	c.2673A>T		missense	0.4	N	*1.000*
	Novel	c.*1465_*1466insT		3'UTR	9.2	N	*0.595*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs41474449	c.*2899_*2897delGTT		3'UTR	7.5	N	*0.475*
	rs74316246	c.*96T>C		3'UTR	0.9	N	*1.000*
	rs771296481	c.*1466delT		3'UTR	9.2	N	*0.595*
	rs33918808	c.3516G>C	p.Glu1172Asp	missense	5.7	N	*0.304*
	rs9282541	c.688C>T	p.Arg230Cys	missense	1.8	D	*1.000*
	Novel	c.*1466_*1467insT		3'UTR	1.8	N	*1.000*
	rs41436749	c.3763A>C	p.Ser1255Arg	missense	0.4	D	*1.000*
	rs567793069	c.-24380A>G		5'UTR	0.4	N	*1.000*
	rs10991377	c.*3220A>G		3'UTR	2.6	N	*1.000*
	rs35819696	c.2320A>C	p.Thr774Pro	missense	0.4	D	*1.000*
	rs1033439149	c.3316C>T	p.His1106Tyr	missense	0.4	D	*1.000*
	rs9282543	c.1196T>C	p.Val399Ala	missense	0.9	N	*1.000*
	rs115216814	c.634T>A	p.Ser212Thr	missense	0.9	N	*1.000*
	rs77877520	c.*3083T>C		3'UTR	0.4	N	*1.000*
	rs111292742	c.-24442C>G		5'UTR	1.3	N	*1.000*
	rs2066715	c.2473G>A	p.Val825Ile	missense	3.5	N	*1.000*
	rs148080589	c.*3251T>C		3'UTR	0.4	N	*1.000*
	Novel	c.6197T>G		missense	0.4	D	*1.000*
	rs143180998	c.3544G>A	p.Ala1182Thr	missense	0.4	D	*1.000*
	rs563665817	c.2419G>A	p.Asp807Asn	missense	0.4	N	*1.000*
	rs34879708	c.6729C>A	p.Asp2243Glu	missense	0.4	N	*1.000*
	rs78086474	c.-24368G>T		5'UTR	0.9	N	*1.000*
	rs41437944	c.*1923A>C		3'UTR	0.4	N	*1.000*
	Novel	c.*321_*320delTT		3'UTR	1.3	N	*1.000*
	rs147743782	c.4022G>C	p.Arg1341Thr	missense	0.4	N	*1.000*
	rs940819544	c.*2737T>C		3'UTR	0.4	N	*1.000*
	Novel	c.*1795_*1792delTACT		3'UTR	0.4	N	*1.000*
ABCG1	rs1044317	c.*399A>G		3'UTR	50.5	N	*0.053*
	rs368753152	c.-40_-35del-		5'UTR	8.2	N	*1.000*
	rs55913235	c.*232_*233insT		3'UTR	13.7	N	*0.355*
	rs9975490	c.-16C>G		5'UTR	12	N	*0.595*
	rs1044317	c.*399A>G		3'UTR	50.5	N	*0.053*
	Novel	c.*786T>C		3'UTR	0.5	N	*1.000*
	rs55913235	c.*232_*233insTT		3'UTR	6.6	N	*1.000*
	rs765506549	c.-40_-38del-		5'UTR	2.7	N	*1.000*
	rs56292133	c.*81C>T		3'UTR	1.1	N	*1.000*
	rs765329833	c.-41_-40insCCGCCG		5'UTR	1.6	N	*1.000*
	rs79961376	c.-78G>A		5'UTR	2.2	N	*1.000*
	rs55913235	c.*232_*233insTTT		3'UTR	4.1	N	*1.000*
	rs547649704	c.*175G>A		3'UTR	1.1	N	*1.000*
	rs369739888	c.*233delT		3'UTR	11.2	N	*1.000*
	rs145032173	c.*56A>G		3'UTR	0.5	N	*1.000*
	rs115605747	c.-52C>T		5'UTR	1.1	N	*1.000*
	rs77603571	g.43627101G>A		splicing	0.5	N	*1.000*
	rs56337741	c.*52G>A		3'UTR	3.8	N	*1.000*
	Novel	c.*233_*234insT		3'UTR	9.8	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	Novel	c.*233_*234insTT		3'UTR	9.8	N	*1.000*
	Novel	c.*233_*234insTTT		3'UTR	9.8	N	*1.000*
	Novel	c.*233_*234insTTTT		3'UTR	9.8	N	*1.000*
	rs66675434	c.*232_*233insTT		3'UTR	7.1	N	*1.000*
	rs66675434	c.*232_*233insTTT		3'UTR	7.1	N	*1.000*
	Novel	c.-37_-35del-		5'UTR	1.1	N	*1.000*
	rs1569204516	c.-35_-34insCCGCCG		5'UTR	1.1	N	*1.000*
	rs145298932	c.-64G>A		5'UTR	0.5	N	*1.000*
	rs1220835545	c.34A>G	p.Lys12Glu	missense	0.5	N	*1.000*
	rs138797997	c.-62G>T		5'UTR	0.5	N	*1.000*
	rs144780823	c.71C>T	p.Thr24Met	missense	0.5	D	*1.000*
	rs541178113	c.-42C>T		5'UTR	0.5	N	*1.000*
	rs558008801	c.-91C>T		5'UTR	0.5	N	*1.000*
	rs115417708	c.-18732T>A		5'UTR	0.5	N	*1.000*
	rs142204098	c.-20C>T		5'UTR	0.5	N	*1.000*
ABCG4	rs71482170	c.-799G>A		5'UTR	34.2	N	*0.347*
	rs71482170	c.-799G>A		5'UTR	34.2	N	*0.347*
	rs398017754	c.*25_*26insC		3'UTR	42.4	N	*0.040*
	rs3802885	c.*874A>C		3'UTR	12	N	*1.000*
	rs796346631	c.-356_-355del-		5'UTR	5.4	N	*0.186*
	rs12271907	c.1035C>G	p.Asn345Lys	missense	3.8	D	*0.090*
	rs55659437	c.-356_-355del-		5'UTR	6	N	*0.018*
	rs1323608174	c.-785G>A		5'UTR	0.5	N	*1.000*
	rs73564404	c.*1495G>C		3'UTR	1.6	N	*1.000*
	rs35060365	c.1055C>T	p.Pro352Leu	missense	1.1	D	*1.000*
ABCG5	rs2278356	c.*380T>G		3'UTR	42.1	N	*0.847*
	rs2278357	c.*416G>A		3'UTR	20.2	N	*0.239*
	rs6720173	c.1810C>G	p.Gln604Glu	missense	19.3	N	*0.239*
	rs2278356	c.*380T>G		3'UTR	42.1	N	*0.847*
	rs4148195	c.*622C>T		3'UTR	22.8	N	*0.595*
	rs78070897	c.785A>G	p.Lys262Arg	missense	2.2	N	*1.000*
	rs77105521	c.*522G>A		3'UTR	16.2	N	*0.490*
	rs141828689	c.593G>A	p.Arg198Gln	missense	0.4	D	*1.000*
	rs6756629	c.148C>T	p.Arg50Cys	missense	6.1	D	*1.000*
	Novel	c.1390A>C		missense	0.4	N	*1.000*
	rs192476318	c.*540C>T		3'UTR	0.4	N	*1.000*
	rs55853083	c.-118A>C		5'UTR	0.9	N	*1.000*
	rs376797531	c.431T>C	p.Val144Ala	missense	0.4	D	*1.000*
	rs77265083	c.*72G>A		3'UTR	0.9	N	*1.000*
	rs79475203	c.*399C>T		3'UTR	1.3	N	*0.013*
	rs144452054	c.*219delT		3'UTR	0.4	N	*1.000*
	rs1014472511	c.*324A>G		3'UTR	0.4	N	*1.000*
	rs145241042	c.1304T>C	p.Met435Thr	missense	0.4	D	*1.000*
	rs140374206	c.1864A>G	p.Met622Val	missense	1.3	N	*1.000*
	rs17031672	c.1550C>G	p.Thr517Ser	missense	1.8	N	*0.026*
	rs72542426	c.139G>T	p.Val47Phe	missense	0.4	D	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs139045335	c.*219delT		3'UTR	0.4	N	*1.000*
	rs575195880	c.*367G>A		3'UTR	0.4	N	*1.000*
ABCG8	rs6544718	c.1895T>C	p.Val632Ala	missense	83.8	N	*0.490*
	rs11887534	c.55G>C	p.Asp19His	missense	6.1	D	*1.000*
	rs6544718	c.1895T>C	p.Val632Ala	missense	83.8	N	*0.490*
	rs3806471	c.-19T>G		5'UTR	28.5	N	*1.000*
	rs4148211	c.161A>G	p.Tyr54Cys	missense	30.3	D	*0.658*
	rs4148217	c.1199C>A	p.Thr400Lys	missense	23.2	N	*0.792*
	rs370422066	c.1476T>A	p.Tyr492X	stopgain	0.4	D	*1.000*
	rs80025980	c.239G>A	p.Cys80Tyr	missense	1.3	D	*1.000*
	rs9282574	c.628G>A	p.Val210Met	missense	0.9	D	*1.000*
	rs142250628	c.154C>G	p.Leu52Val	missense	0.4	D	*1.000*
	rs72647315	c.-15A>C		5'UTR	1.8	N	*1.000*
	rs9282573	c.1963A>G	p.Met655Val	missense	0.4	N	*1.000*
	rs144200355	c.1201A>T	p.Thr401Ser	missense	0.4	N	*1.000*
	rs137852991	c.1234C>T	p.Arg412X	stopgain	0.4	D	*1.000*
	rs148370122	c.94A>G	p.Ser32Gly	missense	0.4	N	*1.000*
APOA2	rs6413453	g.161192316G>A		splicing	5.3	N	*1.000*
APOA4	rs35211609	c.*71_*68delTGTC		3'UTR	21.5	N	*0.000*
	rs5104	c.440G>A	p.Ser147Asn	missense	74.1	N	*0.231*
	rs675	c.1099A>T	p.Thr367Ser	missense	17.5	N	*0.518*
	rs35211609	c.*71_*68delTGTC		3'UTR	21.5	N	*0.000*
	rs146353487	c.1057T>G	p.Ser353Ala	missense	0.4	N	*1.000*
	rs5091	c.-98G>A		5'UTR	2.2	N	*1.000*
	rs539176882	c.145_1146insACAGCAGCAGG1>A	p.Glu382delinsEQQQGlu	nonframeshift insertion	1.3	LD	*1.000*
	rs675	c.1099A>G	p.Thr367Ala	missense	7	N	*0.432*
	rs9282602	c.*71_*68delTGTC		3'UTR	12.7	N	*0.000*
	rs5110	c.1140G>T	p.Gln380His	missense	3.5	N	*0.119*
	rs746344058	c.461G>A	p.Arg154Gln	missense	0.4	N	*1.000*
	rs775236625	c.689C>T	p.Thr230Met	missense	0.4	N	*1.000*
	rs142050734	c.598C>T	p.Arg200Cys	missense	0.4	D	*1.000*
	rs12721041	c.37G>A	p.Val13Met	missense	2.2	D	*1.000*
	rs12721043	c.481G>T	p.Ala161Ser	missense	0.9	N	*1.000*
	rs12721040	c.*103C>T		3'UTR	0.4	N	*1.000*
	rs755577773	c.334C>T	p.Arg112Trp	missense	0.4	D	*1.000*
	rs1181852696	c.533C>T	p.Ser178Leu	missense	0.4	N	*1.000*
APOA5	rs2266788	c.*158C>T		3'UTR	90.4	N	*0.000*
	rs651821	c.-3G>A		5'UTR	86	N	*0.457*
	rs2266788	c.*158C>T		3'UTR	90.4	N	*0.000*
	rs619054	c.*31C>T		3'UTR	19.3	N	*0.562*
	rs889100545	c.*418A>G		3'UTR	0.4	N	*1.000*
	rs148759216	c.*289_*290insAG		3'UTR	3.1	N	*1.000*
	rs3135507	c.457G>A	p.Val153Met	missense	4.4	N	*1.000*
	rs33984246	c.*394T>C		3'UTR	5.3	N	*1.000*
	rs34089864	c.*76C>T		3'UTR	3.9	N	*1.000*
	rs45596738	c.*289_*290insAG		3'UTR	2.2	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs3135506	c.56C>G	p.Ser19Trp	missense	11.8	N	*0.652*
	rs114627122	c.*172C>T		3'UTR	0.4	N	*1.000*
	rs34282181	c.111C>A	p.Asp37Glu	missense	0.4	N	*1.000*
	rs2075291	c.553G>T	p.Gly185Cys	missense	0.4	D	*1.000*
	rs186726407	c.-539C>T		5'UTR	0.9	N	*1.000*
	rs45611741	c.-552G>A		5'UTR	0.4	N	*1.000*
	rs143292359	c.944C>T	p.Ala315Val	missense	0.4	D	*1.000*
APOB	rs1042031	c.12541G>A	p.Glu4181Lys	missense	17.1	N	*0.520*
	rs1042034	c.13013G>A	p.Ser4338Asn	missense	76.8	N	*0.016*
	rs1367117	c.293C>T	p.Thr98Ile	missense	32	D	*0.290*
	rs584542	c.6937A>G	p.Ile2313Val	missense	94.3	N	*0.000*
	rs679899	c.1853C>T	p.Ala618Val	missense	37.3	D	*0.550*
	rs1801701	c.10913G>A	p.Arg3638Gln	missense	14.5	N	*1.000*
	rs61744153	c.11477C>T	p.Thr3826Met	missense	0.4	D	*1.000*
	rs12714192	c.2222C>A	p.Thr741Asn	missense	2.2	N	*1.000*
	rs61736761	c.3634C>A	p.Leu1212Met	missense	3.1	N	*1.000*
	rs6752026	c.433C>T	p.Pro145Ser	missense	2.2	D	*1.000*
	rs1042023	c.10294C>G	p.Gln3432Glu	missense	0.9	N	*1.000*
	rs1801702	c.12809G>C	p.Arg4270Thr	missense	6.6	N	*1.000*
	rs61742331	c.10061C>G	p.Ala3354Gly	missense	0.9	N	*1.000*
	rs61744288	c.10780T>C	p.Trp3594Arg	missense	0.9	D	*1.000*
	rs12714225	c.1223T>C	p.Ile408Thr	missense	0.4	N	*1.000*
	rs1800480	c.-115C>G		5'UTR	0.9	N	*1.000*
	rs676210	c.8216C>T	p.Pro2739Leu	missense	19.3	D	*0.562*
	rs977664488	c.4830G>T	p.Arg1610Ser	missense	0.4	D	*1.000*
	rs1801695	c.13441G>A	p.Ala4481Thr	missense	3.9	N	*0.007*
	rs12713675	c.7367C>A	p.Ala2456Asp	missense	2.2	D	*1.000*
	rs12720855	c.9880T>C	p.Ser3294Pro	missense	2.2	D	*1.000*
	rs61743299	c.12697T>A	p.Ser4233Thr	missense	2.2	N	*1.000*
	rs72654423	c.12940A>G	p.Ile4314Val	missense	0.4	N	*1.000*
	rs1801699	c.5741A>G	p.Asn1914Ser	missense	4.4	D	*1.000*
	rs181737266	c.434C>T	p.Pro145Leu	missense	0.4	D	*1.000*
	rs12713450	c.13451C>T	p.Thr4484Met	missense	1.8	N	*1.000*
	rs144034290	c.4187T>C	p.Val1396Ala	missense	0.4	N	*1.000*
	rs140877474	c.4375A>G	p.Ser1459Gly	missense	0.4	D	*1.000*
	rs17240441	c.5_43de3>l	p.12_15del	nonframeshift deletion	2.2	N	*1.000*
	rs12691202	c.2188G>A	p.Val730Ile	missense	1.8	N	*1.000*
	rs886055597	c.-71C>T		5'UTR	0.4	N	*1.000*
	rs61743502	c.12794T>C	p.Val4265Ala	missense	0.9	N	*1.000*
	rs12720854	c.9835A>G	p.Ser3279Gly	missense	0.9	N	*1.000*
	rs533617	c.5768A>G	p.His1923Arg	missense	1.3	D	*1.000*
	rs562574661	c.3480_13482de1>l	p.Gln4494del	nonframeshift deletion	0.4	N	*1.000*
	rs767810570	c.4274C>T	p.Ser1425Phe	missense	0.4	D	*1.000*
	rs761311695	c.5743G>A	p.Gly1915Arg	missense	0.4	D	*1.000*
	rs1801703	c.12382G>A	p.Val4128Met	missense	0.9	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs12714097	c.2630C>T	p.Pro877Leu	missense	0.4	D	*1.000*
	rs752149683	c.2950G>A	p.Ala984Thr	missense	0.4	D	*1.000*
	rs12713540	c.11401T>A	p.Ser3801Thr	missense	0.4	N	*1.000*
	rs766573431	c.1910A>G	p.Tyr637Cys	missense	0.4	D	*1.000*
	rs377429190	c.6223G>A	p.Glu2075Lys	missense	0.4	N	*1.000*
	rs72654430	c.*229A>G		3'UTR	0.4	N	*1.000*
	rs371224295	c.6125T>C	p.Met2042Thr	missense	0.4	N	*1.000*
APOC1	rs12721054	c.*100A>G		3'UTR	1.8	N	*1.000*
	rs72654453	c.48C>G	p.Ile16Met	missense	0.9	N	*1.000*
	rs1064725	c.*74T>G		3'UTR	1.8	N	*0.026*
	rs12721054	c.*100A>G		3'UTR	1.8	N	*1.000*
APOC2	rs148343756	c.8C>T	p.Thr3Ile	missense	0.4	D	*1.000*
	rs74500990	g.45451954G>C		splicing	0.4	N	*1.000*
	rs5126	c.229A>C	p.Lys77Gln	missense	0.9	D	*1.000*
APOC3	rs4225	c.*71G>T		3'UTR	45.2	N	*0.708*
	rs5128	c.*40G>C		3'UTR	83.8	N	*0.000*
	rs4225	c.*71G>T		3'UTR	45.2	N	*0.708*
	rs187628630	c.*139C>G		3'UTR	0.9	N	*1.000*
	rs897418559	c.-659A>T		5'UTR	0.4	N	*1.000*
	rs138326449	g.116701354G>A		splicing	0.9	D	*1.000*
APOC4	rs1132899	c.107T>C	p.Leu36Pro	missense	50	N	*1.000*
	rs5167	c.287T>G	p.Leu96Arg	missense	36.4	N	*0.046*
	rs1132899	c.107T>C	p.Leu36Pro	missense	50	N	*1.000*
	rs12691089	c.155G>A	p.Gly52Asp	missense	0.9	N	*1.000*
APOE	rs429358	c.388T>C	p.Cys130Arg	missense	12.3	N	*0.370*
	rs7412	c.526C>T	p.Arg176Cys	missense	2.6	D	*1.000*
	rs267606661	c.805C>G	p.Arg269Gly	missense	0.4	D	*1.000*
	rs121918396	c.683G>A	p.Trp228X	stopgain	0.4	D	*1.000*
CETP	rs1801706	c.*84G>A		3'UTR	12.3	N	*1.000*
	rs5882	c.1084G>A	p.Val362Ile	missense	61	N	*1.000*
	rs34065661	c.44C>G	p.Ala15Gly	missense	1.8	N	*1.000*
	rs1801706	c.*84G>A		3'UTR	12.3	N	*1.000*
	rs34716057	c.460C>T	p.Arg154Trp	missense	0.9	D	*1.000*
	rs770008221	c.1004A>G	p.Lys335Arg	missense	0.4	D	*1.000*
	rs5880	c.988G>C	p.Ala330Pro	missense	5.7	D	*1.000*
	rs1800777	c.1223G>A	p.Arg408Gln	missense	3.9	N	*1.000*
	rs34855278	c.973G>A	p.Val325Met	missense	0.4	N	*1.000*
	rs1331344801	c.*25G>A		3'UTR	0.9	N	*1.000*
CLMN	Novel	c.*2581_*2580delTT		3'UTR	3.5	N	*1.000*
	rs540351557	c.*157_*158insT		3'UTR	32	N	*0.000*
	rs1054195	c.*9244G>T		3'UTR	46.1	N	*0.850*
	rs3829946	c.*6312A>G		3'UTR	46.1	N	*0.850*
	rs8005908	c.*5776A>G		3'UTR	25.4	N	*0.805*
	rs1054196	c.*9477A>C		3'UTR	42.5	N	*0.443*
	rs142407833	c.*5989G>A		3'UTR	1.3	N	*1.000*
	rs45492302	c.*2125T>G		3'UTR	17.5	N	*0.332*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs561763514	c.*2581_*2582insT		3'UTR	11.8	N	*0.359*
	rs779597760	c.*142_*143insG		3'UTR	16.2	N	*0.041*
	rs879180742	c.*2581delT		3'UTR	12.8	N	*0.209*
	rs5810715	g.95670813del		splicing	16.7	N	*0.015*
	rs116472671	c.*1773G>A		3'UTR	2.6	N	*1.000*
	rs12893595	c.*8498C>T		3'UTR	12.3	N	*1.000*
	rs57061680	c.*8856C>T		3'UTR	12.3	N	*1.000*
	rs66493670	c.*8111G>A		3'UTR	20.6	N	*0.779*
	rs8020060	c.*6283G>A		3'UTR	7	N	*1.000*
	rs75541050	c.*3810C>G		3'UTR	2.6	N	*1.000*
	Novel	c.*2580_*2581insT		3'UTR	12.7	N	*0.209*
	Novel	c.*157_*158insTT		3'UTR	17.1	N	*0.040*
	Novel	c.*2581delT		3'UTR	12.7	N	*0.209*
	Novel	c.*9401delA		3'UTR	0.4	N	*1.000*
	rs148497172	c.735A>C	p.Glu245Asp	missense	0.4	D	*1.000*
	rs11844624	c.*4973G>C		3'UTR	23.2	N	*0.792*
	rs17091868	c.*9118C>T		3'UTR	6.6	N	*0.389*
	rs3814816	c.*6136C>T		3'UTR	6.6	N	*0.389*
	rs61217816	c.*6854A>G		3'UTR	6.6	N	*0.389*
	rs779597760	c.*143T>G		3'UTR	6.6	N	*1.000*
	rs114567749	c.*5635T>C		3'UTR	1.8	N	*1.000*
	rs116779805	c.*3047A>G		3'UTR	1.8	N	*1.000*
	rs116794212	c.*4201A>C		3'UTR	2.2	N	*1.000*
	rs61750771	c.2698A>T	p.Ile900Phe	missense	1.8	D	*1.000*
	rs7155470	c.*2956T>C		3'UTR	6.1	N	*0.346*
	rs7156866	c.*2992A>G		3'UTR	6.1	N	*0.346*
	rs111596735	c.*9128T>C		3'UTR	4.8	N	*0.224*
	rs112366105	c.*5194C>A		3'UTR	3.5	N	*0.119*
	rs7155222	c.*3121T>C		3'UTR	3.9	N	*0.151*
	rs149843418	c.*1330A>G		3'UTR	1.8	N	*1.000*
	rs768809029	c.2882A>G	p.His961Arg	missense	0.4	N	*1.000*
	Novel	c.*2581_*2580delTT		3'UTR	3.5	N	*1.000*
	rs75063901	c.*5035G>A		3'UTR	2.6	N	*1.000*
	rs779597760	c.*142_*143insGT		3'UTR	1.8	N	*1.000*
	rs954588181	c.*3408G>T		3'UTR	0.4	N	*1.000*
	rs61976556	c.*5174C>G		3'UTR	2.6	N	*1.000*
	rs1032856681	c.*50T>G		3'UTR	0.4	N	*1.000*
	rs1896559413	c.*70T>G		3'UTR	0.4	N	*1.000*
	rs10149705	c.2888C>T	p.Pro963Leu	missense	0.9	D	*1.000*
	rs13379182	c.*3160T>C		3'UTR	2.6	N	*1.000*
	rs28707051	c.*1405A>G		3'UTR	3.1	N	*1.000*
	rs55869249	c.*8832C>T		3'UTR	3.5	N	*1.000*
	rs56817762	c.*9223G>C		3'UTR	3.5	N	*1.000*
	rs59940917	c.*7946G>A		3'UTR	0.9	N	*1.000*
	rs7157746	c.*8516A>C		3'UTR	3.5	N	*1.000*
	rs73333229	c.*1025C>G		3'UTR	0.9	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs74079240	c.*5293C>T		3'UTR	0.4	N	*1.000*
	Novel	c.*648_*649insT		3'UTR	0.4	N	*1.000*
	rs547591026	c.*5470C>T		3'UTR	0.4	N	*1.000*
	rs35010297	c.1069G>A	p.Glu357Lys	missense	0.9	D	*1.000*
	rs56119341	c.*985A>G		3'UTR	1.8	N	*1.000*
	rs58411401	c.*2581_*2582insT		3'UTR	0.4	N	*1.000*
	rs143029831	c.*505G>A		3'UTR	0.4	N	*1.000*
	rs143612295	c.*3226C>T		3'UTR	0.9	N	*1.000*
	rs144692084	c.*7537C>A		3'UTR	0.9	N	*1.000*
	rs199567429	c.*7234_*7235insT		3'UTR	0.4	N	*1.000*
	rs537343229	c.*3270G>A		3'UTR	0.4	N	*1.000*
	rs551337617	c.*3767C>T		3'UTR	0.4	N	*1.000*
	Novel	c.*157delT		3'UTR	0.4	N	*1.000*
	rs115304392	c.*9145G>A		3'UTR	0.4	N	*1.000*
	rs148831726	c.2783A>G	p.Tyr928Cys	missense	0.9	D	*1.000*
	rs767493473	c.*977_*975delAGG		3'UTR	0.4	N	*1.000*
	rs558763349	c.*6989G>C		3'UTR	0.4	N	*1.000*
	rs116654567	c.1465G>T	p.Val489Phe	missense	0.9	D	*1.000*
	rs1031568855	c.*5151C>A		3'UTR	0.4	N	*1.000*
	rs114588605	c.*90C>T		3'UTR	0.4	N	*1.000*
	rs149311951	c.*3846C>T		3'UTR	0.4	N	*1.000*
	rs531898727	c.*6460A>T		3'UTR	0.4	N	*1.000*
	rs560028865	c.*6514C>A		3'UTR	0.4	N	*1.000*
	rs531221357	c.*4716T>C		3'UTR	0.4	N	*1.000*
	rs545900155	c.*8901_*8902insACTCAAAAAGGCTTCTGAAATTCTACTCAGAATCG		3'UTR	0.4	N	*1.000*
	rs139780666	c.*7659A>G		3'UTR	0.4	N	*1.000*
	rs183284283	c.*1060C>T		3'UTR	0.4	N	*1.000*
	rs573108459	c.*5030_*5027delAACA		3'UTR	0.4	N	*1.000*
	rs531876967	c.*6421G>T		3'UTR	0.4	N	*1.000*
COQ10A	rs77131854	c.*130C>T		3'UTR	4.8	N	*0.224*
	rs1274498	c.-210A>G		5'UTR	2.6	N	*1.000*
	rs60542959	c.3G>T	p.Met1Ile	missense	2.2	D	*1.000*
	rs77131854	c.*130C>T		3'UTR	4.8	N	*0.224*
	rs60542959	c.3G>T	p.Ala2_M44del	startloss	2.2	D	*1.000*
	rs74603322	c.*332T>C		3'UTR	0.4	N	*1.000*
CYP7A1	rs8192879	c.*458G>A		3'UTR	31.5	N	*0.083*
	rs8192879	c.*458G>A		3'UTR	31.5	N	*0.083*
	rs561226849	c.*539A>C		3'UTR	0.5	N	*1.000*
	rs1004963084	c.-29C>A		5'UTR	0.5	N	*1.000*
	rs142956490	c.*330A>G		3'UTR	0.5	N	*1.000*
	rs8192875	c.1039G>A	p.Asp347Asn	missense	1.1	D	*1.000*
	rs117214002	c.*451C>T		3'UTR	2.2	N	*1.000*
	rs567109509	c.*1001_*1000delAT		3'UTR	0.5	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
HMGCR	rs12916	c.*372T>C		3'UTR	36.8	N	*0.687*
	rs5909	c.*8G>A		3'UTR	10.5	N	*1.000*
	rs12916	c.*372T>C		3'UTR	36.8	N	*0.687*
	rs17238554	c.*1485C>G		3'UTR	0.9	N	*1.000*
	rs17244932	c.*497T>C		3'UTR	1.8	N	*1.000*
	rs150429867	c.*385_*401delAAATGGATTTTTAAATT		3'UTR	0.9	N	*1.000*
	rs6805	c.*1117T>A		3'UTR	1.3	N	*1.000*
	rs5908	c.1753A>G	p.Ile585Val	missense	0.9	N	*1.000*
	rs17883498	c.*385_*401delAAATGGATTTTTAAATT		3'UTR	1.3	N	*1.000*
	rs377093901	c.*34T>C		3'UTR	0.4	N	*1.000*
	rs189370032	c.*1282G>A		3'UTR	1.3	N	*1.000*
	rs906837651	c.*165T>C		3'UTR	0.4	N	*1.000*
	rs17244722	c.-5350T>C		5'UTR	0.9	N	*1.000*
	rs10474435	c.*1113T>C		3'UTR	0.9	N	*1.000*
	rs151001406	c.*424T>C		3'UTR	0.4	N	*1.000*
	rs142563098	c.-5417T>C		5'UTR	1.3	N	*1.000*
	rs112915543	c.*1146C>T		3'UTR	0.4	N	*1.000*
	rs113929238	c.*238A>G		3'UTR	0.4	N	*1.000*
	rs112757256	c.*678C>T		3'UTR	0.4	N	*1.000*
KIF6	rs139304973	c.*968delA		3'UTR	10.5	N	*0.019*
	rs1887716	c.*1476G>A		3'UTR	6.1	N	*0.004*
	rs20455	c.508T>C	p.Trp170Arg	missense	44.3	D	*0.185*
	rs6904582	c.*553C>T		3'UTR	39	N	*0.169*
	rs9462531	c.*714C>T		3'UTR	33.3	N	*0.673*
	rs10947807	c.*501G>A		3'UTR	20.2	N	*0.041*
	rs11756686	c.*342C>T		3'UTR	5.7	N	*0.304*
	rs11758639	c.*468A>G		3'UTR	2.6	N	*1.000*
	rs3823213	c.*438G>A		3'UTR	20.2	N	*0.041*
	rs61748649	c.5G>A	p.Arg2Lys	missense	1.8	N	*1.000*
	rs72858468	c.*1432G>A		3'UTR	2.6	N	*1.000*
	rs72858469	c.*1142T>A		3'UTR	2.6	N	*1.000*
	rs72858477	c.*205A>G		3'UTR	2.6	N	*1.000*
	rs113412831	c.-107T>A		5'UTR	14	N	*0.693*
	rs3734621	c.*97T>G		3'UTR	9.2	N	*0.049*
	rs114269617	c.103A>G	p.Ser35Gly	missense	0.9	N	*1.000*
	rs139304973	c.*968delA		3'UTR	10.5	N	*0.019*
	rs144747535	c.*1169C>T		3'UTR	0.4	N	*1.000*
	rs2273063	c.1535G>A	p.Arg512His	missense	1.8	N	*1.000*
	rs115025619	c.*1001G>A		3'UTR	1.8	N	*1.000*
	rs116706958	c.*430C>T		3'UTR	1.8	N	*1.000*
	rs74659777	c.*296T>A		3'UTR	1.8	N	*1.000*
	rs114951361	c.1564A>T	p.Met522Leu	missense	0.9	N	*1.000*
	rs34059104	c.187A>G	p.Ile63Val	missense	0.9	N	*1.000*
	rs114582772	c.*1370G>A		3'UTR	0.9	N	*1.000*
	rs139767998	c.*160G>T		3'UTR	0.9	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs142944273	c.1087C>T	p.Arg363Cys	missense	0.4	D	*1.000*
	rs918723940	c.*940G>A		3'UTR	0.4	N	*1.000*
	rs190137715	c.*1081T>G		3'UTR	0.4	N	*1.000*
	rs927108958	c.1181A>C	p.Gln394Pro	missense	0.4	D	*1.000*
	Novel	c.*182G>T		3'UTR	0.4	N	*1.000*
	Novel	c.433C>A		missense	0.4	D	*1.000*
	rs564418655	c.*668A>G		3'UTR	0.4	N	*1.000*
	rs897242351	c.*1205T>C		3'UTR	0.4	N	*1.000*
LDLR	rs121908031	c.1539C>A	p.Cys513X	stopgain	1.8	D	*1.000*
	rs1433099	c.*666T>C		3'UTR	71.9	N	*0.487*
	rs17243011	c.*223G>A		3'UTR	1.8	N	*1.000*
	rs2738464	c.*315G>C		3'UTR	85.1	N	*1.000*
	rs2738467	c.*1743C>T		3'UTR	36	N	*0.546*
	rs397844005	c.*2196_*2197delTA		3'UTR	29.8	N	*0.261*
	rs5742911	c.*1453A>G		3'UTR	31.6	N	*1.000*
	rs11669576	c.667G>A	p.Ala223Thr	missense	4.8	N	*1.000*
	rs752596535	c.378C>A	p.Cys126X	stopgain	0.9	D	*1.000*
	Novel	c.514T>C		missense	0.4	D	*1.000*
	rs14158	c.*52G>A		3'UTR	28.1	N	*0.817*
	rs17242683	c.*1168G>A		3'UTR	23.2	N	*0.607*
	rs17249057	c.*1510T>C		3'UTR	28.9	N	*0.649*
	rs17249064	c.*1600G>T		3'UTR	28.9	N	*0.649*
	rs2738465	c.*504G>A		3'UTR	33.8	N	*0.834*
	rs2738466	c.*773A>G		3'UTR	28.9	N	*0.649*
	rs35921663	c.*1406_*1407insA		3'UTR	6.1	N	*1.000*
	rs875989902	c.410A>T	p.Asp137Val	missense	0.4	D	*1.000*
	rs28941776	c.1142G>A	p.Gly381Asp	missense	0.9	D	*1.000*
	rs7254521	c.*1430C>T		3'UTR	8.3	N	*1.000*
	rs397762834	c.*2195_*2196insTATA		3'UTR	7.6	N	*1.000*
	rs72658879	c.*2016G>A		3'UTR	5.3	N	*0.027*
	rs753707206	c.1297G>C	p.Asp433His	missense	0.9	D	*1.000*
	rs10409044	c.*982G>C		3'UTR	2.2	N	*1.000*
	rs28398082	c.*2054G>A		3'UTR	2.2	N	*1.000*
	Novel	c.*2199_*2200insTA		3'UTR	27.2	N	*0.000*
	Novel	c.*2197_*2198insTATA		3'UTR	8.3	N	*1.000*
	Novel	c.*2199_*2200insTATA		3'UTR	27.2	N	*0.000*
	Novel	c.*2198_*2199delTA		3'UTR	27.2	N	*0.000*
	Novel	c.*2196_*2199delTATA		3'UTR	34.9	N	*0.000*
	rs137853964	c.1975G>A	p.Val659Ile	missense	0.4	D	*1.000*
	rs137929307	c.1271G>A	p.Gly424Glu	missense	0.9	D	*1.000*
	rs112029328	g.11213463G>A		splicing	0.9	D	*1.000*
	rs376207800	c.185C>T	p.Thr62Met	missense	0.4	D	*1.000*
	rs3826810	c.*141G>A		3'UTR	5.7	N	*1.000*
	rs28942079	c.787G>A	p.Ala263Thr	missense	0.4	D	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs121908026	c.407C>T	p.Ser136Leu	missense	0.9	D	*1.000*
	Novel	c.*2195_*2196insTA		3'UTR	2.9	N	*1.000*
	rs121908039	c.428G>A	p.Cys143Tyr	missense	0.4	D	*1.000*
	rs373371572	c.1279C>T	p.Arg427Trp	missense	0.4	D	*1.000*
	Novel	c.454del1>T		frameshift deletion	0.4	D	*1.000*
	rs142697277	c.*2126G>A		3'UTR	0.4	N	*1.000*
	rs5928	c.1937G>A	p.Arg646Gln	missense	0.4	D	*1.000*
	rs72658874	c.*965C>T		3'UTR	0.9	N	*1.000*
	rs869054445	c.*2210_*2211insAC		3'UTR	0.4	N	*1.000*
	rs1042897688	c.*1491G>A		3'UTR	0.4	N	*1.000*
	rs113972139	c.1808C>T	p.Ala603Val	missense	0.4	N	*1.000*
	rs879255131	c.573_1574de1>l	p.Lys525Vfs*22	frameshift deletion	0.4	D	*1.000*
	rs879254797	c.614G>A	p.Gly205Asp	missense	0.9	D	*1.000*
	rs879254913	c.959T>C	p.Ile320Thr	missense	1.3	D	*1.000*
	rs148054434	c.*2111G>A		3'UTR	0.4	N	*1.000*
	rs545860269	c.*2069_*2070insC		3'UTR	0.4	N	*1.000*
	rs72658880	c.*2319C>G		3'UTR	0.9	N	*1.000*
	rs931426690	c.*1401C>T		3'UTR	0.4	N	*1.000*
	rs121908031	c.1539C>A	p.Cys513X	stopgain	1.8	D	*1.000*
	rs7258146	c.*1354T>C		3'UTR	0.9	N	*1.000*
	rs143587805	c.*1550A>T		3'UTR	1.8	N	*1.000*
	rs750649426	c.672C>A	p.Cys224X	stopgain	0.4	D	*1.000*
	rs116405216	g.11221324G>A		splicing	0.9	N	*1.000*
	rs869054445	c.*2210T>0		3'UTR	1.8	N	*0.026*
	rs1035917105	c.*1123C>T		3'UTR	0.4	N	*1.000*
	Novel	c.103del1>G		frameshift deletion	0.4	D	*1.000*
	rs72658860	c.466G>A	p.Gly156Ser	missense	0.4	D	*1.000*
	Novel	c.*2195_*2196insTATATA		3'UTR	1.5	N	*1.000*
	rs28942078	c.781G>A	p.Val261Met	missense	0.4	D	*1.000*
	rs993011316	c.*1912C>T		3'UTR	0.4	N	*1.000*
	rs1135402774	c.970G>A	p.Asp324Asn	missense	0.4	D	*1.000*
	rs387906307	c.-138del-		5'UTR	0.4	N	*1.000*
	rs375312185	c.*1477G>A		3'UTR	0.4	N	*1.000*
	rs879254687	c.818-2A>G		splicing	0.4	D	*1.000*
	rs875989887	c.-140C>A		5'UTR	0.9	N	*1.000*
	rs1135402768	c.364C>T	p.Gln122X	stopgain	0.4	D	*1.000*
	rs3180023	c.*1217C>G		3'UTR	0.4	N	*1.000*
	rs34113544	c.*1215_*1216insA		3'UTR	0.4	N	*1.000*
	rs1266961929	c.*1128G>A		3'UTR	0.4	N	*1.000*
	rs56270417	c.*19G>A		3'UTR	0.9	N	*1.000*
	rs1135402774	c.70delG	p.Asp324fs	frameshift deletion	0.4	D	*1.000*
LDLRAP1	rs10635955	c.*985_*986insTG		3'UTR	99.6	N	*0.000*
	rs11563	c.*1370G>T		3'UTR	54.4	N	*0.130*
	rs397860393	c.*445delT		3'UTR	46.1	N	*0.345*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs7491	c.*1755C>T		3'UTR	54.8	N	*0.185*
	rs10635955	c.*985_*986insTG		3'UTR	99.6	N	*0.000*
	rs4537542	c.*1794G>A		3'UTR	7.9	N	*0.136*
	rs62619761	c.*63G>A		3'UTR	1.3	N	*1.000*
	rs1057515539	c.*765_*766insT		3'UTR	1.3	N	*1.000*
	rs76969561	c.*1222T>C		3'UTR	2.6	N	*1.000*
	rs114583297	c.653C>T	p.Thr218Ile	missense	2.6	N	*1.000*
	rs528624038	c.*1677G>A		3'UTR	0.9	N	*1.000*
	rs41291058	c.712C>T	p.Arg238Trp	missense	2.2	D	*1.000*
	rs186747548	c.*430G>T		3'UTR	0.9	N	*1.000*
	rs529005321	c.397G>A	p.Ala133Thr	missense	0.4	D	*1.000*
	rs149951294	c.*765_*766insT		3'UTR	1.8	N	*1.000*
	rs148579379	c.713G>A	p.Arg238Gln	missense	0.9	D	*1.000*
	rs10062	c.*1793C>T		3'UTR	1.8	N	*1.000*
	rs41307931	c.*1115C>T		3'UTR	0.4	N	*1.000*
	rs768454420	c.796C>T	p.Arg266Trp	missense	0.4	D	*1.000*
LIPA	rs13500	c.*1093C>T		3'UTR	11.8	N	*0.048*
	rs1051338	c.46A>C	p.Thr16Pro	missense	33.3	N	*1.000*
	rs1051339	c.67G>A	p.Gly23Arg	missense	13.6	N	*0.123*
	rs1332326	c.-23656A>C		5'UTR	39.9	N	*0.000*
	rs1332327	c.-23645G>A		5'UTR	19.7	N	*0.374*
	rs2297472	g.90984990G>A		splicing	13.6	N	*0.123*
	rs1131706	c.*909T>A		3'UTR	21.5	N	*0.401*
	rs78931290	c.*1187C>A		3'UTR	2.2	N	*1.000*
	rs13500	c.*1093C>T		3'UTR	11.8	N	*0.048*
	rs41284116	c.*744C>G		3'UTR	0.4	N	*1.000*
	rs571012707	c.*647G>A		3'UTR	0.4	N	*1.000*
	rs116074523	c.*841C>T		3'UTR	0.4	N	*1.000*
	rs1211198607	c.-23594A>G		5'UTR	0.4	N	*1.000*
	rs543830356	c.-23579G>A		5'UTR	0.4	N	*1.000*
	rs2228159	c.335T>C	p.Phe112Ser	missense	0.4	D	*1.000*
	rs9664201	c.*608C>T		3'UTR	0.4	N	*1.000*
	rs1589558414	c.254A>G	p.Gln85Arg	missense	0.4	D	*1.000*
	rs1044857442	c.-4065_-4072delCGCGGCGC		5'UTR	0.4	N	*1.000*
LIPC	rs3829462	c.1068C>A	p.Phe356Leu	missense	94.3	N	*0.304*
	rs3829462	c.1068C>A	p.Phe356Leu	missense	94.3	N	*0.304*
	rs6083	c.644A>G	p.Asn215Ser	missense	41.7	N	*0.700*
	rs6078	c.283G>A	p.Val95Met	missense	5.7	N	*1.000*
	rs148828229	c.1430G>A	p.Arg477His	missense	0.4	N	*1.000*
	rs182603751	c.317C>T	p.Ala106Val	missense	0.4	D	*1.000*
LPA	rs1801693	c.5036T>C	p.Met1679Thr	missense	69.3	N	*0.659*
	rs1853021	g.11213463A>G		splicing	20.6	N	*0.000*
	rs3124784	c.6046C>T	p.Arg2016Cys	missense	27.6	D	*1.000*
	rs143431368	g.160969693T>C		splicing	0.4	D	*1.000*
	rs1801693	c.5036T>C	p.Met1679Thr	missense	69.3	N	*0.659*
	rs139145675	c.5311C>T	p.Arg1771Cys	missense	2.2	D	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs41267807	c.6068A>G	p.Tyr2023Cys	missense	1.3	D	*1.000*
	rs41267809	c.5882T>C	p.Leu1961Pro	missense	2.2	N	*1.000*
	rs41272110	c.4195A>C	p.Thr1399Pro	missense	10.5	D	*0.608*
	rs7765781	c.4114C>G	p.Leu1372Val	missense	36	N	*0.841*
	rs7765803	c.4072C>G	p.Leu1358Val	missense	35.5	N	*1.000*
	rs1800769	c.-21G>A		5'UTR	18.4	N	*0.530*
	rs76062330	c.5468G>T	p.Gly1823Val	missense	3.5	D	*0.119*
	rs201200716	c.4046C>T	p.Thr1349Met	missense	0.4	N	*1.000*
	rs41267817	c.3917A>G	p.His1306Arg	missense	0.4	N	*1.000*
	rs41272114	g.161006077C>T		splicing	2.6	D	*1.000*
	rs41264308	c.4793T>C	p.Met1598Thr	missense	1.3	N	*1.000*
	rs41272112	c.4262G>A	p.Arg1421Gln	missense	3.1	N	*1.000*
	rs113020022	c.3178C>A	p.Gln1060Lys	missense	0.4	N	*1.000*
	rs140720828	c.4522C>T	p.Arg1508Trp	missense	0.4	D	*1.000*
	rs3798220	c.5673A>G	p.Ile1891Met	missense	3.1	D	*0.090*
	rs41259144	c.2969G>A	p.Arg990Gln	missense	1.8	D	*1.000*
	rs142720914	c.3428C>T	p.Thr1143Met	missense	0.4	D	*1.000*
	rs59566810	c.4971T>G	p.Asn1657Lys	missense	1.3	N	*1.000*
	rs201013584	c.4607G>A	p.Arg1536Lys	missense	0.4	N	*1.000*
	rs41265936	c.5465G>C	p.Gly1822Ala	missense	1.3	N	*1.000*
	rs200802664	c.2782C>G	p.Gln928Glu	missense	0.4	N	*1.000*
	rs191762721	c.182A>G	p.Asn61Ser	missense	0.4	N	*1.000*
	rs144281871	c.5236G>T	p.Ala1746Ser	missense	0.4	N	*1.000*
	rs756764319	g.160962134C>T		splicing	0.4	N	*1.000*
	rs147235826	c.4523G>A	p.Arg1508Gln	missense	0.4	N	*1.000*
	rs62621433	c.3296C>G	p.Thr1099Ser	missense	0.4	N	*1.000*
	rs114322360	c.6092C>A	p.Thr2031Asn	missense	0.4	N	*1.000*
	rs577363233	c.-2118G>A		5'UTR	0.4	N	*1.000*
	rs76144756	c.4283C>T	p.Pro1428Leu	missense	0.4	D	*1.000*
	rs981155235	c.3889T>C	p.Ser1297Pro	missense	0.4	N	*1.000*
	rs889335800	c.4918T>C	p.Trp1640Arg	missense	0.4	D	*1.000*
LPL	rs1059507	c.*1142C>T		3'UTR	13.2	N	*1.000*
	rs11570892	c.*796A>G		3'UTR	16.2	N	*0.490*
	rs13702	c.*1671T>C		3'UTR	32.5	N	*0.831*
	rs15285	c.*1846C>T		3'UTR	32.5	N	*0.831*
	rs3200218	c.*1250A>G		3'UTR	18	N	*0.757*
	rs3208305	c.*827A>T		3'UTR	32.5	N	*0.831*
	rs3866471	c.*1848C>A		3'UTR	16.7	N	*0.510*
	rs4922115	c.*9G>A		3'UTR	14.5	N	*0.701*
	rs1059507	c.*1142C>T		3'UTR	13.2	N	*1.000*
	rs1800590	c.-281T>G		5'UTR	7.9	N	*1.000*
	rs17091815	c.*1783A>T		3'UTR	1.8	N	*1.000*
	rs5934	c.1279G>A	p.Ala427Thr	missense	0.9	N	*1.000*
	rs1801177	c.106G>A	p.Asp36Asn	missense	2.2	D	*1.000*
	rs3289	c.*371T>C		3'UTR	3.9	N	*1.000*
	rs1059611	c.*1742T>C		3'UTR	9.6	N	*0.595*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs10645926	c.*1805_*1806insTT		3'UTR	9.6	N	*0.595*
	rs1803924	c.*853C>T		3'UTR	7.5	N	*1.000*
	rs328	c.1421C>G	p.Ser474X	stopgain	7.5	D	*1.000*
	rs3735964	c.*1224C>A		3'UTR	7.9	N	*1.000*
	rs146978295	c.-176_-175insCC		5'UTR	1.3	N	*1.000*
	rs147116359	c.*1886G>A		3'UTR	0.4	N	*1.000*
	rs1803923	c.*1387T>C		3'UTR	1.8	N	*1.000*
	rs139240067	c.*1291G>A		3'UTR	1.3	N	*1.000*
	rs80351041	c.-283G>T		5'UTR	0.4	N	*1.000*
	rs300	c.1135A>G	p.Thr379Ala	missense	0.4	N	*1.000*
	rs7818177	c.*29G>A		3'UTR	0.4	N	*1.000*
	rs58998793	c.*1416T>C		3'UTR	1.8	N	*1.000*
	rs150960886	c.*1106G>A		3'UTR	0.4	N	*1.000*
	rs540525285	c.-241G>C		5'UTR	0.4	N	*1.000*
	rs79756214	c.*1928T>C		3'UTR	0.4	N	*1.000*
	rs1365389587	c.*1643C>G		3'UTR	0.4	N	*1.000*
	rs190991033	c.*30G>A		3'UTR	0.4	N	*1.000*
	rs572077788	c.*1877G>A		3'UTR	0.4	N	*1.000*
	rs374509929	c.*412_*416delTACTC		3'UTR	0.4	N	*1.000*
	rs915452684	c.*1217T>A		3'UTR	0.4	N	*1.000*
	rs268	c.953A>G	p.Asn318Ser	missense	0.4	D	*1.000*
	rs1464971282	c.*1038C>T		3'UTR	0.4	N	*1.000*
MYLIP	rs185701087	c.*995G>C		3'UTR	1.3	N	*0.013*
	rs2205794	c.*1194G>A		3'UTR	4.4	N	*0.186*
	rs2205795	c.*1076G>T		3'UTR	21.1	N	*1.000*
	rs35112615	c.*116_*117insA		3'UTR	11	N	*0.355*
	rs3765234	c.-56G>T		5'UTR	8.3	N	*0.558*
	rs9370867	c.1025A>G	p.Asn342Ser	missense	58.8	N	*0.177*
	rs185701087	c.*995G>C		3'UTR	1.3	N	*0.013*
	Novel	c.*117delA		3'UTR	25.9	N	*0.000*
	rs574992262	c.*665delT		3'UTR	0.4	N	*1.000*
	rs2072781	c.*367T>C		3'UTR	7.9	N	*1.000*
	rs35112615	c.*116_*117insAA		3'UTR	0.9	N	*1.000*
	rs560855721	c.*97C>T		3'UTR	0.9	N	*1.000*
	Novel	c.*117_*118insA		3'UTR	9.2	N	*0.595*
	Novel	c.*117_*118insAA		3'UTR	9.2	N	*0.595*
	Novel	c.*117_*118insAAAAA		3'UTR	9.2	N	*0.595*
	rs397971095	c.*116_*117insAA		3'UTR	1.8	N	*1.000*
	rs144304196	c.*627C>G		3'UTR	0.4	N	*1.000*
	rs142596337	c.*248_*249insT		3'UTR	1.8	N	*1.000*
	rs113117363	c.*220G>T		3'UTR	0.9	N	*1.000*
	rs114004922	c.*858A>G		3'UTR	1.8	N	*1.000*
	rs73724995	c.*871A>G		3'UTR	0.9	N	*1.000*
	rs79714658	c.*687C>T		3'UTR	0.4	N	*1.000*
	rs79992066	c.604A>C	p.Ile202Leu	missense	0.9	N	*1.000*
	rs148485764	c.*993_*994insG		3'UTR	2.2	N	*0.044*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs541642474	c.*1170T>C		3'UTR	0.4	N	*1.000*
PCSK9	rs505151	c.2009G>A	p.Gly670Glu	missense	85.5	N	*0.000*
	rs562556	c.1420G>A	p.Val474Ile	missense	76.3	N	*0.000*
	rs662145	c.*571C>T		3'UTR	71.9	N	*0.021*
	rs2495477	g.55518467A>G		splicing	18.4	N	*0.208*
	rs28362201	c.-245G>T		5'UTR	2.6	N	*0.065*
	rs557622245	c.*171C>T		3'UTR	2.6	N	*1.000*
	rs11583680	c.158C>T	p.Ala53Val	missense	10.1	N	*1.000*
	rs45448095	c.-64C>T		5'UTR	9.2	N	*1.000*
	rs145886902	c.169G>A	p.Glu57Lys	missense	0.4	N	*1.000*
	rs28362288	c.*444G>C		3'UTR	2.2	N	*1.000*
	rs182138201	c.*234C>T		3'UTR	0.9	N	*1.000*
	rs17111557	c.*614C>T		3'UTR	3.9	N	*1.000*
	rs35574083	c.2_43insCTG		nonframeshift insertion	14.0	LD	*0.346*
	rs775707869	c.884G>A	p.Arg295His	missense	0.4	D	*1.000*
	rs28362202	c.-26G>A		5'UTR	0.4	N	*1.000*
	rs141502002	c.1405C>T	p.Arg469Trp	missense	0.4	D	*1.000*
	rs72646509	c.835C>A	p.Pro279Thr	missense	0.4	D	*1.000*
	rs13376071	c.*414C>T		3'UTR	2.2	N	*1.000*
	rs28362270	c.1658A>G	p.His553Arg	missense	0.9	N	*1.000*
	rs72646533	c.*442_*443insG		3'UTR	0.9	N	*1.000*
	rs756500786	c.*887C>T		3'UTR	0.4	N	*1.000*
	rs17111555	c.*345C>T		3'UTR	1.3	N	*1.000*
	rs28362287	c.*75C>T		3'UTR	1.3	N	*1.000*
	rs72646535	c.*537delT		3'UTR	1.3	N	*1.000*
	rs1557510084	c.*225T>C		3'UTR	0.4	N	*1.000*
	rs28362201	c.-245G>C		5'UTR	1.8	N	*0.026*
	rs149837083	c.*1052C>T		3'UTR	0.4	N	*1.000*
	rs28362263	c.1327G>A	p.Ala443Thr	missense	0.9	N	*1.000*
	rs181453	c.*413G>T		3'UTR	0.4	N	*1.000*
	rs917249802	c.-112A>G		5'UTR	0.9	N	*1.000*
	rs772677312	c.1399C>G	p.Pro467Ala	missense	0.4	N	*1.000*
	rs1346795665	c.2039G>A	p.Arg680Gln	missense	0.4	N	*1.000*
	rs28362292	c.*849T>C		3'UTR	0.4	N	*1.000*
	rs868163847	c.-117C>T		5'UTR	0.4	N	*1.000*
	rs148195424	c.709C>T	p.Arg237Trp	missense	0.4	D	*1.000*
	rs1277652244	c.*1148T>C		3'UTR	0.4	N	*1.000*
SCAP	rs12487736	c.1627G>A	p.Val543Ile	missense	40.4	N	*0.083*
	rs12487736	c.1627G>A	p.Val543Ile	missense	40.4	N	*0.083*
	rs111762817	c.-49785A>G		5'UTR	14.5	N	*1.000*
	rs45453398	c.*83C>T		3'UTR	3.5	N	*1.000*
	rs150166851	c.*49_*50insGGGGC		3'UTR	0.4	N	*1.000*
SCARB1	rs5891	c.403G>A	p.Val135Ile	missense	2.2	N	*1.000*
	rs4238001	c.4G>A	p.Gly2Ser	missense	7.5	N	*0.475*
	rs10396211	c.*688G>C		3'UTR	2.6	N	*1.000*
	rs58032386	c.*504C>T		3'UTR	4.8	N	*0.018*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs5891	c.403G>A	p.Val135Ile	missense	2.2	N	*1.000*
	rs901958835	c.*332C>T		3'UTR	0.4	N	*1.000*
	rs184715678	c.*497C>A		3'UTR	1.3	N	*1.000*
	rs701103	c.1495G>A	p.Gly499Arg	missense	3.1	N	*1.000*
	rs150512235	c.*759T>C		3'UTR	0.9	N	*1.000*
	rs150222965	c.386C>T	p.Ser129Leu	missense	0.9	N	*1.000*
	rs546100832	c.*234C>A		3'UTR	0.4	N	*1.000*
	rs539157321	c.-206_-205insC		5'UTR	0.4	N	*1.000*
	rs943358614	c.*93T>C		3'UTR	0.4	N	*1.000*
SREBF1	rs2297508	c.*619G>C		3'UTR	60.5	N	*0.173*
	rs60282872	c.-34delG		5'UTR	19.7	N	*0.000*
	rs11868035	c.*835C>T		3'UTR	30.3	N	*0.375*
	rs13306736	c.-150G>A		5'UTR	42.1	N	*0.704*
	rs2297508	c.*619G>C		3'UTR	60.5	N	*0.173*
	rs11304210	c.*1085delC		3'UTR	13.2	N	*1.000*
	rs141503556	c.*159G>C		3'UTR	0.4	N	*1.000*
	rs143430327	c.*736C>G		3'UTR	0.4	N	*1.000*
	rs73981076	c.*521G>T		3'UTR	1.3	N	*1.000*
	Novel	c.*1084delC		3'UTR	14.5	N	*0.247*
	rs1022633114	c.*1086A>C		3'UTR	14.5	N	*0.247*
	rs747735223	c.*1085_*1084delCC		3'UTR	14.5	N	*0.247*
	rs796641934	c.-34delG		5'UTR	17.5	N	*0.006*
	rs74520623	c.820G>A	p.Val274Ile	missense	0.9	N	*1.000*
	rs879617029	c.353G>A	p.Gly118Glu	missense	0.4	N	*1.000*
	rs764217259	c.2594G>A	p.Arg865Gln	missense	0.4	N	*1.000*
	rs73981075	c.*870T>G		3'UTR	1.3	N	*1.000*
	rs554897947	c.-147G>C		5'UTR	1.8	N	*1.000*
	rs36215896	c.1666G>A	p.Val556Met	missense	0.9	D	*1.000*
	rs7214136	c.1757G>A	p.Arg586Gln	missense	2.2	N	*1.000*
	rs8064706	c.*267C>A		3'UTR	2.6	N	*1.000*
	rs114001633	c.518C>T	p.Pro173Leu	missense	1.3	N	*0.013*
	rs768028319	c.*1085delC		3'UTR	0.9	N	*1.000*
	rs2229590	c.1177G>A	p.Val393Met	missense	0.9	N	*1.000*
	rs73981078	c.*255C>T		3'UTR	0.4	N	*1.000*
	rs539120019	c.*446C>T		3'UTR	0.4	N	*1.000*
	rs903846087	c.*974A>C		3'UTR	0.4	N	*1.000*
	rs115855236	c.260C>T	p.Pro87Leu	missense	0.9	D	*1.000*
	rs192087293	c.*337A>G		3'UTR	1.8	N	*1.000*
	rs59995125	c.*559T>C		3'UTR	0.4	N	*1.000*
	rs45567732	g.17718146C>A		splicing	0.4	N	*1.000*
	Novel	c.2483C>T		missense	0.4	N	*1.000*
	rs137899785	c.*937C>T		3'UTR	0.4	N	*1.000*
SREBF2	rs2228314	c.1784G>C	p.Gly595Ala	missense	36	N	*0.221*
	rs2229442	c.*84A>G		3'UTR	14.9	N	*0.459*
	rs569655423	c.3418G>A	p.Ala1140Thr	missense	0.4	D	*1.000*
	rs2157590	c.*48T>C		3'UTR	14	N	*1.000*

Gene	rs code	NT change	AA change	Type	MAF (%)	Prediction	HWE p -value
	rs2228314	c.1784G>C	p.Gly595Ala	missense	36	N	*0.221*
	rs4822067	c.*647G>A		3'UTR	14	N	*1.000*
	rs2228313	c.2580G>C	p.Arg860Ser	missense	7.5	N	*1.000*
	rs2269664	c.*279C>T		3'UTR	8.8	N	*1.000*
	rs376482369	c.1991G>T	p.Arg664Leu	missense	0.4	D	*1.000*
	rs2229440	c.1867G>A	p.Val623Met	missense	3.1	D	*1.000*
	rs183045818	c.*206G>A		3'UTR	0.9	N	*1.000*
	rs191835473	c.*579A>G		3'UTR	0.9	N	*1.000*
	rs537096033	c.*686T>A		3'UTR	1.3	N	*1.000*
	rs182758758	c.*669A>G		3'UTR	2.2	N	*1.000*
	rs576372173	c.*205C>T		3'UTR	0.4	N	*1.000*
	rs143615881	c.03_205de2>l	p.Ser74del	nonframeshift deletion	0.4	LD	*1.000*
	rs2229439	c.1112G>A	p.Arg371Lys	missense	0.9	D	*1.000*
	rs1018819294	c.*527G>A		3'UTR	0.9	N	*1.000*
	rs73431000	c.*333C>T		3'UTR	0.9	N	*1.000*
	rs779626156	c.85_193de1>l	p.62_65del	nonframeshift deletion	0.4	LD	*1.000*
	rs568275502	c.*621_*622insGGTGGGAGGCA		3'UTR	1.8	N	*1.000*
	rs199735149	c.3239G>A	p.Arg1080Gln	missense	0.4	D	*1.000*

[a]*In silico* functionality prediction was performed either using PolyPhen-2, Mutation Assessor, SIFT, PROVEAN, CADD, DANN, and FATHMM for missense, stop gain and stop loss variants or dbNSFP v4.2 *in silico* algorithm for splice variants. Frameshift variants were considered deleterious and inframe variants were considered likely deleterious. AA: amino acid; NT nucleotide; D: deleterious; HWE: Hardy-Weinberg equilibrium; MAF: minor allele frequency; N: neutral; PD: pharmacodynamics.

Supplementary table 15 Influence of variants in PD-related genes and non-genetic factors on LDL-c reduction of FH patients. Univariate linear regression analysis (MAF > 1.0%)

Gene	rs code	NT change	Allele	β	SE	p-*value*	Adjusted p-value
All statins							
ABCA1	rs769705621	g.107556793insA	A allele	-3.0	5.3	*0.578*	*1.000*
	rs769705621	g.107556793insAA	AA allele	0.5	3.9	*0.903*	*1.000*
	rs769705621	g.107556793insAAA	AAA allele	0.7	3.9	*0.851*	*1.000*
	rs769705621	g.107556793insAAAA	AAAA allele	1.9	6.5	*0.772*	*1.000*
	rs769705621	g.107556793insAAAAA	AAAAA / AAAAAA / AAAAAAA allele	14.9	9.8	*0.131*	*1.000*
ABCG4	rs12271907	c.1035C>G	G allele	5.4	8.5	*0.524*	*1.000*
ABCG5	rs6756629	c.148C>T	T allele	1.6	5.5	*0.780*	*1.000*
ABCG8	rs4148211	c.161A>G	G allele	5.1	3.6	*0.161*	*1.000*
	rs80025980	c.239G>A	A allele	-4.6	11.4	*0.687*	*1.000*
APOB	rs12713675	c.7367C>A	A allele	11.3	8.8	*0.202*	*1.000*
	rs12720855	c.9880T>C	C allele	11.3	8.8	*0.202*	*1.000*
	rs1801699	c.5741A>G	G allele	3.8	6.4	*0.551*	*1.000*
	rs533617	c.5768A>G	G allele	-2.7	11.4	*0.812*	*1.000*
	rs6752026	c.433C>T	T allele	-5.1	8.9	*0.569*	*1.000*
	rs676210	c.8216C>T	T allele	0.4	3.8	*0.925*	*1.000*
APOE	rs7412	c.526C>T	T allele	-10.4	8.1	*0.202*	*1.000*
	rs429358	c.388T>C	C allele	-10.4	8.1	*0.202*	*1.000*
CETP	rs5880	c.988G>C	C allele	-7.4	5.7	*0.198*	*1.000*
CLMN	rs61750771	c.2698A>T	T allele	0.8	9.9	*0.934*	*1.000*
COQ10A	rs60542959	c.3G>T	T allele	0.5	8.9	*0.957*	*1.000*
KIF6	rs20455	c.2155T>C	C allele	7.9	3.8	***0.039***	*1.000*
LDLR	rs121908031	c.2043C>A	A allele	0.6	9.9	*0.955*	*1.000*
	rs879254913	c.1463T>C	C allele	1.0	11.4	*0.930*	*1.000*
LDLRAP1	rs41291058	c.712C>T	T allele	-1.7	8.9	*0.851*	*1.000*
LPA	rs139145675	c.5311C>T	T allele	-8.1	8.9	*0.363*	*1.000*
	rs3124784	c.6046C>T	T allele	-5.2	3.6	*0.152*	*1.000*
	rs41259144	c.2969G>A	A allele	13.6	9.8	*0.170*	*1.000*
	rs41267807	c.6068A>G	G allele	19.7	11.2	*0.082*	*1.000*
	rs41272114	g.161006077C>T	T allele	3.3	8.2	*0.689*	*1.000*
	rs76062330	c.5468G>T	T allele	-8.0	7.6	*0.293*	*1.000*
LPL	rs328	c.1421C>G	G allele	6.8	5.1	*0.185*	*1.000*
SREBF2	rs2229440	c.1867G>A	A allele	-4.2	7.6	*0.577*	*1.000*
Atorvastatin							
ABCA1	rs769705621	g.107556793insA	A allele	-2.3	5.1	*0.646*	*1.000*
	rs769705621	g.107556793insAA	AA allele	2.7	4	*0.506*	*1.000*
	rs769705621	g.107556793insAAA	AAA allele	2.9	4	*0.470*	*1.000*
	rs769705621	g.107556793insAAAA	AAAA allele	3.7	6.2	*0.551*	*1.000*
	rs769705621	g.107556793insAAAAA /AAAAAA	AAAAA allele or AAAAAA allele	14.3	10.7	*0.185*	*1.000*
ABCG4	rs12271907	c.1035C>G	G allele	2.4	9.6	*0.805*	*1.000*

Gene	rs code	NT change	Allele	β	SE	p-*value*	Adjusted p-value
ABCG5	rs6756629	c.148C>T	T allele	5.3	5.3	*0.322*	*0.466*
ABCG8	rs4148211	c.161A>G	G allele	6.5	3.7	*0.079*	*0.737*
	rs80025980	c.239G>A	A allele	-4.7	10.8	*0.661*	*1.000*
APOB	rs12713675	c.7367C>A	A allele	5.5	9.4	*0.560*	*1.000*
	rs12720855	c.9880T>C	C allele	5.5	9.4	*0.560*	*1.000*
	rs1801699	c.5741A>G	G allele	1.6	6.8	*0.819*	*1.000*
	rs6752026	c.433C>T	T allele	-4.4	9.4	*0.641*	*1.000*
	rs676210	c.8216C>T	T allele	-1.4	4	*0.724*	*1.000*
APOE	rs7412	c.526C>T	T allele	-10.6	8.4	*0.209*	*1.000*
	rs429358	c.388T>C	C allele	-3.6	4.5	*0.433*	*1.000*
CETP	rs5880	c.988G>C	C allele	-6.9	5.8	*0.241*	*1.000*
CLMN	rs61750771	c.2698A>T	T allele	0.7	9.4	*0.940*	*1.000*
COQ10A	rs60542959	c.3G>T	T allele	6.4	8.4	*0.447*	*1.000*
KIF6	rs20455	c.2155T>C	C allele	10.4	3.8	***0.007***	*0.196*
LDLR	rs879254913	c.1463T>C	C allele	9.5	10.7	*0.377*	*1.000*
LDLRAP1	rs41291058	c.712C>T	T allele	-5.7	9.4	*0.544*	*1.000*
LPA	rs139145675	c.5311C>T	T allele	-8.3	8.4	*0.327*	*1.000*
	rs3124784	c.6046C>T	T allele	-5.3	3.7	*0.155*	*1.000*
	rs41259144	c.2969G>A	G allele	-2.8	10.8	*0.793*	*1.000*
	rs41272114	g.161006077C>T	T allele	0.7	8.4	*0.933*	*1.000*
	rs76062330	c.5468G>T	T allele	-15.2	7.6	***0.048***	*0.672*
LPL	rs328	c.1421C>G	G allele	3.1	5.3	*0.555*	*1.000*
SREBF2	rs2229440	c.1867G>A	A allele	-7.6	8.4	*0.366*	*1.000*

AA: amino acid; NT nucleotide; FH: familial hypercholesterolemia; PD: pharmacodynamics; β: linear coefficient; SE: standard error.

Supplementary table 16 Association of variants in PD-related genes with statin response in FH patients. Univariate logistic regression analysis.

Gene	rs code	NT change	Allele	RE	NRE	OR (CI 95%)	*p-value*	Adjusted p-value
All statins								
ABCA1	rs769705621	g.107556793insA	A allele	20 (8)	17.4 (8)	0.8 (0.3 - 2.5)	*0.757*	*1.000*
	rs769705621	g.107556793insAA	AA allele	34.5 (19)	42.9 (21)	1.4 (0.6 - 3.2)	*0.385*	*1.000*
	rs769705621	g.107556793insAAA	AAA allele	35.2 (19)	45.3 (24)	1.5 (0.7 - 3.3)	*0.288*	*1.000*
	rs769705621	g.107556793insAAAA	AAAA allele	8.7 (4)	14 (6)	1.7 (0.5 - 7.1)	*0.436*	*1.000*
ABCG4	rs12271907	c.1035C>G	G allele	4.4 (2)	8.5 (4)	2 (0.4 - 15)	*0.437*	*1.000*
ABCG5	rs6756629	c.148C>T	T allele	10.3 (6)	14.3 (8)	1.4 (0.5 - 4.7)	*0.523*	*1.000*
ABCG8	rs4148211	c.161A>G	G allele	44.8 (26)	60.7 (34)	1.9 (0.9 - 4)	*0.091*	*1.000*
	rs80025980	c.239G>A	A allele	1.7 (1)	3.6 (2)	2.1 (0.2 - 46.2)	*0.547*	*1.000*
APOB	rs12713675	c.7367C>A	A allele	3.4 (2)	5.4 (3)	1.6 (0.3 - 12.4)	*0.621*	*1.000*
	rs12720855	c.9880T>C	C allele	3.4 (2)	5.4 (3)	1.6 (0.3 - 12.4)	*0.621*	*1.000*
	rs1801699	c.5741A>G	G allele	5.2 (3)	12.5 (7)	2.6 (0.7 - 12.7)	*0.180*	*1.000*
	rs533617	c.5768A>G	G allele	1.7 (1)	3.6 (2)	2.1 (0.2 - 46.2)	*0.547*	*1.000*
	rs6752026	c.433C>T	T allele	5.2 (3)	3.6 (2)	0.7 (0.1 - 4.3)	*0.678*	*1.000*
	rs676210	c.8216C>T	T allele	31 (18)	37.5 (21)	1.3 (0.6 - 2.9)	*0.467*	*1.000*
APOE	rs7412	c.526C>T	T allele	8.6 (5)	1.8 (1)	0.2 (0 - 1.2)	*0.139*	*1.000*
	rs429358	c.388T>C	C allele	20.7 (12)	23.2 (13)	1.2 (0.5 - 2.8)	*0.745*	*0.828*
CETP	rs5880	c.988G>C	C allele	13.8 (8)	8.9 (5)	0.6 (0.2 - 2)	*0.417*	*1.000*
CLMN	rs61750771	c.2698A>T	T allele	3.4 (2)	3.6 (2)	1 (0.1 - 8.9)	*0.972*	*1.000*
COQ10A	rs60542959	c.3G>T	T allele	5.2 (3)	3.6 (2)	0.7 (0.1 - 4.3)	*0.678*	*1.000*
KIF6	rs20455	c.2155T>C	C allele	58.6 (34)	73.2 (41)	1.9 (0.9 - 4.3)	*0.103*	*1.000*
LDLR	rs121908031	c.2043C>A	A allele	3.4 (2)	3.6 (2)	1 (0.1 - 8.9)	*0.972*	*1.000*
	rs879254913	c.1463T>C	C allele	1.7 (1)	3.6 (2)	2.1 (0.2 - 46.2)	*0.547*	*1.000*
LDLRAP1	rs41291058	c.712C>T	T allele	5.2 (3)	3.6 (2)	0.7 (0.1 - 4.3)	*0.678*	*1.000*
LPA	rs139145675	c.5311C>T	T allele	6.9 (4)	1.8 (1)	0.2 (0 - 1.7)	*0.216*	*1.000*
	rs3124784	c.6046C>T	T allele	56.9 (33)	37.5 (21)	0.5 (0.2 - 1)	***0.039***	*1.000*
	rs41259144	c.2969G>A	A allele	1.7 (1)	5.4 (3)	3.2 (0.4 - 66.3)	*0.317*	*1.000*
	rs41272114	g.161006077C>T	T allele	3.4 (2)	7.1 (4)	2.2 (0.4 - 16)	*0.387*	*1.000*
	rs76062330	c.5468G>T	T allele	10.3 (6)	1.8 (1)	0.2 (0 - 1)	*0.092*	*1.000*
LPL	rs328	c.1421C>G	G allele	13.8 (8)	16.1 (9)	1.2 (0.4 - 3.4)	*0.733*	*1.000*
SREBF2	rs2229440	c.1867G>A	A allele	6.9 (4)	5.4 (3)	0.8 (0.1 - 3.6)	*0.733*	*1.000*
Atorvastatin								
ABCA1	rs769705621	g.107556793insA	A allele	22.2 (8)	22.9 (8)	1.0 (0.3 - 3.2)	*0.949*	*0.949*
	rs769705621	g.107556793insAA	AA allele	36 (18)	47.4 (18)	1.6 (0.7 - 3.8)	*0.284*	*0.852*
	rs769705621	g.107556793insAAA	AAA allele	38.8 (19)	47.6 (20)	1.4 (0.6 - 3.3)	*0.396*	*0.972*
	rs769705621	g.107556793insAAAA	AAAA allele	11.9 (5)	12.5 (4)	1.1 (0.2 - 4.4)	*0.938*	*0.974*
	rs769705621	g.107556793insAAAAA / AAAAAA	AAAAA / AAAAAA allele	1.9 (1)	4.4 (2)	2.4 (0.2 - 53.1)	*0.477*	*0.859*
ABCG4	rs12271907	c.1035C>G	G allele	2.4 (1)	8.1 (3)	3.5 (0.4 - 73.2)	*0.284*	*0.958*
ABCG5	rs6756629	c.148C>T	T allele	11.3 (6)	17.8 (8)	1.7 (0.5 - 5.6)	*0.366*	*0.988*
ABCG8	rs4148211	c.161A>G	G allele	39.6 (21)	62.2 (28)	2.5 (1.1 - 5.8)	***0.027***	*0.364*
	rs80025980	c.239G>A	A allele	3.8 (2)	2.2 (1)	0.6 (0 - 6.2)	*0.660*	*0.990*

Gene	rs code	NT change	Allele	RE	NRE	OR (CI 95%)	*p-value*	Adjusted p-value
APOB	rs12713675	c.7367C>A	A allele	3.8 (2)	4.4 (2)	1.2 (0.1 - 10.2)	*0.867*	*1.000*
	rs12720855	c.9880T>C	C allele	3.8 (2)	4.4 (2)	1.2 (0.1 - 10.2)	*0.867*	*1.000*
	rs1801699	c.5741A>G	G allele	3.8 (2)	13.3 (6)	3.9 (0.9 - 27.8)	*0.105*	*0.709*
	rs6752026	c.433C>T	T allele	3.8 (2)	4.4 (2)	1.2 (0.1 - 10.2)	*0.867*	*0.975*
	rs676210	c.8216C>T	T allele	28.3 (15)	33.3 (15)	1.3 (0.5 - 3)	*0.591*	*0.939*
APOE	rs7412	c.526C>T	T allele	7.5 (4)	2.2 (1)	0.3 (0 - 2)	*0.261*	*1.000*
	rs429358	c.388T>C	C allele	20.8 (11)	22.2 (10)	1.1 (0.4 - 2.9)	*0.860*	*1.000*
CETP	rs5880	c.988G>C	C allele	15.1 (8)	6.7 (3)	0.4 (0.1 - 1.5)	*0.199*	*1.000*
CLMN	rs61750771	c.2698A>T	T allele	3.8 (2)	4.4 (2)	1.2 (0.1 - 10.2)	*0.867*	*0.936*
COQ10A	rs60542959	c.3G>T	T allele	5.7 (3)	4.4 (2)	0.8 (0.1 - 4.9)	*0.786*	*1.000*
KIF6	rs20455	c.2155T>C	C allele	58.5 (31)	75.6 (34)	2.2 (0.9 - 5.4)	*0.078*	*0.702*
LDLR	rs879254913	c.1463T>C	C allele	1.9 (1)	4.4 (2)	2.4 (0.2 - 53.1)	*0.477*	*0.991*
LDLRAP1	rs41291058	c.712C>T	T allele	5.7 (3)	2.2 (1)	0.4 (0 - 3.1)	*0.408*	*0.918*
LPA	rs139145675	c.5311C>T	T allele	7.5 (4)	2.2 (1)	0.3 (0 - 2)	*0.261*	*1.000*
	rs3124784	c.6046C>T	T allele	62.3 (33)	35.6 (16)	0.3 (0.1 - 0.8)	***0.009***	*0.243*
	rs41259144	c.2969G>A	A allele	1.9 (1)	4.4 (2)	2.4 (0.2 - 53.1)	*0.477*	*0.920*
	rs41272114	g.161006077C>T	T allele	3.8 (2)	6.7 (3)	1.8 (0.3 - 14.3)	*0.522*	*0.881*
LPL	rs328	c.1421C>G	G allele	13.2 (7)	15.6 (7)	1.2 (0.4 - 3.8)	*0.741*	*1.000*
SREBF2	rs2229440	c.1867G>A	A allele	5.7 (3)	4.4 (2)	0.8 (0.1 - 4.9)	*0.786*	*1.000*

AA: amino acid; NT nucleotide; FH: familial hypercholesterolemia; PD: pharmacodynamics; RE: responder; NRE: non-responder; OR: odds ratio; CI: confidende interval.

Supplementary table 17 Association of deleterious variants in PD-related genes with SRAE in FH patients. Univariate logistic regression analysis.

Variable	rs code	NT change	Allele	No SRAE	SRAE	OR (95% CI)	p-*value*	Adjusted p-value
ABCA1	rs769705621	g.107556793insA	A allele	14.3 (10)	40 (6)	4 (1.1 - 13.8)	***0.027***	*0.648*
	rs769705621	g.107556793insAA	AA allele	35.8 (29)	50 (11)	1.8 (0.7 - 4.7)	*0.229*	*0.687*
	rs769705621	g.107556793insAAA	AAA allele	42.4 (36)	33.3 (7)	0.7 (0.2 - 1.8)	*0.453*	*0.777*
	rs769705621	g.107556793insAAAA	AAAA allele	8.8 (6)	20 (4)	2.6 (0.6 - 10.2)	*0.177*	*0.850*
ABCG4	rs12271907	c.1035C>G	G allele	5.2 (4)	14.3 (2)	3 (0.4 - 17.5)	*0.227*	*0.778*
ABCG5	rs6756629	c.148C>T	T allele	11.2 (10)	16.7 (4)	1.6 (0.4 - 5.3)	*0.476*	*0.672*
ABCG8	rs4148211	c.161A>G	G allele	56.2 (50)	37.5 (9)	0.5 (0.2 - 1.2)	*0.108*	*0.864*
	rs80025980	c.239G>A	A allele	1.1 (1)	8.3 (2)	8 (0.7 - 176.8)	*0.096*	*1.000*
APOB	rs12713675	c.7367C>A	A allele	4.5 (4)	4.2 (1)	0.9 (0 - 6.6)	*0.945*	*1.000*
	rs12720855	c.9880T>C	C allele	4.5 (4)	4.2 (1)	0.9 (0 - 6.6)	*0.945*	*0.986*
	rs1801699	c.5741A>G	G allele	10.1 (9)	4.2 (1)	0.4 (0 - 2.2)	*0.379*	*0.700*
	rs6752026	c.433C>T	T allele	4.5 (4)	4.2 (1)	0.9 (0 - 6.6)	*0.945*	*0.945*
	rs676210	c.8216C>T	T allele	31.5 (28)	41.7 (10)	1.6 (0.6 - 3.9)	*0.350*	*0.764*
APOE	rs7412	c.526C>T	T allele	4.5 (4)	8.3 (2)	1.9 (0.3 - 10.6)	*0.464*	*0.742*
	rs429358	c.388T>C	C allele	24.7 (22)	12.5 (3)	0.4 (0.1 - 1.4)	0.210	00.75
CETP	rs5880	c.988G>C	C allele	12.4 (11)	8.3 (2)	0.6 (0.1 - 2.6)	*0.586*	*0.781*
CLMN	rs61750771	c.2698A>T	T allele	3.4 (3)	4.2 (1)	1.2 (0.1 - 10.3)	*0.852*	*0.974*
KIF6	rs20455	c.2155T>C	C allele	69.7 (62)	54.2 (13)	0.5 (0.2 - 1.3)	*0.158*	*0.948*
LDLR	rs121908031	c.2043C>A	A allele	2.2 (2)	8.3 (2)	4 (0.5 - 34.5)	*0.181*	*0.724*
LPA	rs139145675	c.5311C>T	T allele	3.4 (3)	8.3 (2)	2.6 (0.3 - 16.7)	*0.310*	*0.744*
	rs3124784	c.6046C>T	T allele	49.4 (44)	37.5 (9)	0.6 (0.2 - 1.5)	*0.301*	*0.803*
	rs41272114	g.161006077C>T	T allele	4.5 (4)	8.3 (2)	1.9 (0.3 - 10.6)	*0.464*	*0.696*
	rs76062330	c.5468G>T	T allele	5.6 (5)	8.3 (2)	1.5 (0.2 - 7.6)	*0.627*	*0.792*
LPL	rs328	c.1421C>G	G allele	13.5 (12)	20.8 (5)	1.7 (0.5 - 5.2)	*0.375*	*0.750*
SREBF2	rs2229440	c.1867G>A	A allele	6.7 (6)	4.2 (1)	0.6 (0 - 3.8)	*0.646*	*0.775*

AA: amino acid; NT nucleotide; FH: familial hypercholesterolemia; PD: pharmacodynamics; SRAE: statin-related adverse events; OR: odds ratio; CI: confidende interval.

9 SCIENTIFIC PRODUCTION

9.1 Published Articles

9.1.1 Articles as first author (related to the thesis)

Dagli-Hernandez C, de Freitas RCC, Marçal EDSR, Gonçalves RM, Faludi AA, Borges JB, Bastos GM, Los B, Mori AA, Bortolin RH, Ferreira GM, de Oliveira VF, Hirata TDC, Hirata MH, Hirata RDC. Late response to rosuvastatin and statin-related myalgia due to *SLCO1B1*, *SLCO1B3*, *ABCB11*, and *CYP3A5* variants in a patient with Familial Hypercholesterolemia: a case report. Ann Transl Med. 2021 Jan;9(1):76. doi: 10.21037/atm-20-5540 **(Appendix 1)**

Dagli-Hernandez C, Zhou Y, Lauschke VM, Genvigir FDV, Hirata TDC, Hirata MH, Hirata RDC. Pharmacogenomics of statins: lipid response and other outcomes in Brazilian cohorts. Pharmacol Rep. 2021 Aug 17. doi: 10.1007/s43440-021-00319-y. Epub ahead of print. **(Appendix 1)**

9.1.2 Articles as co-author

Borges JB, Oliveira VF, Ferreira GM, Los B, Barbosa TKAA, Marçal EDSR, **Dagli-Hernandez C**, de Freitas RCC, Bortolin RH, Mori AA, Hirata TDC, Nakaya HTI, Bastos GM, Thurow HS, Gonçalves RM, Araujo DB, Zatz HP, Bertolami A, Faludi AA, Bertolami MC, Sousa AGMR, França JÍD, Jannes CE, Pereira ADC, Nakazone MA, Souza DRS, Carmo TS, Sampaio MF, Gorjão R, Pithon-Curi TC, Moriel P, Silbiger VN, Luchessi AD, de Araújo JNG, Naslavsky MS, Wang JYT, Kronenberger T, Cerda A, Lin-Wang HT, Garofalo AR, Fajardo CM, Hirata RDC, Hirata MH. Genomics, epigenomics and pharmacogenomics of Familial Hypercholesterolemia (FHBGEP): A study protocol. Res Social Adm Pharm. 2021 Jul;17(7):1347-1355. doi: 10.1016/j.sapharm.2020.10.007.

Zhou Y, **Dagli Hernandez C**, Lauschke VM. Population-scale predictions of DPD and TPMT phenotypes using a quantitative pharmacogene-specific ensemble classifier. Br J Cancer. 2020 Dec;123(12):1782-1789. doi: 10.1038/s41416-020-01084-0.

Hirata TDC, **Dagli-Hernandez C**, Genvigir FDV, Lauschke VM, Zhou Y, Hirata MH, Hirata RDC. Cardiovascular Pharmacogenomics: An Update on Clinical Studies of Antithrombotic Drugs in Brazilian Patients. Mol Diagn Ther. 2021 Aug 6. doi: 10.1007/s40291-021-00549-z. Epub ahead of print.

Los B, Borges JB, Oliveira VF, Freitas RC, **Dagli-Hernandez C**, Bortolin RH, Gonçalves RM, Faludi AA, Rodrigues AC, Bastos GM, Jannes CE, Pereira AC, Hirata RD, Hirata MH. Functional analysis of *PCSK9* 3'UTR variants and mRNA-miRNA interactions in patients with familial hypercholesterolemia. Epigenomics. 2021 May;13(10):779-791. doi: 10.2217/epi-2020-0462.

9.2 Articles under review

9.2.1 Articles as co-author

Genvigir FDC, **Carolina Dagli-Hernandez,** Hirata TDC, Lauschke VM, Zhou Y, Hirata MH, Hirata RDC Pharmacogenomics of Antihypertensive Drugs in Brazil: recent progress and clinical implications.

Bruna Los, Glaucio Monteiro Ferreira, Jéssica Bassani Borges, Thales Kronenberger, Victor Fernandes de Oliveira, **Carolina Dagli-Hernandez**, Rodrigo Marques Gonçalves, Andre Arpad Faludi, Raul Hernandes Bortolin, Renata Caroline Costa de Freitas, Thais Kristini Almendros Afonso Barbosa, Cinthia Elim Jannes, Alexandre da Costa Pereira, Gisele Medeiros Bastos, Rosario Dominguez Crespo Hirata, Mario Hiroyuki Hirata. *In silico* and *in vitro* functional studies of *PCSK9* missense variants in patients with Familial Hypercholesterolemia.

Jéssica Bassani Borges, Victor Fernandes Oliveira, **Carolina Dagli-Hernandez**, Glaucio Monteiro Ferreira, Thais Kristini Almendros Afonso Barbosa, et al. Exon targeted gene sequencing and identification of pathogenic variants in the FHBGEP Brazilian cohort.

9.3 Articles in preparation

9.3.1 Articles as first author

Carolina Dagli-Hernandez, Jéssica Bassani Borges, Elisangela de Oliveira Rodrigues Marçal, Renata Caroline Costa de Freitas, Victor Fernandes de Oliveira, Bruna Los, Rodrigo Marques Gonçalves, Andre Arpad Faludi, Gisele Medeiros Bastos, Yitian Zhou, Volker M. Lauschke, Mario Hiroyuki Hirata, Rosario Dominguez Crespo Hirata. Genetic variants in ABC transporters are associated with increased response to statins in patients with Familial Hypercholesterolemia.

This article reports the results obtained during the BEPE internship in Karolinska Institutet and is expected to be submitted by December 2021.

APPENDIX 1 – Articles as first author (related to the thesis)

Case Report

Late response to rosuvastatin and statin-related myalgia due to *SLCO1B1*, *SLCO1B3*, *ABCB11*, and *CYP3A5* variants in a patient with Familial Hypercholesterolemia: a case report

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Abstract: Statins are the most widely used cholesterol-lowering drugs for cardiovascular diseases prevention. However, some patients are refractory to treatment, whereas others experience statin-related adverse events (SRAE). It has been increasingly important to identify pharmacogenetic biomarkers for predicting statin response and adverse events. This case report describes a female patient with familial hypercholesterolemia (FH) who showed late response to rosuvastatin and experienced myalgia on statin treatment. In the first visit (V1), the patient reported myalgia to rosuvastatin 40 mg, which was interrupted for a 6-week wash-out period. In V2, rosuvastatin 20 mg was reintroduced, but her lipid profile did not show any changes after 6 weeks (V3) (LDL-c: 402 *vs.* 407 mg/dL). Her lipid profile markedly improved after 12 weeks of treatment (V4) (LDL-c: 208 mg/dL), suggesting a late rosuvastatin response. Her adherence to treatment was similar in V1 and V3 and no drug interactions were detected. Pharmacogenetic analysis revealed that the patient carries low-activity variants in *SLCO1B1*1B and*5*, *SLCO1B3* (rs4149117 and rs7311358), and *ABCB11* rs2287622, and the non-functional variant in *CYP3A5*3*. The combined effect of variants in pharmacokinetics-related genes may have contributed to the late response to rosuvastatin and statin-related myalgia. Therefore, they should be considered when assessing a patient's response to statin treatment. To the best of our knowledge, this is the first report of a pharmacogenetic analysis on a case of late rosuvastatin response.

Keywords: Pharmacogenetics; precision medicine; familial hypercholesterolemia (FH); statins; myalgia

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Introduction

Familial hypercholesterolemia (FH) is a genetic metabolic disease that leads to increased high low-density lipoprotein (LDL) cholesterol, which is a risk factor for early atherosclerosis and cardiovascular diseases (1). FH is usually treated with high-dose statins, which are inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGR), a key enzyme in cholesterol biosynthesis pathway. Rosuvastatin is one of the most effective statins, probably due its hydrophilicity, that confers selectivity to hepatic cells, higher affinity to HMGR, and lower rates of statin-related adverse events (SRAE) compared to other statins. It is poorly metabolized by CYP2C9 and CYP2C19, while 72% of the non-metabolized molecules are excreted via biliary system. Therefore, rosuvastatin blood levels rely on the activity of membrane transporters, mainly of solute

carrier (SLC) and ATP-binding cassette (ABC) families, highly expressed in intestine, liver, and kidney (2).

Pharmacogenetic studies have shown that loss-of-function variants in genes encoding OATPs, such as *SLCO1B1*, *SLCO2B1*, and *SLCO1B3*, and ABCs have been associated with variability in low-density lipoprotein cholesterol (LDL-c) reduction and higher risk of SRAE (3). The importance of considering the combined effect of variants in key genes for pharmacogenetic analyses has been increasingly evident (4). In this case report, we discuss how variants in genes participating in different stages of statin pharmacokinetics pathway possibly affected the time to response to rosuvastatin and the risk of SRAE in a female FH patient. To the best of our knowledge, this is the first report of a pharmacogenetic analysis on a case of late rosuvastatin response. This case is reported in accordance with the CARE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-5540).

Case presentation

A 26-year-old Caucasian female patient with definite diagnosis of FH according to Dutch Lipid Clinic Network MEDPED criteria (5) was invited to participate in an intervention study in June 2019. She was previously included in a FH sequencing study (May 2018), in which a panel of 84 genes involved in lipid homeostasis and drug metabolism was sequenced using exon-targeted gene sequencing (NGS). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committees and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for both studies.

The patient carries the variant *LDLR* rs28941776 (c.1646G>A, p.Gly549Asp), which has been associated with FH and is classified as pathogenic according to the American College of Medical Genetics (ACMG) guidelines (6).

Her clinical history included high levels of total cholesterol and LDL-c since childhood. In 2008, at the age of 15 years, she had an abnormal lipid profile even under a daily treatment with simvastatin 10 mg and ezetimibe 10 mg. Laboratory analyses showed a total cholesterol of 324 mg/dL, LDL-c 264 mg/dL, high-density lipoprotein cholesterol (HDL-c) 46 mg/dL, and triglycerides 71 mg/dL. In 2014, she was diagnosed with hypothyroidism and treated with levothyroxine 25 µg/day, which was gradually increased to 100 µg/day in 2019. She also had a pregnancy history in January 2017.

Her therapy history included simvastatin, which led to severe myopathy in 2008, with marked increase in serum creatine kinase (CK) to 1,080 U/L (4.7-fold the upper reference value). The cholesterol-lowering therapy was changed to pravastatin 20 mg and ezetimibe 10 mg daily until May 2011, when she reported another episode of myalgia. Pravastatin was withdrawn and atorvastatin 20 mg was introduced, also associated with ezetimibe 10 mg. Three months later, in August 2011, she reported interrupting atorvastatin treatment due to myalgia. Rosuvastatin 10 mg was then introduced, also associated with ezetimibe 10 mg, after which she showed an LDL-c level of 125 mg/dL and never reported myalgia again. However, her lipid profile worsened throughout the years even under rosuvastatin treatment, with her LDL-c reaching 194 mg/dL with rosuvastatin 20 mg.

The patient had no history of liver or kidney impartment, HIV, coronary artery disease (CAD), diabetes, obesity, cardiovascular events, and did not smoke or drink. Her mother and grandmother had a history of FH, but not CAD or cardiovascular events, while her father had hypertension and type 2 diabetes.

In the intervention study, the patient was seen four times (V1 to V4) in 5 months, and clinical history and therapy data were obtained. The protocol consisted of a 6-week rosuvastatin wash-out period, after which rosuvastatin was reintroduced for additional 6 weeks, when treatment response was evaluated. Adherence to treatment was assessed in each timepoint using the translated and validated version of the Brief Medication Questionnaire (BMQ) (7) and blood samples were taken in each visit for laboratory testing.

The lipid profile during the follow-up is shown in *Figure 1*. In April 2019 (V1), the patient was taking rosuvastatin 40 mg, ezetimibe 10 mg, and levothyroxine 88 µg daily. She reported experiencing muscle pain after recently increasing rosuvastatin dose from 20 to 40 mg/day. Her lipid profile was altered (total cholesterol 376 mg/dL, LDL-c 263 mg/dL, HDL-c 67 mg/dL, triglycerides 234 mg/dL) without increase in CK levels. She reported being active, running 2 km 2–3 times a week, and had a healthy diet, eating more than five portions of vegetables daily. Her TSH and T4 levels were normal. Rosuvastatin 40 mg was then discontinued for wash-out, ezetimibe was maintained, and levothyroxine dose was increased to 100 µg/day.

In June 2019 (V2), after undergoing a 6-week

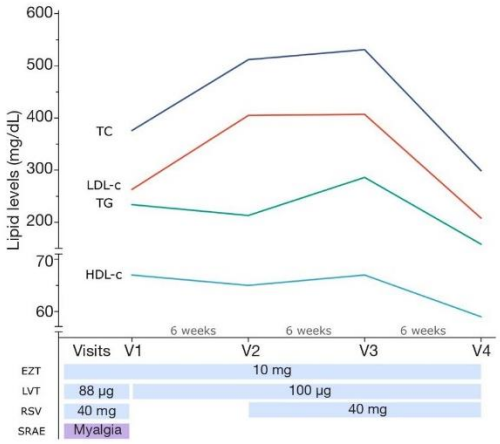

Figure 1 Plasma lipid profile and pharmacotherapy of the FH patient throughout the study period. EZT, ezetimibe; LVT, levothyroxine; RSV, rosuvastatin; SRAE, statin-related adverse events.

rosuvastatin wash-out period between V1 and V2, her lipid profile worsened (total cholesterol 512 mg/dL, LDL-c 405 mg/dL, HDL-c 65 mg/dL, triglycerides 213 mg/dL). Because the patient reported myalgia in V1 (rosuvastatin 40 mg), the physician prescribed rosuvastatin 20 mg/day for six weeks. Surprisingly, in August 2019 (V3), the lipid profile (total cholesterol 531 mg/dL, LDL-c 407 mg/dL, HDL-c 67 mg/dL, triglycerides 286 mg/dL) did not change compared to V2. The patient reported experiencing no myalgia to rosuvastatin 20 mg. In September 2019 (V4), her lipid profile improved (total cholesterol 299 mg/dL, LDL-c 208 mg/dL, HDL-c 59 mg/dL, triglycerides 158 mg/dL) and she continued not experiencing myalgia to rosuvastatin.

During the follow-up period, serum TSH and T4 levels remained unchanged, suggesting that her hypothyroidism was controlled and did not influence the lipid profile. Moreover, serum CK did not show any abnormality, which indicates no muscle damage due to statin treatment.

The patient also reported being adherent to treatment. In the BMQ adherence questionnaire, she reported forgetting the lipid-lowering medications 2 days in the week before V1 (71.4% adherence) and 1 day in the week before V3 (85.7% adherence).

The genetic profile of the patient is shown in *Table 1*. She carries five missense variants in *SLCO1B1*, *SLCO1B3*, and *ABCB11*. She is also homozygote for the *CYP3A5*3* (rs776746) splicing variant. No other missense variants described as impacting rosuvastatin response were found in *CYP3A4*, *CYP2C9*, *CYP2C19*, or other drug transporters, such as *ABCG2* (data not shown).

Discussion

In heterozygous FH patients, LDL-c level reductions of 47.1% have been observed after a 6-week treatment with rosuvastatin 20 mg (8). The patient, however, did not experience any changes in LDL-c levels at week 6 (V3) of rosuvastatin 20 mg treatment, with a 48.9% LDL-c reduction only at week 12 (V4) of therapy.

The delayed rosuvastatin response could be explained by modifications in the therapy scheme during the follow-up period. However, the only change was in levothyroxine dose, that was increased from 88 to 100 µg in V1. It is unlikely that the late response is due to an adaptation to the new levothyroxine dose. The patient was already on treatment with levothyroxine 88 µg before V1; moreover, changes in cholesterol due to an adaptation period should be reflected in her lipid profile in V3, not only in V4. Another possible explanation is a lack of adherence from V2 to V3; however, the patient showed a similar treatment adherence in V3 and V1, which should lead to a similar lipid profile between visits. Furthermore, drug interactions between rosuvastatin, levothyroxine, and ezetimibe that could affect treatment response were not detected, excluding this possibility.

Pharmacokinetics-related genes may have contributed to the late response to rosuvastatin (*Figure 2*). The patient carries two variants in *SLCO1B1*, c.388A>G (*SLCO1B1*1B*) and c.521T>C (*SLCO1B1*5*), that are important determinants of rosuvastatin response. *SLCO1B1*5* is a loss-of-function variant that decreases the hepatic uptake and increases blood levels of statins (9) (*Table 1*). *SLCO1B1*1B* has shown comparable activity to the functional **1A* variant in *in vitro* functional studies (10). *SLCO1B1*1B* and **5* variants are in linkage disequilibrium (LD) and form the *SLCO1B1*15* haplotype, that also reduced rosuvastatin uptake in functional studies with HEK293 and HeLa cells (11). The decreased liver uptake caused by these *SLCO1B1* variants has been associated with increased plasma levels of rosuvastatin in pharmacokinetics studies (9) (*Table 1*).

SLCO1B3 is also an important gene that encodes an influx transporter for rosuvastatin. The patient was homozygous for both *SLCO1B3* c.334T>G and c.699G>A, which are in strong LD (12). In an *in vitro* study, HeLa cells transfected with *SLCO1B3* c.334G and c.699A haplotype showed a 13% decrease in rosuvastatin uptake, while for

Table 1 Variants in pharmacokinetic-related genes of the FH patient with late response to rosuvastatin

Gene	Variant code	Variant type	Nucleotide change (Amino acid change)	Patient genotype	Allele frequency (1,000 genomes, %)	Functional impact	Effects on rosuvastatin pharmacokinetics	References
SLCO1B1	rs2306283 (*SLCO1B1*1B*)	Missense	c.388A>G (p.Asn130Asp)	AG	**1B: 54.4*	Comparable to **1A*	No effect on plasma rosuvastatin levels	Ho *et al.*, 2006; Lee *et al.*, 2013
SLCO1B1	rs4149056 (*SLCO1B1*5*)	Missense	c.521T>C p.(Val174Ala)	TC	**5: 8.8*	Reduced activity	Increased rosuvastatin plasma levels; Reduced hepatic uptake	Kameyama *et al.*, 2005; Lee *et al.*, 2013
SLCO1B1	rs2306283, rs4149056 (*SLCO1B1*15*)	Missense	c.388A>G, c.521T>C (p.Asn130Asp, p.Val174Ala)	AG, TC	**15: 7.8*	Reduced activity	Increased rosuvastatin plasma levels; reduced hepatic uptake	Kameyama *et al.*, 2005; Birmingham *et al.*, 2015
SLCO1B3	rs4149117	Missense	c.334T>G (p.Ser112Ala)	GG	*G: 70.2*	Reduced activity	Reduced hepatic uptake	Schwarz *et al.* 2011
SLCO1B3	rs7311358	Missense	c.699G>A (p.Met233Ile)	AA	*A: 70.2*	Reduced activity	Reduced hepatic uptake	Schwarz *et al.* 2011
ABCB11	rs2287622	Missense	c.1331T>C (p.Val444Ala)	TC	*C: 58.9*	Reduced activity	Increased rosuvastatin plasma levels	Soko *et al.* 2019
CYP3A5	rs776746 (CYP3A5*3)	Splicing	c. 6986A>G	GG	**3: 62.1*	No activity	No rosuvastatin metabolism; Reduced LDL-c response	Bailey *et al.* 2010

FH, familial hypercholesterolemia; LDL-c, low-density lipoprotein cholesterol.

other substrates, such as cholecystokinin-8, an even more marked decrease of 57% was observed (13) (*Table 1*).

Although the effect of *SLCO1B3* c.334G and c.699A haplotype in rosuvastatin uptake is not sufficient to explain the delayed response, it might be significant when combined with the effect of the decreased function haplotype *SLCO1B1*15*. While *SLCO1B1*5* and *SLCO1B1*15* are associated with higher plasma levels of rosuvastatin, previous studies failed to find an association between these variants and LDL-c reduction in response to short- and long-term rosuvastatin treatments (9). Therefore, the simultaneous presence of decreased function *SLCO1B1* and *SLCO1B3* haplotypes possibly caused a marked reduction of rosuvastatin intrahepatic concentration, resulting in the lack of response observed in V3.

ABCB11 encodes the efflux protein ABCB11, which plays an important role in rosuvastatin bile excretion. In a recent study, *ABCB11* c.1331C allele has been associated to increased plasma rosuvastatin levels in healthy subjects (14) (*Table 1*). This variant possibly causes lower rosuvastatin excretion via bile, which in turn would increase intrahepatic rosuvastatin concentrations. Therefore, this mechanism could explain why even in the presence of low function SLC variants, the patient showed a late but evident LDL-c reduction after 12 weeks of rosuvastatin treatment.

The patient also carries the homozygous form of *CYP3A5*3*, an intronic variant that results in undetectable expression of *CYP3A5* (15). The GEOSTAT-1 study reported that dyslipidemic patients carrying *CYP3A5*3/*3* had lower LDL-c reduction after three-month rosuvastatin 10 mg treatment compared to carriers of **1/*1* or **1/*3* (*Table 1*). It was suggested that the metabolite produced by CYP3A5 also plays a role in HMGR inhibition, potentiating the response to rosuvastatin, which is why *CYP3A5* non-expressors have reduced LDL-c response to rosuvastatin (16). *CYP3A5*3* possibly impaired the patient's response time to rosuvastatin, but in lower extent, as *CYP3A5* does not participate markedly in rosuvastatin metabolism.

In addition to the delayed response to rosuvastatin, the patient experienced myalgia associated with rosuvastatin 40 mg/day and other statins, as previously commented. This SRAE may be due to *SLCO1B1* variants. *SLCO1B1*5* and *SLCO1B1*15* have been extensively associated with myopathy to simvastatin. A systematic review and meta-analysis reported that carriers of the C allele of *SLCO1B1*5* (c.521T>C) showed a higher risk of myotoxicity (17). Additionally, *SLCO1B1*5* has been associated to rosuvastatin myotoxicity in previous studies (18,19). It has been

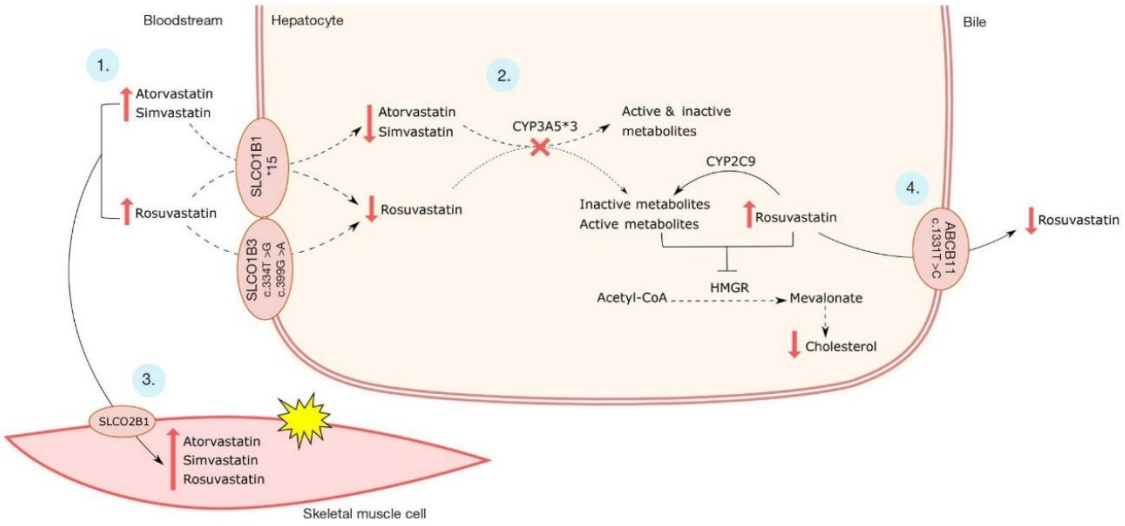

Figure 2 Proposed mechanism for patient's late rosuvastatin response and myalgia. 1. The hepatic uptake of rosuvastatin occurs through SLCO1B1 and SLCO1B3 influx transporters, while atorvastatin and simvastatin are internalized through SLCO1B1. The presence of deleterious variants in these transporters (*SLCO1B1*15* and *SLCO1B3* c.334T>G and c.699G>A) decreases statin uptake, therefore decreasing their concentration inside the hepatocyte and increasing statin plasma levels. 2. The lack of expression of CYP3A5 due to *CYP3A5*3* also decreases atorvastatin and simvastatin metabolization, which contributes to increasing their plasma levels. This enzyme does not participate markedly in rosuvastatin metabolism. 3. The resulting higher blood statin levels increased the patient's muscular exposure to statins, that are internalized through SLCO2B1 transporter into the skeletal muscle cell. The high concentrations in the skeletal muscle cell possibly caused patient's myalgia. 4. Rosuvastatin's bile excretion occurs through ABCB11 efflux protein. *ABCB11* c.1331T>C variant results in a reduced activity ABCB11, which decreases rosuvastatin efflux; this increases rosuvastatin intrahepatic levels and blood levels. Although the patient had reduced function influx transporters, we suggest that the small portion of rosuvastatin absorbed in the beginning of the treatment accumulated due to the loss of function of the *ABCB11* variant. This, together with rosuvastatin active metabolites generated by the normal function CYP2C9, allowed HMGR inhibition and therefore cholesterol lowering in the last visit.

suggested that it causes higher efflux of statins, increasing statin exposure and, therefore, the risk of myalgia (20). Also, a recent case report showed that variants in *SLCO1B3* (c.334T>G and c.699G>A) and *ABCB11* (c.1331T>C) and the interaction between rosuvastatin and ticagrelor led to rhabdomyolysis in a patient with chronic kidney disease and other chronic conditions (21), but no other reports were found.

*CYP3A5*3* may also have contributed to statin myotoxicity, since it has been associated with increased risk to atorvastatin and rosuvastatin-related myalgia in South-Indian dyslipidemic patients (22). However, this variant was not associated to statin intolerance in another study (23). Most studies have evaluated the effect of individual variants in SRAE, and not the interaction between a group of variants in key genes in statin pharmacokinetics pathway. Therefore, we suggest that the combined effect of the low-activity variants in *SLCO1B1* and *SLCO1B3*, the high-activity variant in *ABCB11*, and the lack of activity of *CYP3A5*3* predisposed the patient to low hepatic uptake, metabolization and efflux, respectively. The resulting higher rosuvastatin plasma concentration increased its systemic exposure, which may have caused myalgia (*Figure 2*).

Importantly, the patient carries *LDLR* rs28941776 (c.1646G>A, p.Gly549Asp), a disruptive-missense variant that showed reduced LDL uptake in an *in vitro* study (24). *LDLR* variants have been associated with variability in statin response in FH patients (25), but we did not find studies that investigated the association between *LDLR* variants and time to statin response or myalgia. Nevertheless, this variant could have played a role in patient's rosuvastatin time to response and it should be considered for further

studies.

A limitation of this study is that plasma concentrations of rosuvastatin and its metabolites were not measured. However, the adherence of the patient to the prescribed treatment was ensured using a validated adherence questionnaire and regular follow-up calls.

In summary, the combination of four low-activity variants in *SLC* genes, a high-activity variant in *ABCB11*, and a non-functional variant in *CYP3A5* may explain the observed late response to rosuvastatin and the statin-related myalgia. With this case report, we have shown the importance of considering a combination of variants in a pharmacogenetic analysis to predict individual responses to statin treatment and prevent adverse drug events. We believe this study contributes to precision medicine in future clinical settings.

Patient perspective

"*I have had high cholesterol since I was a child and it has been an issue because of the delayed response to treatments and of many adverse reactions to medications, especially simvastatin. The authors have been very attentive towards me throughout the whole study and discovered possible variants that may delay my response to rosuvastatin and influence the pain that I have felt when using statins. I am very happy for knowing the cause of my problem and I would like to thank the authors for this possible diagnosis. This has improved my perspectives of cholesterol treatment.*"

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-5540). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The intervention study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The DNA sequencing study was approved by the Ethics Committees of the Institute Dante Pazzanese of Cardiology (CAAE #4618713.0.1001.5462) and the School of Pharmaceutical Sciences of the University of Sao Paulo (CAAE #24618713.0.3001.0067), Sao Paulo, Brazil. The intervention study was approved by the Ethics Committee of the Institute Dante Pazzanese of Cardiology (CAAE #05234918.4.0000.5462). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committees. The patient signed the written informed consents before her enrollment in the studies. In the written informed consent of the DNA sequencing study, the patient was informed that clinical data and blood samples would be collected for laboratory tests and genetic analyses. As for the intervention study, the patient was informed on the intervention protocol and sample collections throughout the visits, and that this data would be used for genetic and epigenetic analyses.

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REVIEW

Pharmacogenomics of statins: lipid response and other outcomes in Brazilian cohorts

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Abstract
Statins are inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase, a key enzyme in cholesterol biosynthesis, that are highly effective in reducing plasma low-density lipoprotein (LDL) cholesterol and decreasing the risk of cardiovascular events. In recent years, a multitude of variants in genes involved in pharmacokinetics (PK) and pharmacodynamics (PD) have been suggested to influence the cholesterol-lowering response. However, the vast majority of studies have analyzed the pharmacogenetic associations in populations in Europe and the USA, whereas data in other populations, including Brazil, are mostly lacking. This narrative review provides an update of clinical studies on statin pharmacogenomics in Brazilian cohorts exploring lipid-lowering response, adverse events and pleiotropic effects. We find that variants in drug transporter genes (*SLCO1B1* and *ABCB1*) positively impacted atorvastatin and simvastatin response, whereas variants in genes of drug metabolizing enzymes (*CYP3A5*) decreased response. Furthermore, multiple associations of variants in PD genes (*HMGCR*, *LDLR* and *APOB*) with statin response were identified. Few studies have explored statin-related adverse events, and only *ABCB1* but not *SLCO1B1* variants were robustly associated with increased risk in Brazil. Statin-related pleiotropic effects were shown to be influenced by variants in PD (*LDLR*, *NR1H2*) and antioxidant enzyme (*NOS3*, *SOD2, MTHFR, SELENOP*) genes. The findings of these studies indicate that statin pharmacogenomic associations are distinctly different in Brazil compared to other populations. This review also discusses the clinical implications of pharmacogenetic studies and the rising importance of investigating rare variants to explore their association with statin response.

Keywords Pharmacogenomics · Statins · Brazil · Lipid response · Adverse events · Pleiotropic effects · Rare variants

Abbreviations

AD	Alzheimer disease
ADR	Adverse drug reactions
Apo	Apolipoprotein
ASCVD	Atherosclerotic cardiovascular disease
AUC	Area under the curve
CAC	Coronary artery calcium
CAR	Constitutive androstane receptor
CD36	Scavenger receptor Class B2
CETP	Cholesteryl ester transfer protein
CK	Creatine kinase
CoQ10	Ubiquinone
CPIC	Clinical Pharmacogenetics Implementation Consortium
CVD	Cardiovascular disease
eNOS	Endothelial nitric oxide synthase
ERα	Estrogen receptor α
GPX	Glutathione peroxidase
HC	Hypercholesterolemia
FH	Familial hypercholesterolemia
HDL	High-density lipoprotein
HL	Hepatic lipase
HMGR	3-Hydroxy-3-methylglutaryl-CoA reductase
GRS	Genetic risk score
LCAT	Lecithin:cholesterol acyltransferase
LDL	Low-density lipoprotein
LDLR	LDL receptor
MACE	Major atherosclerotic cardiovascular events

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MTHFR	Methylenetetrahydrofolate reductase
MYLIP	Myosin regulatory light chain interacting protein
PCSK9	Proprotein convertase subtilisin/kexin type 9
PD	Pharmacodynamics
PK	Pharmacokinetics
PON1	Paraoxonase 1
PPARα	Peroxisome proliferator-activated receptor
PXR	Pregnane X receptor
RXRα	Retinoid X receptor alpha
SAMS	Statin-associated muscle symptoms
SCAP	SREBP cleavage-activating protein
Se	Selenium
SNVs	Single nucleotide variations
SOD2	Manganese-dependent superoxide dismutase
SRAE	Statin-related adverse events
SR-B1	Scavenger receptor class B1
SREBP	Sterol regulatory element-binding proteins
VLDL	Very low-density lipoprotein

Introduction

Statins are potent 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) inhibitors that are highly effective in reducing plasma low-density lipoprotein (LDL) cholesterol, delay the progression of atherosclerosis and prevent life-threatening cardiovascular events [1]. The response to statins, however, is considerably variable among individuals and a multitude of genetic and non-genetic factors, such sex, age, smoking status, diabetes and ethnics, have been reported as predictors of LDL cholesterol-lowering response to statins [2].

Variants in genes involved in pharmacokinetics (PK) and pharmacodynamics (PD) have been studied for their impact on statin response and the risk of statin-related adverse events (SRAE) [3]. Most genetic associations with statin response have been found in PK genes, such as drug metabolizing enzymes and transporters, which can alter statins plasma concentrations with impact on their efficacy and safety.

The influence of genetic variants on statin response has been assessed primarily in populations in Europe and the USA for which recent reviews summarized variants in PK (Fig. 1) and PD (Fig. 2) genes associated with statin efficacy and safety, particularly *SLCO1B1* and other drug transporter genes with higher effects [4–6]. However, clinical studies on statin pharmacogenomics in Brazilian patients are generally underrepresented [7, 8].

This narrative review provides an update of pharmacogenomic studies of statins performed in Brazilian cohorts. Original articles that explored genetic variants associated with response to statins were searched in the PubMed database, using the key words: (1) statins, pharmacogenetics (or gene polymorphism) and Brazil; (2) statins, gene polymorphism, lipids, adverse events or pleotropic effects, and Brazil. Thirty-nine clinical studies published from 2005 to 2021 were selected and information on gene, variant, allele frequency, study design, Brazilian cohort, statin regimen, and effects and outcomes (drug response, SRAE risk and others) were extracted. Data on significant associations of genetic variants with statin response and other outcomes

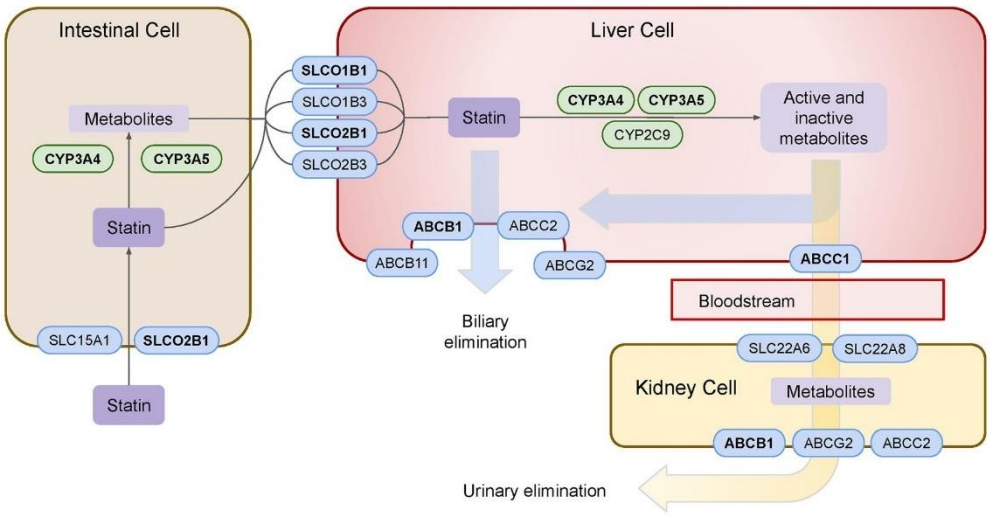

Fig. 1 Genes involved in statin pharmacokinetics. In bold, genes studied in Brazilian cohorts

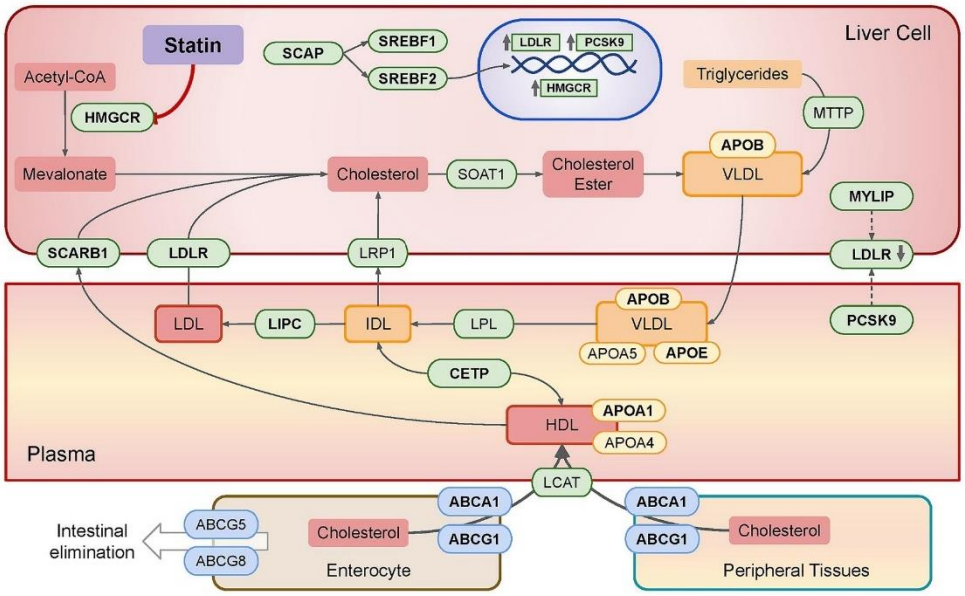

Fig. 2 Genes involved in statin pharmacodynamics and cholesterol metabolism. In bold, genes studied in Brazilian cohorts

are provided in Tables 1 and 2, while variants without significant association are shown in Tables S1 to S3. Clinical implications of the pharmacogenomic studies and the relevance of rare variants in pharmacogenetic studies are also discussed in this review.

Pharmacokinetics genes and statin response

SLC transporters

OATP1B1 and OATP2B1 (encoded by *SLCO1B1* and *SLCO2B1*, respectively) are influx transporters with a critical role in statin uptake by liver cells (Fig. 1) and have important contributions in statin PK and response [4, 9]. The effects of *SLCO1B1* variants, mainly *SLCO1B1*1B* (rs2306283, c.388A>G, p.Asn130Asp) and **5* (rs4149056, c.521T>C, p.Val174Ala) and **15* haplotype (rs2306283 and rs4149056) have been extensively studied.

*SLCO1B1*5* and **15* are low function variants that result in reduced uptake of pravastatin, atorvastatin, and cerivastatin in vitro, whereas **1B* did not alter transporter function [10]. Although *SLCO1B1*5* did not affect simvastatin uptake in vitro [10], healthy volunteers carrying the **5* allele had up to twice the simvastatin exposure compared to non-carriers [11]. *SLCO1B1*15* has also been shown to impact atorvastatin, pitavastatin, and rosuvastatin plasma levels in homozygous carriers [12]. However, *SLCO1B1*5* and **15* had low effect in statin response (LDL cholesterol reduction < 5%) [13]. Other studies reported no impact of *SLCO1B1*5* on LDL cholesterol response to statins in patients with hypercholesterolemia (HC) [14, 15].

The influence of *SLCO1B1*1B* on statin pharmacokinetics is still controversial. *SLCO1B1*1B* carriers showed a reduction of pravastatin plasma area under the curve (AUC) by 65% compared to **1A/*1A* carriers, suggesting **1B* allele as a determinant of pravastatin pharmacokinetics [16]. Another study observed an association between *SLCO1B1*1B* and higher plasma atorvastatin AUC, which was probably due to a higher hepatic *SLCO1B1* activity [17]. Conversely, *SLCO1B1*1B* did not impact the LDL cholesterol response to atorvastatin or simvastatin response in HC patients [14, 15].

Variants in *SLCO1B1* and *SLCO2B1* were explored in three Brazilian HC cohorts (Table 1). *SLCO1B1*1B* (G allele) was associated with increased total and LDL cholesterol response to atorvastatin (10 mg/day) [18], as well as LDL cholesterol reduction in response to simvastatin (20 mg/day) [19].

*SLCO1B1*5* and *SLCO1B1*15*, as well as *SLCO1B1*4* (rs11045819, c.463C>A) and *SLCO1B1*14* (**1B/*4*) haplotype were also investigated in Brazilian HC patients, but no association was found with the response to atorvastatin or simvastatin [18–20] (Table S1). Although similar results were observed in other populations [14, 15], the low frequency of **5* or **15* in the Brazilian studies

Table 1 Genetic variants significantly associated with statin response in Brazilian cohorts

Gene	Variant	NT change	Cohort	Statin	Allele, genotype or haplotype associated	Effect on statin response	References
Drug transporters and metabolizing enzymes							
SLCO1B1	rs2306283 (*1B)	c.388A>G	136 HC	Atorvastatin	*1B allele	↑ TC and LDL-c reduction	[18]
			216 HC[a]	Simvastatin	*1B allele	↑ LDL-c reduction	[19]
ABCB1	rs1128503	c.1236C>T	116 HC[a]	Simvastatin	T allele	↑ TC and LDL-c reduction	[27]
	rs2032582	c.2677T>G/A	116 HC[a]	Simvastatin	GG genotype	↑ TC reduction	[27]
			136 HC	Atorvastatin	A allele	↑ LDL-c reduction	[25]
CYP3A5	rs776746 (*3) rs15524 (*1D)	c.6986A>G c.31611C>T	139 HC	Atorvastatin	*3/*1D haplotype	↓ TC, LDL-c, and HDL-c reduction (non-African descent)	[42]
Cholesterol homeostasis and metabolism							
HMGCR	rs17244841	g.14863A>T	157 HC	Atorvastatin	AT genotype	↑ apo AI/apo B ratio	[20]
					TT genotype	↑ CK levels	[20]
	rs2303151	g.27459C>T	157 HC	Atorvastatin	CT genotype	↑ apo AI and apo AI/apo B ratio	[20]
LDLR	rs5925	c.1959T>C	55 HC	Fluvastatin	CC genotype	↓ TC, LDL-c, and apo B reduction	[57]
			157 HC	Atorvastatin	CC genotype	↑ HDL-c increase	[20]
	rs2569542	g.24716A>G	55 HC	Fluvastatin	GG genotype	↓ TC, LDL-c, and apo B reduction	[57]
	Null/defective mutations	–	156 FH	Atorvastatin + ezetimibe	Null/defective mutation	↓ LDL-c reduction	[59]
	Null/defective mutations	–	206 FH	Statins + ezetimibe	Null mutation	↑ CAC score	[60]
APOB	rs17240441(*Indel*)	–	54 CHD	Fluvastatin	*Del* allele	↓ LDL-c reduction	[62]
MYLIP	rs9370867	c.1025G>A	156 FH	Atorvastatin + ezetimibe	G allele	↓ LDL-c reduction	[71]
Transcription regulators of cholesterogenic genes							
SCAP	rs12487736	c.2392G>A	99 HC[a]	Simvastatin	G allele	↑ TC and TG reduction	[74]
NR1I3	rs2501873	g.8463A>G	240 HC[a]	Simvastatin/atorvastatin	G allele	↑ LDL-c reduction	[80]
ESR1	rs2234693	g.190510T>C	495 HC[a]	Simvastatin/atorvastatin	CC genotype	↑ HDL-c increase (women)	[82]
	rs3798577	g.448305T>C	495 HC[a]	Simvastatin/atorvastatin	T allele	↑ TC and TG reduction (women)	[82]
HDL and reverse cholesterol transport							
APOA1	rs1799837 rs5069	c.-21+68G>A c.-21+67C>T	150 HC[a]	Atorvastatin	rs1799837GG/rs5069CC haplotype	↓ TG and VLDL-c levels (women)	[86]
CETP	rs708272	g.5454G>A	99 HC[a]	Simvastatin	AA genotype	↑ HDL-c increase	[65]
LIPC	rs1800588	c.-514C>T	157 HC	Atorvastatin	C allele	↑ LDL-c reduction	[20]
					TT genotype	↑ apo AI and apo AI/apo B ratio	
SCARB1	rs5888	c.1050C>T	147 HC	Atorvastatin	C allele	↓ TC, LDL-c and apo B reduction	[88]
	rs61932577	c.726+54C>T	147 HC	Atorvastatin	T allele	↑ LDL-c and apo B levels	[88]
PON1	rs662	c.575A>G	433 HC	Simvastatin/atorvastatin	A allele (192Gln)	↑ likelihood of reaching HDL-c goal	[89]

Table 1 (continued)

Gene	Variant	NT change	Cohort	Statin	Allele, genotype or haplotype associated	Effect on statin response	References
	rs854560	c.163T>A	433 HC	Simvastatin/atorvastatin	A allele (55Met)	↑ likelihood of reaching HDL-c goal	[89]
Antioxidant proteins							
NOS3	rs2070744	g.6933C>T	30 HM	Atorvastatin	CC genotype	↓ lower TG levels	[92]
SOD2	rs4880	c.47T>C	122 HC	Rosuvastatin	CC genotype	↓ TC and TG reduction ↓ HDL-c increase	[94]
SELENOP	rs3877899	C>T	32 HC	Simvastatin/atorvastatin Se suppl (Brazil nut)	CC genotype	↑ LDL-c and TG reduction	[95]

ADR adverse drug reaction, *Apo AI* apolipoprotein AI, *Apo B* apolipoprotein B, *CHD* coronary heart disease, *FH* familial hypercholesterolemia patients, *HC* hypercholesterolemic patients, *HM* healthy males, *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *NT* nucleotide, *TC* total cholesterol, *TG* triglycerides, *VLDL-c* very low-density lipoprotein cholesterol

[a]Studies with patients of European ancestry only

possibly prevented a clear assessment of their effects on statin response. We recently discussed the possible role of *SLCO1B1*15* on lipid response to rosuvastatin in a case report of a Brazilian FH patient. *SLCO1B1*15,* combined with other variants in *SLCO1B3* and *ABCB11*, was suggested to delay the response to rosuvastatin (20 mg/day), but did not impact LDL cholesterol reduction in the long term [21].

SLCO2B1 rs2851069 (c.-71T>C), a 5′-untranslated region (UTR) variant, was investigated in two Brazilian studies, which showed no significant association with atorvastatin response in HC patients [18, 20] (Table S1).

ABC transporters

MDR1 (encoded by *ABCB1*) is an efflux transporter with an important role in biliary and urinary elimination of statins and metabolites (Fig. 1). The missense variant *ABCB1* rs2032582 (c.2677T>G/A, p.Ser893Ala/Thr) and two synonymous variants rs1045642 (c.3435T>C, p.Ile1145=) and rs1128503 (c.1236C>T, p.Gly412=), which are in linkage disequilibrium, have been extensively studied [9]. *ABCB1* rs1045642 was associated with increased LDL cholesterol response to atorvastatin in high-risk vascular patients from Australia [22]. A meta-analysis also reported the association of *ABCB1* rs1045642 with lipid-lowering response in hypercholesterolemic patients on statins [23]. Haplotypes of *ABCB1* rs2032582, rs1045642 and rs1128503 also influence statin response. In the Rotterdam study, Dutch males carrying the reference haplotype rs1128503C/rs2032582G/rs1045642C showed lower total and LDL cholesterol reductions to simvastatin treatment [24].

The effects of *ABCB1* variants on statin response were studied in three Brazilian studies with HC patients. *ABCB1* rs2032582 A allele carriers had higher LDL cholesterol reduction after atorvastatin treatment [25] (Table 1). Conversely, *ABCB1* rs2032582 and rs1045642 did not influence the cholesterol response to atorvastatin, although carriers of the *ABCB1* rs1045642T/rs2032582T haplotype showed higher baseline total and LDL cholesterol [26] (Table S1). In another cohort of HC patients, *ABCB1* rs1128503 T allele and *ABCB1* rs2032582 GG genotype were associated with higher total or LDL cholesterol reduction after simvastatin treatment [27] (Table 1), suggesting a positive impact of these variants on response to simvastatin.

MRP1 (encoded by *ABCC1*) promotes the efflux of statin metabolites from hepatocytes to the bloodstream. The association of *ABCC1* variants with response to statins has been less studied. The missense variant *ABCC1* rs45511401 (c.2012G>T, p.Gly671Val) was previously associated with lower LDL cholesterol reduction after atorvastatin treatment in Iranian dyslipidemic patients [28]. However, in Brazilian HC patients, *ABCC1* rs45511401 did not influence cholesterol-lowering response to atorvastatin [25] (Table S1).

Drug metabolizing enzymes

CYP3A4 and CYP3A5 are the main metabolizing enzymes of statins in liver cells (Fig. 1). Variants in genes encoding these enzymes have been implicated in the variability of statin PK and response [4, 29].

The variants *CYP3A4*1B* (rs2740574, g.4713G>A, −290A>G) and *CYP3A4*22* (rs35599367, g.20493C>T) have been mostly assessed in pharmacogenetic studies. *CYP3A4*1B* was associated with low risk of atorvastatin or simvastatin dose decrease or withdrawal, particularly in women, showing that **1B* did not influence significantly

Table 2 Genetic variants significantly associated with statin-related adverse events and pleiotropic effects in Brazilian cohorts

Gene	Variant	NT change	Cohort	Statin	Allele, genotype or haplotype associated	Effect and outcomes	References
Adverse events							
ABCB1	rs1128503 rs2032582 rs1045642	c.1236C>T, c.2677T>G/A, c.3435T>C	116 HC[a]	Simvastatin	rs1128503C/ rs2032582G/ rs1045642C haplotype	↑ Myalgia and other ADR	[27]
HMGCR	rs17244841	g.14863A>T	157 HC	Atorvastatin	TT genotype	↑ CK levels	[20]
NR1I3	rs2307424	c.540C>T	240 HC[a]	Simvastatin/atorvastatin	TT genotype	↓ Risk of ADR	[80]
SELENOP	rs3877899	C>T	32 HC	Simvastatin/atorvastatin Se suppl (Brazil nut)	CC genotype	↓ Plasma CK reduction	[95]
	rs7579	G>A	32 HC	Simvastatin/atorvastatin Se suppl (Brazil nut)	GG genotype	↓ Plasma CK reduction	[95]
Pleiotropic effects							
SOD2	rs4880	c.47T>C	122 HC	Rosuvastatin	CC genotype	↓ Inflammatory and fibrinolytic biomarkers	[94]
NOS3	rs2070744	g.6933C>T	30 HM	Atorvastatin	CC genotype	↓ Plasma malondialdehyde ↑ Blood nitrite	[92]
	rs2070744	g.6933C>T	30 HM	Atorvastatin	CC genotype	↓ sCD40L, sVCAM-1, sP-selectin and MMP-9	[109]
	rs2070744	g.6933C>T	30 HM	Atorvastatin	CC genotype	↓ Erythrocyte membrane fluidity ↑ Erythrocyte cholesterol	[110]
	rs2070744	g.6933C>T	25 OW	Simvastatin	C allele	↑ Plasma nitrite	[111]
MTHFR	rs1801133	c.665C>T, 677C>T	25 OW	Simvastatin	T allele	↑ Plasma nitrite ↓ Plasma homocysteine	[113]
SELENOP	rs3877899	C>T	32 HC	Simvastatin/atorvastatin Se suppl (Brazil nut)	CC genotype	↑ Erythrocyte GPX activity increase	[95]
	rs7579 G > A		32 HC	Simvastatin/atorvastatin Se suppl (Brazil nut)	GG genotype	↑ Erythrocyte GPX activity increase	[95]
LDLR	rs5930	c.1413A>G	193 AD	Antihypertensives/statins	rs5930 GA	↓ Blood pressure reduction	[114]
	rs11669576 rs5930	c.1171G>A c.1413A>G	193 AD	Antihypertensives/statins	rs11669576GG/ rs5930GA haplotype	↓ Systolic blood pressure reduction	[114]
	rs11669576		179 AD	Atorvastatin/simvastatin	rs11669576GA	↓ Worsening of functional decline	[115]
	rs5930		179 AD	Atorvastatin/simvastatin	rs5930 AA	↑ Caregiver burden (*APOE*-ε4 carriers)	[115]
	rs5925	c.1959T>C	179 AD	Atorvastatin/simvastatin	rs5925 TT	↓ Cognitive decline (*APOE*-ε4 non-carriers)	[115]
NR1H2	rs2695121	g.50880741T>C	193 AD	Antihypertensives/statins	CT genotype	↑ Creatinine clearance reduction	[114]

AD Alzheimer disease patients, *ADR* adverse drug reaction, *CAC* coronary artery calcium, *CK* creatine kinase, *FH* familial hypercholesterolemia patients, *GPX* glutathione peroxidase, *HC* hypercholesterolemic patients, *HM* healthy males, NT nucleotide, *OW* obese women, *Se* selenium, *Suppl* supplementation

[a]Studies with patients of European ancestry only

statin pharmacokinetics [30]. *CYP3A4*1B* was also associated with higher LDL cholesterol levels after atorvastatin treatment in patients with primary hypercholesterolemia, but no association was found with statin response [31]. *CYP3A4*22* was reported to reduce *CYP3A4* expression [32] and, consequently, *CYP3A4*22* has been associated with higher LDL cholesterol reduction in simvastatin users [33].

*CYP3A4*1B* and *CYP3A4*22* variants were studied in three Brazilian cohorts, and both variants did not influence the lipid-lowering response to simvastatin [27] or atorvastatin [20, 34] in HC patients (Table S1).

Variants in *CYP3A5*, such as **3* (rs776746, c.6986A>G, c.219-237A>G), **6* (rs10264272, c.14690G>A) and **1D* (rs15524, c.31611T>C), have been explored in pharmacogenomic studies of statins [35–37]. *CYP3A5*3* and *CYP3A5*6* are located within splicing regions and impair gene expression [38].

The effect of *CYP3A5* variants on statin response remains controversial. Carriers of the *CYP3A5*3/*3* genotype (non-expressor) showed enhanced LDL cholesterol response to lovastatin, simvastatin and atorvastatin [39] and higher plasma simvastatin levels compared to *CYP3A5*1* allele (expressor) carriers [40]. In contrast, *CYP3A5*3* showed no effect in atorvastatin response in Chilean dyslipidemic patients [41]. Since CYP3A5 does not participate expressively in the metabolism of statins, it is likely that *CYP3A5* variants have a minor effect on statin pharmacokinetics and response.

CYP3A5 variants were assessed in four Brazilian studies with HC patients taking statins. *CYP3A5*3/*3* genotype was associated with lower reduction in total and LDL cholesterol in patients of non-African descent, but not of African descent [42] probably due to the low frequency of *CYP3A5*3* in African populations [43]. *CYP3A5*3/*3* genotype was also negatively correlated with LDL cholesterol response to atorvastatin treatment in admixed HC patients, suggesting a potential effect of *CYP3A5*3* allele on atorvastatin response [34]. Conversely, *CYP3A5*3* did not influence simvastatin or atorvastatin response in HC patients [20, 27] (Table S1). *CYP3A5*6* was also explored in a Brazilian study, but no associations were found with lipid-lowering response to atorvastatin [42] (Table S1).

Until now, the genetic variability of *CYP2C9* was not investigated in Brazilian individuals treated with statins. In other populations, *CYP2C9*2* (rs1799853, p.Arg144Cys) and *CYP2C9*3* (rs1057910, p.Ile359Leu) variants have been associated with increased plasma concentrations of both fluvastatin enantiomers, 3R,5S- and 3S,5R-fluvastatin [44]. However, contradictory to expectations, these variants do not seem to influence the lipid-lowering response to fluvastatin [45].

Pharmacodynamics genes and statin response

Cholesterol homeostasis and metabolism

HMGCR encodes the target of statins HMGR (Fig. 2) and, therefore, has a relevant role in statin PD. The intronic variants *HMGCR* rs17244841 (g.14863A>T) and rs3846662 (c.1564-106A>G) have been associated with reduced lipid response to statins in patients with HC and familial hypercholesterolemia (FH) [46–48].

In an HC Brazilian study, *HMGCR* rs17244841 AT and rs2303151 AG genotypes were associated with increased apolipoprotein (apo) AI or apo AI/apo B ratio after atorvastatin treatment, suggesting a beneficial effect of these variants on statin response [20] (Table 1). However, *HMGCR* rs17244841, rs2303151 and rs5908 did not influence LDL cholesterol response (Table S2).

Other proteins involved in cholesterol intracellular homeostasis, such as LDL receptor (LDLR), apo B and proprotein convertase subtilisin/kexin type 9 (PCSK9) (Fig. 2), have been also proposed as important biomarkers of statin response. LDLR has high affinity for apo B (structural protein of LDL) and interaction of LDLR with apo B-LDL is responsible for removal of LDL particles from blood circulation [49, 50]. PCSK9 is a serine protease that interacts and directs LDLR to lysosomes to be cleaved instead of returning to the cell surface and consequently reduces LDLR intracellular levels and increases plasma LDL cholesterol [49].

Variants in *LDLR*, *APOB* and *PCSK9* have been associated with variability in response to statins [51–55]. A genome-wide association study with European patients found that the *LDLR* rs688 (c.1773C>T, p.Asn591=) variant was associated with lower LDL cholesterol reduction [56]. In *PCSK9*, loss-of-function variants have been associated with higher LDL reduction [52], whereas gain-of-function variants had the opposite effect [55].

The association of *LDLR* variants with response to statins was assessed in four Brazilian cohorts. A prospective study explored the *LDLR* synonymous variants rs688 and rs5925 (c.1959T>C, p.Val653=) and the intronic variant rs2569542 (g.24716A>G) in 55 HC patients treated with fluvastatin for 16 weeks [57]. Carriers of *LDLR* rs5925 CC and rs2569542 GG genotypes showed lower reduction of total and cholesterol and apo B compared to non-carriers (Table 1), but this effect was not observed for rs688. Moreover, all variants were associated with high baseline total and LDL cholesterol and apo B levels. Another study investigated *LDLR* rs688 and rs5925 in HC patients treated with atorvastatin, and rs5925 CC genotype was associated with increase of high-density lipoprotein (HDL) cholesterol after treatment (Table 1), but no association with LDL cholesterol response was found [20]. *LDLR* rs14158 (c.*52G>A) was also assessed in HC patients, but no association with response to atorvastatin was found [58] (Table S2).

One study investigated *LDLR* null (large deletions or frameshift mutations) and defective mutations Brazilian heterozygous FH patients treated with atorvastatin. Carriers of *LDLR* null and defective mutations showed reduced LDL cholesterol after treatment (Table 1) and increased risk of not reaching LDL cholesterol target levels (OR: 9.07, 95% CI 1.41–58.16, $p=0.02$) [59]. Patients carrying null mutations also showed higher baseline and post-treatment total

and LDL cholesterol compared to carriers of defective mutations and non-carriers.

The impact of *LDLR* null and defective mutations on cardiovascular outcomes was also explored in Brazilian FH patients on long-term statin therapy. *LDLR* null mutations were associated with high coronary artery calcium (CAC) score (Table 1), but not with major atherosclerotic cardiovascular events (MACE) [60]. *LDLR* null or defective mutations were not major determinants of atherosclerotic cardiovascular disease (ASCVD) in older FH patients [61] (Table S2).

APOB variants rs693 (c.7545C>T), rs1042031 (c.12541G>T, p.Glu4181*) and rs17240441 (*Indel*) were also studied in Brazilian HC patients. Lower LDL cholesterol reduction was observed in carriers of *APOB* rs17240441 *Del* allele after treatment with fluvastatin for 16 weeks [62] (Table 1). Conversely, *APOB* rs693 and rs1042031 did not influence the response to atorvastatin or fluvastatin in two independent cohorts [20, 62] (Table S2).

Two studies explored *PCSK9* variants in Brazilian HC patients. The missense variants rs505151 (c.2009A>G, p.Gly670Glu), rs562556 (c.1420A>G, p.Val474Ile) and rs11591147 (c.137G>T, p.Arg46Leu) did not influence the lipid response to atorvastatin, even though rs505151 G allele was associated with high baseline LDL cholesterol [63]. The *PCSK9* 3'UTR variant rs17111557 (c.*614C>T) was also assessed, but no association was found with atorvastatin response [58] (Table S2).

Apo E is a glycoprotein produced mainly by the liver that is present mostly in triglyceride-rich lipoproteins, such as chylomicrons and very low-density lipoprotein (VLDL) (Fig. 2). LDLR has high affinity by apo E, which has a key role in the clearance of apo B-containing lipoproteins from bloodstream [4, 64]. The variants *APOE* rs7412 (c.526C>T) and rs429358 (c.388T>C) give rise to three haplotypes: ε2 (rs7412 T, rs429358 T), ε3 (rs7412 C, rs429358 T), and ε4 (rs7412 C, rs429358 C), which have been extensively studied for their impact on LDL cholesterol levels and statin response [4, 64].

In three independent cohorts of Brazilian HC patients, *APOE* variants did not influence the cholesterol-lowering response to simvastatin [65] or atorvastatin [66, 67] (Table S2). In postmenopausal women, ε3/ε3 genotype was associated with higher reduction of *APOE* mRNA expression in peripheral blood mononuclear cells (PBMC) after atorvastatin treatment, suggesting that atorvastatin modulates *APOE* mRNA expression in a genotype-dependent manner [67].

The myosin regulatory light chain interacting protein (MYLIP, also named IDOL) is an E3 ubiquitin ligase, which mediates ubiquitination of the LDLR at the cell membrane, leading to its destruction in lysosomes and indirectly affecting LDL uptake and cholesterol homeostasis [68]. The missense variant *MYLIP* rs9370867 (c.1025G>A, p.Asn342Ser) has been associated with dyslipidemia in different populations [69, 70]. Further functional characterization in vitro showed that rs9370867 increased LDLR degradation, which was consistent with the increased lipid levels [69].

MYLIP rs9370867 was assessed in Brazilian heterozygous FH patients taking atorvastatin plus ezetimibe. Carriers of rs9370867 G allele showed lower LDL cholesterol reduction and higher post-treatment LDL cholesterol levels [71] (Table 1).

Transcription regulators of cholesterogenic genes

Sterol regulatory element-binding proteins (SREBPs) are a small family of transcription factors that regulate the expression of more than 30 genes involved in the uptake and synthesis of cholesterol, fatty acids, and triglycerides. There are three isoforms of SREBPs: SREBP-1a and SREBP-1c, encoded by *SREBF1*; and SREBP-2, encoded by *SREBP-2*. After synthesis, SREBPs are bound to the endoplasmic reticulum in an inactive state. Their activation depends on the complexation with SREBP cleavage-activating protein (SCAP). The SREBP–SCAP complex is translocated from the endoplasmic reticulum to the Golgi apparatus, where the terminal region of SREBP is cleaved by Golgi proteases and translocated to the nucleus, activating the transcription of *LDLR*, *HMGCR* and other cholesterogenic genes [72, 73].

Two studies also explored the effects of *SREBF1* and *SREBF2* variants on statin response in Brazilian cohorts. *SREBF1* rs60282872 (g.5161delC), an *indel* in the promoter region, and the missense variant *SREBF2* rs2228314 (c.1784G>C, p.Gly595Ala) were not associated with lipid changes after simvastatin treatment in HC patients [74]. Similar results were observed for *SREBF1* rs60282872 in HC patients treated with atorvastatin [75] (Table S2).

The missense variant *SCAP* rs12487736 (c.2392G>A, p.Val798Ile) was also assessed in Brazilian HC patients and G allele carriers showed higher total cholesterol and triglycerides reduction after treatment with simvastatin [74] (Table 1). Another study did not find an association of rs12487736 with lipid response to atorvastatin; however, rs12487736 AA carriers had lower *SCAP* mRNA expression in PBMC after treatment [75] (Table S2).

Nuclear receptors are a superfamily of transcription factors that activate the expression of genes involved in many metabolic pathways. *NR1I3*, *NR1I2,* and *PPARA* encode constitutive androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator-activated receptor α (PPARα), respectively. These molecules regulate the expression of genes in drug metabolism and transport, as well as in lipid metabolism [76–78]. Their activation depends on the

dimerization with the retinoid X receptor alpha (RXRα), encoded by *RXRA* [79].

A prospective cohort study investigated variants in *NR1I2, NR1I3*, *PPARA,* and *RXRA* and statin response in HC patients [80]. *NR1I3* rs2501873 G allele carriers had a higher LDL cholesterol reduction after treatment with simvastatin or atorvastatin (Table 1). CAR (*NR1I3)* regulates the expression of *CYP3A4* and *ABCB1*, which are involved in statin pharmacokinetics; therefore variants in *NR1I3* may impact statin bioavailability and, consequently, the therapeutic response. *NR1I2* rs1523130 (c.-1663T>C), *PPARA* rs1800206 (c.484C>G) and *RXRA* rs11381416 (-/A indel) did not influence statin response in this cohort (Table S2).

ESR1 encodes the estrogen receptor α (ERα), a transcription factor that activates the expression of genes associated with estrogen response, including those involved in lipid metabolism. Variants in *ESR1* have been shown to increase the risk of cardiovascular disease (CVD) [81]. The association between 13 *ESR1* variants and simvastatin or atorvastatin response was studied in Brazilians [82]. In women, *ESR1* rs2234693 (g.190510T>C) CC genotype was associated with higher HDL cholesterol increase and rs3798577 (g.448305T>C) T allele with higher total cholesterol and triglycerides reduction after treatment (Table 1).

HDL and reverse cholesterol transport

APOA1, ABCA1, CETP, and *SCARB1* are genes encoding proteins involved in different steps of the reverse cholesterol transport, in which cholesterol is removed from peripheral tissues, transported to the liver through HDL particles, and excreted via bile [83]. Briefly, plasma apo AI (structural protein of the HDL) interacts with ABCA1, which promotes free cholesterol and phospholipids efflux from the cell membrane to the nascent HDL. Further HDL cholesterol is esterified by lecithin:cholesterol acyltransferase (LCAT) and lipids are exchanged with other lipoproteins by cholesteryl ester transfer protein (CETP). Cholesterol is also transferred to mature HDL via interaction with ABCG1. After removing the cholesterol from peripheral tissues, HDL returns to the liver and the contents of triglycerides and phospholipids are hydrolyzed by hepatic lipase (HL), resulting in the assembly of small HDL which is taken up by the hepatic scavenger receptor class B1 (SR-B1, encoded by *SCARB1*) [83, 84]. Paraoxonase1 (PON1) is a plasma enzyme that prevents HDL and LDL oxidation. Increased PON1 levels have been associated with higher HDL cholesterol levels [85].

The association of variants in genes involved in reverse cholesterol transport with statin response was investigated in Brazilian cohorts. The variants rs1799837 (c.-21+68G>A) and rs5069 (c.-21+67C>T) in *APOA1* were assessed in HC patients, and women carrying rs1799837GG/rs5069CC haplotype had lower reduction of plasma triglycerides and VLDL cholesterol after atorvastatin treatment [86] (Table 1). *ABCA1* rs2230806 (c.656G>A, p.Arg219Lys), rs56064613 (c.-418C>T) and rs1800977 (c.-390C>T) were investigated in another HC cohort, but these variants did not influence the LDL cholesterol response to atorvastatin [87] (Table S2). In HC patients, *CETP* rs708272 (g.5454G>A, *Taq* IB) was associated (AA genotype) with high HDL cholesterol increase after simvastatin treatment [65] (Table 1).

Variants in *LIPC*, which encodes HL, were also explored in Brazilian cohorts. *LIPC* rs1800588 C allele predicted a lower LDL cholesterol reduction after atorvastatin treatment, and TT genotype was associated with post-treatment levels of apo AI and apo AI/apo B ratio in admixed HC patients [20] (Table 1). Conversely, *LIPC* rs2070895, rs690 (c.465G>A) and rs3829462 (c.1104C>A) were not associated with lipid changes in Brazilian HC patients treated with simvastatin [65] or atorvastatin [20] (Table S2).

The variants rs5888 (c.1050C>T), rs4238001 (c.4G>A) and rs61932577 (c.726+54C>T) in *SCARB1*, which encodes SR-B1, were also assessed in HC patients taking atorvastatin [88]. *SCARB1* rs5888 C allele was associated with lower reduction of total and LDL cholesterol and apo B after treatment, whereas the rs61932577 T allele was associated with higher post-treatment LDL cholesterol and apo B levels (Table 1). *SCARB1* rs4238001 did not influence baseline and post-treatment plasma lipids (Table S2).

In Brazilian HC patients, *PON1* rs662 (c.575A>G, p.Gln192Arg) and rs854560 (c.163T>A, p.Leu55Met) missense variants were associated with lower HDL cholesterol after statin treatment [89]. *PON1* rs662A (192Gln) and rs854560A (55Met) carriers had high likelihood of reaching HDL cholesterol post-treatment goal (OR: 2.81, 95% CI 1.35–5.85, $p=0.006$) (Table 1).

The scavenger receptor class B2 (CD36) is a transmembrane protein expressed in macrophages and other cells. Its extracellular domain can recognize multiple molecules, including fatty acids, LDL and HDL. Variants in *CD36* have been associated with many processes such as endothelial dysfunction, foam cell formation and atherosclerosis [90]. In Brazilian HC patients, *CD36* rs1984112 (g.16417A>G) was associated with a higher risk of dyslipidemia (OR: 3.55, 95% CI 1.88–6.70, $p=0.0002$), but not with lipid response to atorvastatin treatment [20] (Table S2).

Antioxidant enzymes

Endothelial nitric oxide synthase (eNOS) produces nitric oxide, an important molecule that increases vasodilation and reduces platelet aggregation, leukocyte adhesion to the endothelium and smooth muscle proliferation [91]. The rs2070744 (g.6933C>T) variant in *NOS3*, which encodes

eNOS, was explored in Brazilian healthy males, and rs2070744 CC genotype was associated with reduction of triglycerides in response to short-term atorvastatin treatment [92] (Table 1).

The manganese-dependent superoxide dismutase (SOD2) is an antioxidant enzyme that converts the superoxide anion into hydrogen peroxide, inactivating this highly damaging anion. Variants in SOD2 have been associated with CVD and other diseases [93]. The effects of *SOD2* rs4880 (c.47T>C) was studied in Brazilian HC patients, and rs4880 CC genotype carriers had lower total and LDL cholesterol reduction and lower HDL cholesterol increase after rosuvastatin treatment [94] (Table 1).

Selenoprotein P is a selenium (Se)-enriched plasma protein with antioxidant activity. A recent study investigated *SELENOP* rs3877899 C>T and rs7579 G>A variants in HC patients taking statins and Se supplementation (Brazilian nuts) [95]. *SELENOP* rs3877899 CC carriers showed higher reduction of triglycerides and LDL cholesterol, after Se supplementation for 3 months (Table 1).

Statin-related adverse events

Adverse drug reactions (ADR) result in significant cost and morbidity and can lead to non-adherence and discontinuation of therapy. Statin-associated muscle symptoms (SAMS) are the most commonly reported ADR associated with statin therapy, whereas statin-induced toxicity in the liver and central nervous system is less frequent [13].

SAMS are one of the major causes of statin discontinuation/non-adherence, which increases the risk of cardiovascular events in dyslipidemic patients [96, 97]. The prevalence of SAMS ranges from 7 to 33% in registries and observational studies [96–98]. In Brazil, a cross-sectional study with national representative sample (8803 patients from 272 cities) reported 0.7% of SAMS in 9.3% of patients taking statins (90.3% simvastatin) [99]. The prevalence of SAMS was significantly higher (50.4%) in a cohort of Brazilian patients on statin therapy with increased serum creatine kinase (CK), a biomarker of muscle damage [100].

Statins block the mevalonate downstream pathway, which results in decreased production of cholesterol, isoprenoids and ubiquinone (CoQ10). CoQ10 is important for mitochondrial function and energy production in muscle cells [101]. Increased exposure of skeletal muscle to statins leads to cell depletion of the mevalonate pathway end products causing a series of intracellular events, including mitochondrial dysfunction, disruption calcium and pro-apoptotic signaling, and reduction of prenylation, which have been proposed as important mechanisms of statin-related myotoxicity [101].

Genetic and non-genetic factors that increase systemic and muscle exposure to statins, and muscle dysfunction have been implicated in statin-related myotoxicity [97, 98, 101]. Variants in PK-related genes, such as *SLCO1B1, SLCO2B1, ABCB1, ABCC2, ABCG2* and *CYP3A4*, were shown to increase statin systemic and muscle exposure and SAMS [4, 22, 97, 101]. Variants in genes involved in CoQ10 biosynthesis and mevalonate pathway have been also associated with statin intolerance and SAMS [4, 101].

Extensive evidence demonstrates the association between *SLCO1B1*5* and simvastatin-induced myopathy [97, 102, 103]. Consequently, *SLCO1B1*5* was included in international guidelines as a risk allele for myopathy, together with two haplotypes containing *5 C allele, *SLCO1B1*15* (*1B and *5 alleles) and *SLCO1B1*17* (*1B, *5 and rs4149015 A alleles) [104].

The association of variants in PK and PD genes with SRAE was explored to a lesser extent in Brazilian cohorts. Only one Brazilian study investigated *SLCO1B1*1B* and **5* and statin-induced myopathy in FH patients treated with atorvastatin (20–80 mg/day), but these variants were not associated with risk of myalgia or increased plasma CK [105] (Table S1). However, the group of patients with myalgia was small, which possibly reduced the statistical power of the analysis. Also, there was no stratification according to atorvastatin doses; this is important because the risk of SRAE in carriers of *SLCO1B1*5* is substantially greater in patients treated with high-intensive statin treatment [97]. We recently reported a case of a Brazilian FH patient who experienced severe myalgia to simvastatin and atorvastatin, and discussed the possible role of *SLCO1B1*15* haplotype, which contains *5 allele, on this SRAE [21].

The *ABCB1* rs1045642 was previously associated with increased risk of myalgia upon atorvastatin in high-risk vascular patients [22]. A meta-analysis also reported that *ABCB1* rs1045642 increased the risk of myopathy for short-term statin therapy, suggesting this variant as a potential pharmacogenomic biomarker for statin-induced myotoxicity [23].

In Brazilian HC patients on simvastatin therapy, *ABCB1* rs1128503 C, rs2032582 G and rs1045642 C alleles and C-G-C haplotype were associated with myalgia and other SRAE, such as abdominal pain and allergy [27] (Table 2). This study also reported lack of association between *CYP3A4*1B* and *CYP3A4*22* alleles and SRAE and other ADR (Table S1).

The influence of variants in PD and other genes on statin-induced myotoxicity in Brazilian cohorts was also explored. *HMGCR* rs17244841 TT genotype was associated with increased CK levels (Table 1), but not with SRAE in HC patients treated with atorvastatin [20]. On the other hand, *LDLR* null or defective mutations did not increase the risk of myalgia in Brazilian patients with heterozygous FH treated with atorvastatin [59] (Table S2).

Another study reported that *NR1I3* rs2307424 TT genotype reduced the risk of SRAE (myalgia, increased CK or hepatic dysfunction) in Brazilian HC patients on simvastatin or atorvastatin therapy [80] (Table 2), whereas variants in *NR1I2* (rs1523130 and rs2472677), *PPARA* (rs1800206), and *RXRA* (rs11381416) lacked association with SRAE (Table S3).

SELENOP rs3877899 C>T and rs7579 G>A were also assessed in HC patients taking statins and Se supplementation (Brazilian nuts) [95]. The rs3877899 CC and rs7579GG genotypes were associated with lower reduction of plasma CK after Se supplementation, suggesting that these genotypes confer a less protective effect on myopathy (Table 1).

Statin-related pleiotropic effects

Statins have been proposed to exert cardiovascular protective effects through cholesterol-independent pleiotropic effects that are important to prevent cardiovascular events [91, 106]. The inhibition of the HMGR by statins reduces the production of isoprenoid intermediates in the mevalonate downstream pathway. These intermediates are essential for prenylation of small GTP-binding proteins and their effectors, which are involved in endothelial and platelet dysfunction, inflammatory process, atherosclerosis, fibrosis and other pathophysiological mechanisms of CVD [91].

Ten studies explored genetic variants and statin-related pleiotropic effects in Brazilian cohorts. A cross-sectional study evaluated *HMGCR* rs17238540, *APOE* rs405509, *CETP* rs708272 and *PON1* rs662 variants in statin-treated HC patients, but no association was found with oxidative stress biomarkers, namely plasma malondialdehyde, oxidized LDL, and total antioxidant activity and plasma tocopherol [107] (Table S3).

SOD2 rs4880 (c.47T>C) was also studied in Brazilian HC patients, and CC genotype was associated with pronounced reduction of pro-inflammatory cytokines (IL-1, IL-6, TNFα and IFNγ) and fibrinolytic markers (α-acid glycoprotein, d-dimer and fibrinogen), as well as marked increase in IL-10, in response to rosuvastatin therapy [94] (Table 2).

Variants in *NOS3* have been associated with statin-related pleiotropic or LDL cholesterol-independent effects that prevent cardiovascular risk [108]. Brazilian healthy males carrying rs2070744 CC genotype had pronounced increase in blood nitrite and decrease in plasma malondialdehyde, sCD40L, sVCAM-1, sP-selectin and MMP-9, and erythrocyte membrane fluidity after treatment [92, 109, 110] (Table 2). In obese women, *NOS3* rs2070744 CC was also associated with higher increase in plasma nitrite after short-term treatment with simvastatin [111] (Table 2).

Methylenetetrahydrofolate reductase (MTHFR) converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine re-methylation to methionine. Polymorphisms in genes of the homocysteine pathway, such as *MTHFR*, were suggested to interact with pravastatin in reducing risk of cardiovascular events [112]. *MTHFR* rs1801133 (677C>T) was investigated in Brazilian obese women, and T allele carriers showed increased plasma nitrite and decreased plasma homocysteine, after simvastatin treatment [113] (Table 2).

Recently, *SELENOP* rs3877899 CC and rs7579 GG genotypes were found to be associated with higher increase in erythrocyte glutathione peroxidase (GPX) activity after Se supplementation in HC Brazilian patients taking statins [95] (Table 2). This study also analyzed the rs1050450 C>T in *GPX1*, which encodes glutathione peroxidase 1, but no influence was found in erythrocyte PGX or plasma or erythrocyte Se (Table S3).

Recent Brazilian studies also explored the influence of common variants in *LDLR*, *APOE* and *NR1H2* on hypertension control and cognitive function in patients with Alzheimer disease (AD) treated with statins. *LDLR* rs5930 GA genotype and rs11669576GG/rs5930GA haplotype were associated with lower reduction of blood pressure in AD patients taking antihypertensives and statins. In these AD patients, *NR1H2* rs2695121 CT genotype was associated with higher reduction of creatinine clearance [114] (Table 2). In AD patients taking lipophilic statins, *LDLR* rs11669576 GA carriers had slower worsening of cognitive function independently of *APOE*-ε4. *LDLR* rs5930 AA and GA genotypes were associated, respectively, with higher caregiver burden (*APOE*-ε4 carriers) and worse prognosis of clinical dementia (*APOE*-ε4 non-carriers). In addition, *LDLR* rs5925 TT was associated with slower cognitive decline in *APOE*-ε4 non-carriers [115] (Table 2).

Clinical implications of statin pharmacogenomics

In Brazil, statins are prescribed according to the cardiovascular risk stratification, varying in statin type and dosage (Fig. 3). Simvastatin, atorvastatin, fluvastatin, lovastatin, and pravastatin are provided by the Brazilian Unified Health System since 2002. At least in part due to the lower cost and bureaucratic administrative burden, simvastatin is the most prescribed statin in Brazil [99].

Both simvastatin and atorvastatin were explored in pharmacogenetic studies in Brazilian cohorts. Variants in *SLCO1B1* and *ABCB1* had a positive impact in LDL cholesterol response to short- and long-term statin treatments, whereas *CYP3A5* variants had a negative effect (Table 1). Most of the studies investigated variants in PD genes, such

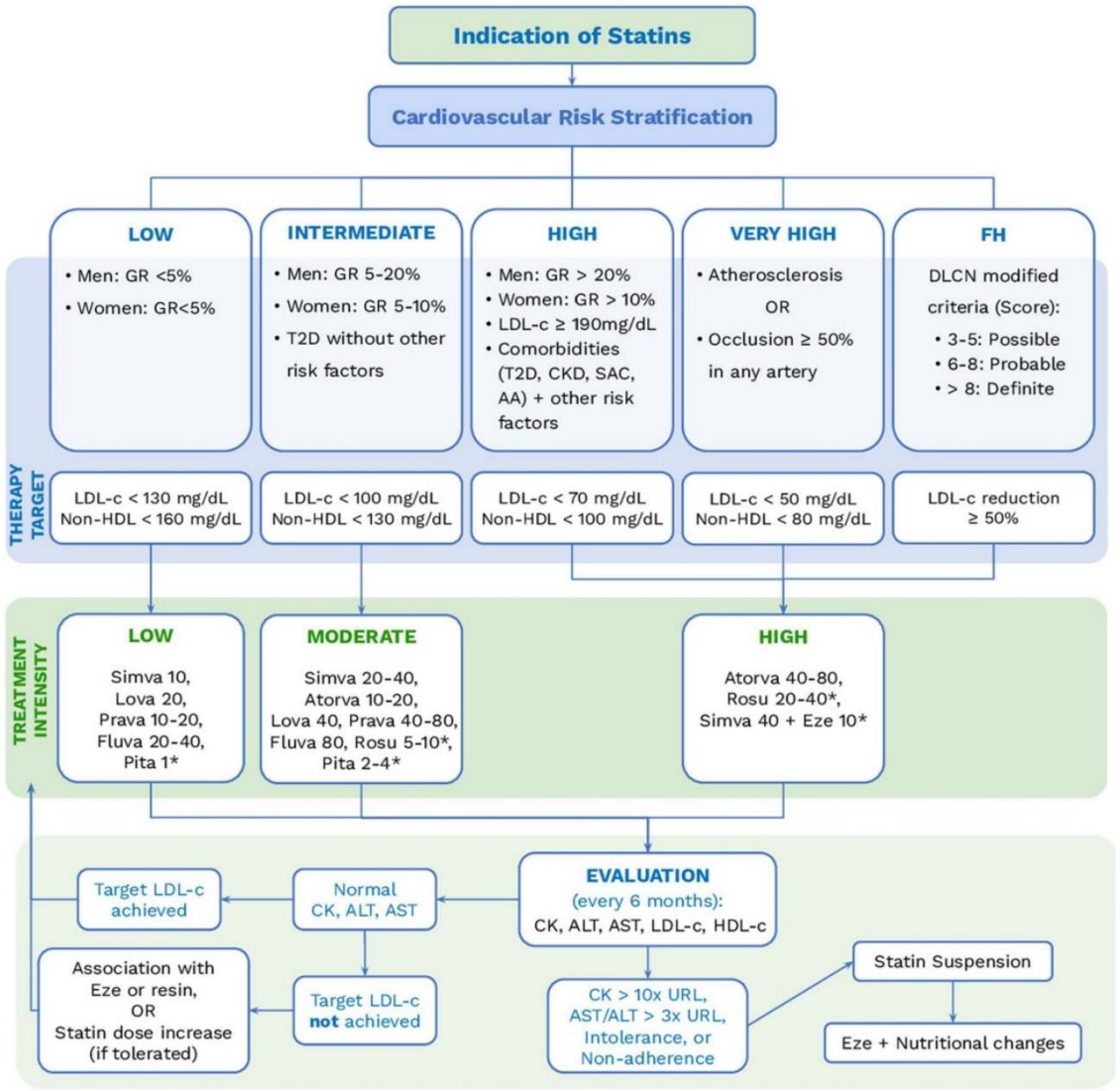

Fig. 3 Statin prescription according to the Brazilian guidelines for the management of dyslipidemia [118]. *Not provided free of cost by the Brazilian Health System. *AA* aorta aneurism, *ALT*: alanine aminotransferase, *AST* aspartate aminotransferase, *Atorva* atorvastatin, *CK* creatine kinase, *CKD* chronic kidney disease, *DLCN* Dutch Lipid Clinic Network, *Eze* ezetimibe, *FH* familial hypercholesterolemia, *Fluva* fluvastatin, *GR* global risk in 10 years, *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *Lova* lovastatin, *Pita* pitavastatin, *Prava* pravastatin, *Rosu* rosuvastatin, *SAC* subclinical atherosclerosis, *Simva* simvastatin, *T2D* type 2 diabetes, *URL* upper reference level

as *HMGCR* and *LDLR*, and significant associations with the lipid response to statins were found (Table 1).

Few studies explored the SRAE in Brazilian cohorts, and only *ABCB1, HMGCR* and *NR1I3* variants were associated with increased CK levels, high risk of myalgia or other ADR (Table 2). These studies are observational, so findings by chance and multiple testing problems might exist leading to false associations.

The effect of genetic variants in statin-induced myopathy is well described. Although many variants have been associated with SAMS, to date, *SLCO1B1* is the only gene included in pharmacogenetic guidelines for statins. In 2014,

the Clinical Pharmacogenetics Implementation Consortium (CPIC) included an updated guideline for simvastatin, recommending dose adjustments for carriers of *SLCO1B1*5, *15* or **17* haplotypes. These haplotypes contain the reduced function variant rs4149056 (p.Val174Ala), which confers elevated risk of simvastatin-induced myotoxicity [104]. Only one Brazilian study explored *SLCO1B1*5* and **15* in FH patients, but no association with atorvastatin-induced myotoxicity was found (Table S1), and the contribution of this variant on simvastatin-related myotoxicity is yet to be investigated. As such, the Brazilian guidelines for dyslipidemia management neither clearly contraindicate simvastatin at high doses (80 mg/day) nor include recommendations for *SLCO1B1* genotyping to predict the risk of simvastatin-induced myopathy [116].

Nevertheless, statin resistance is still an issue for patients and clinicians. EAS/ESC [117] and Brazilian [116, 118] (Fig. 3) guidelines rely on LDL cholesterol treatment targets that are set according to the cardiovascular risk, and for FH patients, the goal is an LDL cholesterol reduction of at least 50%. Still, this is not achieved by 26% of rosuvastatin 40 mg users and up to 42% of atorvastatin 80 mg users, the highest doses for these statins [119].

Although several variants have been associated with lower statin efficacy, there are still no clear conclusions on a biomarker for statin response. This could be due to many factors. First, cholesterol metabolism is extremely complex. Punctual variations of any of the numerous genes involved in cholesterol metabolism may individually have a low-magnitude effect on statin response, as shown by previous studies [56, 120]. Therefore, it is challenging to find key genes—or a combination of genes—that could harbor strong biomarkers of statin response. Second, statin efficacy studies must consider a series of confounding factors that often are difficult to control. Statin response is challenging to evaluate due to the fluctuation of cholesterol levels caused by external factors, such as diet and comorbidities [49]. Also, the adherence to statins should be considered, as adherence was shown to be relatively low in statin users [121]. SRAE, particularly muscle symptoms, was reported as the reason for 65% of cases of low adherence [122]. FH and high CVD risk patients often are prescribed high-dose statins, which are associated with higher myopathy risk [97]. Finally, sample size issues possibly cause discrepant associations between variants and statin response between studies and populations.

Besides *SLCO1B1* variants, PharmGKB also reports an impact of *APOE* rs7412 (c.526C>T, ε2) on atorvastatin response, with ε2 carriers showing higher LDL cholesterol lowering (Level 2B of evidence). This finding was not observed in Brazilian patients on simvastatin or atorvastatin [65–67], as shown in Table S2. A possible solution for this gap is considering the burden of deleterious variants in pharmacogenes that could impair statin response. An approach should be studying also if rare, deleterious variants impact statin response.

Another important aspect is that Brazilian population is very diverse and heterogeneous due to its historical admixture between hundreds of Amerindian groups, Europeans (mainly Portuguese, Italians, Spanish, Dutch, and Germans), sub-Saharan Africans from the slave trade, and East Asians (mainly Japanese). The ethnicity in Brazil is mostly defined by self-declared skin color [123], which does not necessarily reflect genetic ancestry. Also, self-declared color in Brazil does not align well with global ethnic groups. For instance, Brazilian blacks have only 51% African ancestry, whereas Africans and African-Americans have at least 80% and "*pardo*" or "brown" does not correspond to any of the major superpopulations [124]. As a further complicating factor, variant frequencies vary according to the geographical region within Brazil even within each self-reported color [125, 126]. This genetic complexity results in unique challenges when studying population-scale pharmacogenetics in Brazil.

The benefit of statin therapy on primary and secondary prevention of CVD have been explored in large clinical trials. Patients at high CVD-associated genetic risk score (GRS) showed greater benefit from statin therapy to prevent a cardiovascular event, despite similar LDL cholesterol lowering [127, 128]. Few studies explored the impact of genetic variants on cardiovascular events in HC patients on statin therapy, limited to *LDLR* null or defective variants. Considering that CVD-GRS were derived from a population of European ancestry, similar studies should be performed in Brazilian cohorts to explore their influence on statin CVD preventive effects.

Rare and population-specific variability

With the advent of large-scale sequencing projects, such as Exome Sequencing Project [129] and 1000 Genomes Project [130], it became clear that human genomes harbor a plethora of rare genetic variants. For *CYP* genes, analysis of the exomes of > 6500 individuals of European and African ethnicity in the USA indicated that the vast majority of all identified variants were rare with allele frequency < 1% [29, 131]. Analyses of even larger data sets (> 130,000 individuals) corroborated these findings and showed that similar genetic complexity was also observed in polymorphic transporter families of relevance for the treatment of dyslipidemias, such as *ABCs*, *SLCs* and *SLCOs* [132–134], as well as in human apolipoprotein genes [135]. Across 208 PK genes, over 69,000 single nucleotide variations (SNVs) have been described, of which 98.5% were rare [136], and 175 out of these genes harbored partial or full gene deletions or duplications [137]. Furthermore, these studies found that

approximately 75% of all SNVs and copy number variations were identified only in a single population.

While these results unequivocally demonstrate the presence of a multitude of rare variants, the functional relevance of these findings remained unclear. Early estimates suggested that rare variants account for approximately 18% of the functional variability in *SLCO1B1* [138] and 30–40% across PK genes [139]. In recent years, there has been increasing interest in the functional interpretation of rare pharmacogenetic variants. Nevertheless, such low-throughput methods are not applicable for rare variant assessments. Thus, current strategies for rare variant evaluations are based on computational tools or high-throughput-compatible deep mutational scanning strategies [140]. Importantly however, conventional *in silico* methods mostly base their functionality predictions on the evolutionary conservation of the respective nucleotide or amino acid sequence and are trained on pathogenic variant sets. However, since evolutionary conservation in pharmacogenes encoding gene products without important endogenous functions is overall low, and PK genes are only rarely associated with disease, the predictive power of computational methods for pharmacogenetic variants is generally much lower than for pathogenic variation [141].

To overcome such limitations, we have previously developed an algorithm specifically trained on PK genes and found that it achieved high sensitivity and specificity (> 90%) for variants in *CYP* and other polymorphic drug-metabolizing enzymes [142, 143], whereas its predictive power was somewhat lower (78–87%) for rare variants in the bile acid transporter SLC10A1 [144].

In recent years, pharmacogenetic studies using different sequencing technologies have identified rare variants with significant impact on dyslipidemia treatment response. Rare genetic variants contribute to altered lipid traits in founder populations, providing insight into the etiology of dyslipidemias and facilitating development of therapies for metabolic disorders [145, 146]. Thus, rare variants may also have a contribution on statin response. When considering SRAE, whole-exome sequencing of individuals having statin-related myopathy revealed that rare variants in *CYP3A5* and other genes were associated with higher risk of mylagia [147], but a later meta-analysis did not find any associations [148]. While the common alleles *SLCO1B1*5* and **15* that are included in CPIC guidelines as markers of statin toxicity do not show significant effects in the Brazilian population, more research is necessary to investigate whether rare variants with small effect sizes might contribute to statin risk.

So far, only few studies have evaluated the cost-effectiveness of preemptive genotyping for statin use. These studies analyzed the value of testing genotypes of *ACE* [149], *CETP* [150] and *KIF6* [151] in Dutch, Australian and American patients. However, the results remain inconclusive [152] and none of the assessed variations have been shown to be robustly linked to statin response in Brazilians. In addition to biomarkers for the efficacy of hypercholesterolemia treatment, the established reduced function variant rs4149056 in *SLCO1B1* might identify individuals at risk for severe statin myotoxicity. While associations of this polymorphism with myotoxicity risk are well established, patient outcomes were not significantly improved by preemptive genotyping [153] and clinical utility of *SLCO1B1* testing was not demonstrated [154]. Given the substantial cost of genotyping and the lack of genetic variants with a high effect size in the Brazilian population, preemptive genotyping to guide statin treatment is thus not likely to be cost-effective in such an admixed and heterogeneous population. With rapid improvements in genotyping technology, it can be expected that test costs will continue to decline. However, as we and others previously showed, the predictive power of testing and the cost differences between first-line and alternative treatments rather than test costs per se are the main parameters governing the cost-effectiveness of preemptive genotyping [155–157]. In the absence of further quantitative information, such as the number of patients needed to test to prevent one case of toxicity or non-response (NNT_{tox} and $NNT_{non\text{-}res}$, respectively), test positive and negative predictive value, effects on quality-adjusted life years, country specific average costs per myotoxicity event and costs as well as efficacy of treatment alternatives, precise estimates about statin cost-effectiveness in Brazil and its dependency on genotyping costs can currently not be provided.

Final remarks and conclusions

Brazilian studies have brought an extensive contribution of pharmacogenomics of statin response, focusing mainly on LDL cholesterol reduction. However, few studies have explored genetic variants associated with SRAE or pleiotropic effects in Brazilian cohorts. *SLCO1B1*5* and **15* variants were not associated with myopathy in atorvastatin users, problably due to small sample size and SRAE self-reports. No Brazilian pharmacogenetic studies of simvastatin-induced myopathy were found, even though simvastatin is the most prescribed statin. Thus, inclusion of *SLCO1B1*5, *15* and **17* genotyping, as recommended by CPIC, into the Brazilian guidelines seems not warranted at this stage. However, given the strength of the association in other populations, further sufficiently powered studies should explore *SLCO1B1* genotyping to predict the risk of myotoxicity in statin users. Clinical studies should also explore further association of genetic variants with statin-related pleiotropic effects to identify patients who could benefit from these effects.

In conclusion, Brazilian studies have focused mainly on LDL cholesterol reduction; however clinical applications

of pharmacogenetic testing to predict statin efficacy is still far from achieving a consensus. Especially in Brazilians, none of the assessed genetic variants have been shown to be robustly linked to statin response. Therefore, preemptive genotyping is currently not a cost-effective strategy to optimize statin response in Brazilian patients. A possible strategy is to explore the contribution of rare genetic variants to cholesterol reduction in a global manner rather than punctual, frequent genetic variants.

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Declarations

Conflict of interest CDH, FDVG, TDCH, MHH, and RDCH declare no conflict of interest. YZ is co-founder and CEO of PersoMedix AB. VML is CEO and shareholder of HepaPredict AB, co-founder and chairman of the board of PersoMedix AB, and consultant for Enginzyme AB.

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APPENDIX 2 – Articles as co-author

ARTICLE IN PRESS

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Genomics, epigenomics and pharmacogenomics of Familial Hypercholesterolemia (FHBGEP): A study protocol

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BJC British Journal of Cancer

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ARTICLE

Genetics and Genomics

Population-scale predictions of DPD and TPMT phenotypes using a quantitative pharmacogene-specific ensemble classifier

Yitian Zhou[1], Carolina Dagli Hernandez[1,2] and Volker M. Lauschke[1]

BACKGROUND: Inter-individual differences in dihydropyrimidine dehydrogenase (*DPYD* encoding DPD) and thiopurine S-methyltransferase (TPMT) activity are important predictors for fluoropyrimidine and thiopurine toxicity. While several variants in these genes are known to decrease enzyme activities, many additional genetic variations with unclear functional consequences have been identified, complicating informed clinical decision-making in the respective carriers.
METHODS: We used a novel pharmacogenetically trained ensemble classifier to analyse *DPYD* and *TPMT* genetic variability based on sequencing data from 138,842 individuals across eight populations.
RESULTS: The algorithm accurately predicted in vivo consequences of *DPYD* and *TPMT* variants (accuracy 91.4% compared to 95.3% in vitro). Further analysis showed high genetic complexity of DPD deficiency, advocating for sequencing-based *DPYD* profiling, whereas genotyping of four variants in *TPMT* was sufficient to explain >95% of phenotypic TPMT variability. Lastly, we provided population-scale profiles of ethnogeographic variability in DPD and TPMT phenotypes, and revealed striking interethnic differences in frequency and genetic constitution of DPD and TPMT deficiency.
CONCLUSION: These results provide the most comprehensive data set of *DPYD* and *TPMT* variability published to date with important implications for population-adjusted genetic profiling strategies of fluoropyrimidine and thiopurine risk factors and precision public health.

British Journal of Cancer https://doi.org/10.1038/s41416-020-01084-0

BACKGROUND

Adverse drug reactions (ADRs) are a common phenomenon in cancer therapy, and the identification of patients at increased risk thus constitutes an important goal of precision oncology. In the last decade, genetic profiling has identified a multitude of variations that can guide selection and dosing of chemotherapeutic drugs.[1] Two of the most important examples of such pharmacogenetic biomarkers that have transitioned from research into clinical practice are germline variations in the dihydropyrimidine dehydrogenase (*DPYD* encoding DPD) and thiopurine S-methyltransferase (*TPMT*) genes.[2–4]

Fluoropyrimidines are cornerstones of oncological therapy used for the treatment of a wide range of solid tumours. Importantly, DPD deficiency is strongly associated with dose-limiting and sometimes life-threatening toxicity with 60–80% of DPD-deficient individuals experiencing severe ADRs compared to 10–20% of patients with normal enzyme function.[5,6] The most extensively studied variation associated with DPD deficiency is *DPYD*2A* (rs3918290), a splice donor variant that results in truncated protein without catalytic activity.[7] Recent meta-analyses moreover confirmed robust associations of *DPYD* I560S, D949V as well as of the intronic splice variant rs75017182 and the associated haplotype HapB3 with fluoropyrimidine toxicity,[8–10] and prospective testing for these variants followed by genotype-guided upfront dose adjustments significantly increased patient safety.[11–13]

Analogously to *DPYD*, individuals deficient in TPMT are more susceptible to life-threatening toxicity of thiopurines.[14] The most important decreased function alleles are *TPMT*2* (rs1800462), **3A* (rs1800460 and rs1142345) and **3C* (rs1142345).[15]

In addition to the well-characterised variants illustrated above, *DPYD* and *TPMT* harbour hundreds of additional rare genetic variations with unclear effects on enzyme function.[16,17] Recent advances in large-scale mutagenesis screens unlock exciting opportunities for the parallel experimental interrogation of the effect of thousands of variants,[18] as exemplified by the simultaneous characterisation of the effects of thousands of *TPMT* variants on intracellular abundance.[19] However, without experimental assessments of variant effects on enzyme activity, their interpretation has to rely on computational tools. In the last two decades, a multitude of computational prediction tools have been developed that consider sequence conservation as an indicator of variant deleteriousness, as well as various mechanistic parameters, such as impacts on physiochemical properties, post-translational modifications and structural features, such as protein stability and the disruption of binding interfaces.[20,21] These algorithms are mostly trained on pathogenic variants for which evolutionary conservation constitutes a suitable proxy.[22] However, evolutionary constraints for *DPYD* and *TPMT* are limited, and conservation scores are thus not the ideal metric to predict variant function. To overcome these problems,

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Cardiovascular Pharmacogenomics: An Update on Clinical Studies of Antithrombotic Drugs in Brazilian Patients

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Affiliations + expand

PMID: 34357562 DOI: 10.1007/s40291-021-00549-z

Abstract

Anticoagulant and antiplatelet drugs effectively prevent thrombotic events in patients with cardiovascular diseases, ischemic stroke, peripheral vascular diseases, and other thromboembolic diseases. However, genetic and non-genetic factors affect the response to antithrombotic therapy and can increase the risk of adverse events. This narrative review discusses pharmacogenomic studies on antithrombotic drugs commonly prescribed in Brazil. Multiple Brazilian studies assessed the impact of pharmacokinetic (PK) and pharmacodynamic (PD) gene variants on warfarin response. The reduced function alleles CYP2C9*2 and CYP2C9*3, and VKORC1 rs9923231 (c.-1639G>A) are associated with increased sensitivity to warfarin and a low dose requirement to prevent bleeding episodes, whereas CYP4F2 rs2108622 (p.Val433Met) carriers have higher dose requirements (warfarin resistance). These deleterious variants and non-genetic factors (age, gender, body weight, co-administered drugs, food interactions, and others) account for up to 63% of the warfarin dose variability. Few pharmacogenomics studies have explored antiplatelet drugs in Brazilian cohorts, finding associations between CYP2C19*2, PON1 rs662 and ABCC3 rs757421 genotypes and platelet responsiveness or clopidogrel PK in subjects with coronary artery disease (CAD) or acute coronary syndrome (ACS), whereas ITGB3 contributes to aspirin PK but not platelet responsiveness in diabetic patients. Brazilian guidelines on anticoagulants and antiplatelets recommend the use of a platelet aggregation test or genotyping only in selected cases of ACS subjects without ST-segment elevation taking clopidogrel, and also suggest CYP2C9 and VKORC1 genotyping before starting warfarin therapy to assess the risk of bleeding episodes or warfarin resistance.

› Epigenomics. 2021 May;13(10):779-791. doi: 10.2217/epi-2020-0462. Epub 2021 Apr 26.

Functional analysis of *PCSK9* 3'UTR variants and mRNA–miRNA interactions in patients with familial hypercholesterolemia

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PMID: 33899508 DOI: 10.2217/epi-2020-0462

Abstract

Aim: Functional analysis of *PCSK9* 3'UTR variants and mRNA-miRNA interactions were explored in patients with familial hypercholesterolemia (FH). **Materials & methods:** *PCSK9* 3'UTR variants were identified by exon-targeted gene sequencing. Functional effects of 3'UTR variants and mRNA-miRNA interactions were analyzed using *in silico* and *in vitro* studies in HEK293FT and HepG2 cells. **Results:** Twelve *PCSK9* 3'UTR variants were detected in 88 FH patients. c.*75C >T and c.*345C >T disrupted interactions with miR-6875, miR-4721 and miR-564. Transient transfection of the c.*345C >T decreased luciferase activity in HEK293FT cells. miR-4721 and miR-564 mimics reduced *PCSK9* expression in HepG2 cells. **Conclusion:** *PCSK9* c.*345C >T has a possible role as loss-of-function variant. miR-4721 and miR-564 downregulate *PCSK9* and may be useful to improve lipid profile in FH patients.

Keywords: 3′UTR variants; PCSK9; epigenomics; familial hypercholesterolemia; functional analysis; microRNAs.

APPENDIX 3 - Ethical approval

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Ultrassequenciamento exômico dos principais genes relacionados com a hipercolesterolemia familiar

Pesquisador: Jéssica Bassani Borges

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 8

CAAE: 24618713.0.1001.5462

Instituição Proponente: Instituto Dante Pazzanese de Cardiologia - SP

Patrocinador Principal: Financiamento Próprio
CNPQ
FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 2.587.235

Apresentação do Projeto:

Nº DO PROTOCOLO DO CEP:4398/ 2013

A hipercolesterolemia familial (HF) é uma doença autossômica dominante com bases genéticas ainda não totalmente esclarecidas. O presente
estudo propõe a análise genômica, epigenômica e farmacogenômica de portadores de HF monogênica e poligênica. Serão recrutados pacientes
com HF diagnosticada fenotipicamente, em seis centros de pesquisa de diferentes regiões do Brasil. Os métodos utilizados incluem: (i)
ultrassequenciamento dos principais genes relacionados à HF e outras dislipidemias primárias utilizando o equipamento MiSeq (Illumina); (ii) análise
funcional de novas variantes nos genes LDLR, APOB e PCSK9 por citometria de fluxo, com estudo de interação com receptores de LDL em
linfócitos primários e com estudo de mutagênese dirigida utilizando CRISPR/Cas9 em células HepG2 e HUVEC; (iii) perfil de expressão diferencial
de miRNAs circulantes em amostras de plasma por PCR array; (iv) perfil de metilação dos genes LDLR, APOB e PCSK9 em leucócitos por

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pirossequenciamento; (v) análise farmacogenômica incluindo genes envolvidos no metabolismo e na resposta a hipolipemiantes. As análises de
bioinformática serão realizadas utilizando-se os programas MiSeq Reporter e CLC Genomic Workbench. Este estudo é pioneiro no país e a sua
realização na população brasileira, altamente miscigenada, é inovadora e desafiadora. Os resultados deste estudo visam contribuir para o
conhecimento das bases moleculares da HF, fornecer elementos para direcionamento no diagnóstico genético e na terapia personalizada de
pacientes afetados, e possibilitar a criação de um banco nacional de dados genômicos que auxilie na orientação da conduta diagnóstica molecular
para pacientes com fenótipo HF e seus familiares. Contribuirá para a formação de recursos humanos, consolidação da pesquisa e integração das
instituições envolvidas.

Objetivo da Pesquisa:

Objetivo Primário:

Identificar as causas genéticas das dislipidemias primárias dos pacientes diagnosticados fenotipicamente no Instituto Dante Pazzanese de
Cardiologia.

Objetivo Secundário:

• Sequenciar os exomas dos genes relacionados a dislipidemias de origem genética e verificar o perfil das novas variantes polimórficas em pacientes
com diagnóstico de hipercolesterolemia familiar.

• Identificar novas variantes nos genes relacionados com alteração do metabolismo do colesterol.

• Avaliar as correlações entre as mutações e as alterações fenotípicas.

• Caracterizar a funcionalidade de variantes do gene LDLR in vitro pelo perfil de captação de LDL, em cultura primária de linfócitos oriundos de
portadores de HF;

• Caracterizar a funcionalidade de variantes do gene APOB in vitro pelo perfil de captação de LDL oriunda de portadores de HF, em células HepG2 e
HUVEC;

• Realizar a mutagênese de variantes dos genes LDLR e PCSK9, encontradas no sequenciamento, em células HepG2 e HUVEC para avaliar sua
funcionalidade independente da presença de outras variantes.

Endereço: Av. Dr. Dante Pazzanese N.º 500, Torre 6º andar
Bairro: Ibirapuera **CEP:** 04.012-909
UF: SP **Município:** SAO PAULO
Telefone: (11)5085-6040 **Fax:** (11)5085-6040 **E-mail:** cep@dantepazzanese.org.br

• Avaliar o perfil de expressão diferencial de miRNAs circulantes entre os diferentes padrões fenotípicos de HF encontrados em nossa população;
• Avaliar o perfil de metilação das ilhas CpG dos genes LDLR, APOB e PCSK9 de portadores de HF com diferentes padrões fenotípicos;
• Avaliar a associação de variantes em genes envolvidos no metabolismo e na resposta a medicamentos hipolipemiantes, em pacientes HF.

Avaliação dos Riscos e Benefícios:

Riscos:

Os participantes deste estudo não se submeterão a procedimentos adicionais, exceto a coleta de material biológico para dosagem dos
biomarcadores, que em alguns serviços fazem parte da rotina do atendimento desses pacientes. Os riscos físicos referentes à coleta de amostra de
sangue para o estudo são: hematoma, flebite, breve dor. Algumas pessoas têm vertigens quando coletam sangue, mas os sintomas desaparecem
quando a pessoa se deita.

Benefícios:

Os participantes deste estudo não poderão receber nenhum benefício direto por fazer parte do Estudo. As informações obtidas deste estudo serão
importantes para melhorar o diagnóstico, prognóstico e a prevenção dos eventos cardiovasculares em pacientes com dislipidemias primárias.

Comentários e Considerações sobre a Pesquisa:

Sem restrições do ponto de vista de ética em pesquisa

Considerações sobre os Termos de apresentação obrigatória:

1_Inclusão de 4 centros participantes;
USP
HC de porto alegre
UNICAMP
Cruzeiro do sul educacional s.a

Conclusões ou Pendências e Lista de Inadequações:

Sem restrições do ponto de vista de ética em pesquisa

Endereço: Av. Dr. Dante Pazzanese N.º 500, Torre 6º andar
Bairro: Ibirapuera **CEP:** 04.012-909
UF: SP **Município:** SAO PAULO
Telefone: (11)5085-6040 **Fax:** (11)5085-6040 **E-mail:** cep@dantepazzanese.org.br

Considerações Finais a critério do CEP:

Diante do exposto, O Comitê de Ética em Pesquisa do Instituto Dante Pazzanese de Cardiologia, de acordo com as atribuições definidas na Resolução CNS nº 466 de 2012, resolução 510/96 e da Norma Operacional nº 001 de 2013 do CNS, em reunião ordinária de 27/03/2018 manifesta-se pela aprovação da emenda.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Outros	emenda_inclusaocentors.pdf	20/03/2018 09:50:50	Pedro Silvio Farsky	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1072404_E6.pdf	16/03/2018 14:26:04		Aceito
Outros	Carta_Emenda.pdf	30/06/2016 16:01:00	Jéssica Bassani Borges	Aceito
Projeto Detalhado / Brochura Investigador	HF_CEP_ultima_versao.pdf	30/06/2016 16:00:32	Jéssica Bassani Borges	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	HF_TCLE2.pdf	30/06/2016 15:59:44	Jéssica Bassani Borges	Aceito
Declaração de Pesquisadores	Justificativa_CEPIDPC_vinculo_Institucional.pdf	11/12/2015 16:40:04	Jéssica Bassani Borges	Aceito
Folha de Rosto	PLATAFORMA BRASIL - JESSICA.pdf	06/03/2015 11:01:08		Aceito
Outros	Troca de Pesquisador.pdf	08/12/2014 10:17:29		Aceito
Outros	Troca pesquisador.pdf	08/12/2014 10:17:29		Aceito
Outros	Carta de mudança de pesquisador.pdf	14/11/2014 12:20:49		Aceito
Outros	DECLARAÇÕES CEP Thiago D C Hirata.pdf	12/11/2013 14:32:38		Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Endereço: Av. Dr. Dante Pazzanese N.º 500, Torre 6º andar
Bairro: Ibirapuera **CEP:** 04.012-909
UF: SP **Município:** SAO PAULO
Telefone: (11)5085-6040 **Fax:** (11)5085-6040 **E-mail:** cep@dantepazzanese.org.br

SAO PAULO, 09 de Abril de 2018

Assinado por:
Pedro Silvio Farsky
(Coordenador)

Endereço: Av. Dr. Dante Pazzanese N.º 500, Torre 6º andar
Bairro: Ibirapuera **CEP:** 04.012-909
UF: SP **Município:** SAO PAULO
Telefone: (11)5085-6040 **Fax:** (11)5085-6040 **E-mail:** cep@dantepazzanese.org.br

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Ultrassequenciamento exômico dos principais genes relacionados com a hipercolesterolemia familiar

Pesquisador: Jéssica Bassani Borges

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 2

CAAE: 24618713.0.3001.0067

Instituição Proponente: Faculdade de Ciências Farmacêuticas da Universidade de São Paulo

Patrocinador Principal: Financiamento Próprio
CNPQ
FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 2.708.638

Apresentação do Projeto:
Trata-se de um projeto destinado à avaliação de genes relacionados à hipercolesterolemia familiar. Serão recrutados 150 indivíduos adultos acompanhados no Instituto Dante Pazzanese de Cardiologia, em São Paulo, sendo 100 indivíduos com dosagem elevada de colesterol e 50 indivíduos controle. Serão coletados 20 ml de sangue dos participantes e amostra de sangue poderá ser armazenada por um período de 5 anos.

Objetivo da Pesquisa:
O objetivo principal é avaliar a presença de genes relacionados com a hipercolesterolemia familiar em pacientes que já receberam o diagnóstico clínico da doença e determinar o perfil genético nesta população.

Avaliação dos Riscos e Benefícios:
Não há benefícios diretos aos participantes e os riscos são os inerentes à coleta de sangue e à punção venosa. Como os pacientes são indivíduos adultos, o volume de sangue a ser coletado é considerado adequado.

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

Comentários e Considerações sobre a Pesquisa:

A pesquisa é importante, pois busca trazer mais conhecimentos sobre as causas da hipercolesterolemia.

Considerações sobre os Termos de apresentação obrigatória:

Trata-se de emenda de projeto de pesquisa já aprovado pelo CEP da Instituição Proponente (Instituto Dante Pazzanese de Cardiologia-SP) e do Centro Colaborador (Faculdade de Ciências Farmacêuticas da USP). A pesquisadora principal solicita a inclusão de 4 outros centros colaboradores:

-Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da USP,

-Hospital de Clínicas de Porto Alegre,

-Faculdade de Ciências Médicas da UNICAMP,

-Cruzeiro do Sul Educacional SA.

Recomendações:

As cartas de anuência das Instituições co-participadoras foram anexadas.

Conclusões ou Pendências e Lista de Inadequações:

Nenhuma

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1109917.pdf	04/06/2018 15:03:35		Aceito
Outros	Carta_de_Anuencia_UNICSUL.pdf	04/06/2018 14:59:34	Jéssica Bassani Borges	Aceito
Outros	Carta_de_Anuencia_UNICAMP.pdf	04/06/2018 14:58:59	Jéssica Bassani Borges	Aceito
Outros	Carta_de_Anuencia_UFRGS.pdf	04/06/2018 14:58:28	Jéssica Bassani Borges	Aceito
Outros	Carta_de_anuencia_INCOR.pdf	04/06/2018 14:58:01	Jéssica Bassani Borges	Aceito
Outros	Carta_resposta_Pendencia_CEP_FCF_USP.pdf	04/06/2018 14:57:23	Jéssica Bassani Borges	Aceito
Outros	emenda_inclusaocentors.pdf	20/03/2018 09:50:50	Pedro Silvio Farsky	Aceito

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

Outros	Carta_Emenda.pdf	30/06/2016 16:01:00	Jéssica Bassani Borges	Aceito
Projeto Detalhado / Brochura Investigador	HF_CEP_ultima_versao.pdf	30/06/2016 16:00:32	Jéssica Bassani Borges	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	HF_TCLE2.pdf	30/06/2016 15:59:44	Jéssica Bassani Borges	Aceito
Outros	Troca de Pesquisador.pdf	08/12/2014 10:17:29		Aceito
Outros	Troca pesquisador.pdf	08/12/2014 10:17:29		Aceito
Outros	Carta de mudança de pesquisador.pdf	14/11/2014 12:20:49		Aceito
Outros	DECLARAÇÕES CEP Thiago D C Hirata.pdf	12/11/2013 14:32:38		Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 12 de Junho de 2018

Assinado por:
Elvira Maria Guerra Shinohara
(Coordenador)

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO EXÔMICA DOS PRINCIPAIS GENES RELACIONADOS COM A HIPERCOLESTEROLEMIA FAMILIAR

Pesquisador: Vivian Nogueira Silbiger

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 24618713.0.2001.5292

Instituição Proponente: Departamento de Análises Clínicas e Toxicológicas

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.144.318

Data da Relatoria: 26/06/2015

Apresentação do Projeto:

Trata-se de um projeto de Doutorado com o titulo AVALIAÇÃO EXÔMICA DOS PRINCIPAIS GENES RELACIONADOS COM A HIPERCOLESTEROLEMIA FAMILIAR,onde será realizada a coleta de sangue, extração de DNA genômico e ultrasequenciamento por MiSeq (illumina)dos genes relacionados à dislipdemias

Objetivo da Pesquisa:

Identificar as causas genéticas das dislipidemias primárias dos pacientes diagnosticados fenotipicamente no Instituto Dante Pazzanese de Cardiologia.

Avaliação dos Riscos e Benefícios:

Os riscos físicos referentes à coleta de amostra de sangue para o estudo são: hematoma, flebite, breve dor. Algumas pessoas têm vertigens quando coletam sangue, mas os sintomas desaparecem quando a pessoa se deita. As informações obtidas deste estudo serão importantes para melhorar o diagnóstico, prognóstico e a prevenção dos eventos cardiovasculares em pacientes com dislipidemias primárias.

Comentários e Considerações sobre a Pesquisa:

O projeto preenche os requisitos fundamentais da Resolução CNS 466 de 12 de Dezembro de

Endereço: Avenida Nilo Peçanha, 620 - 3º subsolo
Bairro: Petrópolis **CEP:** 59.012-300
UF: RN **Município:** NATAL
Telefone: (84)3342-5003 **Fax:** (84)3202-3941 **E-mail:** cep_huol@yahoo.com.br

2012, sobre as Diretrizes e Normas Regulamentadoras de Pesquisa Envolvendo Seres Humanos, do Conselho Nacional de Saúde / Agência Nacional de Vigilância Sanitária.

Considerações sobre os Termos de apresentação obrigatória:

Apresentou toda documentação exigida

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

O trabalho por ser Multicêntrico já foi aprovado pelo CEP DO INSTITUTO DANTE PAZZANESE DE CARDIOLOGIA

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Trata-se de um projeto Multicêntrico aprovado pelo CEP DO INSTITUTO DANTE PAZZANESE DE CARDIOLOGIA com parecer favorável

NATAL, 09 de Julho de 2015

Assinado por:
HELIO ROBERTO HEKIS
(Coordenador)

Endereço: Avenida Nilo Peçanha, 620 - 3º subsolo
Bairro: Petrópolis **CEP:** 59.012-300
UF: RN **Município:** NATAL
Telefone: (84)3342-5003 **Fax:** (84)3202-3941 **E-mail:** cep_huol@yahoo.com.br

APPENDIX 4 – Educational history

USP Universidade de São Paulo

FICHA DO ALUNO

Janus

9142 - 4316757 / 1 - Carolina Dagli Hernandez

Email:	carolina.hernandez@usp.br
Data de Nascimento:	12/05/1991
Cédula de Identidade:	RG - 38.219.402-0 - SP
Local de Nascimento:	Estado de São Paulo
Nacionalidade:	Brasileira
Graduação:	Farmacêutica-Bioquímica - Faculdade de Ciências Farmacêuticas - Universidade de São Paulo - São Paulo - Brasil - 2015

Curso:	Doutorado Direto
Programa:	Farmácia (Fisiopatologia e Toxicologia)
Área:	Fisiopatologia
Data de Matrícula:	06/12/2016
Início da Contagem de Prazo:	06/12/2016
Data Limite para o Depósito:	01/12/2021
Orientador:	Prof(a). Dr(a). Rosario Dominguez Crespo Hirata - 06/12/2016 até o presente Email: rosariohirata@usp.br

Proficiência em Línguas:

Inglês, Aprovado em 06/12/2016

Data de Aprovação no Exame de Qualificação:	Aprovado em 19/12/2018
Estágio no Exterior:	Karolinska Institutet, Suécia - Período de 03/01/2020 até 17/12/2020

USP Universidade de São Paulo

FICHA DO ALUNO

Janus

Data do Depósito do Trabalho:

Título do Trabalho:

Data Máxima para Aprovação da Banca:

Data de Aprovação da Banca:

Data Máxima para Defesa:

Data da Defesa:

Resultado da Defesa:

Histórico de Ocorrências: Primeira Matrícula em 06/12/2016
Aluno matriculado no Regimento da Pós-Graduação USP (Resolução nº 6542 em vigor de 20/04/2013 até 28/03/2018).

Última ocorrência: Matrícula de Acompanhamento em 09/07/2021

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
MCP5835-3/6	Princípios de Análise de Dados e de Bioestatística (Faculdade de Medicina - Universidade de São Paulo)	06/03/2017	19/03/2017	60	0	-	-	N	Matrícula cancelada
FBF5820-1/2	Segurança do Paciente no Uso de Medicamentos	06/03/2017	19/03/2017	60	0	-	-	N	Matrícula cancelada
PSP5121-1/4	Bioestatística (Faculdade de Saúde Pública - Universidade de São Paulo)	07/03/2017	23/05/2017	90	6	95	A	N	Concluída
FBC5792-4/1	Tópicos em Fisiopatologia e Toxicologia III	07/03/2017	19/06/2017	15	0	-	-	N	Matrícula cancelada
BMF5881-1/1	Medicina Personalizada: Contribuições da Farmacogenômica e da Nanotecnologia (Instituto de Ciências Biomédicas - Universidade de São Paulo)	01/06/2017	12/07/2017	90	6	100	A	N	Concluída
MCP5871-1/4	Tratamento de Dados em Estudo Científico (Faculdade de Medicina - Universidade de São Paulo)	26/06/2017	02/07/2017	30	0	-	-	N	Matrícula cancelada
FBC5780-2/4	Análise de Dados Aplicados às Pesquisas Biológicas	07/08/2017	17/09/2017	90	6	95	A	N	Concluída
FBC5708-7/1	Farmacogenômica Cardiovascular	07/08/2017	17/09/2017	90	0	-	-	N	Turma cancelada

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
MAG5001-1/3	Genética Humana (Instituto de Biociências - Universidade de São Paulo)	10/08/2017	22/11/2017	120	0	-	-	N	Matrícula cancelada
FBC5766-6/1	Tópicos em Fisiopatologia e Toxicologia IV	15/08/2017	27/11/2017	15	1	85	A	N	Concluída
FBC5792-5/1	Tópicos em Fisiopatologia e Toxicologia III	05/03/2018	19/06/2018	15	1	75	A	N	Concluída
VPS5717-7/2	Preparação Pedagógica (Faculdade de Medicina Veterinária e Zootecnia - Universidade de São Paulo)	12/03/2018	25/03/2018	30	2	100	A	N	Concluída
FBA5728-4/6	Aprimoramento Pedagógico	03/04/2018	30/04/2018	60	0	-	-	N	Matrícula cancelada
BIE5782-4/3	Uso da Linguagem R para Análise de Dados em Ecologia (Instituto de Biociências - Universidade de São Paulo)	09/04/2018	29/04/2018	60	0	-	-	N	Matrícula cancelada
TIC5021-1/1	Genética em Cardiologia (Instituto Dante Pazzanese de Cardiologia - Universidade de São Paulo)	16/04/2018	06/05/2018	90	6	100	A	N	Concluída
EPI5713-2/1	Introdução ao R para a Análise de Dados (Faculdade de Saúde Pública - Universidade de São Paulo)	04/06/2018	09/07/2018	30	0	-	-	N	Pré-matrícula indeferida
FBC5757-8/2	Tópicos em Fisiopatologia e Toxicologia II	02/08/2018	14/11/2018	15	1	75	A	N	Concluída
FLS6397-3/1	Introdução à Programação e Ferramentas Computacionais para as Ciências Sociais (Faculdade de Filosofia, Letras e Ciências Humanas - Universidade de São Paulo)	22/03/2019	13/06/2019	120	8	91	A	N	Concluída
VPS5741-1/3	Manipulação e Visualização de Dados no R (Faculdade de Medicina Veterinária e Zootecnia - Universidade de São Paulo)	01/04/2019	14/04/2019	60	0	-	-	N	Matrícula cancelada
EPI5713-2/2	Introdução ao R para a Análise de Dados (Faculdade de Saúde Pública - Universidade de São Paulo)	03/06/2019	07/07/2019	30	0	-	-	N	Pré-matrícula indeferida
FBC5957-1/1	Farmacogenômica e Epigenômica	09/09/2019	07/10/2019	60	4	100	A	N	Concluída

Universidade de São Paulo

FICHA DO ALUNO

Janus

	Créditos mínimos exigidos		Créditos obtidos
	Para exame de qualificação	Para depósito de tese	
Disciplinas:	0	25	41
Estágios:			
Total:	0	25	41

Créditos Atribuídos à Tese: 167

Conceito a partir de 02/01/1997:

A - Excelente, com direito a crédito; B - Bom, com direito a crédito; C - Regular, com direito a crédito; R - Reprovado; T - Transferência.

Um(1) crédito equivale a 15 horas de atividade programada.

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Documento emitido às 18:23:31 horas do dia 22/11/2021 (hora e data de Brasília)

Código de controle: HWN5-P43Z-DW6C-QKF2

Código de controle válido até: 22/12/2021